

BIOMARKER WITH THERAPEUTIC IMPLICATIONS FOR CARCINOMATOSIS

CROSS-REFERENCE TO RELATED APPLICATIONS

5 [001] This application claims the benefit of priority of Singapore provisional application no. 10201902763U, filed 27 March 2019, the contents of it being hereby incorporated by reference in its entirety for all purposes.

FIELD OF THE INVENTION

[002] The present invention relates generally to the field of molecular biology. In particular, the present
10 invention relates to the use of biomarkers for the detection, diagnosis and subsequent treatment of cancer.

BACKGROUND OF THE INVENTION

[003] Colorectal cancer is the third most common cancer and the fourth most common cause of cancer death globally, accounting for 1.4 million new cases and 600 000 deaths per year. Deaths from colorectal
15 cancer are largely due to metastasis with peritoneal carcinomatosis (PC) occurring in 15% of all patients and accounting for up to 30% of all metastases. Compared to other forms of metastatic colorectal cancer without peritoneal involvement, colorectal peritoneal carcinomatosis has consistently demonstrated to have significantly shorter overall survival despite palliative systemic chemotherapy.

[004] Cytoreductive surgery (CRS) and hyperthermic intraperitoneal chemotherapy (HIPEC) have
20 revolutionised the treatment of peritoneal carcinomatosis. Cytoreductive surgery refers to a series of visceral resections and peritonectomy procedures that remove all macroscopic disease. Remaining viable microscopic disease is then eradicated with the instillation of hyperthermic intraperitoneal chemotherapy. The combined treatment modalities of cytoreductive surgery and hyperthermic intraperitoneal chemotherapy have greatly improved survival in patients with peritoneal carcinomatosis of colorectal
25 origin. The median survival of patients treated with cytoreductive surgery and hyperthermic intraperitoneal chemotherapy was 33 months, compared to 6 to 12 months in patients treated with systemic chemotherapy alone. However, despite this dramatic improvement, much more needs to be done to further improve the outcome of treatment for patients with colorectal peritoneal carcinomatosis by improving the hyperthermic intraperitoneal chemotherapy regimen, as surgery is unlikely to improve
30 patient outcome further.

[005] Hence, there is a need for improved patient stratification in order to improve treatment.

SUMMARY

[006] In one aspect, the present disclosure refers to a use of a “plasminogen activator inhibitor 1” (PAI-
35 1) inhibitor in the manufacture of a medicament for treating a subject suffering from peritoneal carcinomatosis, wherein the subject is to be identified by determining the concentration of PAI-1 and determining the level of phosphorylation of “signal transducer and activator of transcript 3” (STAT3) in a

sample obtained from the subject; wherein if the subject shows (a) an increase in PAI-1 concentration and an increase in STAT3 phosphorylation, or (b) a decrease in PAI 1 concentration and an increase in STAT3 phosphorylation, the subject is to be administered the medicament; wherein the increase and/or decrease of the concentration of PAI-1 and STAT3 phosphorylation is compared to a reference value; wherein the sample is selected from the group consisting of ascites, tumour samples, and biopsy samples; wherein the reference values are obtained from a reference group comprising subjects suffering from peritoneal carcinomatosis. In one example, there is described a method of treating a subject suffering from peritoneal carcinomatosis with a “plasminogen activator inhibitor 1” (PAI-1) inhibitor, the method comprising determining the concentration of PAI-1 and determining the level of phosphorylation of “signal transducer and activator of transcript 3” (STAT3) in a sample obtained from the subject; administering the PAI-1 inhibitor to the subject showing (a) an increase in PAI-1 concentration and an increase in STAT3 phosphorylation, or (b) a decrease in PAI 1 concentration and an increase in STAT3 phosphorylation; wherein the increase and/or decrease of the concentration of PAI-1 and STAT3 phosphorylation is compared to a reference value.

[007] In another aspect, the present disclosure refers to a method of detecting or determining susceptibility of a subject suffering from peritoneal carcinomatosis to a treatment with a “plasminogen activator inhibitor 1”(PAI-1) inhibitor, the method comprising determining the concentration of PAI-1 and determining the level of phosphorylation of “signal transducer and activator of transcript 3” (STAT3) in a sample obtained from a subject; wherein the subject is susceptible to the treatment if the subject shows (a) an increase in PAI 1 concentration and an increase in STAT3 phosphorylation, or (b) a decrease in PAI 1 concentration and an increase in STAT3 phosphorylation; wherein the subject is not considered to be susceptible to treatment if the subject shows (c) a decrease in PAI-1 concentration and a decrease in STAT3 phosphorylation; wherein the increase and/or decrease of the concentration of PAI-1 and the level of STAT3 phosphorylation is compared to a reference value; wherein the sample is selected from ascites, tumour samples, and biopsy samples; wherein the reference values are obtained from a reference group comprising subjects suffering from peritoneal carcinomatosis.

[008] In one aspect, the present disclosure refers to a panel of markers for treating a patient suffering from peritoneal carcinomatosis with a “plasminogen activator inhibitor 1” (PAI-1) inhibitor, or for detecting or determining susceptibility of a subject suffering from peritoneal carcinomatosis to a treatment with a “plasminogen activator inhibitor 1” (PAI-1) inhibitor, wherein the panel of markers comprises PAI-1, and two or more surrogate markers of STAT3 phosphorylation, or p-STAT3, wherein the two or more surrogate markers are selected from the group consisting of IL6, IL10, CCL2, MMP9, ANGPT1, TGFB1, POSTN, VSIG4, CD44, and CXCL10, wherein one of the at least two surrogate markers is MMP9, wherein the panel of markers is to be measured in a sample selected from ascites, tumour samples, and biopsy samples.

[009] In another aspect, the present disclosure refers to the use of a panel of markers in the use or the method disclosed herein, wherein the panel comprises PAI-1 and two or more surrogate markers of STAT3 phosphorylation, wherein at least one of the surrogate markers of STAT3 phosphorylation is MMP9, or PAI-1 and p-STAT3; wherein the at two or more surrogate markers are selected from the group consisting of IL6, IL10, CCL2, MMP9, ANGPT1, TGFB1, POSTN, VSIG4, CD44, and CXCL10, wherein one of two or more surrogate markers is MMP9.

BRIEF DESCRIPTION OF THE DRAWINGS

[0010] The invention will be better understood with reference to the detailed description when considered in conjunction with the non-limiting examples and the accompanying drawings, in which:

[0011] **Fig. 1** shows data indicating that the presence of ascites leads to a poorer prognosis in patients regardless of histological subtype. (A) Kaplan-Meier survival curve of all patients with peritoneal carcinomatosis ($P = 0.002$). (B) Kaplan-Meier survival curve of colorectal patients with peritoneal carcinomatosis ($P = 0.001$). (C) Kaplan-Meier survival curve of ovarian patients with peritoneal carcinomatosis ($P = 0.077$).

[0012] **Fig. 2** (A) Addition of cell-free ascites increased cancer cells proliferation in a dose-dependent manner. 0.1% of cell-free ascitic fluid was sufficient to maintain cell viability without proliferation. (B) Treatment with cell-free ascites significantly increased cancer cells migration. (C) Treatment with cell-free ascites dramatically increased the cell settlement of cancer cells *in vitro* independently of serum supplemented media (As: Ascites, SFM: Serum-free media, FBS: Foetal bovine serum).

CLAIMS

1. Use of a “plasminogen activator inhibitor 1” (PAI-1) inhibitor in the manufacture of a medicament for treating a subject suffering from peritoneal carcinomatosis, wherein the subject is to be identified by

determining the concentration of PAI-1 and determining the level of phosphorylation of “signal transducer and activator of transcript 3” (STAT3) in a sample obtained from the subject;

wherein if the subject shows (a) an increase in PAI-1 concentration and an increase in STAT3 phosphorylation, or (b) a decrease in PAI-1 concentration and an increase in STAT3 phosphorylation, the subject is to be administered the medicament;

wherein the increase and/or decrease of the concentration of PAI-1 and STAT3 phosphorylation is compared to a reference value;

wherein the sample is selected from the group consisting of ascites, tumour samples, and biopsy samples;

wherein the reference values are obtained from a reference group comprising subjects suffering from peritoneal carcinomatosis.

2. A method of detecting or determining susceptibility of a subject suffering from peritoneal carcinomatosis to a treatment with a “plasminogen activator inhibitor 1” (PAI-1) inhibitor, the method comprising

determining the concentration of PAI-1 and determining the level of phosphorylation of “signal transducer and activator of transcript 3” (STAT3) in a sample obtained from a subject;

wherein the subject is susceptible to the treatment if the subject shows (a) an increase in PAI-1 concentration and an increase in STAT3 phosphorylation, or (b) a decrease in PAI-1 concentration and an increase in STAT3 phosphorylation;

wherein the subject is not considered to be susceptible to treatment if the subject shows (c) a decrease in PAI-1 concentration and a decrease in STAT3 phosphorylation;

wherein the increase and/or decrease of the concentration of PAI-1 and the level of STAT3 phosphorylation is compared to a reference value;

wherein the sample is selected from ascites, tumour samples, and biopsy samples;

wherein the reference values are obtained from a reference group comprising subjects suffering from peritoneal carcinomatosis.

3. The use of claim 1 or the method of claim 2, wherein the concentration of PAI-1 is determined by measuring the concentration of PAI-1 directly, and/or in one or more complexes.

4. The use of claim 1 or 3, or the method of claims 2 to 3, wherein the level of STAT3 phosphorylation is determined by measuring the concentration of two or more surrogate markers, and/or

by measuring the concentration of STAT3 phosphorylation directly, wherein the two or more surrogate markers are selected from the group consisting of IL6, IL10, CCL2, MMP9, ANGPT1, TGFB1, POSTN, VSIG4, CD44, and CXCL10, wherein one of the two or more surrogate markers is MMP9.

5 5. The use or the method of claim 4, wherein the two or more surrogate markers are selected from the group consisting of IL6, IL10, CCL2, MMP9 and ANGPT1.

6. The use or the method of any one of claims 4 to 5, wherein the two or more surrogate markers are selected from the group consisting of IL6, IL10, CCL2, and MMP9.

10 7. The use or the method of any one of claims 4 to 6, wherein the two or more surrogate markers comprise IL6, IL10, CCL2, MMP9 and ANGPT1; or wherein the surrogate markers comprise at least IL6, IL10, CCL2, and MMP9.

15 8. The use of claim 1 or 3 to 7, or the method of any one of claims 2 to 7, wherein the PAI-1 inhibitor is an anti-cancer drug or an anti-cancer treatment.

9. The use or the method of claim 8, wherein the anti-cancer drug or the anti-cancer therapy is selected from the group consisting of a small molecule, a chemotherapeutic agent, a peptide, an antibody, combinations thereof, and combination therapy.

10. The use or the method of any one of claims 8 to 9, wherein the anti-cancer drug is selected from the group consisting of TM5441 (5-Chloro-2-[[2-[2-[[3-(3-furanyl)phenyl]amino]-2-oxoethoxy]acetyl]amino]benzoic acid sodium salt; CAS 1190221-43-2), TM5007 (N, N-bis [3,3'-carboxy-4,4'-(2,2'-thienyl)-2,2'- thienyl] hexanedicarboxamide; CAS 342595-05-5), TM5275 (5-Chloro-2-[[2-[2-[4-(diphenylmethyl)-1-piperazinyl]-2-oxoethoxy]acetyl]amino]-benzoic acid sodium salt; CAS 1103926-82-4), tiplaxtinin (2-(1-Benzyl-5-(4-(trifluoromethoxy)phenyl)-1H-indol-3-yl)oxoacetic acid; CAS 393105-53-8), and ZK4044.

30 11. The use of claim 1 or claims 3 to 10, wherein the PAI-1 inhibitor is to be administered intra-peritoneally.

12. The use of claim 1 or claims 3 to 11, or the method according to any one of claims 2 to 10, wherein the peritoneal carcinomatosis is selected from the group consisting of colorectal peritoneal carcinomatosis, small bowel peritoneal carcinomatosis, mesothelioma, endometrial peritoneal carcinomatosis, gastric peritoneal carcinomatosis, ovarian peritoneal carcinomatosis, appendiceal peritoneal carcinomatosis, pancreatic peritoneal carcinomatosis, urothelial carcinomatosis and Pseudomyxoma peritonei (PMP).

13. The use of claim 1 or claims 3 to 12, or the method according to any one of claims 2 to 10 and 12, wherein the treatment is to be performed in a treatment setting selected from the groups consisting of neoadjuvant setting, adjuvant setting, palliative setting and prophylactic setting.
- 5 14. The use of claim 1 or claims 3 to 13, or the method of any of claims 2 to 10 and 12 to 13, wherein administration of the PAI-1 inhibition leads to inhibition of PAI-1 activity compared to patients suffering from the same disease.
- 10 15. A panel of markers for treating a patient suffering from peritoneal carcinomatosis with a “plasminogen activator inhibitor 1” (PAI-1) inhibitor, or for detecting or determining susceptibility of a subject suffering from peritoneal carcinomatosis to a treatment with a “plasminogen activator inhibitor 1” (PAI-1) inhibitor, wherein the panel of markers comprises PAI-1, and two or more surrogate markers of STAT3 phosphorylation or p-STAT3, wherein the two or more surrogate markers are selected from the group consisting of IL6, IL10, CCL2, MMP9, ANGPT1, TGFB1, POSTN, VSIG4, CD44, and CXCL10, 15 wherein one of the at least two surrogate markers is MMP9, wherein the panel of markers is to be measured in a sample selected from ascites, tumour samples, and biopsy samples.
- 20 16. Use of a panel of markers in the use of claim 1 or claims 3 to 14, or the method of any one of claims 2 to 10 and 12 to 14, wherein the panel comprises PAI-1 and two or more surrogate markers of STAT3 phosphorylation, wherein at least one of the surrogate markers of STAT3 phosphorylation is MMP9, or PAI-1 and p-STAT3; wherein the at two or more surrogate markers are selected from the group consisting of IL6, IL10, CCL2, MMP9, ANGPT1, TGFB1, POSTN, VSIG4, CD44, and CXCL10, wherein one of two or more surrogate markers is MMP9.
- 25 17. The panel of claim 15 or the use of claim 16, wherein the panel comprises PAI-1, and two or more or all of IL6, IL10, CCL2, and MMP9.
18. The panel of claims 15 and 17 or the use of claims 16 to 17, wherein the panel comprises PAI-1, and one or more or all of IL6, IL10, CCL2, MMP9 and ANGPT1.