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(54) Titre : METHODES DE DIAGNOSTIC ET DE TRAITEMENT DE CANCER
(54) Title: METHODS FOR DIAGNOSING AND TREATING CANCER

(57) Abrégé/Abstract:
Disclosed is a method for identifying a subject at risk of developing a recurrent or metastatic cancer, comprising detecting PD-L1$^+$ circulating tumor cells in a blood sample, a tissue fluid sample or a specimen of the subject. Also disclosed is method for treating a cancer comprising identifying a subject having one ore more PD-L1$^+$ circulating tumor cells by detecting PD-L1$^+$ circulating tumor cells in a blood sample, a tissue fluid sample or a specimen of the subject, and administering a treatment to the subject.

ABSTRACT OF THE DISCLOSURE

Disclosed is a method for identifying a subject at risk of developing a recurrent or metastatic cancer, comprising detecting PD-L1$^+$ circulating tumor cells in a blood sample, a tissue fluid sample or a specimen of the subject. Also disclosed is method for treating a cancer comprising identifying a subject having one ore more PD-L1$^+$ circulating tumor cells by detecting PD-L1$^+$ circulating tumor cells in a blood sample, a tissue fluid sample or a specimen of the subject, and administering a treatment to the subject.

TITLE OF THE INVENTION

METHODS FOR DIAGNOSING AND TREATING CANCER

FIELD OF THE INVENTION

[0001] The present invention pertains to methods for diagnosing and treating cancer.

BACKGROUND OF THE INVENTION

[0002] Circulating tumor cells (CTCs) presence in circulation play active roles in mediating metastasis[1]. Enumeration of CTCs was reported as a prognostic predictor for metastatic colorectal cancer (mCRC) patients [2]. In most of the previous studies, the number of CTCs was enumerated from blood drawn by venipuncture of the forearm [3, 4]. It was suggested that the presence of circulating tumor cells expressing PD-L1 in non-small cell lung cancer patients at 6 months after the treatment of Nivolumab (PD-1 inhibitor) corresponds to a therapy escape or poor prognosis [5].

[0003] Until now, there is no promising method or kit for early detection or diagnosis of a metastatic cancer.

BRIEF SUMMARY OF THE INVENTION

[0004] The present invention is based on the unexpected finding that in patients having a gastrointestinal cancer or head and neck squamous-cell carcinoma, the presence/level of circulating tumor cells expressing PD-L1 (called as "PD-L1$^+$ circulating tumor cells") before a therapy for, or during a surgery of curative resection of the gastrointestinal cancer or head and neck squamous-cell carcinoma, correlates with the metastasis of cancers or the prognosis of patients.

[0005] Accordingly, in one aspect, the present invention provides a method for identifying a subject at risk of developing a recurrent or metastatic cancer, comprising detecting PD-L1$^+$ circulating tumor cells in a blood sample, a tissue fluid sample or a specimen of the subject, wherein the presence of one or more PD-L1$^+$ circulating tumor cells indicates that the subject is at risk of developing a recurrent or metastatic cancer, and wherein the subject has a gastrointestinal cancer or head and neck squamous-cell carcinoma and the blood sample, the tissue fluid sample or the specimen is derived from the subject before a therapy for, or during a

surgery of curative resection of the gastrointestinal cancer or head and neck squamous-cell carcinoma.

[0006] In one embodiment of the present invention, the cancer is a gastrointestinal cancer, particularly a colorectal cancer. In another embodiment, the cancer is head and neck squamous-cell carcinoma.

[0007] In another aspect, the present invention provides a method for treating a cancer comprising: (a) identifying a subject having one or more PD-L1$^+$ circulating tumor cells by detecting PD-L1$^+$ circulating tumor cells in a blood sample, a tissue fluid sample or a specimen of the subject, wherein the subject has a gastrointestinal cancer or head and neck squamous-cell carcinoma and the blood sample, the tissue fluid sample or the specimen is derived from the subject before a therapy for, or during a surgery of curative resection of the gastrointestinal cancer or head and neck squamous-cell carcinoma, and (b) administering a treatment to the subject.

[0008] In a further aspect, present invention provides a method for predicting prognosis of a patient having gastrointestinal cancer or head and neck squamous-cell carcinoma, comprising detecting PD-L1$^+$ circulating tumor cells in a blood sample, a tissue fluid sample or a specimen of the patient, wherein the presence of one or more PD-L1$^+$ circulating tumor cells indicates poor prognosis or overall survival of the patient, wherein the blood sample, the tissue fluid sample or the specimen is derived from the patient before a therapy for, or during a surgery of curative resection of the gastrointestinal cancer or head and neck squamous-cell carcinoma.

[0009] According to certain preferred embodiments of the present invention, said treatment is an immunotherapy. In some other embodiments, the subject is administered with an immunotherapy, in combination with a chemotherapy or a targeted therapy.

[0010] According to certain embodiments of the present invention, the cancer is a recurrent or metastatic cancer.

[0011] In one embodiment of the present invention, the cancer is a gastrointestinal cancer, particularly a colorectal cancer. In another embodiment, the cancer is head and neck squamous-cell carcinoma.

[0012] It is to be understood that both the foregoing general description and the following detailed description are exemplary and explanatory only and are not restrictive of the invention.

BRIEF DESCRIPTION OF THE SEVERAL VIEWS OF THE DRAWINGS

[0013] The foregoing summary, as well as the following detailed description of the invention, will be better understood when read in conjunction with the appended drawings. For the purpose of illustrating the invention, there are shown in the drawings embodiments which are presently preferred.

[0014] In the drawings:

[0015] **Fig. 1** illustrates the sample preparation for circulating tumor cell (CTC) enumeration via MiSelect R System.

[0016] **Fig. 2** shows typical CTC images.

[0017] **Fig. 3** shows the comparison of CTC counts in peripheral blood (PB) and mesenteric venous blood (MVB).

[0018] **Fig. 4A** shows the results on CTC counts in PB of colorectal cancer (CRC) patients at various stages. **Fig. 4B** shows the results on CTC counts in MVB of CRC patients at various stages.

[0019] **Fig. 5** shows the heterogeneity of PD-L1 expressions on CTC.

[0020] **Fig. 6** shows PD-L1$^+$ CTC counts in PB and MVB.

[0021] **Fig. 7** shows PD-L1$^+$ CTC counts in MVB at various stages.

[0022] **Fig. 8** shows the frequency of occurrence of PD-L1$^+$ CTC at various stages.

[0023] **Fig. 9** shows the heterogeneity of PD-L1 expressions on CTC

DETAILED DESCRIPTION OF THE INVENTION

[0024] In one aspect, the present invention provides a method for identifying a subject at risk of developing a recurrent or metastatic cancer, comprising detecting PD-L1$^+$ circulating tumor cells in a blood sample, a tissue fluid sample or a specimen of the subject, wherein the presence of PD-L1$^+$ circulating tumor cells indicates that the subject is at risk of developing a recurrent or metastatic cancer, and wherein the subject has a gastrointestinal cancer or head and neck squamous-cell carcinoma and the blood sample, the tissue fluid sample or the specimen is derived from the subject before a therapy for, or during a surgery of curative resection of the gastrointestinal cancer or head and neck squamous-cell carcinoma.

[0025] According to the present invention, a higher level of the PD-L1$^+$ circulating tumor cells indicates a higher risk of developing a recurrent or metastatic cancer.

[0026] In one embodiment of the present invention, the cancer is a gastrointestinal cancer, particularly a colorectal cancer. For gastrointestinal cancers, the blood sample is preferably a mesenteric venous blood sample. The mesenteric venous blood sample may be derived during surgeries of curative resection of the gastrointestinal cancer. The gastrointestinal cancer includes but is not limited an esophageal cancer, a gastric cancer, a gastrointestinal stromal tumor, a pancreatic cancer, a liver cancer, a gallbladder cancer, a colorectal cancer, and an anal cancer. In a particular example of the present invention, the cancer is a colorectal cancer.

[0027] In another embodiment, the cancer is head and neck squamous-cell carcinoma.

[0028] In another aspect, the present invention provides a method for treating a cancer comprising: (a) identifying a subject having PD-L1$^+$ circulating tumor cells by detecting PD-L1$^+$ circulating tumor cells in a blood sample, a tissue fluid sample or a specimen of the subject, wherein the subject has a gastrointestinal cancer or head and neck squamous-cell carcinoma and the blood sample, the tissue fluid sample or the specimen is derived from the subject before a therapy for, or during a surgery of curative resection of the gastrointestinal cancer or head and neck squamous-cell carcinoma, and (b) administering a treatment to the subject.

[0029] In a further aspect, present invention provides a method for predicting prognosis of a patient having a gastrointestinal cancer or head and neck squamous-cell carcinoma, comprising detecting PD-L1$^+$ circulating tumor cells in a blood sample, a tissue fluid sample or a specimen of the patient, wherein the presence of one or more PD-L1$^+$ circulating tumor cells indicates poor prognosis or overall survival of the patient, and wherein the blood sample, the tissue fluid sample or the specimen is derived from the patient before a therapy for, or during a surgery of curative resection of the gastrointestinal cancer or head and neck squamous-cell carcinoma.

[0030] According to the present invention, a higher level of the PD-L1$^+$ circulating tumor cells indicates a worse prognosis of the patient.

[0031] The treatment includes but is not limited to immunotherapy, chemotherapy, radiation therapy, hormone therapy, or a combination thereof.

[0032] According to certain preferred embodiments of the present invention, said treatment is an immunotherapy. In some other embodiments, the subject is administered with an immunotherapy, in combination with a chemotherapy or a targeted therapy.

[0033] According to certain embodiments of the present invention, the cancer is a recurrent or metastatic cancer.

[0034] In one embodiment of the present invention, the cancer is a gastrointestinal cancer, particularly a colorectal cancer. In another embodiment, the cancer is head and neck squamous-cell carcinoma.

[0035] For gastrointestinal cancers, the blood sample is preferably a mesenteric venous blood sample. The mesenteric venous blood sample may be derived during surgeries of curative resection of the gastrointestinal cancer.

[0036] In certain embodiments of the present invention, the cancer is a gastrointestinal cancer, which includes, but is not limited to, an esophageal cancer, a gastric cancer, a gastrointestinal stromal tumor, a pancreatic cancer, a liver cancer, a gallbladder cancer, a colorectal cancer, and an anal cancer. In a particular example of the present invention, the cancer is a colorectal cancer.

[0037] In some other embodiments of the present invention, the cancer is head and neck squamous-cell carcinoma.

[0038] The present invention is further illustrated by the following examples, which are provided for the purpose of demonstration rather than limitation.

[0039] **Examples**

[0040] **Materials and Methods**

[0041] 1. Patient characteristics

[0042] A total of 116 patients who underwent curative surgical resection at Taipei Veterans General Hospital between April 2016 and September 2017 were enrolled. A total 26 HNSCC, 9 HCC, 5 UC and 2 RCC patients who underwent receiving immunotherapy at Taipei Veterans General Hospital between October 2016 and September 2017 were also enrolled. The enrollment procedures followed the protocols approved by the Internal Review Board of Taipei Veterans General Hospital. All patients provided written informed consent. Patients who prior to colonoscopy examination or suspected of having colorectal cancer (CRC) with unconfirmed clinical stages were recruited. Six patients who had histological diagnosed tubular adenoma were also enrolled. The average (±SD) age of the assessable patients was 63.6 ± 12.5 years (median, 64) (see **Table 1** below). Primary tumor staging was confirmed by histologic examination of the resected primary tumor. Based on histologic examination, the subjects consisted of 116 CRC patients (24 stage I, 38 stage II, 42 stage III and 12 stage IV, respectively).

[0043] **Table 1.** Clinicopathological characteristics of the study population

	Stage I (n=24)	Stage II (n=38)	Stage III (n=42)	Stage IV (n=12)
Age Median (range)	64 (37-79)	68 (38-92)	64 (37-84)	59 (47-90)
Gender				
Male	10	28	22	8
Female	14	10	20	4
Tumor location				
Colon	20	27	29	9
Rectum	4	11	13	3
T stage				
T1	14	0	3	0
T2	10	0	5	1
T3	0	24	21	4
T4	0	14	13	7
N stage				
N0	24	38	0	2
N1	0	0	31	4
N2	0	0	11	6
CEA (≥ 5 ng/mL)	3	17	17	10
CA 19-9 (≥ 37 ng/mL)	1	7	8	5

[0044] 2. Blood sample collection

[0045] Blood samples for CTC analysis were obtained from CRC patients before curative resection of tumor. Sampling of blood from the antecubital veins of patients with CRC was conducted before surgery. During surgery, mesenteric venous blood samples were drawn from the main drainage vein of the diseased segment of the colon, for example, the inferior mesenteric vein for cancer of the sigmoid colon or rectum or the ileocolic vein if the tumor was located on the right side of the colon. To minimize the possibility of releasing CTCs by mechanical manipulation, colonoscopy was scheduled at least 1 day before the surgery. The surgical approach sought vascular control first, that is, ligation of the feeding artery at the beginning, followed by mesenteric vein cannulation and blood drawing. The tumor was left untouched until late in the surgery. Peripheral blood (PB) and mesenteric venous blood (MVB) samples for CTC analysis were obtained from 116 patients. Blood samples for CTC analysis were also obtained from other cancer patients before drug administrations.

[0046] 3. CTC enumeration and PD-L1 expression test on CTC

[0047] The sample preparation is shown in **Fig. 1**. Transfer two 4-ml aliquots of blood from K_2EDTA tube into two correspondingly labeled 50 ml conical centrifuge tubes. Samples incubate with Sorting Reagent (PE-conjugated anti-EpCAM antibody) of SelectKit for 20 minutes at room temperature. After staining, spilt each 4ml blood to two 2-ml aliquots into two correspondingly labeled 50 ml conical centrifuge tubes, add 24 ml ISOTON Diluent into each tube. Centrifuge the sample at 800 x g for a full 10 minutes with the brake off using a swing bucket centrifuge at room temperature. Following centrifugation, remove 24 ml supernatant of each tube and mix the samples following recovery of two 2-ml aliquots into 4ml aliquots for CTC analysis. Process on the MiSelect R System within 1 hour of sample preparation.

[0048] MiSelect R System with SelectChip Dual (MiCareo Taiwan Co., Ltd) can sort and enrich CTC. Once the aliquots containing CTCs have been collected in SelectChip, blood cells, especially RBCs, are removed from the CTCs by an on-chip filtration system. After enrichment of CTCs, Fixation and Staining Reagent of SelectKit are automated added for identification and enumeration of CTCs. Anti-panCK APC is specific targeting for the intracellular protein cytokeratin, DAPI stains for the cell nucleus and anti-CD45 FITC is specific for leukocytes. An event is classified as a tumor cell when its morphological features are consistent with that of a tumor cell and it exhibits the phenotype $EpCAM^+$, CK^+, $DAPI^+$, and $CD45^-$.

[0049] The CTC number will be counted and analyzed by operators and recorded directly. For further immunostaining on CTCs, anti-PD-L1 will be automated injecting into SelectChip for labeling CTC on MiSelect R System. The fluorescence images of each biomarkers on CTC will be taken and intensity of the biomarkers will be recorded for further analysis.

[0050] 4. Statistical analyses

[0051] All data were statistically analyzed with SPSS software (v19.0) and GraphPad Prism (v5.0). The distributions of continuous variables were described as median values and ranges. The Mann–Whitney U test and the Wilcoxon-signed ranks test were performed to evaluate the differences between groups, as appropriate. All P-values were two-sided. P-values of less than 0.05 indicated statistical significance.

[0052] **Results**

[0053] 1. Prevalence of CTC in PB and MVB

[0054] CTC was defined as a cell with intact nucleus, expressing EpCAM and cytokeratin, but absence of CD45 expression. Typical CTC images were demonstrated in **Fig. 2**. The EpCAM expression showed heterogeneity among CTCs even within the same CRC patient. CTCs were barely found in PB (mean, 0.17 ± 0.89 per 8 ml of whole blood; range = 0–8, n = 116) but more abundant in MVB (mean, 7.1 ± 48.1 per 8 ml of whole blood; range = 0–515, n = 116) (**Fig. 3**; P < 0.001).

[0055] 2. Distribution of CTC counts and detection rates

[0056] The CTC counts for CRC patients are presented in **Table 2** below and in **Fig. 4**. CTC count ranged from 0 to 8 in non-metastatic CRC and 0 to 4 in metastatic CRC in PB; In MVB, CTC count ranged from 0 to 515 in non-metastatic CRC and 0 to 20 in metastatic CRC. The overall detection rate of CTC is 6% and 40.5% in PB and MVB, respectively. Within each subgroup, the detection rate increased with the severity of the subgroup's condition. In MVB, CTC detection rate was 20.8%, 42.1%, 45.2% and 58.3% for stage I, II, III, and IV respectively. Besides, the amount of CTC was significantly more abundant in late stages than early stages (**Fig. 4**).

[0057] **Table 2.** CTC count in various stages of PB and MVB

	No. of cases N = 116	Detection rate in MVB %	Range of CTC number in 8mL
Stage I	24	20.8% (5/24)	0-9
Stage II	38	42.1% (16/38)	0-20
Stage III	42	45.2% (19/42)	0-515
Stage IV	12	58.3% (7/12)	0-20
P-value = 0.0298			

	No. of cases N = 116	Detection rate in PB %	Range of CTC number in 8mL
Stage I	24	4.2% (1/24)	0-1
Stage II	38	10.5% (4/38)	0-8

Stage III	42	2.4% (1/42)	0-1
Stage IV	12	16.6% (2/12)	0-4
P-value = 0.7053			

[0058] 3. Relationships between CTC number and clinicopathological characteristics

[0059] We explored the bivariate relationship between CTC numbers (present or absent) versus various clinical and pathological parameters. The results are shown in **Table 3** below. The clinical staging (TNM) positively correlated to CTC number in MVB (**Table 2**) and pre-operative serum CEA level and tumor invasion depth (pT) positively correlated to CTC levels in MVB. No association was noted between CTC numbers and presence of liver or lung metastases, primary CRC differentiation, histology, nodal status (N), lymphatic/venous invasion/perineural invasion, inflammatory change around carcinoma, invasion pattern of cancer tissue and pre-operative serum CA-19-9 level.

[0060] **Table 3.** Correlation between clinicopathological parameter and the present of CTCs

Clinicopathological variables	CTC present %	CTC absent %	P value
T stage T1/T2 T3/T4	 17% (6/34) 48%(41/84)	 83% (28/34) 52% (43/84)	0.018
N stage N0 N1	 34% (22/65) 49% (25/51)	 66%(43/65) 51% (26/51)	0.13
CEA ≥ 5mg/mL < 5mg/mL	 53% (23/44) 33% (23/71)	 47% (21/44) 67% (48/71)	0.049

CA-199 ≥ 37mg/mL < 37mg/mL	 50% (11/22) 37%(35/94)	 50%(11/22) 63% (49/94)	0.33

[0061] 4. PD-L1 biomarker assessment on CTCs in CRC patients

[0062] CTCs isolated from PB and MVB were examined for PD-L1 protein expression. The PD-L1 biomarker expression showed heterogeneity among isolated CTCs between patients and within the same blood sample (**Fig. 5**). PD-L1 status on CTC in PB was evaluated in 8 patients with detectable CTC (see **Table 4** below). PD-L1 status on CTC in MVB was evaluated in 47 patients with detectable CTC. Among these 47 patients, 31 (65.9%) showed a subpopulation of PD-L1$^+$ CTCs (see **Fig. 6 and Table 5** below). The number of PD-L1$^+$ CTCs varied from 1 to 33 (median = 3) and the fraction of PD-L1$^+$ CTCs ranged from 16 to 100% of the whole number of detectable CTCs. The PD-L1$^+$ CTC number gradually increased with stages (**Fig. 7**) and the frequency of PD-L1$^+$ CTCs among CTCs also increased with stages (**Fig. 8**).

[0063] **Table 4.** Number of PD-L1$^+$ CTC in PB of various stages

Patients number	Stage	CTC number	PD-L1$^+$CTC number
0031	I	1	0
0009	II	1	0
0078	II	1	0
0090	II	2	1
0113	II	3	0
0057	III	1	0
0004	IV	1	0

0075	IV	4	3

[0064] **Table 5.** Number of PD-L1$^+$ CTC in MVB of various stages

Patients number	Stage	CTC number	PD-L1$^+$CTC number
0011	I	1	0
0021	I	3	2
0031	I	1	0
0123	I	2	0
0129	I	9	1
0001	II	5	0
0009	II	2	2
0016	II	1	0
0037	II	2	1
0046	II	2	1
0051	II	2	0
0055	II	8	4
0060	II	1	0
0089	II	1	0

0091	II	1	0
0094	II	11	6
0096	II	2	0
0097	II	1	0
0103	II	15	8
0118	II	3	1
0139	II	20	14
0006	III	1	0
0012	III	4	4
0025	III	1	0
0030	III	1	1
0057	III	1	1
0069	III	5	5
0076	III	1	0
0077	III	3	3
0082	III	45	33
0084	III	21	12

0085	III	5	3
0086	III	2	1
0098	III	2	1
0100	III	1	1
0102	III	1	1
0104	III	2	1
0106	III	1	1
0134	III	1	0
0141	III	6	2
0004	IV	5	0
0017	IV	17	16
0028	IV	13	2
0034	IV	5	3
0038	IV	2	2
0068	IV	2	2
0075	IV	10	9

[0065] 5. Relationships between PD-L1$^+$ CTC number and clinicopathological characteristics in CRC patients

[0066] We explored the bivariate relationship between PD-L1$^+$CTC numbers (present or absent) versus various clinical and pathological parameters. The results are shown in **Table 6** and **Table 7** below. The clinical staging (TNM) positively correlated to PD-L1$^+$CTC number in MVB (**Table 6**) and pre-operative serum CEA level, tumor invasion depth (pT), nodal status (N), positively correlated to PD-L1$^+$CTC levels in MVB. Besides, the primary CRC lymphatic and venous invasion were correlated to PD-L1$^+$CTC levels in MVB (**Table 7**). No association was noted between PD-L1$^+$CTC numbers and primary CRC differentiation, perineural invasion, inflammatory change around carcinoma, invasion pattern of cancer tissue and pre-operative serum CA-19-9 level.

[0067] **Table 6.** Correlation between PD-L1(+) CTC presence and clinical stage

	Detection rate of PD-L1(+) CTC N = 116	PD-L1(+) rate in CTC detected patients N = 47
Stage I	8.3% (2/24)	40.0% (2/5)
Stage II	21.1% (8/38)	50.0% (8/16)
Stage III	35.7% (15/42)	78.9% (15/19)
Stage IV	50.0% (6/12)	85.7% (6/7)
	P-value = 0.0017	P-value = 0.0178

[0068] **Table 7.** Correlation between clinicopathological parameter and the present of PD-L1$^+$ CTCs

Clinicopathological variables	PD-L1(+) CTC present (%)	PD-L1(+) CTC absent (%)	P value
T stage T1/T2 T3/T4	 6% (2/33) 48% (23/83)	 94% (31/33) 52% (60/83)	0.016
N stage N0 N1	 12% (8/65) 33% (17/51)	 88% (57/65) 67% (34/51)	0.013

CEA ≥ 5mg/mL < 5mg/mL	 36% (17/47) 10% (7/67)	 64% (30/47)90%(7/67)	0.002
CA-199 ≥ 37mg/mL < 37mg/mL	 33% (7/21) 17% (16/92)	 67% (14/21) 83% (76/92)	0.13
Blood Vascular Invasion (+) (-)	 42% (11/26) 17% (14/84)	 58% (15/26) 83% (70/84)	0.014
Lymphatic Vascular Invasion (+) (-)	 36% (11/30) 18% (14/80)	 64% (19/30) 82% (66/80)	0.0042

[0069] 6. PD-L1 biomarker assessment on CTCs in other cancer patients

[0070] The overall detection rate of CTC is 31%, 55% and 40% in patients with head and neck squamous-cell carcinoma (HNSCC), hepatocellular carcinoma (HCC) and uterine cancer (UC), respectively (see **Table 8** below). CTCs isolated from HNSCC, HCC and UC patients were examined for PD-L1 protein expression. The PD-L1 biomarker expression showed heterogeneity among isolated CTCs between patients and within the same blood sample (**Fig. 9**). PD-L1 status on CTC was evaluated in patients with detectable CTCs. Among these patients, 100% of HNSCC, 50% of HCC and 100% of UC showed a subpopulation of PD-L1$^+$ CTCs (**Table 8**). The presence of PD-L1(+) CTC significantly correlates with disease progression in HNSCC patients (see **Table 9** below).

[0071] **Table 8.** CTC and PD-L1(+) CTC detection rate in advanced cancers

Cancer type	CTC detection rate %	PD-L1(+) rate in CTC detected patients %	PD-L1 positive rate in CTC (Range)
HNSCC N = 26	31% (8/26)	100% (8/8)	12.5-100%
HCC N = 9	55% (4/9)	50% (2/4)	50-100 %
UC N = 5	40% (2/5)	100% (2/2)	100%

RCC N = 2	0/2	0%	N.A.

[0072] **Table 9.** Correlation between CTC or PD-L1(+) CTC presence and disease progression

CTC/PD-L1(+) CTC Clinical outcome	present	absent	P-value
Progression disease	7	4	0.034
Complete response/ Partial response/ Stable disease	0	6	

[0073] References:

1. Pantel, K., C. Alix-Panabieres, and S. Riethdorf, Nat Rev Clin Oncol, 2009. **6**(6): p. 339-51.
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3. Thorsteinsson, M., G. Soletormos, and P. Jess, Anticancer Res, 2011. **31**(2): p. 613-7.
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5. Nicolazzo, C. et al., Sci Rep. 2016 Aug 24;6:31726.

CLAIMS

What is claimed is:

1. A method for identifying a subject at risk of developing a recurrent or metastatic cancer, comprising detecting PD-L1$^+$ circulating tumor cells in a blood sample, a tissue fluid sample or a specimen of the subject, wherein the presence of one or more PD-L1$^+$ circulating tumor cells indicates that the subject is at risk of developing a recurrent or metastatic cancer, and wherein the subject has a gastrointestinal cancer or head and neck squamous-cell carcinoma and the blood sample, the tissue fluid sample or the specimen is derived from the subject before a therapy for, or during a surgery of curative resection of the gastrointestinal cancer or head and neck squamous-cell carcinoma.

2. A method for treating a cancer comprising:

identifying a subject having PD-L1$^+$ circulating tumor cells by detecting PD-L1$^+$ circulating tumor cells in a blood sample, a tissue fluid sample or a specimen of the subject, wherein the subject has a gastrointestinal cancer or head and neck squamous-cell carcinoma and the blood sample, the tissue fluid sample or the specimen is derived from the subject before a therapy for, or during a surgery of curative resection of the gastrointestinal cancer or head and neck squamous-cell carcinoma; and

administering a treatment to the subject.

3. The method of claim 2, wherein the cancer is a recurrent or metastatic cancer.

4. The method of claim 2, wherein the treatment includes an immunotherapy.

5. The method of claim 3, wherein the treatment includes an immunotherapy.

6. The method of claim 1, wherein the cancer is a gastrointestinal cancer.

7. The method of claim 2, wherein the cancer is a gastrointestinal cancer.

8. The method of claim 6, wherein the gastrointestinal cancer is a colorectal cancer.

9. The method of claim 7, wherein the gastrointestinal cancer is a colorectal cancer.

10. The method of claim 6, wherein the blood sample is a mesenteric venous blood sample.

11. The method of claim 7, wherein the blood sample is a mesenteric venous blood sample.

12. A method for predicting prognosis of a patient having gastrointestinal cancer or head and neck squamous-cell carcinoma, comprising detecting PD-L1$^+$ circulating tumor cells in a blood sample, a tissue fluid sample or a specimen of the patient, wherein the presence of one or more PD-L1$^+$ circulating tumor cells indicates poor prognosis of the patient, and wherein the tissue fluid sample or the specimen is derived from the patient before a therapy for, or during a surgery of curative resection of the gastrointestinal cancer or head and neck squamous-cell carcinoma.

13. The method of claim 12, wherein the patient has a gastrointestinal cancer.

14. The method of claim 13, wherein the gastrointestinal cancer is a colorectal cancer.

15. The method of claim 13, wherein the blood sample is a mesenteric venous blood sample.

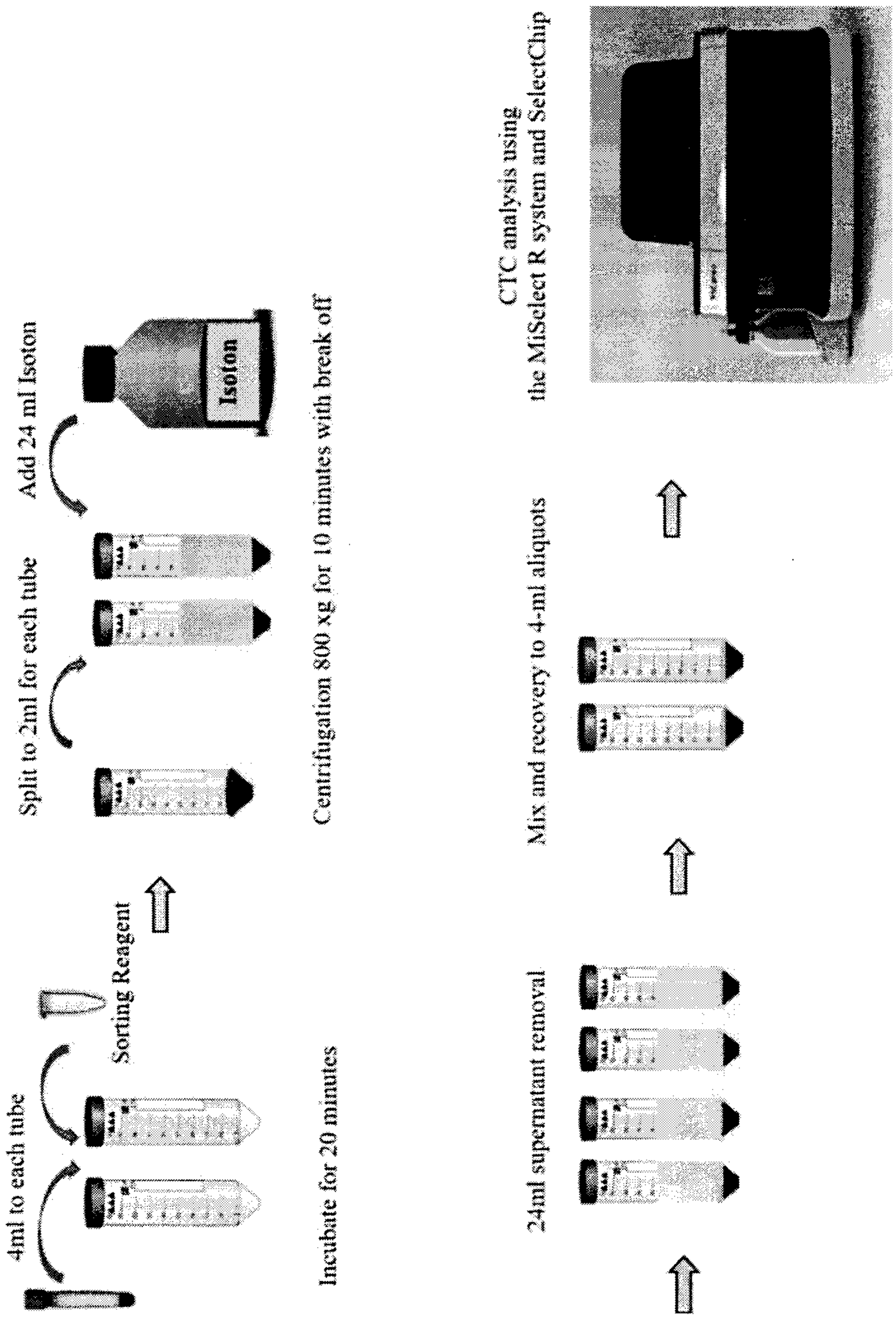
Isolon
Add 24 ml Isoton
Centrifugation 800 xg for 10 minutes with break off
Split to 2ml for each tube
4ml to each tube
Sorting Reagent
Incubate for 20 minutes
24ml supernatant removal
Mix and recovery to 4-ml aliquots
Centrifugation 800 xg for 10 minutes with break off
CTC analysis using
the MiSelect R system and SelectChip

Fig. 1

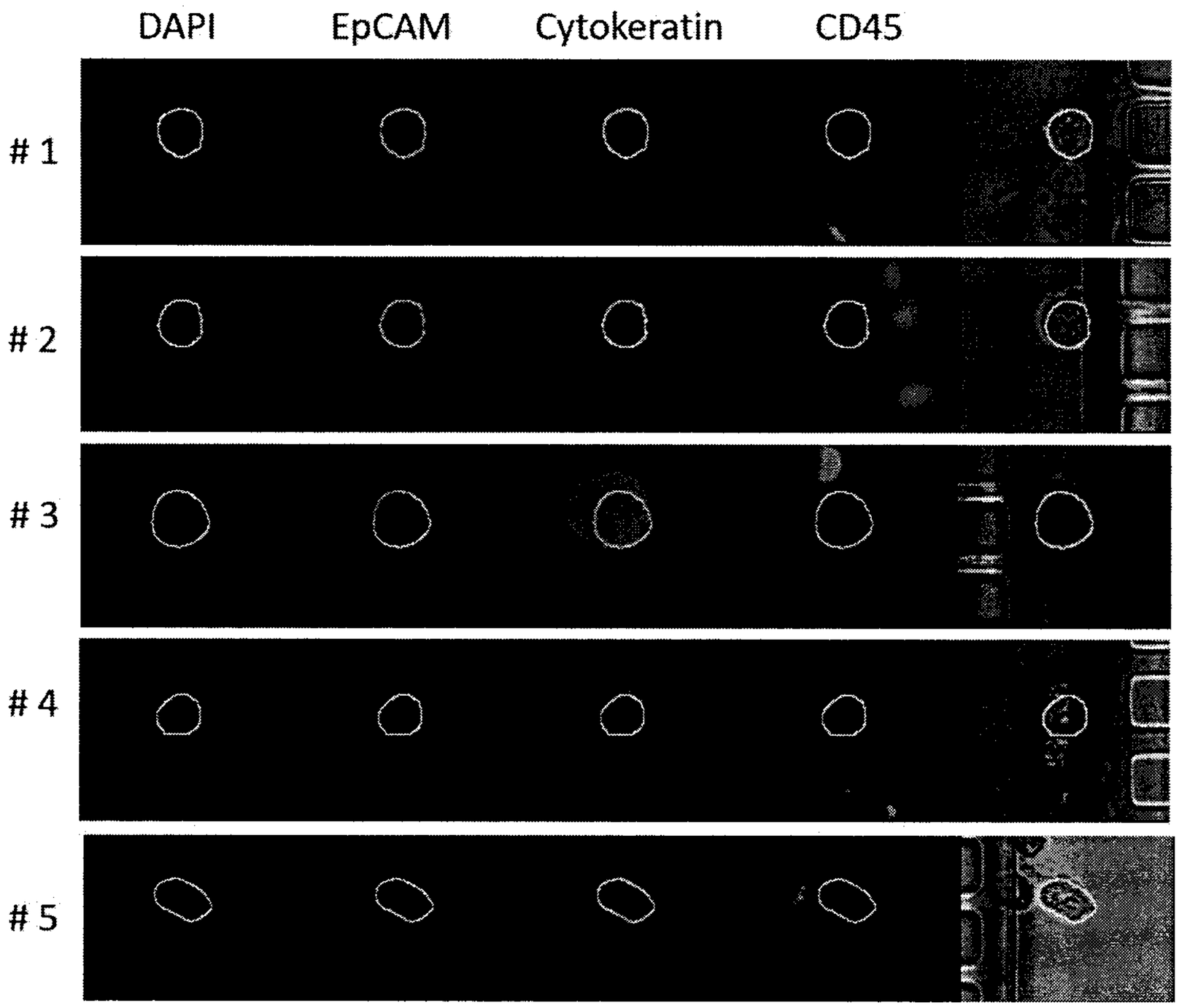
DAPI
EpCAM
Cytokeratin
CD45
1
2
3
4
5

Fig. 2

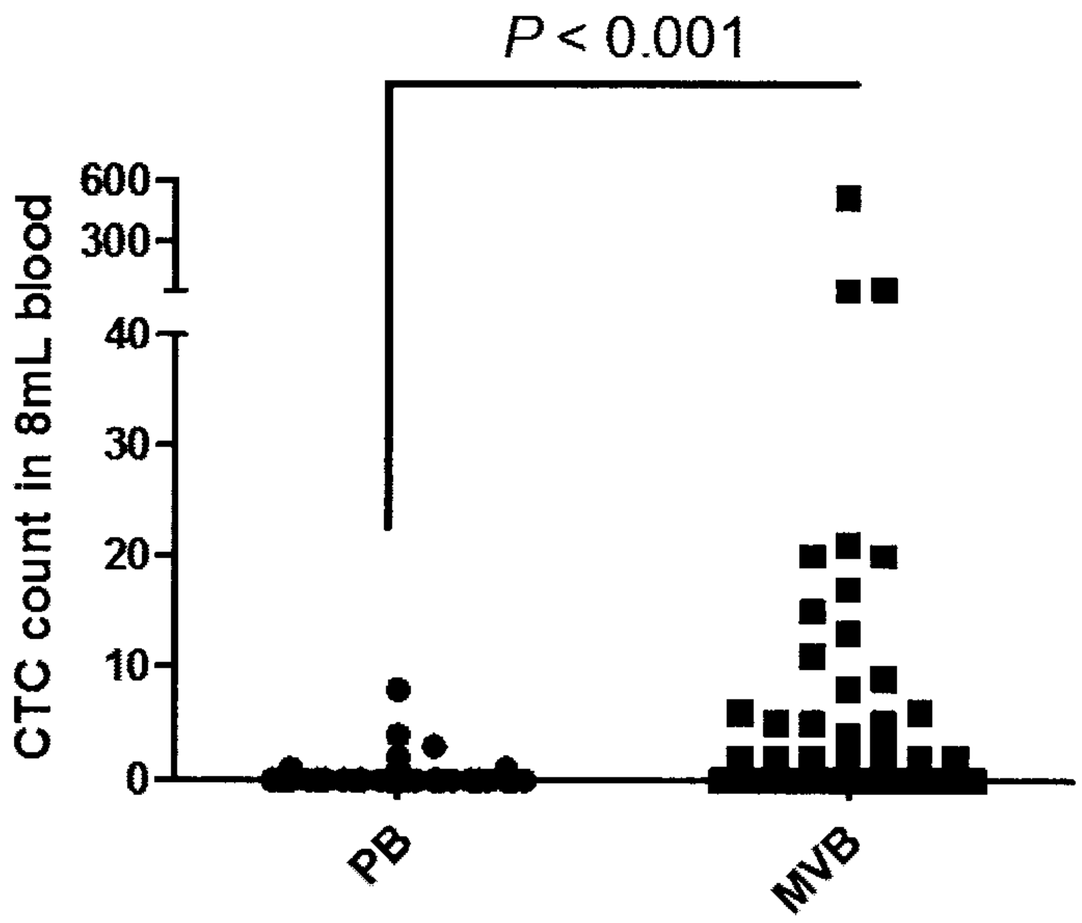
P < 0.001
CTC count in 8mL blood
600
300
40
30
20
10
0
PB
MVB

Fig. 3

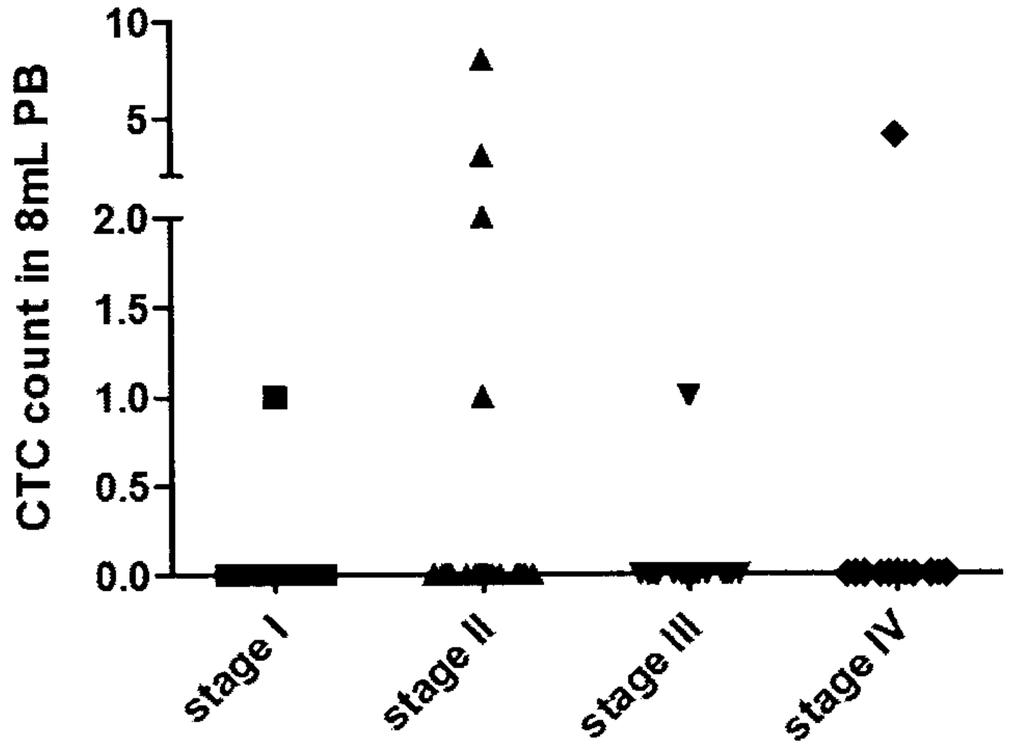
CTC count in 8mL PB
10
5
2.0
1.5
1.0
0.5
0.0
stage I
stage II
stage III
stage IV

Fig. 4A

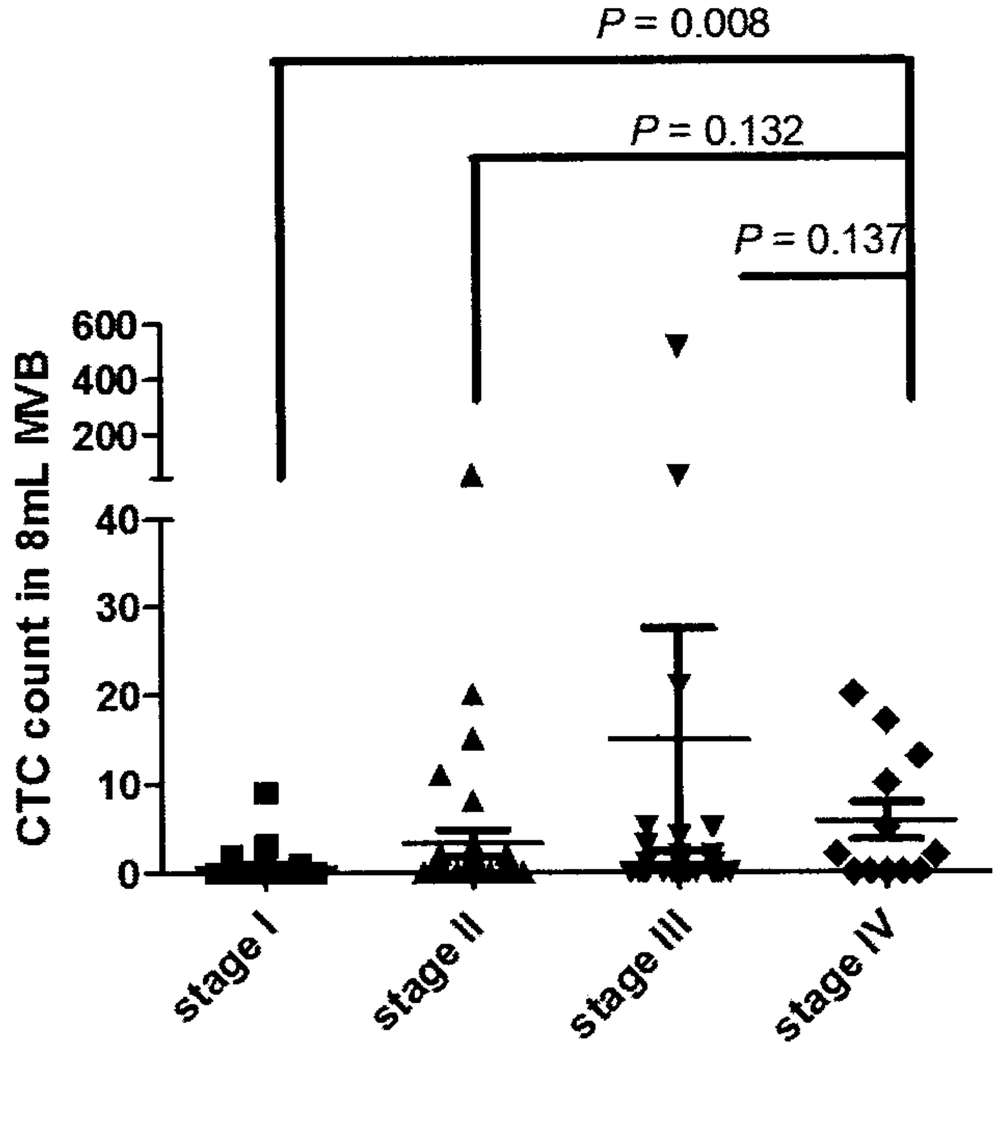
P = 0.008
P = 0.132
P = 0.137
CTC count in 8mL MVB
600
400
200
40
30
20
10
0
stage I
stage II
stage III
stage IV

Fig. 4B

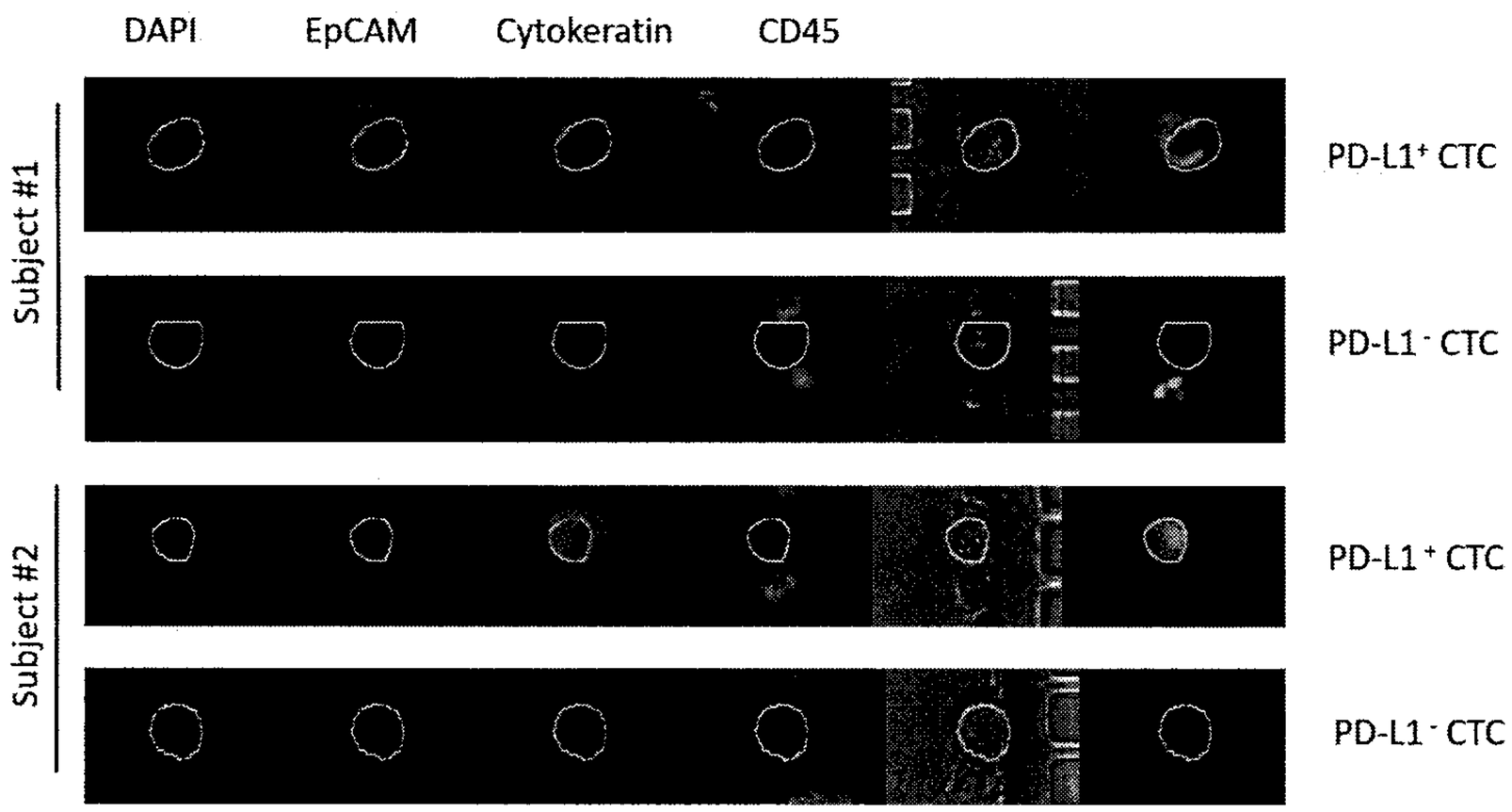
DAPI
EpCAM
Cytokeratin
CD45
Subject #1
PD-L1+ CTC
PD-L1- CTC
Subject #2
PD-L1+ CTC
PD-L1- CTC

Fig. 5

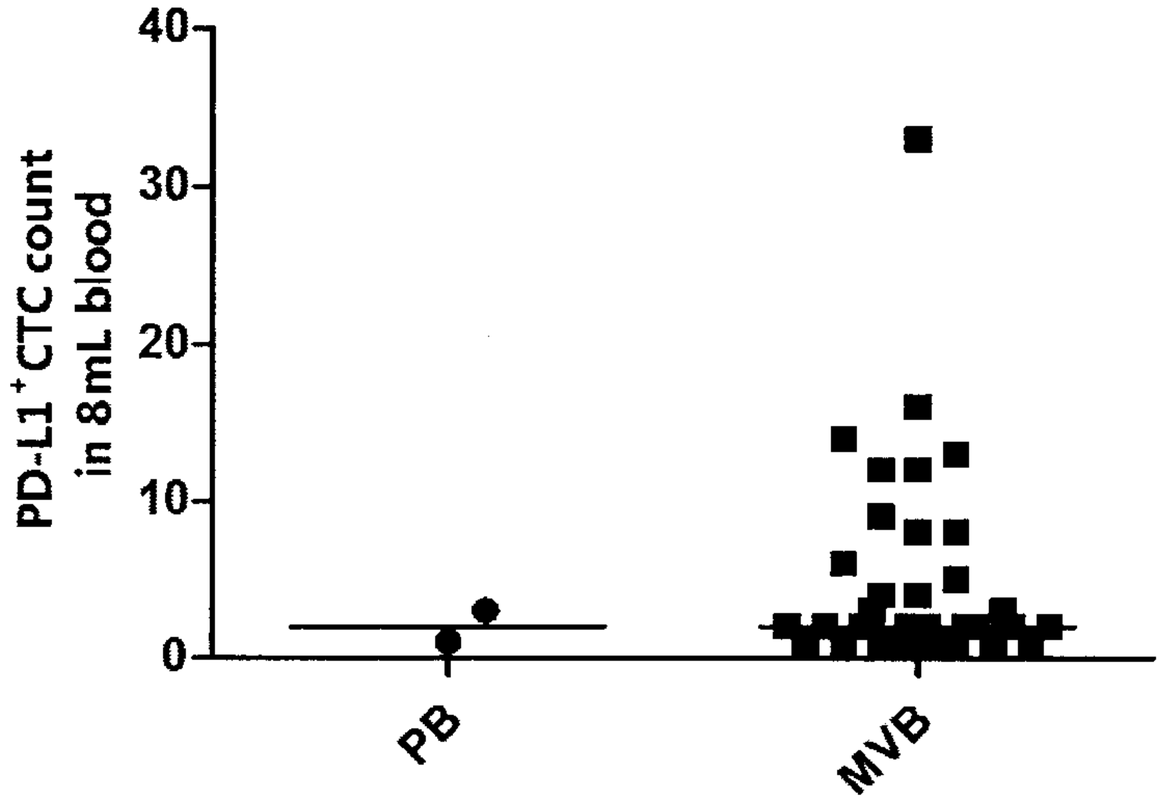
PD-L1+CTC count in 8mL blood
40
30
20
10
0
PB
MVB

Fig. 6

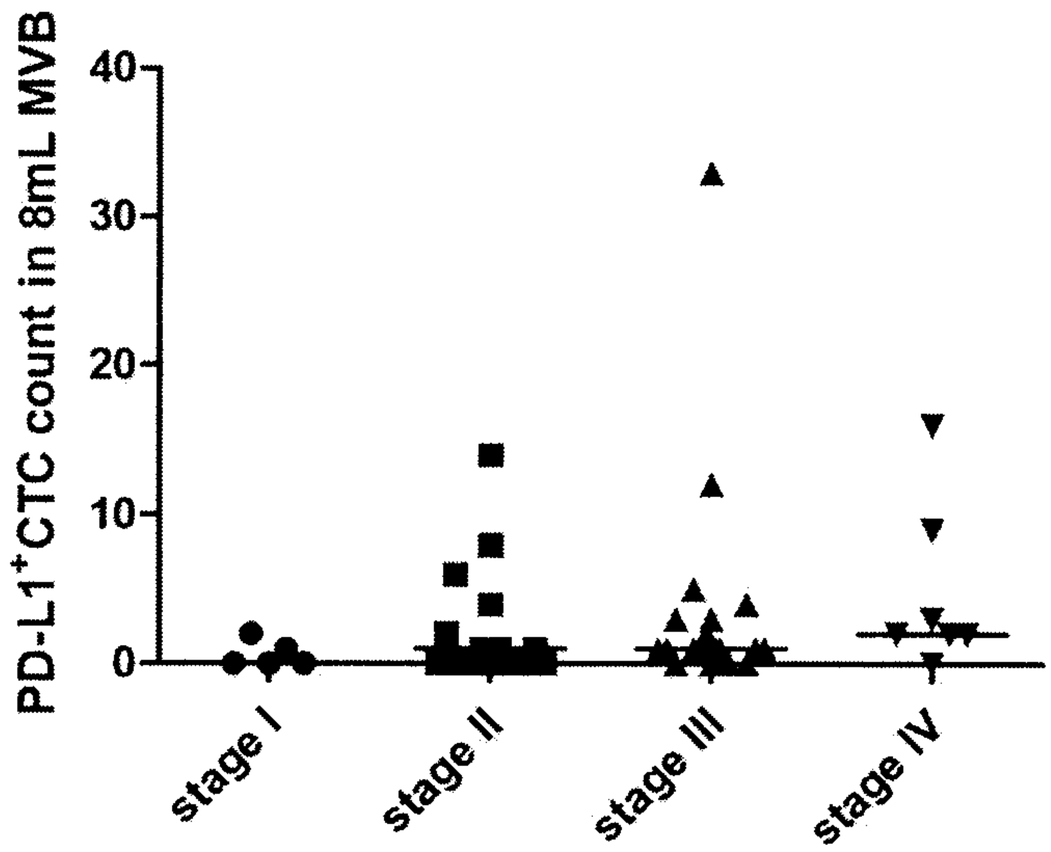
PD-L1+CTC count in 8mL MVB
40
30
20
10
0
stage I
stage II
stage III
stage IV

Fig. 7

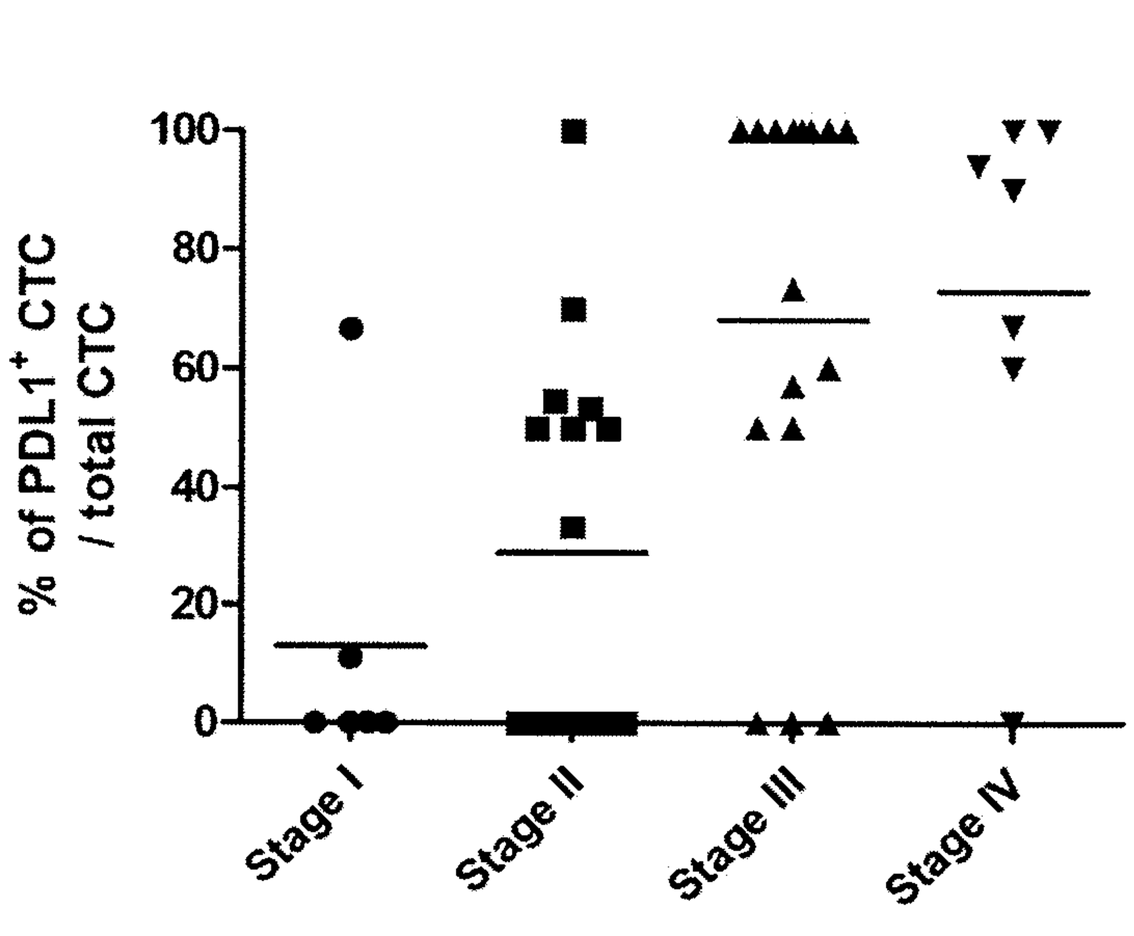
% of PDL1+ CTC / total CTC
100
80
60
40
20
0
Stage I
Stage II
Stage III
Stage IV

Fig. 8

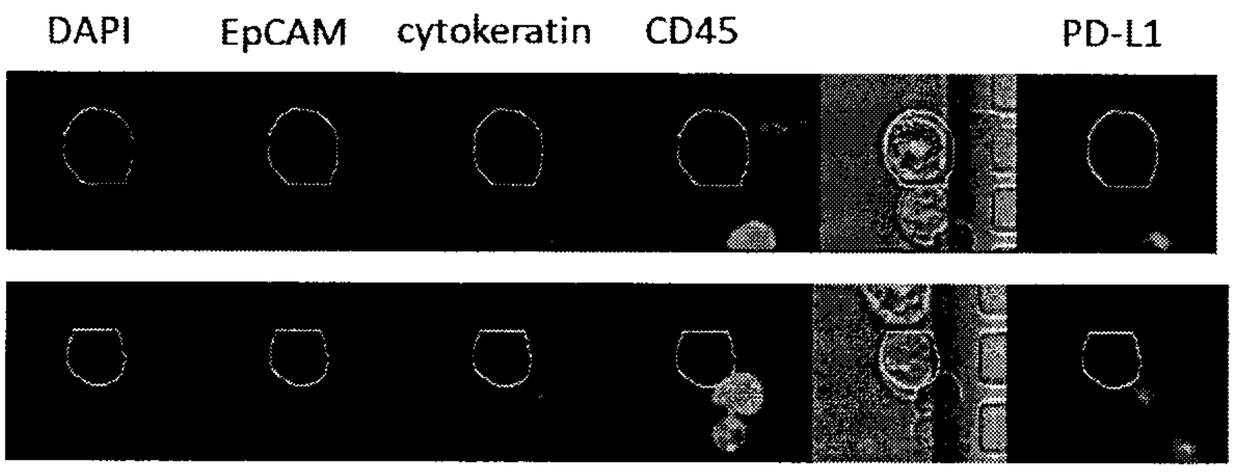

Fig. 9