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(54) Title: MARKERS FOR IDENTIFYING TUMOR CELLS, METHODS AND KIT THEREOF

(57) Abstract: The present disclosure relates to a combination of biological markers (including CD44, CD24, ABCG2, ESA, ABCC4, CD133, Oct-4, Sox-2, APC, β-catenin and P-cadherin) for identification of prognosis of cancer. The present disclosure further relates to a method of identifying the said markers, a method of predicting prognosis and a method of planning personalized treatment for cancer. The present disclosure further relates to a kit/test comprising the antibodies against/other methods of detecting said markers for the said prediction.

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"MARKERS FOR IDENTIFYING TUMOR CELLS, METHODS AND KIT THEREOF"

TECHNICAL FIELD

The present disclosure relates to a combination of biological markers for identification of prognosis of cancer. The present disclosure further relates to a method of identifying the said markers, a method of predicting prognosis and a method of planning personalized treatment for cancer. The present disclosure further relates to a kit/test comprising the antibodies against/other methods of detecting said markers for the said prediction.

BACKGROUND AND PRIOR ART OF THE DISCLOSURE

Chemotherapy/ Radiotherapy:

In the field of oncology, the detection, identification and characterization of cancer cells is an important aspect of diagnosis. Of the many challenges of medicine, none has had a more controversial beginning or has experienced more hard-fought progress than the treatment and cure of cancer. Effective treatment for most patients needed to reach every organ in the body to pin down the metastatic disease. More than 70% of cancer patients undergo chemo/radio therapy.

Despite the path breaking progress in oncology therapy from multiple angles, cancer cure still remains elusive. Advanced solid malignancies remain therapeutic challenges despite maximal therapy, in part, due to the development of resistance to radiation and chemotherapy. Eg Glioblastomas are among the most lethal of cancers with current therapies offering only palliation. Standard-of-care for glioblastoma consists of surgical resection, ionizing external beam irradiation, and chemotherapy. Radiotherapy has been the most effective nonsurgical treatment modality yet recurrence is essentially universal.

However, majority of patients undergoing chemo/radio therapy suffer from un-necessary severe side effects of the treatment. In addition many patients also show resistance to the treatment resulting in treatment failure.

Thus, there exists a need to develop diagnostic tests that can determine *a priori* the effectiveness of the prescribed chemo/radio therapy. Today, decisions for treatment are made on the basis of clinical parameters such as tumor histology, tumor volume as well as tumor stage and increasingly biological imaging techniques. Radiation/chemotherapy doses and schedules as well as combinations with drugs are prescribed as empirical class solutions under consideration of the tolerance limits of surrounding normal tissues. Since the current standard treatments prescriptions do not take in to account the heterogeneity/individuality of a tumor the therapies fail often and the patient undergoes un-necessary side effects.

Tumors typically have two kinds of cells, CSCs and tumor cells. CSCs constitute only a part of tumors and usually in minority. The bulk of tumor is made up of tumor cells which constantly divide and make the solid big mass called as 'tumor'.

On the other hand CSCs are quiescent cells which hardly divide and have the ability to self-renew, i.e divide in such a way that they make a daughter cell which is a perfect copy of them-selves plus make a cells which can go and differentiate into various cells of a particular tumor (Ref: The Biology of Cancer Stem Cells, Neethan A. Lobo et al, Annu. Rev. Cell Dev. Biol. 2007. 23:675–99 and The theoretical basis of cancer-stem-cell-based therapeutics of cancer: can it be put into practice?, Isidro Sa´ nchez-Garcı´a et al, BioEssays 29:1269–1280)

Chemo/radiotherapies work towards curtailing tumor size by targeting and killing fast dividing cells. Since CSCs hardly divide they do not get killed by these therapies and survive to make a tumor again, which is called as 'relapse' of a cancer. (Ref: Chemotherapy and Cancer Stem Cells, Jeremy N. Rich et al, Cell Stem Cell 1, October 2007; Identification of Selective Inhibitors of Cancer Stem Cells by High-Throughput Screening, Piyush B. Gupta et al, Cell 138, 1–15, August 21, 2009; Identification and targeting of cancer stem cells, Tobias Schatton et al, BioEssays 31:1038–1049; TUMOUR STEM CELLS AND DRUG RESISTANCE, Michael Dean et al, Nature Reviews, cancer, Volume 5, April 2005; Cancer stem cells in solid tumors, Patrick C.

Hermann et al, *Seminars in Cancer Biology* (2008))

From the above references it can be seen that the CSCs have been isolated from fresh tumors based on certain cell surface markers that they have, using FACS (Fluorescent Activated Cell Sorting). These isolated CSCs as few as 100-200 cells were sufficient to initiate a new tumor as against up to 10000-20000 non CSCs/ bulk tumor cells. (Ref: Prospective identification of tumorigenic breast cancer cells, Muhammad Al-Hajj et al, PNAS, April 1, 2003, vol. 100 _ no. 7 _ 3983–3988; Identification of a Cancer Stem Cell in Human Brain Tumors, Sheila K. Singh et al, CANCER RESEARCH 63, 5821–5828, September 15, 2003; Identification of human brain tumour initiating cells, Sheila K. Singh et al, NATURE | VOL 432 | 18 NOVEMBER 2004). CSCs were identified in mid 90s in blood cancers and first time in solid tumors in 2003 from breast cancers, followed by brain and all other cancers)

Presence of CSCs and lack of drugs directed against them could be one reason cancer cure has been eluding us. Multiple pharmas and biotechs are directing their efforts to invent drugs which will specifically target these CSCs in a tumor to prevent cancer relapse etc. Many of these drugs are in clinical trials.

The resistance to chemo/radio therapy and the treatment failure is due to presence of what are called Cancer Stem Cells (CSC) in the tumor. Tumors are heterogeneous in nature and contain 2 kinds of cells, cancer stem cells and tumor cells which form the bulk of the tumor. While it has been recognized for a long time that not all tumor cells have the potential to initiate a new tumor, or a recurrence after treatment, only recently methodological advances have emerged that eventually allowed identification of CSCs and to investigate their biology. CSCs have been prospectively isolated from a growing number of human cancers, including leukemias and tumors of the breast, brain, colon, head & neck and pancreas. For different tumors it has been shown that transplantation of CSC subpopulations led to higher tumor take rates when compared to unsorted populations from the same tumor.

A CSC is defined as a cell within the tumor that possesses the capacity to self-renew and to generate the heterogeneous lineages of all cancer cells that comprise a tumor. This implies that CSCs are possibly a small subpopulation of tumor cells which are able to expand the CSC pool or differentiate into cancer progenitor cells by symmetric or asymmetric division. However, this point is tumor dependent and in melanomas CSCs contributed to a significantly higher percentage of total tumor cells. The non-stem cells constitute the bulk of all cancer cells within the tumor, but have only limited proliferative potential and are non-tumorigenic.

Properties of CSCs: CSCs are quiescent cells present in the tumor and thus are functionally different from the rapidly proliferating tumor cells which make up the bulk of the tumor. CSCs by virtue of being quiescent in nature are able to resist the 'desirable effects' chemo/radio therapy well as these therapies target the rapidly dividing cells of the tumor. In addition CSCs are endowed with multiple ion channels/transports, higher hypoxia tolerance and differential gene expression, etc which contribute to their chemo/radio resistance phenomenon. An increasing body of data suggests biological differences of CSCs and non-CSCs are crucial to respond to the standard therapies and most pharmas are using these differences to design rational drugs against CSCs. For failure of radiotherapy and chemotherapy treatments, one underlying reason might be a low efficacy of current treatments on eradication of cancer stem cells (CSCs). Growing evidence indicating that CSCs are resistant to cytotoxic/radiation therapies and may thus contribute to treatment failure.

The current technologies that exist in the field similar to the instant disclosure include Oncotype Dx, and Mammaprint. These are similar but they do not detect presence of CSCs. They assess presence of ER/PR and Her-2-neu pathways in patients to assess if a patient needs post-operative chemotherapy. Currently there are no diagnostic tests which detect presence of CSCs in tumors and hence cannot predict relapse time and usefulness of chemo and radiotherapy. In addition, current methods do not offer help in choosing a particular chemotherapy drug/combination.

From a clinical point of view, the direct consequence of this concept is that cancer therapy can cure a patient only if all CSCs are eliminated and that a single surviving CSC can cause a recurrence or metastasis. In addition it also implies that if the tumor is assessed for presence of CSCs before prescribing the chemo/radio therapy there is a fair chance that the oncologist can predict the effectiveness of the treatment, manage the cancer treatment better and reduce the unwanted side effects of the treatment to patients in cases when the therapy is ineffective. Since the discovery of first solid tumor CSCs in 2004 there have been great advances in CSC biology. Predictive tests for content, distribution and sensitivity of CSCs, microenvironmental CSC niches and signatures should be used to allow CSC-based individualized tailoring of therapy within the class solutions.

STATEMENT OF THE DISCLOSURE

Accordingly, the present disclosure relates to biological marker selected from a group comprising CD44, CD24, ABCG2, ESA, ABCC4, CD133, Oct-4, Sox-2, APC, β-catenin and P-cadherin or any combination thereof for prognosis of cancer; a kit for prognosis of a subject having cancer or suspected of having cancer, said kit comprising antibody against biological marker selected from a group comprising CD44, CD24, ABCG2, ESA, ABCC4, CD133, Oct-4, Sox-2, APC, β-catenin and P-cadherin or any combination thereof, optionally along with organic solvent, reagent, secondary antibody, enzyme for performing immunohistochemistry and instruction manual; a method of identifying biological marker on cells in a biological sample being or suspected of being a tumor, said method comprising acts of a) collecting, fixing, sectioning and treating the biological sample with organic solvent, followed by antigen retrieval using predetermined sample dilutions and b) adding primary antibody against biological marker selected from a group comprising CD44, CD24, ABCG2, ESA, ABCC4, CD133, Oct-4, Sox-2, APC, β-catenin and P-cadherin or any combination thereof and adding secondary antibody conjugated with an enzyme and reagents for obtaining a colored reaction or fluorescence for identifying the biological marker; a method of prognosis of a subject having cancer or suspected of having cancer, said method comprising acts of a) collecting biological sample from the subject and identifying expression or absence of receptors selected from

a group comprising estrogen receptor, progesterone receptor and Her-2-neu receptor or any combination thereof in cells of the sample to obtain expression identified cells, b) carrying out immunohistochemistry analysis on the cells of step (a) with antibody against biological marker selected from a group comprising CD44, CD24, ABCG2, ESA, ABCC4, CD133, Oct-4, Sox-2, APC, β-catenin and P-cadherin or any combination thereof and c) identifying expression or absence of the receptors and the one or more biological markers in cells of the sample and correlating the identification of the marker with predictive outcome reference table for predicting the prognosis of the subject; and a method of treating cancer, said method comprising acts of a) identifying biological marker selected from a group comprising CD44, CD24, ABCG2, ESA, ABCC4, CD133, Oct-4, Sox-2, APC, β-catenin and P-cadherin or any combination thereof, on tumor cells in a biological sample and predicting prognosis of a subject having cancer or suspected of having cancer and b) based on the prediction, designing a cancer therapy for suppression of the cancer.

DETAILED DESCRIPTION OF THE DISCLOSURE

The present disclosure relates to, biological marker selected from a group comprising CD44, CD24, ABCG2, ESA, ABCC4, CD133, Oct-4, Sox-2, APC, β-catenin and P-cadherin or any combination thereof for prognosis of cancer.

In an embodiment of the present disclosure, the cancer is breast cancer.

In another embodiment of the present disclosure, the marker is located on tumor cells of the cancer at locations selected from a group comprising cell membrane, cytoplasm, nucleus, and nuclear membrane or any combination thereof.

The present disclosure, further relates to a kit for prognosis of a subject having cancer or suspected of having cancer, said kit comprising antibody against biological marker selected from a group comprising CD44, CD24, ABCG2, ESA, ABCC4, CD133, Oct-4, Sox-2, APC, β-catenin and P-cadherin or any combination thereof, optionally along with organic solvent, reagent, secondary antibody, enzyme for performing

immunohistochemistry and instruction manual.

The present disclosure, further relates to a method of identifying biological marker on cells in a biological sample being or suspected of being a tumor, said method comprising acts of:

a) collecting, fixing, sectioning and treating the biological sample with organic solvent, followed by antigen retrieval using predetermined sample dilutions and adding primary antibody against biological marker selected from a group comprising CD44, CD24, ABCG2, ESA, ABCC4, CD133, Oct-4, Sox-2, APC, β-catenin and P-cadherin or any combination thereof; and

b) adding secondary antibody conjugated with an enzyme and reagents for obtaining a colored reaction or fluorescence for identifying the biological marker.

In an embodiment of the present disclosure, the cancer is breast cancer.

In another embodiment of the present disclosure, the organic solvent is selected from a group comprising alcohol and xylene or any combination thereof.

In yet another embodiment of the present disclosure, the collecting, fixing, sectioning and treating is carried out under predetermined conditions by conventional immunohistochemistry technique.

The present disclosure further relates to a method of prognosis of a subject having cancer or suspected of having cancer, said method comprising acts of:

a) collecting biological sample from the subject and identifying expression or absence of receptors selected from a group comprising estrogen receptor, progesterone receptor and Her-2-neu receptor or any combination thereof in cells of the sample to obtain expression identified cells;

b) carrying out immunohistochemistry analysis on the cells of step (a) with

antibody against biological marker selected from a group comprising CD44, CD24, ABCG2, ESA, ABCC4, CD133, Oct-4, Sox-2, APC, β-catenin and P-cadherin or any combination thereof; and

c) identifying expression or absence of the receptors and the one or more biological markers in cells of the sample and correlating the identification of the marker with predictive outcome reference table for predicting the prognosis of the subject.

In an embodiment of the present disclosure, the immunohistochemistry analysis is carried out by conventional method and wherein the identification of markers is carried out by visualizing a colored reaction or fluorescence obtained at completion of the method due to staining of the cells from the sample.

In another embodiment of the present disclosure, the correlating is based on parameters selected from a group comprising percentage of staining, intensity of staining and location of staining or any combination thereof; and wherein the location of the staining is selected from a group comprising cell membrane, cytoplasm, nucleus, and nuclear membrane or any combination thereof.

In yet another embodiment of the present disclosure, the correlating comprises multiplying the percentage of staining with the intensity of staining to arrive at a predictive score in order to predict the prognosis as being good or bad depending on the location of expression of the biological marker.

In still another embodiment of the present disclosure, the predictive score is selected from a group comprising low score ranging from about 1 to about 80, moderate score ranging from about 81 to about 150 and high score ranging from about 150 to about 300.

In still another embodiment of the present disclosure, the predictive outcome reference table is individually or a combination of tables selected from a group comprising 1, 1A, 2, 2A, 3, 3A, 4, 4A, 5, 6, 6A, 7, 7A, 8, 9 and 10 or any combination of tables thereof.

The present disclosure further relates to a method of treating cancer, said method comprising acts of:

a) identifying biological marker selected from a group comprising CD44, CD24, ABCG2, ESA, ABCC4, CD133, Oct-4, Sox-2, APC, β-catenin and P-cadherin or any combination thereof, on tumor cells in a biological sample and predicting prognosis of a subject having cancer or suspected of having cancer; and

b) based on the prediction, designing a cancer therapy for suppression of the cancer.

The present disclosure is a diagnostic test that assesses the tumor sample using certain CSC specific markers using immunohistochemistry and reverse transcription polymerase reaction (RT-PCR) as a technique to find out the presence of CSCs/drug resistant cells in the given tumor which are indicative of the response of the patient to standard therapies. Examples of markers which includes but is not limited to by the following markers: CD44, CD133, CD24, Oct 4, Sox2, and ion transporters/channels present on CSCs such as the ABC family of transporters namely ABCG2 and ABCC4, ESA, APC, P-cadherin, B-catenin (phospho, total and unphospho).

The present disclosure has utility in the field of oncology for the early detection of tumors. The diagnosis/prognosis of a possible cancer will help oncologist in planning the chemo and prescribing alternate targeted treatment. The patient will be further spared from unwanted side effects of the expensive treatment.

The present disclosure also relates to markers used to identify the CSCs and a combination of these markers and methodologies to detect such Cancer Stem Cells (CSCs).

A more complete understanding can be obtained by reference to the following specific examples, which are provided for purposes of illustration only and are not intended to limit the scope of the disclosure.

The following examples represent various markers which may be used either individually or in combination with each other for prognosis of breast cancer. The examples provided herein illustrate the kinds of combinations which are possible for analysing and arriving at prognosis of a subject having or suspected of having cancer. The tables provided herein are combined for the sake of representation and clarity as to how to arrive at an interpretation by perceiving either a combination of markers or the markers individually. Any table from any combination illustrated herein may be used either single or in combination with any other marker provided herein for an interpretation which may not have been explicitly illustrated by way of the examples herein. All such possible combinations and interpretations of such combinations fall within the scope of the instant disclosure. A person skilled in the art would therefore be able to envisage the prognosis of a subject by way of examining the sample, arriving at results, and comparing the results with the interpretation of the markers provided herein, for accurate prognosis and strategize the course of further treatment based on such prognosis.

EXAMPLES

In the present disclosure, IHC (immunohistochemistry) is performed with two antibodies against CD44 and CD24 to understand if any CSC signature in these patients can be seen. The signature being looked for is $CD44^{+}/CD24^{-/low}$

However, these two markers are known to be present on CSCs based on FACS analysis, which only detects surface/membrane associated expression. FACS can only detect membrane expression, so even though CD24 expression is seen in cytoplasm in many cases, it is considered as negative or it is marked as cytoplasmic. However, IHC can detect presence of CD24 in both membrane and cytoplasm and in many cases expression can be seen in the cytoplasm in addition to membrane or at times in cytoplasm only. Therefore, in cases where the expression is entirely cytoplasmic as disclosed in some of the tables of the instant disclosure, the results in them can be considered as CD24- (CD24

negative) if detected using FACS, unlike using IHC in the instant disclosure. Because of detection based on IHC in the instant disclosure, membrane as well as cytoplasmic, and nuclear membrane expression is detected.

In an embodiment of the present disclosure, it is to be noted that cancer/tumour node status/stage N0 also at times reflects bad outcome. However, as common practice, usually increase in node stage Eg: N2 or N1 reflect bad outcome, whereas N0 is considered to have no metastasis to the adjoining nodes and thus resulting in good outcome/having less severe form of cancer/tumor. Nonetheless, table 10 (patient history) disclosed in the instant disclosure clearly depicts that there are bad outcomes with lower node stage tumors and vice versa as well. Hence, the node status cannot be completely relied upon and thus a deeper analysis of the sample is required since good or bad prognosis cannot be solely carried out based on such priorly known techniques, as node status identification. Therefore, the instant disclosure clearly reflects that need and importance of identification of markers present on cancer cells which may be combined with previously known techniques such as the tumor node status. Thus, as is apparent from the instant disclosure, a combination of the % of cells stained, intensity of staining and location of the respective markers is to be considered for arriving at the correct prognosis of a patient sample.

In another embodiment of the present disclosure, it is also to be noted that the markers reflected below are to be identified, not only in the cancer stem cells/cancer initiating cells/tumor initiating cells of the patient sample, but instead also to encompass all the tumor cells of the sample.

Example 1

The technique used for the identification of markers in a patient sample in the present disclosure involves Immunohistochemistry (IHC) or RT-PCR.

The process of identifying the Cancer Stem Cells from the tumor is carried out using the technique as below:

Tumor sample is collected from the patient. The sample is formalin fixed by embedding the sample in paraffin to make a block

↓

The block is sectioned on a slide

↓

The slide is passed through a series of organic solvents to dehydrate the water

↓

Antigen retrieval is carried out under controlled experimental conditions, using specific sample dilutions

↓

This is followed by cooling the section on the slide to room temperature (RT)

↓

Followed by adding blocking agent

↓

Followed by adding of primary antibody against the antigen

↓

Followed by adding of secondary antibody conjugated with an enzyme

↓

Followed by adding of reagents for obtaining a coloured reaction

↓

Observing staining for identification of % of staining, intensity and location of staining. Correlating all these results to arrive at a conclusion for identifying presence of CSC's in the tumour sample and arriving at a resulting good/bad outcome.

In an embodiment of the present disclosure, the organic solvents, reagents, secondary antibody and enzymes mentioned in the detailed protocol below are only for the purposes of illustration and should not be construed to be limiting in nature. The instant disclosure envisages and encompasses all possible combinations and alternatives of such solvents, reagents, secondary antibody and enzymes known and commonly used by a person skilled in the art.

To further provide clarity on the process employed in the present disclosure, the detailed protocol of the immunohistochemistry is provided as below:

IHC Protocol

A] Coating Slides and cutting a section of FFPE block:

1. Wash new glass slides with tap water.
2. Dip the slides with 1% acid alcohol solution (1ml HCl+99 ml of 70% ethanol) for 5 mins.
3. Wash the slides with distilled water once to make sure the acid alcohol is washed off.
4. Dry the slides.
5. Dip the slides in10% Poly –L-lysine solution (PLL) in water for 15 minutes at room temperature (RT).
6. Remove the slides and dry them at RT.
7. Take a 3-5 micron section of the FFPE tumor block on these PLL coated slides using Leica Microteome.
8. Incubate the section at 60C for 3hrs or over night.

B] De-paraffinization protocol:

1. Dip the slides with tumor section in Xylene-I for 10 mins at RT
2. Dip the slides in Xylene-II for 10 mins at RT.
3. Dip the slides in Xylene-III for 10 mins at RT.
4. Dip the slides in 100% alcohol for 5 mins at RT.
5. Dip the slides in 100% alcohol for 5 mins at RT.
6. Dip the slides in 70% alcohol for 5 mins at RT.
7. Dip the slides in 70% alcohol for 5 mins at RT.
8. Dip the slides in D/W for 5 mins at RT.
9. Dip the slides in 3% H2O2 in methanol at RT for 20-30 mins.

C] Antigen retrieval and immunostaining:

This step will be different for each antibody/marker. Following the protocol for antibody A and B. The buffer for antigen retrieval for both antibodies is as follows:

1mM EDTA + 10mM Tris-Cl buffer at pH 7.4

1. For antibody A: The antigen is best retrieved by using pressure cooker conditions in Tris-EDTA buffer 3 whistles (depending on your pressure cooker, the number whistles can be adjusted. Please avoid over cooking).

For antibody B: The antigen is best retrieved by using microwave conditions as follows:

800 Watts for 6 mins

800 watts for 6 mins

200 watts for 15 mins

However, one can also try pressure cooker method as above.

2. After the antigen retrieval, cool the slides to RT in the buffer itself for 30 mins

3. Wash the slides in distilled water for 2 mins.

4. Wash the slides in TBST (Tris-Buffered Saline with 0.1% Tween 20) for 5 mins

5. Wash the slides in TBST for 5 mins.

6. Block the slides with 3% BSA solution/ if you are using a kit for the secondary antibody then use the blocking given by the kit as appropriate.

7. Add primary antibody diluted in buffer (see below) for 60 mins at RT.

8. Wash the slides in TBST for 5 mins at RT.

9. Repeat step # 8 twice more.

10. Add secondary antibody i.e HRP conjugated anti-mouse antibody at the appropriate dilution as per kit. Incubate at RT for 30-60 mins.

11. Wash in TBST for 5 mins at RT.

12. Repeat step # 11 twice more.

13. Add the substrate DAB etc as per the recommendation by the kit.

14. Wash off the excess substrate as per kit.

15. Counter stain with haematoxylin.

16 Wash off excess stain by dipping in 100% alcohol for 5 mins

17. Repeat step # 16.

18. Dry the slides.

19. Wash in xylene once for 5 mins at RT.
20. Mount in DPX/appropriate mounting medium.
21. Score/grade the slides as per the sheet.

Recipes for solutions:

1. Acid alcohol: 1ml of conc HCl + 99 ml of 70% alcohol
2. Antigen retrieval buffer: 10mMEDTA pH 8.0 + 10mM Tris-Cl pH 7.4
3. Primary Antibody dilution buffer: 10mM Tris-Cl pH7.4 + 0.9% NaCl + 0.5% BSA
4. Blocking buffer: 3-5% BSA in Tris-Cl pH 7.4 **OR** use blocking solution given by the kit **OR** as per your protocol.
5. TBST: 10mM Tris-Cl pH 7.4 + 0.9% NaCl + 0.1% Tween-20

Further, the specific sample dilutions, organic solvents and experimental conditions employed for antigen retrieval are provided as below, only for the purposes of illustration. A person skilled in the art would be able to comprehend the various parameters and conditions that are employed and thus all the various alternatives and substitutes known to the person having skill in the art for arriving at a protocol for such analysis are also under the purview of the instant disclosure:

Antibody A- Pressure cooker (PC), Tris-EDTA (TE) pH 7.4 buffer, 2 whistles at setting 2 and 1 whistle at setting 1; 1:1400 dilution

Antibody B- Microwave, 50mM Citrate buffer pH 6.0; Microwave/Cit 6.0/7 min at 'high' setting-700W; 5 min at High-750W;15min at 'defrost' setting-200W; Dilution 1:50

Antibody C- 2N Hcl, RT; 1:300 dilution

Antibody E- Water bath at 90C for 30 mins in 50mM Citrate buffer pH 6.0; Dilution 1:600

Antibody F- Pressure Cooker, Citrate buffer pH-6; 1 whistle at 1 setting on the heater and boil 20 mins at 1 setting; Dilution 1:200

Antibody G- 2N HCl, RT 15 minutes, 1:50 dilution

Antibody H- PC/Cit 6.0/2 whistles at 2 setting & 1whistle at 1 setting; Dilution 1:1000

Antibody I- Pressure cooker (2 whistles at 2 minutes, 1 whistle at 1setting), Cit 6.0;

Dilution 1:1000

Antibody J- Pressure Cooker, Cit 6.0 - Pressure cooker (2 whistles at 2 minutes, 1 whistle at 1 setting).; Antibody dilutions of 1:50

Antibody K- Water bath at 90C for 30 minutes in Citrate, pH 6.0; 1:100 dilutions

Antibody L- Pressure cooker (2 whistles at 2 minutes, 1 whistle at 1minute), TE buffer at 7.4 pH, prediluted antibody from company

Antibody O- Pressure cooker in Citrate buffer pH 6.0 at 2 whistles at 2 setting and 1 whistle at 1 setting

Example 2

The antibodies being used in the instant disclosure are selected from one or more of the following:

CD44: (Ref: CD44 Std. / HCAM Ab-4 (Clone 156-3C11))

CD24: (Ref : (CD24 (GPI-linked surface mucin) Ab-2 (Clone SN3b)

ABCG2: (Ref: NB-11093511 from Novus Biologicals)

ESA: (Ref: Novocastra Epithelial Specific Antigen)

ABCC4: (Ref: ABCC4 monoclonal antibody (M03), clone 1B2)

CD133: (Ref: PAB-12663 from Abnova)

Oct-4: (Ref: OCT4 Antibody (NB110-90606))

Sox-2: (E-18600 from Spring Biosciences)

APC: (Ref: Adenomateous Polyposis Coli gene product)

P-cadherin: (Ref: Anti-P cadherin antibody [56C1], prediluted (ab75442))

B-catenin: (Ref: Anti-Active-β-Catenin (anti-ABC), clone 8E7) JBC-1870057.

In all the tables below from tables 1-9, the intensity of cells stained, have the values ranging from 1 to 3. Here, intensity 1 = very light brown colour of slide stained, intensity 2 = medium brown colour of slide stained and intensity 3 = dark brown colour of slide stained. Intermediate colour observation is referred as 1-2 or 2-3 etc.

Further, the tables provided herein selected from a group comprising table 1, 1A, 2, 2A, 3, 3A, 4, 4A, 5, 6, 6A, 7, 7A, 8, 9 and 10, either individually or any combination of tables

thereof may be used by a person skilled in the art to interpret and arrive at relevant results for prediction of prognosis for a subject having or suspected of having cancer. These tables individually or in combination may also be referred to as predictive outcome reference table in the instant disclosure.

TABLE 1	Serial No of Patients	Antibody A			Antibody B		
		CD44			CD24		
		% of cells	intensity	location	% of cells	intensity	location
ER+/PR+ GOOD OUTCOME	1	20	1-2	M	60 80	1.5 1.5	M C
	2	20	2-3	M	10 40	1.5 1.5	M C
	3	Negative			55	1.5	C
	4	35	2	M	65-70	1.5	C
	5	5	3	M	85 50	3 2	M C
	6	40	2-3	M	80 20	2-3 1-2	C M
	7	40	3	M	35-40 25-30	3 2	C M
	8	10	1.5	C	75 35-40	2 1.5	C M
	9	20	3	M	25 15	1.5 EACH	M C
ER+/PR+ BAD OUTCOME	10	20 60	1.5 2	M C	95	1.5	C
	11	95	3	M	<10 90	1 1	M C
	12	70	3	M	50 70	2-3 1-2	M C
	13	90	2.5	M	95	2.5	C
	14	75	1.5	M	95 80	1.5 1.5	C M
	15	60	2.5	M	70	1.5	C
	16	80-85	3	M	10 20	1.5 2	C M

	17	65-70	3	M	55	1.5	C
	18	90	3	M	50 50	1 1	M C
ER-/PR- GOOD OUTCOME	19	100	2-3	M	70 70	1 1	M C
	20	90	3	M	50 70	1-2 3	M C
	21	80	3	M	60 70	1-2 1	M C
	22	40	2.5	M	95 60	1.5 1.5	C M
	23	95	3	M	60 90	2-3 2-3	M C
	24	95	3	M	50 20	1.5 1.5	C M
	25	90	3	M	Negative		
	26	95	3	M	10	1.5	M
	27	80	3	M	45 35	1.5 1.5	C M
ER-/PR- BAD OUTCOME	28	<5	1	M	60-70 40	1-2 1-2	M C
	29	75 20	3 EACH	M C	20	1-2	C
	30	5	1-2	M	50 90	2-3 2-3	M C
	31	30 50	2.5 2	M C	40 60	2-3 2-3	M C
	32	70 40	1.5 1.5	M C	95 55	2.5 1.5	C M
	33	60	2.5	M	95	2	C

		40	1.5	C	50	2	M
	34	45 30	2.5 1.5	M C	90 45	1.5 1.5	C M
	35	30	2.5	M	Negative		
	36	35 15	3 1-2	M C	50 30	2 1.5	C M

Table 1A
Expression of A/CD44 should be scored at the invasive edge and not at DCIS edge
M: cell membrane
C: cytoplasm
N: nucleus
NM: Nuclear membrane

ER/PR Status	Outcome	Expression level and location		Potential use
		Marker A	Marker B	
P/P	Good	Low expression in M **65**	Moderately high when expression in C alone **91.5** When expressed in MC, C is higher than M **M-65** **C-108**	Less aggressive follow-up
	Bad	High expression in	Moderate when	More aggressive

		M **213**	expression in C alone **142** When expressed in MC, C is higher than M **M-57** **C-82**	treatment
N/N	Good	High expression in M **238**	When expressed in MC, C is higher than M **M-82** **C-119**	Less aggressive treatment
	Bad	Low when expression in M alone **20** When expressed in MC, M is much higher than C **M-131** **C-58.5**	When expressed in MC, C is much higher than M **M-91** **C-148**	Clues for better follow-up

Ranges: Low: 1-80; Moderate: 81-150; High 150-onwards

Formula Applied for scoring:

Total of Column A (% of cells) / number of patient samples = score A

Total of Column B (intensity) / number of patient samples = score B

Score A multiplied by Score B = Final score for that Marker in that category.

The above scoring and formula has been used keeping in mind the entities only in a specific field [location of the marker] and not across all the patient samples of that category. For Example: in ER-/PR- status of patients with bad outcome for Marker A, the scoring for predicting the prognosis is carried out in the following manner:

For arriving at an interpretation of expression of Marker A in Membrane and Cytoplasm together, only 6 out of the 9 samples are considered, as the remaining 3, constitutes samples having expression only in the Membrane.

Similarly, for arriving at an interpretation of expression of Marker A in Membrane alone, only 3 out of the 9 samples are considered, as the remaining 6, constitutes samples having expression both in Cytoplasm and Membrane, and not in Membrane alone, which is the required criteria for the prediction in this category.

Similar strategy and conversion is employed for interpreting results of all the samples, across all the markers and ER/PR status.

Note: ER+/PR+ Good outcome:

It is critical to assess the presence or absence of marker A at invasive edge. It is possible that the entire tumor with DCIS focus has HIGH expression of A but the invasive edge has low or no expression and therefore it is the actual expression at the invasive edge that is very important for the interpretation of results for Marker A.

Table 1 and 1A interpretation:

Patient sample is segregated based on ER+/PR+ status as one group and ER-/PR- status as another group. On selection and segregation of such expression, the test sample is checked for the presence of either individual markers or a combination of markers (for example as captured here: CD44 in combination with CD24 is taken into account) mentioned in Table 1.

The result for good and bad prognosis/outcome should ideally show the marker status after taking into account the expression of staining intensity in conjunction with location of the marker and % of staining as disclosed in Table 1.
A pattern of good/bad outcome as depicted in Table 1A can be arrived at, by correlating the 3 entities namely % of staining, staining intensity and location of the marker in the sample after carrying out the process steps disclosed as aforementioned under Example 1. It is to be noted that it is critical to assess the presence or absence of marker A at invasive edge. It is possible that the entire tumor with DCIS focus has HIGH expression of A but the invasive edge has low or no expression and therefore, this aspect is very important for the interpretation of results for Marker A.

If the results obtained after conducting the aforementioned process steps on the test sample coincide with the marker expression results for good outcome (as disclosed in Table 1), the treatment module followed for the patients having good outcome as per Table 10 (patient history) can be referred to and treatment strategy for such patients will therefore require lesser follow up or less aggressive treatment strategy. However, in case of sample results coinciding with marker expression results for bad outcome (as disclosed in Table 1), then such patients will require more aggressive follow ups along with strategic treatment module to be followed OR an alternate treatment approach needs to be employed or conceived which can essentially comprise developing and administering antibodies specific to these combination of markers (here CD44 and CD24) to curtail its expression

Increasingly the world over it has been realised that there is a subset of patients in ER+/PR+ group who tend to have bad outcome. Our above mentioned results can segregate ER+/PR+ women with potential good and bad outcome based on

expression of CD44 and CD24. However, a person skilled in the art would be able to comprehend that based on the above mentioned results, one way to treat these patients is to prescribe anti-CD44 antibodies since CD44 is highly expressed in the cell-membrane in these patients with a hope of long term disease free survival. On the other hand, in ER-/PR- patients with potential bad outcome, there is low over all expression of CD44, and so antibodies to CD44 will not help much. Nevertheless, it is important to identify them and treat them appropriately with more targeted drugs, frequent and thorough follow-ups etc to ensure long term disease free survival. Similar strategy can be employed for treating cancer patients with varied marker expressions, some of which are illustrated in the tables below.

It is also to be noted that Good outcome from across ER/PR status cannot be combined. As aforementioned, typically +/+ patients are considered good prognosis cases and hence are need not be treated aggressively. Typically, follow ups for +/+ patients can be carried out every 6-12 months which can be too long for the +/+ bad outcome group. Thus, with the aforementioned interpretation of outcome, it can be inferred that there can be more frequent follow-ups to reduce cancer recurrences between two follow-ups, called interval recurrences, especially for the +/+ bad outcome cases/patients. On the other hand -/- cancers are aggressive cancers and hence patients with good outcome need not be aggressively treated. Whereas, -/- patients with bad outcomes, can have more detailed, effective and innovative follow-ups to catch the metastasis as early as possible and start the treatment.

TABLE 2	Serial No of Patients	Antibody A			Antibody B			Antibody F		
		CD44			CD24			ABCC4		
		% of cells	intensity	location	% of cells	intensity	location	% of cells	intensity	location
ER+/PR+ GOOD OUTCOME	1	20	1-2	M	60 80	1.5 1.5	M C	20	1-2	M
	2	20	2-3	M	10 40	1.5 1.5	M C	30	1.5	M
	3	Negative			55	1.5	C	Negative		
	4	35	2	M	65-70	1.5	C	60 35	1.5 EACH	C M
	5	5	3	M	85 50	3 2	M C	50	2	M
	6	40	2-3	M	80 20	2-3 1-2	C M	20 20	2 2	M C
	7	40	3	M	35-40 25-30	3 2	C M	20 30	2 2	M C
	8	10	1.5	C	75 35-40	2 1.5	C M	50 each	1-2 1-2	C M
	9	20	3	M	25 15	1.5 EACH	M C	15 15	1.5	M
ER+/PR+ BAD OUTCOME	10	20 60	1.5 2	M C	95	1.5	C	50	1.5	C
	11	95	3	M	<10 90	1 1	M C	Negative		
	12	70	3	M	50 70	2-3 1-2	M C	70	2	M
	13	90	2.5	M	95	2.5	C	90 50	3 2	C M
	14	75	1.5	M	95 80	1.5 1.5	C M	80	1.5	M

	15	60	2.5	M	70	1.5	C	Negative		
	16	80-85	3	M	10 20	1.5 2	C M	Negative		
	17	65-70	3	M	55	1.5	C	40	1.5	C
	18	90	3	M	50 50	1 1	M C	Negative		
ER-/PR- GOOD OUTCOME	19	100	2-3	M	70 70	1 1	M C	20	1.5	M
	20	90	3	M	50 70	1-2 3	M C	40- 50	1.5	M
	21	80	3	M	60 70	1-2 1	M C	Negative		
	22	40	2.5	M	95 60	1.5 1.5	C M	Negative		
	23	95	3	M	60 90	2-3 2-3	M C	70	2	M
	24	95	3	M	50 20	1.5 1.5	C M	Negative		
	25	90	3	M	Negative			Negative		
	26	95	3	M	10	1.5	M	25	1.5	M
	27	80	3	M	45 35	1.5 1.5	C M	85	3	M
ER-/PR- BAD OUTCOME	28	<5	1	M	60-70 40	1-2 1-2	M C	30	1.5	M
	29	75 20	3 EACH	M C	20	1-2	C	60 20	2 2	M C
	30	5	1-2	M	50 90	2-3 2-3	M C	Negative		
	31	30 50	2.5 2	M C	40 60	2-3 2-3	M C	20	1.5	M

	32	70 40	1.5 1.5	M C	95 55	2.5 1.5	C M	60	1.5	M
	33	60 40	2.5 1.5	M C	95 50	2 2	C M	80	2.5	M
	34	45 30	2.5 1.5	M C	90 45	1.5 1.5	C M	60 35	1.5 each	C M
	35	30	2.5	M	Negative			50	1.5	C
	36	35 15	3 1-2	M C	50 30	2 1.5	C M	75 55	1.5 EACH	C M

Table 2A

ER/PR Status	Outcome	Expression level and location			Potential use
		Marker A	**Marker B**	**Marker F**	
P/P	Good	Low expression in M **65**	Moderately high when expression in C alone **91.5** When expressed in MC, C is higher than M **M-65** **C-108**	Low when expression in M alone **49** When expressed in MC, C is higher than M **M-56** **C-72**	Less aggressive follow-up
	Bad	High expression in M **213**	Moderate when expression in C alone **142** When expressed in	Low when expression in C alone **67.5** Moderate when	More aggressive treatment

			MC, C is higher than M **M-57** **C-82**	expression in M alone **135** When expressed in MC, C is much higher than M **M-100** **C-270** When compared to ER+GOOD	
N/N	Good	High expression in M **238**	When expressed in MC, C is higher than M **M-82** **C-119**	Moderate expression in M **98** OR No Expression at all	Less aggressive treatment
	Bad	Low when expression in M alone **20** When expressed in MC, M is much higher than C **M-131** **C-58.5**	When expressed in MC, C is much higher than M **M-91** **C-148**	Moderate when expression in M alone **86** When expressed in MC, C is higher than M **M-85** **C-88**	Clues for better follow-up

Expression of A/CD44 should be scored at the invasive edge and not at DCIS edge
M: cell membrane
C: cytoplasm
N: nucleus

NM: Nuclear membrane

Table 2 and 2A interpretation:

Patient sample is segregated based on ER+/PR+ status as one group and ER-/PR- status as another group. On selection and segregation of such expression, the test sample is checked for the presence of either individual markers or a combination of markers (for example as captured here: CD44 and CD24 in combination with ABCC4 is taken into account) mentioned in Table 2.

The result for good and bad prognosis/outcome should ideally show the marker status after taking into account the expression of staining intensity in conjunction with location of the marker and % of staining as disclosed in Table 2.

A pattern of good/bad outcome as depicted in Table 2A can be arrived at, by correlating the 3 entities namely % of staining, staining intensity and location of the marker in the sample after carrying out the process steps disclosed as aforementioned under Example 1. It is to be noted that it is critical to assess the presence or absence of marker A at invasive edge. It is possible that the entire tumor with DCIS focus has HIGH expression of A but the invasive edge has low or no expression and therefore, this aspect is very important for the interpretation of results for Marker A.

If the results obtained after conducting the aforementioned process steps on the test sample coincide with the marker expression results for good outcome (as disclosed in Table 2), the treatment module followed for the patients having good outcome as per Table 10 (patient history) can be referred to and treatment strategy for such patients will therefore require lesser follow up or less aggressive treatment strategy. However, in case of sample results coinciding with marker expression results for bad outcome (as disclosed in Table 2), then such patients will require more aggressive follow ups along with

strategic treatment module to be followed OR an alternate treatment approach needs to be employed or conceived which can essentially comprise developing and administering antibodies specific to these combination of markers (here CD44 and CD24 in combination with ABCC4) to curtail its expression.

Increasingly the world over it has been realised that there is a subset of patients in ER+/PR+ group who tend to have bad outcome. Our above mentioned results can segregate ER+/PR+ women with potential good and bad outcome based on expression of CD44 and CD24. However, a person skilled in the art would be able to comprehend that based on the above mentioned results, one way to treat these patients is to prescribe anti-CD44 antibodies since CD44 is highly expressed in the cell-membrane in these patients with a hope of long term disease free survival. On the other hand, in ER-/PR- patients with potential bad outcome, there is low over all expression of CD44, and so antibodies to CD44 will not help much. Nevertheless, it is important to identify them and treat them appropriately with more targeted drugs, frequent and thorough follow-ups etc to ensure long term disease free survival. Similar strategy can be employed for treating cancer patients with varied marker expressions, some of which are illustrated in the tables below.

It is also to be noted that Good outcome from across ER/PR status cannot be combined. As aforementioned, typically +/+ patients are considered good prognosis cases and hence are need not be treated aggressively. Follow ups for +/+ patients can be carried out every 6-12 months which can be too long for the +/+ bad outcome group. Thus, with the aforementioned interpretation of outcome, it can be inferred that there can be more frequent follow-ups to reduce cancer recurrences between two follow-ups, called interval recurrences, especially for the +/+ bad outcome cases/patients. On the other hand -/- cancers are aggressive cancers and hence patients with good outcome need not be aggressively treated. Whereas, -/- patients with

bad outcomes, can have more detailed, effective and innovative Follow-ups to catch the metastasis as early as possible and start the treatment.

ABCC4 is a membrane transporter. Cells use it to get the chemotherapy drugs pumped out, hence when the expression of this marker is high the cells tend to be more resistant to CT (chemotherapy). Also, it is been noted that the cytoplasmic expression of this transporter also helps the cells to pump out the drugs which should be kept in mind while treating the patients. Patients with high M or M+C expression of ABCC4 will be more resistant to drugs and hence perhaps are going to have bad prognosis/outcome and therefore should be treated accordingly.

TABLE 3	Serial No of Patients	Antibody I			Antibody J		
		Oct-4			Sox-2		
		% of cells	Intensity	location	% of cells	intensity	location
ER+/PR+ GOOD OUTCOME	1	90	3	N	20	3	N
	2	Negative			Negative		
	3	Negative			Negative		
	4	Negative			15	2.5	N
	5	Negative			40	3	N
	6	Negative			40	3	N
	7	60	3	N	Negative		
	8	Negative			Negative		
	9	Negative			Negative		
ER+/PR+ BAD OUTCOME	10	70	2-3	N	Negative		
	11	90	3	N	Negative		
	12	70	2-3	N	Negative		
	13	65	1-2	C	Negative		
	14	30	1-2	N	Negative		
	15	75	1-2	N	Negative		
	16	30-35	3	N	Negative		
	17	25-30	1-2	N	Negative		
	18	80	3	N	Negative		
ER-/PR- GOOD	19	65	1-2	N	25	3	N
	20	Negative			Negative		
	21	Negative			10	3	N
	22	Negative			10	3	N
	23	Negative			Negative		
	24	Negative			Negative		
	25	75	1-2	N	Negative		
	26	Negative			80	3	N

OUTCOME	27	15	1-2	N	Negative		
	28	80	2.5	N	45	3	N
	29	40	1-2	N	25	3	N
	30	Negative			50	3	N
ER-/PR-	31	Negative			Negative		
	32	25	1-2	N	Negative		
BAD OUTCOME	33	20	1-2	N	Negative		
	34	25	2	N	Negative		
	35	20	3	N	10	3	N
	36	55	1-2	N	Negative		

Table 3A

ER/PR Status	Outcome	Expression level and location		Potential use
		Marker I	**Marker J**	
P/P	Good	**No expression at all**	Moderate expression in N **84** **Or NO expression at all**	Less aggressive follow-up
	Bad	Moderate expression in N **118**	**No expression at all**	More aggressive treatment
N/N	Good	Low expression in N **78**	Moderate expression in N **93**	Less aggressive treatment

		Or NO expression at all	**Or NO expression at all**	
	Bad	Low expression in N **76**	Moderate expression in N **98** **Or NO expression at all**	Clues for better follow-up

M: cell membrane
C: cytoplasm
N: nucleus
NM: Nuclear membrane

Table 3 and 3A interpretation:

Patient sample is segregated based on ER+/PR+ status as one group and ER-/PR- status as another group. On selection and segregation of such expression, the test sample is checked for the presence of either individual markers or a combination of markers (for example as captured here: Oct-4 in combination with Sox-2 is taken into account) mentioned in Table 3.

The result for good and bad prognosis/outcome should ideally show the marker status after taking into account the expression of staining intensity in conjunction with location of the marker and % of staining as disclosed in Table 3.

A pattern of good/bad outcome as depicted in Table 3A can be arrived at, by correlating the 3 entities namely % of staining,

staining intensity and location of the marker in the sample after carrying out the process steps disclosed as aforementioned under Example 1.

If the results obtained after conducting the aforementioned process steps on the test sample coincide with the marker expression results for good outcome (as disclosed in Table 3), the treatment module followed for the patients having good outcome as per Table 10 (patient history) can be referred to and treatment strategy for such patients will therefore require lesser follow up or less aggressive treatment strategy. However, in case of sample results coinciding with marker expression results for bad outcome (as disclosed in Table 3), then such patients will require more aggressive follow ups along with strategic treatment module to be followed OR an alternate treatment approach needs to be employed or conceived which can essentially comprise developing and administering antibodies specific to these combination of markers (here Oct-4 and Sox-2) to curtail its expression.

It is also to be noted that Good outcome from across ER/PR status cannot be combined. As aforementioned, typically +/+ patients are considered good prognosis cases and hence are need not be treated aggressively. Follow ups for +/+ patients can be carried out every 6-12 months which can be too long for the +/+ bad outcome group. Thus, with the aforementioned interpretation of outcome, it can be inferred that there can be more frequent follow-ups to reduce cancer recurrences between two follow-ups, called interval recurrences, especially for the +/+ bad outcome cases/patients. On the other hand -/- cancers are aggressive cancers and hence patients with good outcome need not be aggressively treated. Whereas, -/- patients with bad outcomes, can have more detailed, effective and innovative Follow-ups to catch the metastasis as early as possible and start the treatment.

TABLE 4	Serial No of Patients	Antibody K APC			Antibody 0 B-Catenin		
		% of cells	Intensity	Location	% of cells	Intensity	Location
ER+/PR+ GOOD OUTCOME	1	60 80 30	2 3 3	M C N	35-40 45	1-2 EACH	N M
	2	70	2	C	15 10	1-2 2	M N
	3	85 50	3 2	C M	Negative		
	4	85 70	2 2-3	C NM	20	1.5	M
	5	60 90	2 2.5	M C	-		
	6	10 90	1-2 3	M C	Negative		
	7	80 30	3 2-3	C M	10	2	N
	8	90 50	3 3	C N	Negative		
	9	75 30	2 1-2	M C	35	1.5	M
ER+/PR+ BAD OUTCOME	10	80 50	3 2-3	C NM	50	1.5	C
	11	65 85 65	2 2.5 2.5	M C NM	80	2	M
	12	60 80	2 2	M C	50 25-30	2 1-2	C M
	13	90	3	C	-		
	14	70	3	C	20	1.5	M

		60	2-3	M			
	15	90 50	2-3 2	C M	-		
	16	70 60	1-2 2	C N	-		
	17	50	1-2	C	40	2	M
	18	80 50	2.5 2.5	C NM	65	3	M
ER-/PR-GOOD OUTCOME	19	30 60	2 1-2	M C	75	1-2	M
	20	70 90	2-3 2-3	M C	60	1-2	M
	21	50 70	2 1.5	M C	20	1-2	M
	22	60 60	3 2	C M	50	1.5	C
	23	90 80	3 2.5	C M	75	1-2	M
	24	85 75	3 2-3	C M	-		
	25	85 30	3 2	C M	-		
	26	80	1-2	C	Negative		
	27	70 70	2 2	C M	60	3	M
ER-/PR-BAD OUTCOME	28	80 55	2-3 1.5	M C	50	1-2	M
	29	40 40	1.5 2	C M	60	2	M
	30	90	2-3	C	60	1-2	M

		40 65	2 2.5	M NM			
	31	95 60	3 2-3	C M	60	1-2	M
	32	80 70 50	3 2-3 2-3	C M NM	75	2	M
	33	90 85	3 2	C M	80 40	1.5 EACH	C M
	34	95 60	3 2	C M	-		
	35	30	1-2	C	-		
	36	90	2	C	Negative		

Table 4A

ER/PR Status	Outcome	Expression level and location		Potential use
		Marker K	**Marker O**	
P/P	Good	When expressed in MC, C is much higher than M **M-86** **C-225** Expression also seen in N in various levels	Low when expression in M alone **41** When expressed in N and M, M and N are almost equally expressed **M-45** **N-43**	Less aggressive follow-up
	Bad	Moderate when	Moderate	More aggressive

		expression in C alone **140** When expressed in MC, C is higher than M **M-114** **C-200** When expressed in C-NM, C is higher than NM **NM-138** **C-246**	expression in M **102** Expression in C is also seen	treatment
N/N	Good	When expressed in MC, C is higher than M **M-116** **C-152**	Moderate expression in M alone **104**	Less aggressive treatment
	Bad	When expressed in MC, C is higher than M **M-130** **C-150** Moderate when expression in C alone **108**	Moderate expression in M alone **104**	Clues for better follow-up

		When expressed in C, M and NM, C is highest, followed by NM and M **M-110** **NM-145** **C-238**		

M: cell membrane
C: cytoplasm
N: nucleus
NM: Nuclear membrane

Table 4 and 4A interpretation:

Patient sample is segregated based on ER+/PR+ status as one group and ER-/PR- status as another group. On selection and segregation of such expression, the test sample is checked for the presence of either individual markers or a combination of markers (for example as captured here: APC in combination with B-Catenin is taken into account) mentioned in Table 4.

The result for good and bad prognosis/outcome should ideally show the marker status after taking into account the expression of staining intensity in conjunction with location of the marker and % of staining as disclosed in Table 4.

A pattern of good/bad outcome as depicted in Table 4A can be arrived at, by correlating the 3 entities namely % of staining, staining intensity and location of the marker in the sample after carrying out the process steps disclosed as aforementioned under Example 1.

If the results obtained after conducting the aforementioned process steps on the test sample coincide with the marker expression results for good outcome (as disclosed in Table 4), the treatment module followed for the patients having good outcome as per Table 10 (patient history) can be referred to and treatment strategy for such patients will therefore require lesser follow up or less aggressive treatment strategy. However, in case of sample results coinciding with marker expression results for bad outcome (as disclosed in Table 4), then such patients will require more aggressive follow ups along with strategic treatment module to be followed OR an alternate treatment approach needs to be employed or conceived which can essentially comprise developing and administering antibodies specific to these combination of markers (here APC and B-Catenin) to curtail its expression.

It is also to be noted that Good outcome from across ER/PR status cannot be combined. As aforementioned, typically +/+ patients are considered good prognosis cases and hence are need not be treated aggressively. Follow ups for +/+ patients can be carried out every 6-12 months which can be too long for the +/+ bad outcome group. Thus, with the aforementioned interpretation of outcome, it can be inferred that there can be more frequent follow-ups to reduce cancer recurrences between two follow-ups, called interval recurrences, especially for the +/+ bad outcome cases/patients. On the other hand -/- cancers are aggressive cancers and hence patients with good outcome need not be aggressively treated. Whereas, -/- patients with bad outcomes, can have more detailed, effective and innovative Follow-ups to catch the metastasis as early as possible and start the treatment.

TABLE 5	**Serial No of Patients**	**Antibody G**		
		CD133		
		% of cells	**Intensity**	**Location**
ER+/PR+ GOOD OUTCOME	1	60 30	2.5 1.5	N M
	2	50	1.5	C
	3	90 20	1.5 EACH	C M
	4	30 -35 50	2 1.5	M C
	5	70 50	2 2	M C
	6	50 60	2 2	M C
	7	40 70	2 2-3	M C
	8	45	1-2	C
	9	65	2	M
ER+/PR+ BAD OUTCOME	10	80	1.5	C
	11	60	1-2	C
	12	25 80	2 2	M C
	13	80	2	C
	14	90 30	2 1.5	M C
	15	70 30	1.5 EACH	M C
	16	15 35	1.5 EACH	M C
	17	30	1.5	M
	18	Negative		

ER-/PR- GOOD OUTCOME ER-/PR-	19	NEGATIVE		
	20	70 60	2 2	M C
	21	25	1-2	M
	22	65 65	1.5 each	C M
	23	55 75	2 2	M C
	24	60 25	2 1-2	M C
	25	60 40	1.5 1.5	C M
	26	20	1.5	M
	27	55 45	1.5 EACH	C M
ER-/PR- BAD OUTCOME ER-/PR-	28	60 30	1-2 1-2	M C
	29	20 20	2 2	M C
	30	50 50	1.5 Each	M C
	31	55 10	1.5 Each	C M
	32	70 45	2 1.5	M C
	33	80 75	2 each	C M
	34	65	2	C
	35	40 20	2 3	C N

	36	50	2	C

TABLE 6	**Serial No of Patients**	**Antibody L**		
		P-Cadherin		
		% of cells	**Intensity**	**Location**
ER+/PR+ GOOD OUTCOME	1	20	2-3	M
	2	Negative		
	3	Negative		
	4	20	2	M
	5	Negative		
	6	Negative		
	7	50	3	M
	8	10	3	M
	9	40	3	M
ER+/PR+ BAD OUTCOME	10	20	3	M
	11	80	3	M
	12	20	3	M
	13	Negative		
	14	Negative		
	15	90	3	M
	16	30	2.5	M
	17	45	2.5	M
	18	90-100	3	M
ER-/PR- GOOD OUTCOME ER-/PR-	19	50	3	M
	20	55	3	M
	21	50	3	M
	22	80	3	M
	23	70	2	M

	24	70	3	M
	25	65-70	3	M
	26	Negative		
	27	80	3	M
ER-/PR- BAD OUTCOME ER-/PR-	28	80	3	M
	29	15-20	3	M
	30	15	3	M
	31	Negative		
	32	20	3	M
	33	75	3	M
	34	<5	3	M
	35	45	2.5	M
	36	Negative		

Table 6A

ER/PR Status	**Outcome**	**Expression level and location**	**Potential use**
		Marker L	
P/P	Good	Moderate expression in M **84**	Less aggressive follow-up
	Bad	High expression in M **162**	More aggressive treatment
N/N	Good	High expression in M **195**	Less aggressive treatment
	Bad	Moderate	Clues for better

		expression in M **111**	follow-up

M: cell membrane
C: cytoplasm
N: nucleus
NM: Nuclear membrane

Table 6 and 6A interpretation:

Patient sample is segregated based on ER+/PR+ status as one group and ER-/PR- status as another group. On selection and segregation of such expression, the test sample is checked for the presence of either individual markers or a combination of markers (for example as captured here: P-cadherin alone is taken into account) mentioned in Table 6.

The result for good and bad prognosis/outcome should ideally show the marker status after taking into account the expression of staining intensity in conjunction with location of the marker and % of staining as disclosed in Table 6.

A pattern of good/bad outcome as depicted in Table 6A can be arrived at, by correlating the 3 entities namely % of staining, staining intensity and location of the marker in the sample after carrying out the process steps disclosed as aforementioned under Example 1.

If the results obtained after conducting the aforementioned process steps on the test sample coincide with the marker expression results for good outcome (as disclosed in Table 6), the treatment module followed for the patients having good

outcome as per Table 10 (patient history) can be referred to and treatment strategy for such patients will therefore require lesser follow up or less aggressive treatment strategy. However, in case of sample results coinciding with marker expression results for bad outcome (as disclosed in Table 6), then such patients will require more aggressive follow ups along with strategic treatment module to be followed OR an alternate treatment approach needs to be employed or conceived which can essentially comprise developing and administering antibodies specific to these combination of markers (here P-cadherin alone) to curtail its expression.

Increasingly the world over it has been realised that there is a subset of patients in ER+/PR+ group who tend to have bad outcome. Our above mentioned results can segregate ER+/PR+ women with potential good and bad outcome based on expression of P-cadherin. However, a person skilled in the art would be able to comprehend that based on the above mentioned results, one way to treat these patients is to prescribe anti- P-cadherin antibodies since P-cadherin is highly expressed in the cell-membrane in these patients with a hope of long term disease free survival. On the other hand, in ER-/PR- patients with potential bad outcome, there is lower over all expression of P-cadherin, and so antibodies to P-cadherin will not help much. Nevertheless, it is important to identify them and treat them appropriately with more targeted drugs, frequent and thorough follow-ups etc to ensure long term disease free survival. Similar strategy can be employed for treating cancer patients with varied marker expressions, some of which are illustrated in the tables below.

It is also to be noted that Good outcome from across ER/PR status cannot be combined. As aforementioned, typically +/+ patients are considered good prognosis cases and hence are need not be treated aggressively. Follow ups for +/+ patients can be carried out every 6-12 months which can be too long for the +/+ bad outcome group. Thus, with the aforementioned

interpretation of outcome, it can be inferred that there can be more frequent follow-ups to reduce cancer recurrences between two follow-ups, called interval recurrences, especially for the +/+ bad outcome cases/patients. On the other hand -/- cancers are aggressive cancers and hence patients with good outcome need not be aggressively treated. Whereas, -/- patients with bad outcomes, can have more detailed, effective and innovative Follow-ups to catch the metastasis as early as possible and start the treatment.

TABLE 7	Serial No of Patients	Antibody A			Antibody B			Antibody I			Antibody J		
		CD44			CD24			Oct-4			Sox-2		
		% of cells	intensity	location	% of cells	intensity	location	% of cells	intensity	location	% of cells	intensity	location
ER+/PR+ GOOD OUTCOME	1	20	1-2	M	60 80	1.5 1.5	M C	90	3	N	20	3	N
	2	20	2-3	M	10 40	1.5 1.5	M C	Negative			Negative		
	3	Negative			55	1.5	C	Negative			<5%	2-3	N
	4	35	2	M	65-70	1.5	C	Negative			15	2.5	N
	5	5	3	M	85 50	3 2	M C	Negative			40	3	N
	6	40	2-3	M	80 20	2-3 1-2	C M	Negative			40	3	N
	7	40	3	M	35-40 25-30	3 2	C M	60	3	N	Negative		
	8	10	1.5	C	75 35-40	2 1.5	C M	Negative			Negative		
	9	20	3	M	25 15	1.5 EACH	M C	Negative			Negative		
ER+/PR+	10	20 60	1.5 2	M C	95	1.5	C	70	2-3	N	Negative		

BAD OUTCOME	11	95	3	M	<10 90	1 1	M C	90	3	N	Negative		
	12	70	3	M	50 70	2-3 1-2	M C	70	2-3	N	5	3	N
	13	90	2.5	M	95	2.5	C	65	1-2	C	Negative		
	14	75	1.5	M	95 80	1.5 1.5	C M	30	1-2	N	Negative		
	15	60	2.5	M	70	1.5	C	75	1-2	N	Negative		
	16	80-85	3	M	10 20	1.5 2	C M	30-35	3	N	Negative		
	17	65-70	3	M	55	1.5	C	25-30	1-2	N	Negative		
	18	90	3	M	50 50	1 1	M C	80	3	N	Negative		
ER-/PR- GOOD OUTCOME	19	100	2-3	M	70 70	1 1	M C	65	1-2	N	25	3	N
	20	90	3	M	50 70	1-2 3	M C	Negative			Negative		
	21	80	3	M	60 70	1-2 1	M C	Negative			10	3	N
	22	40	2.5	M	95 60	1.5 1.5	C M	Negative			10	3	N
	23	95	3	M	60 90	2-3 2-3	M C	Negative			Negative		
ER-/PR- GOOD OUTCOME	24	95	3	M	50 20	1.5 1.5	C M	Negative			<5	2-3	N
	25	90	3	M	Negative			75	1-2	N	Negative		
	26	95	3	M	10	1.5	M	Negative			80	3	N
	27	80	3	M	45 35	1.5 1.5	C M	15	1-2	N	Negative		

ER-/PR-	28	<5	1	M	60-70 40	1-2 1-2	M C	80	2.5	N	45	3	N
	29	75 20	3 EACH	M C	20	1-2	C	40	1-2	N	25	3	N
	30	5	1-2	M	50 90	2-3 2-3	M C	Negative			50	3	N
	31	30 50	2.5 2	M C	40 60	2-3 2-3	M C	Negative					
	32	70 40	1.5 1.5	M C	95 55	2.5 1.5	C M	25	1-2	N	Negative	<5%	-
BAD OUTCOME	33	60 40	2.5 1.5	M C	95 50	2 2	C M	20	1-2	N	Negative		
	34	45 30	2.5 1.5	M C	90 45	1.5 1.5	C M	25	2	N	Negative		
	35	30	2.5	M	Negative			20	3	N	10	3	N
	36	35 15	3 1-2	M C	50 30	2 1.5	C M	55	1-2	N	Negative		

Table 7A

ER/PR Status	Outcome	Expression level and location				Potential use
		Marker A	**Marker B**	**Marker I**	**Marker J**	
P/P	Good	Low expression in M **65**	Moderately high when expression in C alone **91.5** When expressed in MC, C is higher than M	**No expression at all**	Moderate expression in N **84** **Or NO expression at all**	Less aggressive follow-up

			M-65 **C-108**			
	Bad	High expression in M **213**	Moderate when expression in C alone **142** When expressed in MC, C is higher than M **M-57** **C-82**	Moderate expression in N **118**	**No expression at all**	More aggressive treatment
N/N	Good	High expression in M **238**	When expressed in MC, C is higher than M **M-82** **C-119**	Low expression in N **78** **Or NO expression at all**	Moderate expression in N **93** **Or NO expression at all**	Less aggressive treatment
	Bad	Low when expression in M alone **20** When expressed in MC, M is much higher than C **M-131** **C-58.5**	When expressed in MC, C is much higher than M **M-91** **C-148**	Low expression in N **76**	Moderate expression in N **98** **Or NO expression at all**	Clues for better follow-up

Expression of A/CD44 should be scored at the invasive edge and not at DCIS edge

M: cell membrane
C: cytoplasm
N: nucleus
NM: Nuclear membrane

Table 7 and 7A interpretation:

Patient sample is segregated based on ER+/PR+ status as one group and ER-/PR- status as another group. On selection and segregation of such expression, the test sample is checked for the presence of either individual markers or a combination of markers (for example as captured here: CD44 in combination with CD24, Oct-4 and Sox-2 is taken into account) mentioned in Table 7.

The result for good and bad prognosis/outcome should ideally show the marker status after taking into account the expression of staining intensity in conjunction with location of the marker and % of staining as disclosed in Table 7.

A pattern of good/bad outcome as depicted in Table 7A can be arrived at, by correlating the 3 entities namely % of staining, staining intensity and location of the marker in the sample after carrying out the process steps disclosed as aforementioned under Example 1. It is to be noted that it is critical to assess the presence or absence of marker A at invasive edge. It is possible that the entire tumor with DCIS focus has HIGH expression of A but the invasive edge has low or no expression and therefore, this aspect is very important for the interpretation of results for Marker A.

If the results obtained after conducting the aforementioned process steps on the test sample coincide with the marker expression results for good outcome (as disclosed in Table 7), the treatment module followed for the patients having good

outcome as per Table 10 (patient history) can be referred to and treatment strategy for such patients will therefore require lesser follow up or less aggressive treatment strategy. However, in case of sample results coinciding with marker expression results for bad outcome (as disclosed in Table 7), then such patients will require more aggressive follow ups along with strategic treatment module to be followed OR an alternate treatment approach needs to be employed or conceived which can essentially comprise developing and administering antibodies specific to these combination of markers (here CD44 in combination with CD24, Oct-4 and Sox-2) to curtail its expression

Increasingly the world over it has been realised that there is a subset of patients in ER+/PR+ group who tend to have bad outcome. Our above mentioned results can segregate ER+/PR+ women with potential good and bad outcome based on expression of CD44 and CD24. However, a person skilled in the art would be able to comprehend that based on the above mentioned results, one way to treat these patients is to prescribe anti-CD44 antibodies since CD44 is highly expressed in the cell-membrane in these patients with a hope of long term disease free survival. On the other hand, in ER-/PR- patients with potential bad outcome, there is low over all expression of CD44, and so antibodies to CD44 will not help much. Nevertheless, it is important to identify them and treat them appropriately with more targeted drugs, frequent and thorough follow-ups etc to ensure long term disease free survival. Similar strategy can be employed for treating cancer patients with varied marker expressions, some of which are illustrated in the tables below.

It is also to be noted that Good outcome from across ER/PR status cannot be combined. As aforementioned, typically +/+ patients are considered good prognosis cases and hence are need not be treated aggressively. Follow ups for +/+ patients can be carried out every 6-12 months which can be too long for the +/+ bad outcome group. Thus, with the aforementioned

interpretation of outcome, it can be inferred that there can be more frequent follow-ups to reduce cancer recurrences between two follow-ups, called interval recurrences, especially for the +/+ bad outcome cases/patients. On the other hand -/- cancers are aggressive cancers and hence patients with good outcome need not be aggressively treated. Whereas, -/- patients with bad outcomes, can have more detailed, effective and innovative Follow-ups to catch the metastasis as early as possible and start the treatment.

TABLE 8	**Serial No of Patients**	**Antibody C**		
		ABCG2		
		% of cells	**Intensity**	**Location**
ER+/PR+ GOOD OUTCOME	1	95 30	3 1.5	N M
	2	75	3	N
	3	95 35	1.5 1.5	C M
	4	90 35	2.5 1.5	C M
	5	50 80	2 2	M C
	6	<5 80	2 2-3	M C
	7	80 60	3 3	N C
	8	90 70 10	3 2 1-2	N C M
	9	45 35	3 2	N M

ER+/PR+ BAD OUTCOME	10	95 45	1.5 EACH	C N
	11	80 90	2 3	C N
	12	20 50 40	1.5 1.5 2.5	M C N
	13	90	2.5	C
	14	90 70	1.5 1.5	C M
	15	95 95 20	1.5 EACH	N C M
	16	30	3	N
	17	60 80	2 3	C N
	18	50 80	1.2 3	C N
ER-/PR- GOOD OUTCOME ER-/PR-	19	80	3	Nu
	20	40 80 75	2 2-3 3	M C Nu
	21	65 40 30	2.5 1.5 2	N C M
	22	75 90 65	1.5 1.5 1.5	N C M
	23	30 65	2 3	M C
	24	85 50	1.5 1.5	C M

	25	75 80 20	2.5 1.5 1.5	N C M
	26	45	2	N
	27	95 20 20	2 2 2	C N M
ER-/PR- BAD OUTCOME ER-/PR-	28	50 65 20	1.5 each	N C M
	29	40 20	2-3 3	C Nu
	30	70	2	C
	31	70 10	2.5 2	C M
	32	30 60 40	2 EACH	N M C
	33	95 10	1.5 1	C M
	34	75	1.5	C
	35	80 30	2 3	C N
	36	85 40	2 2	C N

TABLE 9	Serial No of Patients	Antibody E		
		ESA		
		% of cells	Intensity	Location
ER+/PR+ GOOD OUTCOME	1	90	3	M
	2	90	3	M
	3	95	3	M
	4	95	3	M
	5	90	3	M
	6	95	3	M
	7	95	3	M
	8	-		
	9	45	3	M
ER+/PR+ BAD OUTCOME	10	95	3	M
	11	95	3	M
	12	75	3	M
	13	95	3	M
	14	95	3	M
	15	95	3	M
	16	90	3	M
	17	55	3	M
	18	100	3	M
ER-/PR- GOOD OUTCOME ER-/PR-	19	85-90	3	M
	20	95	3	M
	21	90	3	M
	22	95	3	M
	23	90	3	M
	24	95	3	M
	25	95	3	M
	26	85	3	M
	27	35	3	M
ER-/PR- BAD	28	100	3	M
	29	50	3	M

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OUTCOME ER-/PR-	30	85	3	M
	31	90	3	M
	32	95	3	M
	33	95	3	M
	34	95 70	3 1.5	M C
	35	75	3	M
	36	90	3	M

Table 10: Patient history	**Serial No of Patients**	**Age**	**Stage**	**Grade**	**ER/PR/Her2**	**MRM/CT/RT**	**Patient status**
ER+/PR+ Good Outcome	1	54,Post	IDC-2, small foci DCIS, L Br	T2N2M0, pT2NaM2	P/P/P	11/03: MRM; 12/03-04/04: CT-FAC x6; 04/04: RT; 10/04 on Tamx, c/o lower back ache	No mets and Alive;
	2	26	IDC-2, L Br	T2N0M0 (Stage-2a)	P/P/N	Quadrectomy in Nov 04, BCT /RT/CT-FAC, on TAM 20γ HS; 2007-Normal, 3yrs of TAM; Dec 2009-Normal, TAM to stop after 5yrs of usage; 2011-All normal	Alive, most likely Ca free
	3	44, Premeno.	IDC-3 lymphatic emboli & perineural invasion; R Br	T2N1M0, pT2N3M0 (Stage-3a)	20%P/20%P	H/o DM, Hypothyroid, HT, Lap. Cholecystectomy; 01/04 Trucut Biopsy, Bone scan-N, MRM; 02-06/04 CT-FEC x6; 04/04 RT; 07/09 on extended Adj Hormonal therapy; 06/10 Anastrozole for 6yrs L-Mammo: some mass present, no intervention adv.; 08/11: R-Mammo - N, Inj Zolodronic acid.	No mets and alive;
	4	48	IDC-2; L Br	pT2N0M0	P/P/P	MRM in 08/2005; Adj CT-FEC x6 in 08-12/2005; 12/2005 Start Tamx 20mg/d x5 yrs; 12/2005-01/2006 RT 45 Gy/25#	Alive
	5	55, Post	IDC, L Br	T2N2M0	P/P	12/02: Exi. Biopsy L Br, MRM, Bone scan-Normal; 01-06/03: High risk CT-	No mets for 6 yrs and alive

						AC x6; 03-05/03: RT; 06/03 Started Tamx; 03/09: Breathless, no wt gain, R Br, L MRM - Normal, adv. PET-CT scan	
	6	54, Post	IDC-3, R Br	T2N0M0, pT2N0	P/P	lump in R br for 2 weeks, MRM 05/03; CT-AC x4 06-08/03; on Tamoxifen for 7 yrs total & anastrazole for 5 yrs; 10/06 all Normal; 09/08 all normal; 07/09 Fatty changes in liver, 02/11 L br Normal; 12/11 BMD-osteoporosis L & R br Normal, on Zoledromide fro 01/10 till 12/11	no mets and alive
	7	52/54, Post	IDC, L Br	multifocal T1N1M0	P/P	R Br Lumpectomy in '99 , again in 2001 which was found Normal, Post surgery CT/RT; Was put on TAM but stopped and started Tab Anastrazole; Mother had Cancer; Cholecystectomy on 05/04/08	No mets and alive, R Br in '91, '00(found to be Normal), Mother had cancer
	8	62	IDC-3	T2N0	P/P	K/c/o Ca Br; L-MRM 03/2005; Adj CT-Taxol x4 ; Pt on Shelcal; Stazonex, inj Zolastra; anastrazole ect tablets and regular F/up since 2005; Last F/up in 07/2011 and next due in 07/2012.	Alive and doing well and no mets
	9	40	IDC-III; R Br	T2N1MX	N/P30%/P	Lump in R breast for 6 months; R-MRM on 08/2005; Adj CT-TACx6 09-12/2005, Adj RT 50.4 Gy/28#s 01-02/2006 ; 2006-2009 on Tamx; Switched to Aromasin in 02/2009; Last F/up 04/2011 all Normal.	All Normal,
ER+/PR+ Bad Outcome	10	41, Premeno.	ILC, R Br	T2N0M0	P/P	04/03 c/o Lump R Br(6mths), H/o Hypothyroid, Trucut Biopsy, 04/2003 MRM, Bone scan-N, 04-06/2003 CT-AC x6; RT; 09/2007 local recurrence R	Mets and alive; Local recurrence within 4 yrs, defaulted on CT

						ch. wall & Wide exi of R chest wall lesion, CT-FECx6 11/07-02/2008; 03/08 No hormonal Rx past 4 yrs, Adv Anastrozole; Not been taking Anastozole; F/up 05 2010 R&L br N & on Anastrazole now; 05/2011 Br N, U/S N continue Anastrazole; all Normal; 09/11 Cardio checkup-N, pain in L neck, No node identified clinically, Sonography, TSH, FT4 - All N, Adv. see Endocrinologist for Thyroxene replacement	
ER+/PR+ Bad Outcome	11	54, Post	IDC-3, R Br	T2N0M0	P/P	H/O Hypothyroid, vomitting atleast 1/day for 15 days(03/01), c/o Thyrotoxicosis in Gastritis; MRM (R Br) 06/04, Post op. Green color urine, No fam history; CT-FACx6 (07-10/04); C/o Seizures, Brain CT, given anticonvulsants 06/08, RT 06/08, completed Palli WBRT 30Gy/10# 2 weeks; 2x CT-Gem+Cis (11 & 30/07/08); Br Ca (Stage 4)+ Intracranial Mets, on Hormonal therapy, refused treatment; completes Palli RT & CT-Gem+Cis 29/08/08; altered sensorium, fever, vomit, bluish color of arm, 2units of FFP + 2 red blood transfusion, discharged for Palli care at home 18/09/08	Diag./MRM 06/04, Bone & Brain Mets, expired in 4 yrs 09/08
	12	43, Post	ILC, R Br	T2N2M0	P/P	04/04: Lump in Br; 05/04: MRM; 05-09/04: CT-FAC x6 09-11/04: RT 50.4Gy/18#; 09/04: Started Tamx; 02/05: came back w pain, on Novoldex;	Expired in 3.5 yrs. Bone, lung, live mets within 1-2 yrs

						10/05 Bone scan- multiple mets adv RT; 06/06: Bone, liver, lung mets; 03/07: CT-Gem+Cis x6; 06/07: RT 30Gy/10#; 09/07: Intracranial CT-Methotrexate; 09/07: Expired	
	13	58, Post	ILC; L Br		P/80%P	02/02 c/o severe back pain, Op. Laminectomy, surg. D9 vertebra, found Br mass; Trucut Biopsy for Br, Diag. ILC w multiple bone mets, recd RT for spial lesions, pan-CK +ive; 02/02 CT-Dexona; 06/06 On Letrozole, c/o pain in R shoulder, lower back ache, recd. Palli RT 20Gy/5#/1 wk	Mets and dead; Came w bone mets, possible met recurrence within 4 years?
ER+/PR+ Bad Outcome	14	66	IDC-3; R Br	T2N1M0 (L Br)	80%P/N/100%P	H/o Hystrectomy('87), Ht, Hypothyroid; 02/03: Lump, MRM+BCS, RT; 04-09/03 On Herceptin & Xeloda, locally recurrent T nodules ++ all over reconstructed Br; 06/03 Nodules regressed, RT; 10/03 L Br- N, skin healthy 3weekly dose of Herceptin; 12/03 R Br noticed, FNAC +ve; 01/04 Local recurrence, recd. FAC, Tamx, Letrozole, started on Palli Gemcitabine; 02/04 C/o Met AdenoCa advanced disease, recd. Palli CT x6, planned CT-5FU+Levoflex/weekly; 04/04 Palli MRM (R) + Exi. L side nodules, Toilet Mast (R); 09/04 only on supportive care, c/o severe pain in neck & chest wall, unconciousness, giddiness duee to locally recurrent disease, adv. Brain CT	Mets and dead; Progressive disease despite CT, RT, Hormonal therapy, local recurrence in <1yr
	15	61	IDC-3, R Br	pT3N2aMx	P/5%P/P	R Br TCB in 09/03; R MRM 09/03; CT-FECx3 09/03-11/03; RT 50.4Gy 11/03-	Recurred in 3 yrs to L br and to

						01/04; CT-FECx3 11/03-01/04; Normal F/U 05/04; Ricurred in L Br 2007; L-MRM 2007; aggressive disease as -/-/+; Bone mets in 03/09; Now metastatic progressive disease; Likely expired as per Dr Patil since metastatic dis in 2009	bone in 5 yrs; Likely expired.
	16	48	IDC3; L Br	T2N2aM0	P/P/N	08/2000 L MRM w ax. Clearance; Adj CT-ACx3 IN 08-10/2000; Defaulted on CT; Locally advanced dis in 2003; 04/2006 Recurrence of IDC of Br in soft tissues of Chest wall; RT 50.4+10 Gy/30#s; Defaulted on CT; 12/2009 Ch wall recurrence; 12/2009 CT-FECx6 in till 04/2010; On Lterozole 2.5 mg x 3 mths; Regular F/up; Last in 10/2011	Alive with recurrent local disease
ER+/PR+ Bad Outcome	17	58	ILC-2A; R Br	pT2N0Mx	P/P/N	Lumpectomy on 05/2005; Neo adj CT-FACx6 06-09/2005; 11/2005 Interstitial brachy of R Br ??/; 02/2006 F/up N	Metastatic disease/Dead
	18	55	IDC-3; L Br	T2N1	P/P(20%)/N	MRM on 02/2005	Mets and dead
ER-/PR- Good Outcome	19	49, Post	IDC-3, L Br, high mitotic case, no embolie or invasion but necrosis present	T2N0M0	N/N	Lumpectomy on 03/03, have lumpectomy sample, MRM 04/03	Alive, 9 yrs since MRM
	20	62,Post	IDC-3, L Br	T4N0Mx	N/N	Mammo, Trucut (01/01/04), Lump, MRM (L Br) 6 & 7/01/04; CT-FACx6 (17/01-10/05/04), RT 50.4Gy/28#	8 yr since MRM (L Br), currently c/o pain in R Br

						(02/06-08/07/04); 08/06-In remission; 08/07- c/o UTI, vomitting, fever, adv CT scan, Mammo; 10/07- Inj Avastin; 04/08- R-Br Normal; 07/09 - lower back pain, bone scan-no Met; 01/12- 8yrs since MRM, now pain in R Br, adv Mammo.	
	21	49, Premeno.	IDC, R Br	T2N2M0	N/N	08/03: Lump R Br(1.5mths), MRM; 09-12/03 CT-FEC x6; 10/03 RT 50.4GY/26# 02/06: All Normal; 11/06: Accident, Rt shoulder bruise	Alive & no mets
ER-/PR-Good Outcome	22	61,	IDC-3 w DCIS foci; R Br	pT2N1Mx	N/N	10/08 F/u L Br, R MRM - N	No mets and alive
	23	51, Premeno.	IDC-3, R Br	T2N1M0, pT3N2M0	N/N/N	91, '00: FNAC for Lump L, R Br-Neg(repectively); H/o Tubectomy; 08/03: c/o Lump in Br(2yrs), pain only during menstural cycle, MRM (R); 08/03-01/04 CT-FEC x6; 12/03 RT; 04/04 Recurrent Br Ca Nodule- exi biopsy; 06/08 5yrs since MRM, All Normal; 06/09: Bone scan-Normal; 06/11: Multiple neck nodules-Met Ca; 07/11: Local RT, start Capecitabine, 09/11: CT, 1 week break then start Capecitabine; 12/11: 5 cycles of Capecitabine; 01/12: ----?	Alive with Mets; 1st FNAC done in '91, then '00, MRM in 03, local recurrence in 1 yr, mets in 8 yrs
	24	43	IDC-3 + DCIS, L Br	T2N1M0	N/N	L-MRM 02/2003; Adj CT-ACx4 03-05/2003; Adj RT 50.4Gy/28 # + Boost RT 14Gy/7# in 07/2003; CT-Pacli x4 08-09/2003; Liver mets 08/2008; Alive with mets	Alive with mets CHECK
	25	47	IDC-3	pT2N0M0	N/N/P	06/2001 L Br surgery & B/L	Alive with local

						oophorectomy; Adj CT-ECx4 07-09/2001; Adj RT 50.4+10 Gy/33#s 10/2001; On Tamx 2002-2004 & all normal F/up; Local recurrence 06/2005; L-MRM 08/2005; Adj CT-Docx6 09-12/2005; Adj RT 50.4Gy/28# 01-02/2006; 11/2008 F/up Normal; 05/2011 F/up normal.	mets
	26	57	IDC; Medullary Ca; R Br	T2N0M0; Stg 2A	N/N/N	R-MRM 02/2006; Adj CT-ACx4 IN 03-05/2006; Adj CT-Paclix12 05-08/2006; Adj RT to R ch wall 50.4Gy/28# 08-10/2006; On regular F/up in 2007-2009, all normal, minor c/o swelling in arms w pains; 05/2010 local recurrence to L breast;	Doing well
ER-/PR-Good Outcome	27	35	IDC-III; L Br	T2N1M0; Stg 2B	N/N/P	Had L BC Surgery 01/2006; Adj CT-TACx6 in 02-05/2006; Adj CT Pacli+ Herceptin x12 in 05-08/2006 ;	Doing well
ER-/PR-Bad Outcome	28	68, Post	IDC-3, R Br	T3N1M0	N/N	06/03: Lump in R Br(3 wks), MRM, Bone scan- possible skeletal mets; 06/03-01/04: CT-AC x6; 06/04 No c/o Mets, Facial (R) Palsy, severe weakness, no other Neuro complaints; 10/04 severe pain L shoulder, Bone scan- multiple skeletal mets, adv. Palli CT-Taxane and RT	Mets and dead; Bone mets within 1 year
	29	50	R Br	T4bN1M1	-	06/2000: Diag Br Ca w pulmonary met in Hyd, 01-04/03 Palli CT-FAC; 04/03: R Br-mass reduced in size; 11/03 c/o vomit, headache, disorientation -> Brain mets, given Palli RT	Brain mets in 3 yrs; dead
	30	42,	IDC-3, R	T2N1M0	N/N	MRM 08/03, adj CT-FEC x1/ FEC x6;	Barin mets in 1.8

		Premeno.	Br			RT taken; 06/05 H/0 headache; Recurred in 22 months in brain, crainiotomy done 06/05	yrs yrs of MRM; Dead
	31	55, Post	IDC-2, R Br	T4N2M0	N/N/P	Neo CT-AC x1/ RT+Tamoxifen x4/ MRM-3c/ Her2+ & Xeloda x1; Met: L Br, Bones in 10 months/ CT- Herpceptin +Vinorelbrine	Mets and dead: L Br, Bones in 10 months
	32	38, Premeno.	ILC+IDC-3+DCIS; L Br		N/N/	02/03 FNAC, Lump, MRM; 02-08/03 CT-AC x6; 03/03 RT 50.4GY/28#; 02/04 - All Normal; 07/05 vomitting, headache, L facial pain, adv Dexona, Hydrocort; 10/05 feels weak, mild pallor, Bone marrow asp & Bx-met Ca, adv CT-Docetaxel/week; 06/06 FNAC R Br- IDC, tiny brain mets, bone marrow-met Ca, started on Xeloda; 07/06 severe headache(1mth), FNAC R Br(swelling) +ve, Mets in contralateral Br, bone marrow, brain, Carcinomatous meningitis, Adv contuinue CT , Palli RT spinal, cranial, femur; 12/06 Bone scan-some lesions regressed, some increased.	Mets and dead; Bone (3yrs) and subsequently brain mets (4yrs)
ER-/PR-Bad Outcome	33	45, Premeno.	IDC-3 w DCIS foci, L Br	T3N1Mx, pT3N2aM0 (Stage-3a)	N/N	c/o Br lump-6 mths, H/o Hashinoto's Thyroiditis, Thyromegaly(2 yrs); 07/03: MRM; 07-12/03: CT-FEC x6; 09/03: RT 50.4 Gy/28# given after 3rd CT; 11/03: cold, anemia w Mycoplasma infection, given washed RBC; 09/03/05: c/o Rt Hemiparesis(1 week), loss of appetite & weakness, Bone, brain, chest scan show mets. Adv Palli RT; 11/03/05: Expired, COD: Multiple brain sec., Pri Br Ca w Liver mets	Mets and dead; Expired within 2 yrs with Brain, liver mets

	34	62	IDC-3, R Br	T3N1Mx; Stg 4	N/N/P	Br lump since 2003; 06/06 lung mets; Neoadj CT-TACx4 in 10/06; T increased in size, CT changed to Gem+Cisplatin x 03/07; on Xeloda since 07/07; T increased in size; Start Capcitabine/Vincristine/Gem+irinotecan by 07/08 -> Palliative R MRM 07/08; Lung mets; cough & itching in 05/09 advised Xeloda+Tykerb; 10/09 endoxan, celecoxib; 07/10 disease progression to LN mets + cough	Had multiple regimes of CT, Mets and dead
ER-/PR- Bad Outcome	35	60	IDC-3b,	T4N2M1; IV	TBD	06/2004 came w lump in R br + bone & brain mets; Neo-adj CT-Doce+epirubicin x3 06-08/2004; Progressive disease thus changed to salvage CT Gemcitabine+Vinorelbine 08/2004; T mass increased, CT changed to palliative CT-TACx4 01-03/2005; Had HT also, Patient passed away on 17.8.2005	Multiple CTs; Metastatic adenocarcinoma, dead
	36	44	IDC-3; R Br	TxN1M1	N/N/P2+	B/L Lump excision in 08/2005; R-MRM on 11/2005; Adj CT-FECx6 11/05-02/06; RT 50.4/28# 03-05/2006; 12/2006 brain mets & crainiotectomy done; 01/2007 RT to brain 45Gy/25#s; 02/2007 c/o cough B/L lung mets; Not willing for injectible CT hence oral CT advised	Mets and dead

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MARKER	ER+/PR+	ER-/PR-	RESULTS
CD44	YES	-	**GOOD OUTCOME** LOW EXPRESSION OF CD44 IN MEMBRANE
	YES	-	**BAD OUTCOME** HIGH EXPRESSION OF CD44 IN MEMBRANE OR MERE CYTOPLASMIC/NUCLEAR EXPRESSION
CD44	-	YES	**GOOD OUTCOME** HIGH EXPRESSION OF CD44 IN MEMBRANE
	-	YES	**BAD OUTCOME** LOW EXPRESSION OF CD44 IN MEMBRANE OR MERE CYTOPLASMIC/NUCLEAR EXPRESSION

TABLE 11

MARKER	ER+/PR+	ER-/PR-	RESULTS
ABCG2	-	YES	**GOOD OUTCOME** HIGH EXPRESSION OF ABCG2 IN NUCLEUS
	-	YES	**BAD OUTCOME** LOW EXPRESSION OF ABCG2 IN NUCLEUS

TABLE 12

MARKER	ER+/PR+	ER-/PR-	RESULTS
	YES	-	**GOOD OUTCOME**

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ABCC4			LOW EXPRESSION OF ABCC4 IN MEMBRANE
	YES	-	**BAD OUTCOME** HIGH EXPRESSION OF ABCC4 IN MEMBRANE

TABLE 13

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MARKER	ER+/PR+	ER-/PR-	RESULTS
CD133	-	YES	**GOOD OUTCOME (HER2 –ve)** LOW EXPRESSION OF CD133 IN MEMBRANE
	-	YES	**BAD OUTCOME (HER2 –ve)** HIGH EXPRESSION OF CD133 IN MEMBRANE
CD133	-	YES	**GOOD OUTCOME (HER2 +ve)** HIGH EXPRESSION OF CD133 IN MEMBRANE
	-	YES	**BAD OUTCOME (HER2 +ve)** LOW EXPRESSION OF CD133 IN MEMBRANE

TABLE 14

MARKER	ER+/PR+	ER-/PR-	RESULTS
P-CADHERIN	YES	-	**GOOD OUTCOME** LOW EXPRESSION OF P-CADHERIN IN MEMBRANE
	YES	-	**BAD OUTCOME** HIGH EXPRESSION OF P-CADHERIN IN MEMBRANE
P-CADHERIN	-	YES	**GOOD OUTCOME** HIGH EXPRESSION OF P-CADHERIN IN MEMBRANE
	-	YES	**BAD OUTCOME**

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			LOW EXPRESSION OF P-CADHERIN IN MEMBRANE

TABLE 15

MARKER	ER+/PR+	ER-/PR-	RESULTS
β-CATENIN	YES	-	**GOOD OUTCOME** LOW EXPRESSION OF β-CATENIN IN MEMBRANE
	YES	-	**BAD OUTCOME** HIGH EXPRESSION OF β-CATENIN IN MEMBRANE

TABLE 16

Wherein, the low expression represents percentage intensity of staining of the cells between 0 to 40%, and the high expression represents percentage intensity of staining of the cells between more than 40% and upto 100%.

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We claim:

1. A combination of
    a) biological markers consisting of CD44, ABCC4, P-cadherin and β-catenin; or
    b) biological markers consisting of CD44, ABCG2, CD133 and P-cadherin optionally along with Oct-4; or
    c) biological markers consisting of CD44, ABCG2 and CD133; or
    d) biological markers consisting of CD44, CD133 and P-cadherin;

    when used for prognosis of breast cancer.
2. The combination as claimed in claim 1, wherein the combination of biological markers consisting of CD44, ABCC4, P-cadherin and β-catenin prognose breast cancer in a subject having cells expressing estrogen receptor or progesterone receptor or both.
3. The combination as claimed in claim 1, wherein the combination of biological markers consisting of CD44, ABCG2, CD133 and P-cadherin optionally along with Oct-4 prognose breast cancer in a subject having cells not expressing estrogen receptor and progesterone receptor.
4. The combination as claimed in claim 1, wherein the combination of biological markers consisting of CD44, ABCG2 and CD133 prognose breast cancer in a subject having cells not expressing Human Epidermal Growth Factor Receptor 2, estrogen receptor and progesterone receptor.
5. The combination as claimed in claim 1, wherein the combination of biological markers consisting of CD44, CD133 and P-cadherin prognose breast cancer in a subject having cells expressing Human Epidermal Growth Factor Receptor 2, and not expressing estrogen receptor and progesterone receptor.
6. The combination as claimed in claim 1, wherein the markers are located on tumor cells of a subject at locations selected from a group comprising cell membrane, cytoplasm, nucleus, and nuclear membrane or any combination thereof.
7. A kit when used for prognosis of a subject having breast cancer or suspected of having breast cancer, said kit comprising antibody against combination of:
    a) biological markers consisting of CD44, ABCC4, P-cadherin and β-catenin; or
    b) biological markers consisting of CD44, ABCG2, CD133 and P-cadherin optionally along with Oct-4; or
    c) biological markers consisting of CD44, ABCG2 and CD133; or

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d) biological markers consisting of CD44, CD133 and P-cadherin; and

optionally along with organic solvent, reagent, secondary antibody, enzyme for performing immunohistochemistry and instruction manual.

8. A method of identifying biological marker on cells in a biological sample of a subject having or suspected of having breast cancer, said method comprising acts of:
    a) collecting, fixing, sectioning and treating the biological sample with organic solvent, followed by antigen retrieval using predetermined sample dilutions and adding primary antibody against a combination of:
        i. biological markers consisting of CD44, ABCC4, P-cadherin and β-catenin; or
        ii. biological markers consisting of CD44, ABCG2, CD133 and P-cadherin optionally along with Oct-4; or
        iii. biological markers consisting of CD44, ABCG2 and CD133; or
        iv. biological markers consisting of CD44, CD133 and P-cadherin; and

    b) adding secondary antibody conjugated with an enzyme and reagents for obtaining a colored reaction or fluorescence for identifying the biological marker.
9. The kit as claimed in claim 7 or the method as claimed in claim 8, wherein the organic solvent is selected from a group comprising alcohol and xylene or any combination thereof.
10. The kit as claimed in claim 7 or the method as claimed in claim 8, wherein the combination of biological markers consisting of CD44, ABCC4, P-cadherin and β-catenin prognose breast cancer in the subject having cells expressing estrogen receptor or progesterone receptor or both.
11. The kit as claimed in claim 7 or the method as claimed in claim 8, wherein the combination of biological markers consisting of CD44, ABCG2, CD133 and P-cadherin optionally along with Oct-4 prognose breast cancer in the subject having cells not expressing estrogen receptor and progesterone receptor.
12. The kit as claimed in claim 7 or the method as claimed in claim 8, wherein the combination of biological markers consisting of CD44, ABCG2 and CD133 prognose breast cancer in the subject having cells not expressing Human Epidermal Growth Factor Receptor 2, estrogen receptor and progesterone receptor.

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13. The kit as claimed in claim 7 or the method as claimed in claim 8, wherein the combination of biological markers consisting of CD44, CD133 and P-cadherin prognose breast cancer in the subject having cells expressing Human Epidermal Growth Factor Receptor 2, and not expressing estrogen receptor and progesterone receptor.
14. The method as claimed in claim 8, wherein the collecting, fixing, sectioning and treating are carried out under predetermined conditions by conventional immunohistochemistry technique.
15. A kit when used for prognosis of a subject having breast cancer or suspected of having breast cancer, said kit comprising:
    a) antibody against combination of at least 3 biological markers selected from a group consisting of CD44, ABCC4, P-cadherin, β-catenin, ABCG2 and CD133, optionally along with Oct-4; and
    b) optionally along with organic solvent, reagent, secondary antibody, enzyme for performing immunohistochemistry and instruction manual.
16. A method of prognosis of a subject having breast cancer or suspected of having breast cancer, said method comprising acts of:
    a) collecting biological sample from the subject and identifying expression or absence of receptors selected from a group comprising estrogen receptor, progesterone receptor and Human Epidermal Growth Factor Receptor 2receptor or any combination thereof in cells of the sample to obtain expression identified cells;
    b) carrying out immunohistochemistry analysis on the cells of step (a) with antibody against a biological marker selected from a group consisting of CD44, ABCG2, ABCC4, CD133, Oct-4, P-cadherin and β-catenin; and
    c) identifying expression or absence of the receptors and the biological markers in cells of the sample and correlating the identification of the marker with corresponding outcome based on predictive outcome reference table nos. 11, 12, 13, 14, 15 and 16, for predicting the prognosis of the subject.
17. A method of prognosis of a subject having breast cancer or suspected of having breast cancer, said method comprising acts of:
    a) collecting biological sample from the subject and identifying expression or absence of receptors selected from a group comprising estrogen receptor,

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progesterone receptor and Human Epidermal Growth Factor Receptor 2receptor or any combination thereof in cells of the sample to obtain expression identified cells;

b) carrying out immunohistochemistry analysis on the cells of step (a) with antibody against a combination of:

i. biological markers consisting of CD44, ABCC4, P-cadherin and β-catenin; or

ii. biological markers consisting of CD44, ABCG2, CD133 and P-cadherin optionally along with Oct-4; or

iii. biological markers consisting of CD44, ABCG2 and CD133; or

iv. biological markers consisting of CD44, CD133 and P-cadherin; and

c) identifying expression or absence of the receptors and the biological markers in cells of the sample and correlating the identification of the markers with a corresponding outcome based on combination of predictive outcome reference table nos. 11, 12, 13, 14, 15 and 16, for predicting the prognosis of the subject.

18. The method as claimed in any of the claims 16 or 17, wherein the immunohistochemistry analysis is carried out by conventional method and wherein the identification of markers is carried out by visualizing a colored reaction or fluorescence obtained at completion of the method due to staining of the cells from the sample.

19. The method as claimed in any of the claims 16 or 17, wherein the correlating is based on parameters selected from a group comprising percentage of staining, intensity of staining and location of staining or any combination thereof; and wherein the location of the staining is selected from a group comprising cell membrane, cytoplasm, nucleus, and nuclear membrane or any combination thereof.

20. The method as claimed in any of the claims 16 or 17, wherein the correlating comprises multiplying the percentage of staining with the intensity of staining to arrive at a predictive score, or wherein the correlating is carried out based on percentage of cells stained in order to predict the prognosis as being good or bad depending on the location of expression of the biological marker.

21. A method of treating breast cancer, said method comprising acts of:

a) identifying a combination of biological markers on tumor cells in a biological sample and predicting prognosis of a subject having breast cancer or suspected

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of having breast cancer; and

b) based on the prediction, designing a cancer therapy for suppression of the breast cancer;

wherein the combination of biological markers are:

i. CD44, ABCC4, P-cadherin and β-catenin; or

ii. CD44, ABCG2, CD133 and P-cadherin optionally along with Oct-4; or

iii. CD44, ABCG2 and CD133; or

iv. CD44, CD133 and P-cadherin.