to spread in the absence of any medical intervention. By the time the patient seeks medical help, the cancer may be out of reach of available clinical treatments. Moreover, unavailability of good biomarkers is another hindrance for cancer treatment.

[005] Cancer can be detected by several ways, including the medical imaging, tissue biopsy, and liquid biopsy. Once a possible cancer is detected it is usually diagnosed by microscopic examination of a tissue sample from a tissue biopsy. Detecting and diagnosing cancers early on is essential when it comes to treatment outcome and survival, especially when it comes to highly malignant tumors. From several decades, the only method for detecting cancer in humans has been to remove surgically a little portion of the tumour and to examine it with a microscope to look for cancer cells in the tissue. Unfortunately, this surgical intervention can only be carried out when the tumour has attained a certain volume or begins to be the cause of functional disorders. As this method can be used only when clinical symptoms are present, it cannot be considered as the best one for an early diagnosis of cancer or, in other words, it cannot be used as a screening test routinely made before any clinical sign of cancer. Therefore, there is a need to develop a simple yet highly sensitive and specific cancer detection systems and methods to overcome the above and other problems.

[006] Biomarkers are not only important for diagnostic purposes but can also be of great prognostic value. With the identification of the right biomarker, the cancer progression and effect and success of chemotherapeutic drugs can be evaluated in great details. There is an urgent need to identify reliable biomarkers for different cancers and also to develop methods for efficient detection and prediction of cancer and/or metabolically altered and/or quiescent cells.

SUMMARY

[007] In an example of the present disclosure, there is provided an *in-vitro* method for detecting presence of metabolically altered cells, said method comprising: (a) obtaining a blood sample; (b) enriching pluripotent stem cells from the blood sample, to obtain a

mixture comprising said pluripotent stem cells; (c) obtaining nucleic acid from the mixture of step (b); (d) performing an assay with the nucleic acid for analysing expression level of at least one biomarker of pluripotent stem cell; and (e) comparing the expression level of the at least one biomarker of pluripotent stem cell in the sample with an expression level of the at least one biomarker in a control sample, wherein an increase in the expression level of the at least one biomarker in the sample as compared to the expression level of the at least one biomarker in the control sample detects the presence of metabolically altered cells. In one aspect, the present disclosure refers to an *in-vitro* method for detecting presence of metabolically altered cells, said method comprising: a) enriching pluripotent stem cells from a blood sample, to obtain a mixture comprising said pluripotent stem cells; b) obtaining nucleic acid from the mixture of step (a); c) performing an assay with the nucleic acid for analysing expression level of at least one biomarker of pluripotent stem cell; and d) comparing the expression level of the at least one biomarker of pluripotent stem cell in the sample with an expression level of the at least one biomarker in a control sample, wherein an increase in the expression level of the at least one biomarker in the sample is at least 2 folds as compared to the expression level of the at least one biomarker in the control sample for detecting the presence of metabolically altered cells.

[008] In a second example of the present disclosure, there is provided an *in-vitro* method for detecting presence of quiescent cells, said method comprising: (a) obtaining a blood sample; (b) enriching pluripotent stem cells from the blood sample, to obtain a mixture comprising said pluripotent stem cells; (c) obtaining nucleic acid from the mixture of step (b); (d) performing an assay with the nucleic acid for analysing expression level of at least one biomarker of pluripotent stem cell; and (e) comparing the expression level of the at least one biomarker of pluripotent stem cell in the sample with an expression level of the at least one biomarker in a control sample, wherein an increase in the expression level of the at least one biomarker in the sample as compared to the expression level of the at least one biomarker in the control sample detects the presence of quiescent cells. In another aspect, the present disclosure refers to an *in-*

vitro method for detecting presence of quiescent cells, said method comprising: a) enriching pluripotent stem cells from a blood sample, to obtain a mixture comprising said pluripotent stem cells; b) obtaining nucleic acid from the mixture of step (a); c) performing an assay with the nucleic acid for analysing expression level of at least one biomarker of pluripotent stem cell; and d) comparing the expression level of the at least one biomarker of pluripotent stem cell in the sample with an expression level of the at least one biomarker in a control sample, wherein an increase in the expression level of the at least one biomarker in the sample is at most 1.9 folds as compared to the expression level of the at least one biomarker in the control sample for detecting the presence of quiescent cells.

[009] In a third example of the present disclosure, there is provided an *in-vitro* method for detecting cancer, said method comprising: (a) obtaining a blood sample; (b) enriching pluripotent stem cells from the blood sample, to obtain a mixture comprising said pluripotent stem cells; (c) obtaining nucleic acid from the mixture of step (b); (d) performing an assay with the nucleic acid for analysing expression level of at least one biomarker of pluripotent stem cell; and (e) comparing the expression level of the at least one biomarker of pluripotent stem cell in the sample with an expression level of the at least one biomarker in a control sample, wherein an increase in the expression level of the at least one biomarker in the sample as compared to the expression level of the at least one biomarker in the control sample detects cancer.

In yet another aspect, the present disclosure refers to an *in-vitro* method for detecting cancer, said method comprising: a) enriching pluripotent stem cells from a blood sample, to obtain a mixture comprising said pluripotent stem cells; b) obtaining nucleic acid from the mixture of step (a); c) performing an assay with the nucleic acid for analysing expression level of at least one biomarker of pluripotent stem cell; and d) comparing the expression level of the at least one biomarker of pluripotent stem cell in the sample with an expression level of the at least one biomarker in a control sample, wherein an increase in the expression level of the at least one biomarker in the sample is at least 10 folds as compared to the expression level of the at least one biomarker in the control sample for detecting cancer.

[0010] In a fourth example of the present disclosure, there is provided an *in-vitro* method for predicting cancer, said method comprising: (a) obtaining a blood sample; (b) enriching pluripotent stem cells from the blood sample, to obtain a mixture comprising said pluripotent stem cells; (c) obtaining nucleic acid from the mixture of step (b); (d) performing an assay with the nucleic acid for analysing expression level of at least one biomarker of pluripotent stem cell; and (e) comparing the expression level of the at least one biomarker of pluripotent stem cell in the sample with an expression level of the at least one biomarker in a control sample, wherein an increase in the expression level of the at least one biomarker in the sample as compared to the expression level of the at least one biomarker in the control sample predicts cancer. In yet another aspect, the present disclosure refers to an *in-vitro* method for predicting cancer, said method comprising: a) enriching pluripotent stem cells from a blood sample, to obtain a mixture comprising said pluripotent stem cells; b) obtaining nucleic acid from the mixture of step (a); c) performing an assay with the nucleic acid for analysing expression level of at least one biomarker of pluripotent stem cell; d) comparing the expression level of the at least one biomarker of pluripotent stem cell in the sample with an expression level of the at least one biomarker in a control sample, wherein an increase in the expression level of the at least one biomarker in the sample

as compared to the expression level of the at least one biomarker in the control sample is at least 6 folds for predicting cancer.

[0011] In a fifth example of the present disclosure, there is provided an *in-vitro* method for monitoring response to cancer treatment, said method comprising: (a) obtaining a blood sample at a time point during an anti-cancer therapy; (b) enriching pluripotent stem cells from the blood sample, to obtain a mixture comprising said pluripotent stem cells; (c) obtaining nucleic acid from the mixture of step (b); (d) performing an assay with the nucleic acid for analysing expression level of at least one biomarker of pluripotent stem cell; and (e) comparing the expression level of the at least one biomarker of pluripotent stem cell with an expression level of the at least one biomarker of pluripotent stem cell in a reference sample to monitor the response to cancer treatment. In yet another aspect, the present disclosure refers to an *in-vitro* method for monitoring response to anti-cancer therapy, said method comprising: a) enriching pluripotent stem cells from a blood sample to obtain a mixture comprising said pluripotent stem cells, wherein the blood sample had been obtained at one time point during an anti-cancer therapy; b) obtaining nucleic acid from the mixture of step (a); c) performing an assay with the nucleic acid for analysing expression level of at least one biomarker of pluripotent stem cell; and d) comparing the expression level of the at least one biomarker of pluripotent stem cell in the sample with an expression level of the at least one biomarker of pluripotent stem cell in a reference that monitors the response to anti-cancer therapy, wherein a decrease in the expression level of the at least one biomarker of pluripotent stem cell as compared to the expression level in the reference is at least 2 folds for indicating a positive response to the anti-cancer therapy, and wherein the reference is at least one selected from a group consisting of: (i) a blood sample obtained prior to administration of anti-cancer therapy; (ii) a blood sample obtained at a previous time point as compared to the time point mentioned in step (a); and (iii) a blood sample obtained from a cancer-free subject.

[0012] In a sixth example of the present disclosure, there is provided an *in-vitro* method for detecting a positive response to cancer treatment, said method comprising:

(a) obtaining a blood sample-I, before administration of an anti-cancer therapy; (b) obtaining a blood sample-II, after administration of the anti-cancer therapy; (c) enriching pluripotent stem cells from the blood sample-I to obtain a mixture-I comprising said pluripotent stem cells; (d) enriching pluripotent stem cells from the blood sample-II to obtain a mixture-II comprising said pluripotent stem cells; (e) obtaining nucleic acid-I from the mixture-I; (f) obtaining nucleic acid-II from the mixture-II; (g) independently performing an assay with the nucleic acid-I and the

nucleic acid-II for analysing expression level of at least one biomarker of pluripotent stem cell; and (h) comparing the expression levels of the at least one biomarker of pluripotent stem cell from the nucleic acid-II with the expression level of the at least one biomarker of pluripotent stem cell from the nucleic acid-I, wherein a decrease in the expression level of the at least one biomarker of pluripotent stem cell from the nucleic acid-II as compared to the expression level of the at least one biomarker of pluripotent stem cell from the nucleic acid-I detects a positive response to the cancer treatment. In yet another aspect, the present disclosure refers to an *in-vitro* method for detecting a positive response to anti-cancer therapy, said method comprising: a) enriching pluripotent stem cells from a blood sample-I to obtain a mixture-I comprising said pluripotent stem cells, wherein the blood sample-I had been obtained before administration of an anti-cancer therapy; b) enriching pluripotent stem cells from the blood sample-II to obtain a mixture-II comprising said pluripotent stem cells wherein the blood sample-II had been obtained after administration of the anti-cancer therapy; c) obtaining nucleic acid-I from the mixture-I; d) obtaining nucleic acid-II from the mixture-II; e) independently performing an assay with the nucleic acid-I and the nucleic acid-II for analysing expression level of at least one biomarker of pluripotent stem cell; and f) comparing the expression levels of the at least one biomarker of pluripotent stem cell from the nucleic acid-II with the expression level of the at least one biomarker of pluripotent stem cell from the nucleic acid-I, wherein a decrease in the expression level of the at least one biomarker of pluripotent stem cell from the nucleic acid-II as compared to the expression level of the at least one biomarker of pluripotent stem cell from the nucleic acid-I is at least 2 folds for detecting a positive response to the cancer treatment.

[0013] In a seventh example of the present disclosure, there is provided an *in-vitro* method for detecting cancer, said method comprising: (a) obtaining a blood sample; (b) enriching pluripotent stem cells from the blood sample, to obtain a mixture comprising said pluripotent stem cells; (c) obtaining nucleic acid from the mixture; (d) performing an assay with the nucleic acid for analysing expression level of at least one

biomarker of pluripotent stem cell; (e) comparing the expression level of the at least one biomarker of pluripotent stem cell in the sample with an expression level of the at least one biomarker in a control sample, wherein an increase in the expression level of the at least one biomarker in the sample as compared to the expression level of the at least one biomarker in the control sample detects presence of cancer; (f) performing sequence-based assays on the nucleic acid and analysing for mutation in at least one cancer-related marker, wherein presence of mutation in the at least one cancer-related marker indicates presence of a specific type of cancer based on the cancer-related marker analysed. In yet another aspect, the present disclosure refers to an *in-vitro* method for detecting cancer, said method comprising: a) enriching pluripotent stem cells from a blood sample, to obtain a mixture comprising said pluripotent stem cells; b) obtaining nucleic acid from the mixture of step (a); c) performing an assay with the nucleic acid for analysing expression level of at least one biomarker of pluripotent stem cell; d) comparing the expression level of the at least one biomarker of pluripotent stem cell in the sample with an expression level of the at least one biomarker in a control sample, wherein an increase in the expression level of the at least one biomarker in the sample as compared to the expression level of the at least one biomarker in the control sample is at least 10 folds for indicating presence of cancer; and e) performing sequence-based assays on the nucleic acid and analysing for mutation in at least one cancer-related marker, wherein presence of mutation in the at least one cancer-related marker indicates presence of a specific type of cancer based on the cancer-related marker analysed.

[0014] In an eighth example of the present disclosure, there is provided a use of a pluripotent stem cell biomarker selected from a group consisting of Oct-4, Sox-2, Nanog, p53, NFκB, Sirt-1, Sirt-6, Sirt-3, NAD, RAS, ERC, erbB-2, ABL, subsets thereof, and combinations thereof, for detecting cancer from a blood sample. In yet another aspect, the present disclosure refers to use of a pluripotent stem cell biomarker selected from a group consisting of Oct-4, Sox-2, Nanog, p53, NFκB, Sirt-1, Sirt-6,

Sirt-3, NAD, RAS, ERC, erbB-2, ABL, subsets thereof, and combinations thereof, for either detecting cancer, or predicting cancer, or for grading stage of cancer, or for monitoring progression of anti-cancer therapy from a blood sample.

[0015] In a ninth example of the present disclosure, there is provided a use of a pluripotent stem cell biomarker selected from a group consisting of Oct-4, Sox-2,

Nanog, p53, NFκB, Sirt-1, Sirt-6, Sirt-3, NAD, RAS, ERC, erbB-2, ABL, subsets thereof, and combinations thereof, for predicting cancer from a blood sample.

[0016] In a tenth example of the present disclosure, there is provided a use of pluripotent stem cell biomarker selected from a group consisting of Oct-4, Sox-2, Nanog, p53, NFκB, Sirt-1, Sirt-6, Sirt-3, NAD, RAS, ERC, erbB-2, ABL, subsets thereof, and combinations thereof, for grading stage of cancer from a blood sample.

[0017] In an eleventh example of the present disclosure, there is provided a use of a pluripotent stem cell biomarker selected from a group consisting of Oct-4, Sox-2, Nanog, p53, NFκB, Sirt-1, Sirt-6, Sirt-3, NAD, RAS, ERC, erbB-2, ABL, subsets thereof, and combinations thereof, for monitoring progression of anti-cancer therapy from a blood sample.

[0018] In a twelfth example of the present disclosure, there is provided a method for treating cancer, said method comprising: (a) obtaining a blood sample from a subject; (b) enriching pluripotent stem cells from the blood sample, to obtain a mixture comprising said pluripotent stem cells; (c) obtaining nucleic acid from the mixture of step (b); (d) performing an assay with the nucleic acid for analysing expression level of at least one biomarker of pluripotent stem cell; (e) comparing the expression level of the at least one biomarker of pluripotent stem cell in the sample with an expression level of the at least one biomarker in a control sample, wherein an increase in the expression level of the at least one biomarker in the sample as compared to the expression level of the at least one biomarker in the control sample detects cancer; and (f) administering an anti-cancer therapy to the subject for treating cancer.

[0019] These and other features, aspects, and advantages of the present subject matter will be better understood with reference to the following description and appended claims. This summary is provided to introduce a selection of concepts in a simplified form. This summary is not intended to identify key features or essential features of the claimed subject matter, nor is it intended to be used to limit the scope of the claimed subject matter.

I/We Claim:

1. An *in-vitro* method for detecting presence of metabolically altered cells, said method comprising:
    a) enriching pluripotent stem cells from a blood sample, to obtain a mixture comprising said pluripotent stem cells;
    b) obtaining nucleic acid from the mixture of step (a);
    c) performing an assay with the nucleic acid for analysing expression level of at least one biomarker of pluripotent stem cell; and
    d) comparing the expression level of the at least one biomarker of pluripotent stem cell in the sample with an expression level of the at least one biomarker in a control sample,

    wherein an increase in the expression level of the at least one biomarker in the sample is at least 2 folds as compared to the expression level of the at least one biomarker in the control sample for detecting the presence of metabolically altered cells.
2. An *in-vitro* method for detecting presence of quiescent cells, said method comprising:
    a) enriching pluripotent stem cells from a blood sample, to obtain a mixture comprising said pluripotent stem cells;
    b) obtaining nucleic acid from the mixture of step (a);
    c) performing an assay with the nucleic acid for analysing expression level of at least one biomarker of pluripotent stem cell; and
    d) comparing the expression level of the at least one biomarker of pluripotent stem cell in the sample with an expression level of the at least one biomarker in a control sample,

    wherein an increase in the expression level of the at least one biomarker in the sample is at most 1.9 folds as compared to the expression level of the at least one biomarker in the control sample for detecting the presence of quiescent cells.
3. An *in-vitro* method for detecting cancer, said method comprising:
    a) enriching pluripotent stem cells from a blood sample, to obtain a mixture comprising said pluripotent stem cells;
    b) obtaining nucleic acid from the mixture of step (a);
    c) performing an assay with the nucleic acid for analysing expression level of at least one biomarker of pluripotent stem cell; and

d) comparing the expression level of the at least one biomarker of pluripotent stem cell in the sample with an expression level of the at least one biomarker in a control sample,

wherein an increase in the expression level of the at least one biomarker in the sample is at least 10 folds as compared to the expression level of the at least one biomarker in the control sample for detecting cancer.

4. An *in-vitro* method for predicting cancer, said method comprising:
    a) enriching pluripotent stem cells from a blood sample, to obtain a mixture comprising said pluripotent stem cells;
    b) obtaining nucleic acid from the mixture of step (a);
    c) performing an assay with the nucleic acid for analysing expression level of at least one biomarker of pluripotent stem cell; and
    d) comparing the expression level of the at least one biomarker of pluripotent stem cell in the sample with an expression level of the at least one biomarker in a control sample,

    wherein an increase in the expression level of the at least one biomarker in the sample as compared to the expression level of the at least one biomarker in the control sample is at least 6 folds for predicting cancer.
5. The method as claimed in claim 1wherein the increase in the expression level of the at least one biomarker of pluripotent stem cell is at least 2 folds, or at least 3 folds, or at least 5 folds, or 10-20 folds, or 20-30 folds, or 30-40 folds, or 40-50 folds as compared to the control.
6. The method as claimed in any one of the claims 3, or 4, wherein the increase in the expression level of the at least one biomarker of pluripotent stem cell is in a range of 10-20 folds, or 20-30 folds, or 30-40 folds, or 40-50 folds as compared to the control.
7. An *in-vitro* method for monitoring response to anti-cancer therapy, said method comprising:
    a) enriching pluripotent stem cells from a blood sample to obtain a mixture comprising said pluripotent stem cells, wherein the blood sample had been obtained at one time point during an anti-cancer therapy;
    b) obtaining nucleic acid from the mixture of step (a);
    c) performing an assay with the nucleic acid for analysing expression level of at least one biomarker of pluripotent stem cell; and

d) comparing the expression level of the at least one biomarker of pluripotent stem cell in the sample with an expression level of the at least one biomarker of pluripotent stem cell in a reference that monitors the response to anti-cancer therapy, wherein a decrease in the expression level of the at least one biomarker of pluripotent stem cell as compared to the expression level in the reference is at least 2 folds for indicating a positive response to the anti-cancer therapy, and wherein the reference is at least one selected from a group consisting of: (i) a blood sample obtained prior to administration of anti-cancer therapy; (ii) a blood sample obtained at a previous time point as compared to the time point mentioned in step (a); and (iii) a blood sample obtained from a cancer-free subject.

8. The method as claimed in claim 7, wherein the reference is at least one selected from a group consisting of: (a) a blood sample obtained prior to administration of anti-cancer therapy; (b) a blood sample obtained at a previous time point as compared to the time point mentioned in step (a) of claim 7; (c) a blood sample obtained at a subsequent time point as compared to the time point mentioned in step (a) of claim 7; and (d) a blood sample obtained from a cancer-free subject.

9. An *in-vitro* method for detecting a positive response to anti-cancer therapy, said method comprising:
   a) enriching pluripotent stem cells from a blood sample-I to obtain a mixture-I comprising said pluripotent stem cells, wherein the blood sample-I had been obtained before administration of an anti-cancer therapy;
   b) enriching pluripotent stem cells from the blood sample-II to obtain a mixture-II comprising said pluripotent stem cells wherein the blood sample-II had been obtained after administration of the anti-cancer therapy;
   c) obtaining nucleic acid-I from the mixture-I;
   d) obtaining nucleic acid-II from the mixture-II;
   e) independently performing an assay with the nucleic acid-I and the nucleic acid-II for analysing expression level of at least one biomarker of pluripotent stem cell; and
   f) comparing the expression levels of the at least one biomarker of pluripotent stem cell from the nucleic acid-II with the expression level of the at least one biomarker of pluripotent stem cell from the nucleic acid-I,

wherein a decrease in the expression level of the at least one biomarker of pluripotent stem cell from the nucleic acid-II as compared to the expression level of the at least one biomarker of pluripotent stem cell from the nucleic acid-I is at least 2 folds for detecting a positive response to the cancer treatment.

10. An *in-vitro* method for detecting cancer, said method comprising:
    a) enriching pluripotent stem cells from a blood sample, to obtain a mixture comprising said pluripotent stem cells;
    b) obtaining nucleic acid from the mixture of step (a);
    c) performing an assay with the nucleic acid for analysing expression level of at least one biomarker of pluripotent stem cell;
    d) comparing the expression level of the at least one biomarker of pluripotent stem cell in the sample with an expression level of the at least one biomarker in a control sample, wherein an increase in the expression level of the at least one biomarker in the sample as compared to the expression level of the at least one biomarker in the control sample is at least 10 folds for indicating presence of cancer; and
    e) performing sequence-based assays on the nucleic acid and analysing for mutation in at least one cancer-related marker,
    wherein presence of mutation in the at least one cancer-related marker indicates presence of a specific type of cancer based on the cancer-related marker analysed.
11. The method as claimed in any one of the claims 1-4, 7, 9, or 10, wherein the at least one biomarker of pluripotent stem cell is selected from a group consisting of Oct-4, Oct-4a, Oct-4b, Oct 4b1, Sox-2, Nanog, p53, Sirt-1, Sirt-6, Sirt-3, NAD, RAS, ERC, erbB-2, ABL, subsets thereof, and combinations thereof.
12. The method as claimed in any one of the claims 1-4, 7, 9, or 10, wherein obtaining the nucleic acid from the mixture is by any one method selected from a group consisting of: (a) guanidinium thiocyanate-phenol-chloroform nucleic acid extraction; (b) cesium chloride gradient centrifugation method; (c) cetyltrimethylammonium bromide nucleic acid extraction; (d) alkaline extraction; (e) resin-based extraction; and (f) solid phase nucleic acid extraction, and wherein performing an assay with the nucleic acid for analysing the expression of the at least one biomarker is done by a technique selected

from a group consisting of: quantitative PCR, flow cytometry, and Next Generation Sequencing (NGS).

13. The method as claimed in any one of the claims 1-4, 7, or 10, wherein the control is the expression level of the at least one biomarker of pluripotent stem cells obtained from a cancer-free subject.
14. The method as claimed in any one of the claims 1-4, 7, 9, or 10, wherein the enriching of the pluripotent stem cells from the blood sample comprises:
    a) contacting the blood sample with a neutral buffer in a ratio range of 1:1 to 1:20, to obtain a first mixture;
    b) contacting at least one salt solution to the first mixture in a ratio range of 1:2 to 1:10, to obtain a second mixture; and
    c) processing the second mixture to obtain enriched pluripotent stem cells.

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15. The method as claimed in claim 14, wherein the processing of the second mixture comprises, at least one method selected from a group consisting of: (a) extraction process; (b) washing process; (c) centrifugation process, and combinations thereof.
16. The method as claimed in any one of the claims 1-4, 7, 9, or 10, wherein the method is independent of invasive techniques.
17. Use of a pluripotent stem cell biomarker selected from a group consisting of Oct-4, Sox-2, Nanog, p53, NFκB, Sirt-1, Sirt-6, Sirt-3, NAD, RAS, ERC, erbB-2, ABL, subsets thereof, and combinations thereof, for either detecting cancer, or predicting cancer, or for grading stage of cancer, or for monitoring progression of anti-cancer therapy from a blood sample.

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