of clarity associated with such studies with respect to the tissue to be studied *vis-à-vis* the condition of a subject, there is a knowledge gap which exists in this area of work.

[006] Stem cells, particularly of embryonic origin, possess pluripotency markers viz. Oct4, Nanog, Sox2 and their isoforms are indicative of varied differentiation potentials into multiple tissues forming organs in development, homeostasis and aging. Since stem cells contribute to tissue development, they act as molecular biosensors implicative of tissue damage and injury, a hallmark of medical conditions.

[007] Thus, there is a dire need in the art to deploy non-invasive methods based on analyzing the expression of stem cell markers, as stem cell marker are prominent biomarkers for determining severity of medical conditions and identification of embryonic-like stem cell markers in body fluids can detect medical condition non-invasively.

SUMMARY OF INVENTION

[007a] In an aspect of the present disclosure, there is provided an *in-vitro* method for detecting a cancer in a subject, the method comprising: (a) adding a salt solution to a blood sample obtained from the subject, wherein the salt solution comprises Dulbecco's Phosphate Buffered Saline (DPBS); (b) layering the blood sample over a neutral buffer, wherein the neutral buffer comprises a Ficoll Hypaque solution; (c) subjecting the blood sample to a density gradient centrifugation at a speed in the range of 200 g and 900 g to obtain a first pellet comprising red blood cells (RBCs); (d) lysing the RBCs in the first pellet to obtain a RBC-lysed solution; (e) subjecting the RBC-lysed solution to centrifugation at a speed in the range of 400 g and 4000 g to obtain a second pellet comprising enriched very small embryonic like stem cells (VSELs); (f) subjecting the second pellet to cell lysis and extracting ribonucleic acid (RNA) molecules from the enriched VSELs; (g) assaying the RNA molecules to determine an expression level of Oct 4A in the VSELs; and (h) comparing the expression level of Oct 4A in the VSELs with a reference expression level of Oct 4A, wherein an increase of 1.1 to 3 folds in the expression level of Oct 4A in the VSELs as compared to the reference expression level of Oct 4A detects the cancer in the subject.

[007b] In an aspect of the present disclosure, there is provided an in-vitro method for predicting an onset of a cancer or predicting a presence of a tumor or the cancer in a subject, the method comprising: (a) adding a salt solution to a blood sample obtained from the subject, wherein the salt solution comprises Dulbecco's Phosphate Buffered Saline (DPBS); (b) layering the blood sample over a neutral buffer, wherein the neutral buffer comprises a Ficoll Hypaque solution; (c) subjecting the blood sample to a density gradient centrifugation at a speed in the range of 200 g and 900 g to obtain a first pellet comprising red blood cells (RBCs); (d) lysing the RBCs in the first pellet to obtain a RBC-lysed solution; (e) subjecting the RBC-lysed solution to centrifugation at a speed in the range of 400 g and 4000 g to obtain a second pellet comprising enriched very small embryonic like stem cells

(VSELs); (f) subjecting the second pellet to cell lysis and extracting ribonucleic acid (RNA) molecules from the enriched VSELs; (g) assaying the RNA molecules to determine an expression level of Oct 4A in the VSELs; and (h) comparing the expression level of Oct 4A in the VSELs with a reference expression level of Oct 4A, wherein an increase of 3 to 5 folds in the expression level of Oct 4A in the VSELs as compared to the reference expression level of Oct 4A predicts the onset of the cancer in the subject, and wherein an increase of at least 5 folds in the expression level of Oct 4A in the VSELs as compared to the reference expression level of Oct 4A indicates a presence of the tumor or the cancer in the subject.

[007c] In an aspect of the present disclosure, there is provided a method for predicting an onset of a cancer or detecting a presence of a tumor or the cancer in a subject, said method comprising: (a) enumerating a number of very small embryonic like stem cells (VSELs) in a blood sample obtained from the subject, wherein the blood sample is diluted with a salt solution, wherein the salt solution comprises Dulbecco's Phosphate Buffered Saline (DPBS), wherein the enumerating is performed by flow cytometry; and (b) comparing the number of VSELs in the blood sample with a number of VSELs in a reference blood sample for determining an increase or a decrease in the number of VSELs, wherein an increase in the number of VSELs in the blood sample in the range of 2 to 5 folds as compared to the number of VSELs in the reference blood sample predicts the onset of the cancer in the subject, and wherein an increase in the number of VSELs in the blood sample by at least 5 folds as compared to the number of VSELs in the reference blood sample detects the presence of the tumor or the cancer in the subject.

In an aspect of the present disclosure, there is provided an *in-vitro* method for detecting a medical condition in a subject, said method comprising: (a) obtaining a blood sample and adding a salt solution to the blood sample; (b) layering the blood sample of step (a) over a neutral buffer and subjecting the blood sample to a density gradient centrifugation at a speed in the range of 200-900 g to obtain a first pellet comprising red blood cells (RBC); (c) lysing the RBC in the first pellet to obtain a RBC-lysed solution; (d) centrifuging the RBC-lysed solution at a speed in the range of 400–4000 g to obtain a second pellet comprising enriched very small embryonic like stem cells; (e) subjecting the second pellet to a lysis for obtaining nucleic acid from the enriched very small embryonic like stem cells; (f) performing an assay with the nucleic acid of step (e) for analysing expression level of Oct 4A in the very small embryonic like stem cells ; and (g) comparing the expression level of Oct 4A in the very small embryonic like stem cells from the blood sample with an expression level of Oct 4A in a control sample for determining an increase or decrease in the

I/We Claim:

1. An *in-vitro* method for detecting a cancer in a subject, the method comprising:

(a) adding a salt solution to a blood sample obtained from the subject, wherein the salt solution comprises Dulbecco's Phosphate Buffered Saline (DPBS);

(b) layering the blood sample over a neutral buffer, wherein the neutral buffer comprises a Ficoll Hypaque solution;

(c) subjecting the blood sample to a density gradient centrifugation at a speed in the range of 200 g and 900 g to obtain a first pellet comprising red blood cells (RBCs);

(d) lysing the RBCs in the first pellet to obtain a RBC-lysed solution;

(e) subjecting the RBC-lysed solution to centrifugation at a speed in the range of 400 g and 4000 g to obtain a second pellet comprising enriched very small embryonic like stem cells (VSELs);

(f) subjecting the second pellet to cell lysis and extracting ribonucleic acid (RNA) molecules from the enriched VSELs;

(g) assaying the RNA molecules to determine an expression level of Oct 4A in the VSELs; and

(h) comparing the expression level of Oct 4A in the VSELs with a reference expression level of Oct 4A,

wherein an increase of 1.1 to 3 folds in the expression level of Oct 4A in the VSELs as compared to the reference expression level of Oct 4A detects the cancer in the subject.

2. An *in-vitro* method for predicting an onset of a cancer or predicting a presence of a tumor or a cancer in a subject, the method comprising:

(a) adding a salt solution to a blood sample obtained from the subject, wherein the salt solution comprises Dulbecco's Phosphate Buffered Saline (DPBS);

(b) layering the blood sample over a neutral buffer, wherein the neutral buffer comprises a Ficoll Hypaque solution;

(c) subjecting the blood sample to a density gradient centrifugation at a speed in the range of 200 g and 900 g to obtain a first pellet comprising red blood cells (RBCs);

(d) lysing the RBCs in the first pellet to obtain a RBC-lysed solution;

(e) subjecting the RBC-lysed solution to centrifugation at a speed in the range of 400 g and 4000 g to obtain a second pellet comprising enriched very small embryonic like stem cells (VSELs);

(f) subjecting the second pellet to cell lysis and extracting ribonucleic acid (RNA) molecules from the enriched VSELs;

(g) assaying the RNA molecules to determine an expression level of Oct 4A in the VSELs; and

(h) comparing the expression level of Oct 4A in the VSELs with a reference expression level of Oct 4A,

wherein an increase of 3 to 5 folds in the expression level of Oct 4A in the VSELs as compared to the reference expression level of Oct 4A predicts the onset of the cancer in the subject, and

wherein an increase of at least 5 folds in the expression level of Oct 4A in the VSELs as compared to the reference expression level of Oct 4A indicates a presence of the tumor or the cancer in the subject.

3. The method of claim 1 or 2, wherein an increase in the expression level of Oct 4A in the VSELs as compared to the reference expression level of Oct 4A in the range of 5 to 10 folds indicates stage I cancer.

4. The method of any one of claims 1 to 3,

further comprising performing a sequence-based assay on the RNA molecules and analyzing the RNA molecules for a mutation in at least one cancer-related marker, wherein a presence of the mutation in the at least one cancer-related marker indicates a presence of a specific type of the cancer.

5. A method for predicting an onset of a cancer or detecting a presence of a tumor or the cancer in a subject, said method comprising:

(a) enumerating a number of very small embryonic like stem cells (VSELs) in a blood sample obtained from the subject, wherein the blood sample is diluted with a salt solution, wherein the salt solution comprises Dulbecco's Phosphate Buffered Saline (DPBS), wherein the enumerating is performed by flow cytometry; and

(b) comparing the number of VSELs in the blood sample with a number of VSELs in a reference blood sample for determining an increase or a decrease in the number of VSELs,

wherein an increase in the number of VSELs in the blood sample in the range of 2 to 5 folds as compared to the number of VSELs in the reference blood sample predicts the onset of the cancer in the subject,

and wherein an increase in the number of VSELs in the blood sample by at least 5 folds as compared to the number of VSELs in the reference blood sample detects the presence of the tumor or the cancer in the subject.

6. The method of any one of claims 1 to 5, wherein the blood sample is a peripheral blood sample.

7. The method of any one of claims 1 to 4, wherein extracting ribonucleic acid from the enriched VSELs is performed by a method selected from the group consisting of: (i) a guanidinium thiocyanate-phenol-chloroform nucleic acid extraction; (ii) a cesium chloride gradient centrifugation method; (iii) a cetyltrimethylammonium bromide nucleic acid extraction; (iv) an alkaline extraction; (v) a resin-based extraction; and (vi) a solid phase nucleic acid extraction.

8. The method of any one of claims 1 to 4, wherein assaying the RNA molecules is performed by a technique selected from the group consisting of: quantitative polymerase chain reaction (qPCR), flow cytometry, and Next Generation Sequencing (NGS).

9. The method of claim 8, wherein the technique is the qPCR.

10. The method of claim 8, wherein the technique is the flow cytometry.

11. The method of claim 8, wherein the technique is the NGS.

12. The method of claim 4, wherein the at least one cancer-related marker is selected from the group consisting of

ABL1, EVI1, MYC, APC, IL2, TNFAIP3, ABL2, EWSR1, MYCL1, ARHGEF12, JAK2, TP53, AKT1, FEY, MYCN, ATM, MAP2K4, TSC1, AKT2, FGFR1, NCOA4, BCL11B, MDM4, TSC2, ATF1, FGFR1OP, NFKB2, BLM, MEN1, VHL, BCL11A, FGFR2, NRAS, BMPR1A, MLH1, WRN, BCL2, FUS, NTRK1, BRCA1,

MSH2, WT1, BCL3, GOLGA5, NUP214, BRCA2, NF1, BCL6, GOPC, PAX8, CARS, NF2, BCR, HMGA1, PDGFB, CBFA2T3, NOTCH1, BRAF, HMGA2, PIK3CA, CDH1, NPM1, CARD11, HRAS, PIM1, CDH11, NR4A3, CBLB, IRF4, PLAG1, CDK6, NUP98, CBLC, JUN, PPARG, CDKN2C, PALB2, CCND1, KIT, PTPN11, CEBPA, PML, CCND2, KRAS, RAF1, CHEK2, PTEN, CCND3, LCK, REL, CREB1, RB1, CDX2, LM02, RET, CREBBP, RUNX1, CTNNB1, MAF, ROS1, CYLD, SDHB, DDB2, MAFB, SMO, DDX5, SDHD, DDIT3, MAML2, SS18, EXT1, SMARCA4, DDX6, MDM2, TCL1A, EXT2, SMARCB1, DEK, MET, TET2, FBXW7, SOCS1, EGFR, MITF, TFG, FH, STK11, ELK4, MLL, TLX1, FLT3, SUFU, ERBB2, MPL, TPR, FOXP1, SUZ12, ETV4, MYB, USP6, GPC3, SYK, ETV6, IDH1, TCF3, and combinations thereof.

13. The method of any one of claims 1 to 4, wherein lysing the RBCs in the first pellet comprises treating the first pellet with an ammonium chloride solution.

14. The method of claim 1 or 2, wherein an increase in the expression level of Oct 4A in the VSELs as compared to the reference expression level of Oct 4A in the range of 10 to 15 folds indicates stage II cancer.

15. The method of claim 1 or 2, wherein an increase in the expression level of Oct 4A in the VSELs as compared to the reference expression level of Oct 4A in the range of 15 to 20 folds indicates stage III cancer.

16. The method of claim 1 or 2, wherein an increase in the expression level of Oct 4A in the VSELs as compared to the reference expression level of Oct 4A of at least 20 folds indicates stage IV cancer.