

biomarkers. However, searching for biomarkers in a sea of noise from healthy cells still remains one of the biggest challenges. In addition, tumors are immensely heterogeneous at the DNA, RNA and epigenetic levels. Patient-specific interactions between cancer cells and the immune system in tumor microenvironment further increased variations from evolutionary and mutational aspects^{17, 18, 19}. Consequently, identifying a set of protein or nucleic acid biomarkers that are highly sensitive and specific to a type of cancer is technically challenging and costly. That is why despite the emergence of successful stories of cancer liquid biopsy, there is still only quite limited number of validated cancer biomarkers available to a few cancer types in clinical setting^{20, 21, 22, 23}. Furthermore, the list of biomarkers for different types of cancer is not exhaustive. Potential cancer patients might need to be diagnosed against hundreds of biomarkers to be free from a specific list of cancers. This might increase the diagnosis costs and involve the withdrawing of excessive amount of patient blood. Thus, a more broad-spectrum cancer diagnostic test that can detect multiple types of cancers simultaneously and does not rely on cancer biomarkers is needed.

[0009] Not only do TEXs contain biomarkers indicative of their parental cancer cells' identity, they also possess functional messenger molecules deployed by tumor cells to influence other cells, especially those in the immune system^{9, 24, 25, 26}. TEXs have been demonstrated to be immunosuppressive. They contain or express various combinations of immunoregulatory molecules such as IL-10, TGF- β , PD-1, PDL-1, TRAIL, FasL, CD39 and CD73 to suppress the function of T-cells, impair T-cells responses to stimulants, promote expansion of regulatory T-cells, or induce apoptosis of cytotoxic T-cells. In the meantime, TEXs can also be immunostimulatory due to their concentrated tumor antigens and heat shock proteins^{27, 25, 26, 28, 29, 30, 31, 32}.

[0009a] SUMMARY

[0009b] In one aspect, the present disclosure refers to a method of (i) detecting a cancer type in a subject having cancer or (ii) simultaneously testing for, or distinguishing between, multiple types of cancer in a subject, said method comprising the step of using a cancer-specific response profile, created based on the measurement of immunological impacts of tumor-derived exosomes on immune cells *in vitro*, to identify the cancer type or types in the subject, wherein said tumor-derived exosomes are harvested from the subject, and wherein said immune cells are T-cells.

[0009c] In another aspect, the present disclosure refers to a method of screening subjects for a cancer type or cancer types, said method comprising the step of using a cancer-specific response profile created for each subject, created based on the measurement of immunological

impacts of tumor-derived exosomes on immune cells *in vitro*, to identify the cancer type or types in each said subject, wherein said tumor-derived exosomes are harvested from the subjects, and wherein said immune cells are T-cells.

[00009d] In yet another aspect, the present disclosure refers to a method of treating a subject with a cancer type, said method comprising the steps of: (1) using a cancer-specific response profile, created based on the measurement of immunological impacts of tumor-derived exosomes on immune cells *in vitro*, to identify the cancer type in the subject, wherein said tumor-derived exosomes are harvested from the subject; and (2) treating the subject if the subject has been found to have the cancer type, wherein said immune cells are T-cells.

[00009e] In yet another aspect, the present disclosure refers to a method of identifying whether a subject having a cancer type is responding to treatment of that cancer type, said method comprising the steps of: (1) using a cancer-specific response profile, created based on the measurement of immunological impacts of tumor-derived exosomes on immune cells *in vitro*, to identify the cancer type in the subject, wherein said tumor-derived exosomes are harvested from the subject; and (2) comparing the respective cancer-specific response profile created before and during and/or after treatment of the cancer type, wherein a change in the cancer-specific response profile identifies the subject as having responded to the treatment of the cancer type, wherein said immune cells are T-cells.

[00009f] In yet another aspect, the present disclosure refers to a tumor-derived exosome-induced immune response or cancer-specific response profile created based on the measurement of immunological impacts of tumor-derived exosomes on immune cells *in vitro* when used for detecting or diagnosing a cancer type in a subject, wherein said immune cells are T-cells.

[00009g] In yet another aspect, the present disclosure refers to a tumor-derived exosome-induced immune response when used for creating a cancer-specific response profile measuring immunological impacts of tumor-derived exosomes on immune cells *in vitro*, wherein said immune cells are T-cells.

[00009h] In yet another aspect, the present disclosure refers to use of a tumor-derived exosome-induced immune response for generating a cancer-specific response profile measuring immunological impacts of tumor-derived exosomes on immune cells *in vitro*, wherein said tumor-derived exosomes are harvested from a subject having cancer and said cancer-specific response profile is indicative of the cancer type in the subject, and wherein said immune cells are T-cells.

[00009i] In yet another aspect, the present disclosure refers to a method of generating a response profile specific for a cancer type, said method comprising the steps of: (1) measuring

immunological impacts of tumor-derived exosomes on immune cells *in vitro*, wherein said tumor-derived exosomes are harvested from a subject having a specific cancer type; and (2) creating a cancer-specific response profile based on the immunological impact specific for the cancer type, wherein said immune cells are T-cells.

[00009j] In yet another aspect, the present disclosure refers to a test, assay, kit, apparatus or device when used for the method as disclosed herein, the response as disclosed herein, the profile as disclosed herein, or the use as disclosed herein.

[00010] DETAILED DESCRIPTION OF THE INVENTION

[00011] The present inventors have now discovered that heterogeneous functional impacts of TEXs on immune cells might serve as another identity signature in addition to the cancer-specific genetic and protein information in TEXs.

[00012] According to a first embodiment of the present invention, there is provided a method of detecting cancer or cancer type in a subject, said method comprising the step of using a cancer-specific response profile, created based on the measurement of functional impacts of tumor-derived exosomes on immune cells *in vitro*, to identify the cancer or the cancer type in the subject, wherein said tumor-derived exosomes are isolated from the subject.

[00013] According to a second embodiment of the present invention, there is provided a method of simultaneously testing for, or distinguishing between, multiple types of cancer in a

CLAIMS

1. A method of (i) detecting a cancer type in a subject having cancer or (ii) simultaneously testing for, or distinguishing between, multiple types of cancer in a subject, said method comprising the step of using a cancer-specific response profile, created based on the measurement of immunological impacts of tumor-derived exosomes on immune cells *in vitro*, to identify the cancer type or types in the subject, wherein said tumor-derived exosomes are harvested from the subject, and wherein said immune cells are T-cells.

2. A method of screening subjects for a cancer type or cancer types, said method comprising the step of using a cancer-specific response profile created for each subject, created based on the measurement of immunological impacts of tumor-derived exosomes on immune cells *in vitro*, to identify the cancer type or types in each said subject, wherein said tumor-derived exosomes are harvested from the subjects, and wherein said immune cells are T-cells.

3. A method of treating a subject with a cancer type, said method comprising the steps of:

(1) using a cancer-specific response profile, created based on the measurement of immunological impacts of tumor-derived exosomes on immune cells *in vitro*, to identify the cancer type in the subject, wherein said tumor-derived exosomes are harvested from the subject; and

(2) treating the subject if the subject has been found to have the cancer type, wherein said immune cells are T-cells.

4. A method of identifying whether a subject having a cancer type is responding to treatment of that cancer type, said method comprising the steps of:

(1) using a cancer-specific response profile, created based on the measurement of immunological impacts of tumor-derived exosomes on immune cells *in vitro*, to identify the cancer type in the subject, wherein said tumor-derived exosomes are harvested from the subject; and

(2) comparing the respective cancer-specific response profile created before and during and/or after treatment of the cancer type, wherein a change in the cancer-specific response profile identifies the subject as having responded to the treatment of the cancer type, wherein said immune cells are T-cells.

5. A tumor-derived exosome-induced immune response or cancer-specific response profile created based on the measurement of immunological impacts of tumor-derived exosomes on immune cells *in vitro* when used for detecting or diagnosing a cancer type in a subject, wherein said immune cells are T-cells.

6. A tumor-derived exosome-induced immune response when used for creating a cancer-specific response profile measuring immunological impacts of tumor-derived exosomes on immune cells *in vitro*, wherein said immune cells are T-cells.

7. Use of a tumor-derived exosome-induced immune response for generating a cancer-specific response profile measuring immunological impacts of tumor-derived exosomes on immune cells *in vitro*, wherein said tumor-derived exosomes are harvested from a subject having cancer and said cancer-specific response profile is indicative of the cancer type in the subject, and wherein said immune cells are T-cells.

8. A method of generating a response profile specific for a cancer type, said method comprising the steps of:

(1) measuring immunological impacts of tumor-derived exosomes on immune cells *in vitro*, wherein said tumor-derived exosomes are harvested from a subject having a specific cancer type; and

(2) creating a cancer-specific response profile based on the immunological impact specific for the cancer type,
wherein said immune cells are T-cells.

9. The method of any one of claims 1-4 and 8, the response of claim 5 or 6, the profile of claim 5, or the use of claim 7, wherein the T-cells are selected from the group consisting of CD8 T-cells and CD4 T-cells.

10. The method, response, profile or use of claim 9, wherein the T-cells are selected from the group consisting of naïve CD8⁺ T-cells, naïve CD4⁺ T-cells, activated (Act) CD8⁺ T-cells and Act CD4⁺ T-cells.

11. The method of any one of claims 1-4 and 8-10, the response of any one of claims 5, 6, 9 and 10, the profile of any one of claims 5, 9 and 10, or the use of any one of claims 7, 9 and 10, wherein creating a cancer-specific response profile comprises measuring one or more of the following immunological impacts: suppression of the function of immune cells; impairment of immune cell responses to stimulants; promotion of expansion of regulatory immune cells; induction of apoptosis of cytotoxic immune cells; or immunostimulation.

12. The method, response, profile or use of claim 11, wherein the immunological impact is immunosuppression due to one or more of the following immunoregulatory molecules: IL-10, TGF- β , PD-1, PDL-1, TRAIL, FasL, CD39 and CD73.

13. The method, response, profile or use of claim 11, wherein the immunological impact is an immunostimulatory effect due to one or more of the following molecules: tumor antigens and heat shock proteins.

14. The method of any one of claims 1-4 and 8-13, the response of any one of claims 5, 6 and 9-13, the profile of any one of claims 5 and 9-13, or the use of any one of claims 7 and 9-13, wherein creating a cancer-specific response profile comprises measuring at least one expression level of a marker on and/or in an immune cell.

15. The method, response, profile or use of claim 14, wherein the marker is selected from an immune cell activation marker, an immune cell proliferation marker, an immune cell exhaustion marker, an immune cell cytotoxicity marker, an immune cell cytotoxicity and apoptosis marker, or an immune cell inhibitory marker.

16. The method, response, profile or use of claim 15, wherein the activation marker is CD69, CD25 or pSTAT5.

17. The method, response, profile or use of claim 15, wherein the proliferation marker is Ki67.

18. The method, response, profile or use of claim 15, wherein the exhaustion marker is Tim3.

19. The method, response, profile or use of claim 15, wherein the cytotoxicity marker is Granzyme B or IFN γ .

20. The method, response, profile or use of claim 15, wherein the cytotoxicity and apoptosis marker is FasL.

21. The method, response, profile or use of claim 15, wherein the inhibitory marker is PD-1 or CTLA4.

22. The method of any one of claims 1-4 and 8-21 the response of any one of claims 5, 6 and 9-21, the profile of any one of claims 5 and 9-21, or the use of any one of claims 7 and 9-21, wherein the cancer is selected from the group consisting of renal carcinoma, colorectal carcinoma (colon cancer and/or rectal cancer), skin cancer (including basal cell carcinoma, cell carcinoma, squamous cell carcinoma and melanoma), leukemia, lymphoma, tumors of the central nervous system, breast cancer, prostate cancer, cervical cancer, uterine cancer, lung cancer, ovarian cancer, testicular cancer, thyroid cancer, astrocytoma, glioma, pancreatic cancer, mesotheliomas, gastric cancer, liver cancer, renal cancer including nephroblastoma, bladder cancer, oesophageal cancer, cancer of the larynx, cancer of the parotid, cancer of the biliary tract, endometrial cancer, adenocarcinomas, small cell carcinomas, neuroblastomas, adrenocortical carcinomas, epithelial carcinomas, desmoid tumors, desmoplastic small round cell tumors, endocrine tumors, Ewing sarcoma family tumors, germ cell tumors, hepatoblastomas, hepatocellular carcinomas, non-rhabdomyosarcome soft tissue sarcomas, osteosarcomas, peripheral primitive neuroectodermal tumors, retinoblastomas, and rhabdomyosarcomas.

23. The method of any one of claims 1-4 and 8-22, the response of any one of claims 5 and 9-22, or the profile of any one of claims 5 and 9-22, comprising the step of comparing the created cancer-specific response profile of the subject with one or more previously created reference cancer-specific response profiles, wherein each said reference profile was created based on a subject diagnosed with a particular type of cancer.

24. The method of any one of claims 1-4 and 8-23, the response of any one of claims 5 and 9-23, or the profile of any one of claims 5 and 9-23, comprising the step of comparing the created cancer-specific response profile of the subject with a prebuilt database of reference cancer-specific response profiles, wherein matching or statistically significantly matching subject and reference profiles indicate the type of cancer that the subject has.

25. The method of any one of claims 1-4 and 8-24, the response of any one of claims 5, 6 and 9-24, the profile of any one of claims 5 and 9-24, or the use of any one of claims 7 and 9-24, wherein a plurality of different immunological impact types are used to create a cancer-specific response profile.

26. The method of any one of claims 1-4 and 8-25, the response of any one of claims 5, 6 and 9-25, the profile of any one of claims 5 and 9-25, or the use of any one of claims 7 and 9-25, wherein measuring the immunological impact of tumor-derived exosomes on immune cells to create a cancer-specific response profile (or reference profile) comprises the step of calculating a first 'Parameter' score based on the immunological impact, normalized with respect to a control, wherein the first Parameter score is calculated by dividing the geometric mean for the immunological impact by an average geometric mean for the control, and then log-2 transforming to obtain the first Parameter score for that immunological impact.

27. The method, response, profile or use of claim 26, wherein the first Parameter score is calculated in accordance with:

$$\text{Parameter Score (M}_i\text{)} = \log_2 \frac{\text{gMFI}_i \text{ of sample}}{\text{Average gMFI}_i \text{ of control}}$$

wherein gMFI_i is a geometric mean of the immunological impact.

28. The method, response, profile or use of claim 27, wherein measuring the immunological impact of tumor-derived exosomes on immune cells to create a cancer-specific response profile (or reference profile) comprises the step of calculating a second ('Exo') score based on a mean absolute value of the first Parameter score.

29. The method, response, profile or use of claim 28, wherein the second Exo score is calculated in accordance with:

$$\text{Exo Score} = \frac{\sum_{i=1}^n |M_i|}{n}$$

wherein the M_i is the first Parameter score and n is the number of Parameter scores.

30. The method, response, profile or use of claim 29, wherein the immunological impact of tumor-derived exosomes on immune cells to create a cancer-specific response profile (or

reference profile) comprises the step of calculating a third 'Deviation' score based on a mean of the absolute value of an average normalized deviation of the first Parameter score.

31. The method, response, profile or use of claim 30, wherein the third Deviation score is calculated in accordance with:

$$\text{Deviation Score} = \frac{\sum_{i=1}^n |\overline{NPD}_i|}{n}$$

wherein the NPD comprises a normalized parameter deviation calculated in accordance with:

$$\text{Normalized parameter deviation (NPD)} = \frac{x_i - M_i}{|M_i|}$$

and wherein x is the Parameter score of a test sample for immunological impact i and M is the identified Parameter score for that immunological impact.

32. The method, response, profile or use of claim 31, wherein the immunological impact is selected for inclusion in the response profile after conducting linear regressions and Spearman's rank-order correlation tests of first Parameter score data.

33. The method, response, profile or use of claim 32, wherein the first Parameter score, the second Exo score and the third Deviation score are based on the fluorescence intensity of the expression level of a marker on an immune cell.

34. The method, response, profile or use of claim 33, wherein the first Parameter score, the second Exo score and/or the third Deviation score are used in creating cancer-specific response profiles.

35. The method, response, profile or use of claim 34, wherein the first Parameter score, the second Exo score and/or the third Deviation score are used in creating reference profiles from subjects known to have cancer.

36. The method, response, profile or use of claim 35, wherein the first Parameter score, the second Exo score and/or the third Deviation score are used when comparing the cancer specific response profile of a subject to reference profiles.

37. The method, response, profile or use of claim 36, wherein the second Exo score is used to give an overall 'yes' or 'no' answer as to whether cancer is present in the subject.

38. The method, response, profile or use of claim 37, wherein the third Deviation score is used to determine the type of cancer present in the subject.

39. The method, response, profile or use of claim 38, wherein the cancer specific response profile of the subject and reference profile are each in the form of an immune response signature barcode.

40. The method, response, profile or use of claim 39, wherein with regard to markers expressed on or in an immune cell, the method comprises:

- (a) calculating a first Parameter score based on the expression level of the marker on the immune cell normalized with the expression level of the marker on a control immune cell;
- (b) calculating a second Exo score based on a mean absolute value of the first Parameter score;
- (c) calculating a third Deviation score based on a mean of the absolute value of an average normalized deviation of the first Parameter score; and
- (d) comparing the first Parameter score, the second Exo score, and/or the third Deviation score of the subject response profile to a set of reference profiles prepared from subjects known to have cancer.

41. The method of any one of claims 1-4 and 8-40, the response of any one of claims 5, 6 and 9-40, the profile of any one of claims 5 and 9-40, or the use of any one of claims 7 and 9-40, wherein the subject is a human.

42. A test, assay, kit, apparatus or device when used for the method of any one of claims 1-4 and 8-41, the response of any one of claims 5, 6 and 9-41, the profile of any one of claims 5 and 9-41, or the use of any one of claims 7 and 9-41.

43. The method of any one of claims 1-4 and 8-41, the response of any one of claims 5, 6 and 9-41, the profile of any one of claims 5 and 9-41, or the use of any one of claims 7 and 9-41, wherein the tumor-derived exosomes are harvested from a liquid biopsy taken from the subject.