However, several studies have demonstrated significant inter-observer variability and technical variability in up to 40% of immunohistochemistry results. Moreover, immunohistochemistry only allows a qualitative or, in some cases, a semi-quantitative statement regarding the respective receptor status.

Therefore, there is a need for a reliable, objective, quantitative and reproducible test system for the molecular subtyping of tumors, e.g. breast tumors, which facilitates the selection of suitable tumor treatment regimens (patient stratification), and allows prognosis and prediction of therapy success and assessment of a patient's risk for distant metastasis. Moreover, such test system should allow for decentralized testing that is suitable for a significant proportion of cancer patients.

SUMMARY

In one aspect, the present invention refers to a method of stratifying a breast cancer patient for adjuvant chemotherapy, said method comprising, as a first step, identifying a molecular subtype of a breast tumor in the breast cancer patient, and, as a second step, deciding in favor of or against adjuvant chemotherapy based on the molecular subtype, wherein the molecular subtype is identified by using an *in vitro* method comprising the steps: (a) determining the expression level of RNA transcript of human epidermal growth factor receptor 2 (HER2) in a sample of the tumor; (b) determining the expression level of RNA transcript of estrogen receptor (ESR1) in a sample of the tumor; (c) determining the expression level of RNA transcript of progesterone receptor (PGR) in a sample of the tumor; and (d) determining the expression level of RNA transcript of proliferation antigen Ki-67 (Ki67) in a sample of the tumor; and, optionally, (e) determining the expression level of RNA transcript of RacGTPase-activating protein 1 (RACGAP1) in a sample of the tumor, wherein the sample is RNA extracted from the tumor, wherein the expression level of RNA transcript is determined by reverse transcription (RT) quantitative PCR, and wherein no expression level of RNA transcript of a gene other than HER2, ESR1, PGR, Ki67 and, optionally, RACGAP1 and, optionally, one or more reference genes is determined.

In another aspect, the present invention refers to the use of a kit useful for identifying a molecular subtype of a breast tumor in a breast cancer patient by means of reverse transcription (RT) quantitative PCR for stratifying a breast cancer patient for adjuvant chemotherapy according to the method of the present invention, said kit comprising a group of primer pairs and probes consisting of: at least one pair of HER2-specific primers and at least one HER2-specific probe; at least one pair of ESR1-specific primers and at least one ESR1-specific probe; at least one pair of PGR-specific primers and at least one PGR-specific probe; and at least one pair of Ki67-specific primers and at least one Ki67-specific probe; and, optionally, at least one pair of RACGAP1-specific primers and at least one RACGAP1-specific probe.

In one example, the disclosure describes an *in vitro* method of identifying a molecular subtype of a tumor in a cancer patient, said method comprising the steps:

(a) determining the expression level of RNA transcript of human epidermal growth factor receptor 2 (HER2) in a sample of the tumor;
(b) determining the expression level of RNA transcript of estrogen receptor (ESR1) in a sample of the tumor;
(c) determining the expression level of RNA transcript of progesterone receptor (PGR) in a sample of the tumor; and
(d) determining the expression level of RNA transcript of proliferation antigen Ki-67 (Ki67) in a sample of the tumor; and/or
(e) determining the expression level of RNA transcript of RacGTPase-activating protein 1 (RACGAP1) in a sample of the tumor.

In one example, determining the expression level of RNA transcript of HER2, ESR1, PGR and Ki67 and/or RACGAP1 comprises determining whether the expression level of RNA transcript of HER2, ESR1, PGR and Ki67 and/or RACGAP1 is lower or higher than a defined expression threshold of RNA transcript of HER2, ESR1, PGR and Ki67 and/or RACGAP1.

In one example, step (a) is performed before steps (b), (c) and (d) and/or (e).

CLAIMS

1. A method of stratifying a breast cancer patient for adjuvant chemotherapy, said method comprising, as a first step, identifying a molecular subtype of a breast tumor in the breast cancer patient, and, as a second step, deciding in favor of or against adjuvant chemotherapy based on the molecular subtype, wherein the molecular subtype is identified by using an *in vitro* method comprising the steps:

(a) determining the expression level of RNA transcript of human epidermal growth factor receptor 2 (HER2) in a sample of the tumor;

(b) determining the expression level of RNA transcript of estrogen receptor (ESR1) in a sample of the tumor;

(c) determining the expression level of RNA transcript of progesterone receptor (PGR) in a sample of the tumor; and

(d) determining the expression level of RNA transcript of proliferation antigen Ki-67 (Ki67) in a sample of the tumor; and, optionally,

(e) determining the expression level of RNA transcript of RacGTPase-activating protein 1 (RACGAP1) in a sample of the tumor,

wherein the sample is RNA extracted from the tumor,

wherein the expression level of RNA transcript is determined by reverse transcription (RT) quantitative PCR, and

wherein no expression level of RNA transcript of a gene other than HER2, ESR1, PGR, Ki67 and, optionally, RACGAP1 and, optionally, one or more reference genes is determined.

2. The method of claim 1, wherein determining the expression level of RNA transcript of HER2, ESR1, PGR and Ki67 and, optionally, RACGAP1 comprises determining whether the expression level of RNA transcript of HER2, ESR1, PGR and Ki67 and, optionally, RACGAP1 is lower or higher than a defined expression threshold of RNA transcript of HER2, ESR1, PGR and Ki67 and, optionally, RACGAP1.

3. The method of claim 1 or 2, wherein step (a) is performed before steps (b), (c) and (d) and, optionally, (e).

4. The method of any one of claims 1 to 3, wherein step (d) and, optionally, step (e) are performed after steps (a), (b) and (c).

5. The method of any one of claims 1 to 4, wherein step (a) is performed before step (b), step (b) is performed before step (c), and step (c) is performed before step (d) and, optionally, step (e).

6. The method of any one of claims 1 to 5, wherein the molecular subtype is selected from the group comprising HER2-positive, triple-negative, luminal A and luminal B.

7. The method of any one of claims 1 to 6, wherein an expression level of RNA transcript of HER2 which is higher than a defined expression threshold of RNA transcript of HER2 identifies the molecular subtype of the tumor as HER2-positive.

8. The method of any one of claims 1 to 6, wherein

- an expression level of RNA transcript of HER2 which is lower than a defined expression threshold of RNA transcript of HER2;
- an expression level of RNA transcript of ESR1 which is lower than a defined expression threshold of RNA transcript of ESR1;
- an expression level of RNA transcript of PGR which is lower than a defined expression threshold of RNA transcript of PGR; and
- an expression level of RNA transcript of Ki67 which is lower or higher than a defined expression threshold of RNA transcript of Ki67

identify the molecular subtype of the tumor as triple-negative.

9. The method of any one of claims 1 to 6, wherein the molecular subtype is luminal A or luminal B.

10. The method of claim 9, wherein

- an expression level of RNA transcript of HER2 which is lower than a defined expression threshold of RNA transcript of HER2;
- an expression level of RNA transcript of ESR1 which is higher than a defined expression threshold of RNA transcript of ESR1;
- an expression level of RNA transcript of PGR which is higher than a defined expression threshold of RNA transcript of PGR; and

- an expression level of RNA transcript of Ki67 which is higher than a defined expression threshold of RNA transcript of Ki67

identify the molecular subtype of the tumor as luminal B.

11. The method of claim 9, wherein

- an expression level of RNA transcript of HER2 which is lower than a defined expression threshold of RNA transcript of HER2;
- an expression level of RNA transcript of ESR1 which is higher than a defined expression threshold of RNA transcript of ESR1;
- an expression level of RNA transcript of PGR which is higher than a defined expression threshold of RNA transcript of PGR; and
- an expression level of RNA transcript of Ki67 which is lower than a defined expression threshold of RNA transcript of Ki67

identify the molecular subtype of the tumor as luminal A.

12. The method of claim 9, wherein

- an expression level of RNA transcript of HER2 which is lower than a defined expression threshold of RNA transcript of HER2;
- an expression level of RNA transcript of ESR1 which is higher than a defined expression threshold of RNA transcript of ESR1;
- an expression level of RNA transcript of PGR which is lower than a defined expression threshold of RNA transcript of PGR; and
- an expression level of RNA transcript of Ki67 which is lower or higher than a defined expression threshold of RNA transcript of Ki67

identify the molecular subtype of the tumor as luminal B.

13. The method of claim 9, wherein

- an expression level of RNA transcript of HER2 which is lower than a defined expression threshold of RNA transcript of HER2;
- an expression level of RNA transcript of ESR1 which is lower than a defined expression threshold of RNA transcript of ESR1;
- an expression level of RNA transcript of PGR which is higher than a defined expression threshold of RNA transcript of PGR; and

- an expression level of RNA transcript of Ki67 which is higher than a defined expression threshold of RNA transcript of Ki67

identify the molecular subtype of the tumor as luminal B.

14. The method of claim 9, wherein

- an expression level of RNA transcript of HER2 which is lower than a defined expression threshold of RNA transcript of HER2;
- an expression level of RNA transcript of ESR1 which is lower than a defined expression threshold of RNA transcript of ESR1;
- an expression level of RNA transcript of PGR which is higher than a defined expression threshold of RNA transcript of PGR; and
- an expression level of RNA transcript of Ki67 which is lower than a defined expression threshold of RNA transcript of Ki67

identify the molecular subtype of the tumor as luminal A.

15. The method of claim 9, wherein

- an expression level of RNA transcript of HER2 which is lower than a defined expression threshold of RNA transcript of HER2;
- an expression level of RNA transcript of ESR1 which is higher than a defined expression threshold of RNA transcript of ESR1;
- an expression level of RNA transcript of PGR which is lower than a defined expression threshold of RNA transcript of PGR; and
- an expression level of RNA transcript of Ki67 which is higher than a defined expression threshold of RNA transcript of Ki67

identify the molecular subtype of the tumor as luminal B.

16. The method of any one of claims 9 to 15, wherein the molecular subtype luminal A is associated with a probability of distant recurrence-free survival 5 years after treatment which is at least 11%, preferably at least 13% higher than the probability of distant recurrence-free survival 5 years after treatment associated with molecular subtype luminal B and/or with a probability of survival 5 years after treatment which is at least 7%, preferably at least 9% higher than the probability of survival 5 years after treatment associated with molecular subtype luminal B.

17. The method of any one of claims 1 to 16, wherein the method comprises steps (d) and (e), and wherein

- an expression level of RNA transcript of Ki67 which is lower than a defined expression threshold of RNA transcript of Ki67 and
- an expression level of RNA transcript of RACGAP1 which is higher than a defined expression threshold of RNA transcript of RACGAP1

indicates an increased risk of poor clinical outcome for the cancer patient.

18. The method of any one of claims 1 to 16, wherein the method comprises steps (d) and (e), and wherein

- an expression level of RNA transcript of Ki67 which is higher than a defined expression threshold of RNA transcript of Ki67 and
- an expression level of RNA transcript of RACGAP1 which is higher than a defined expression threshold of RNA transcript of RACGAP1

indicates a further increased risk of poor clinical outcome for the cancer patient.

19. The method of any one of claims 17 or 18, wherein poor clinical outcome comprises a relative reduction in or more of survival, recurrence-free survival and distant recurrence-free survival.

20. The method of any one of claims 1 to 19, wherein the tumor is a solid tumor.

21. The method of any one of claims 1 to 20, wherein the quantitative PCR is fluorescence-based quantitative real-time PCR.

22. The method of any one of claims 1 to 21, comprising the use of ESR1-specific primers having a length of 15 to 30 nucleotides and comprising at least 10 contiguous nucleotides of the sequences of SEQ ID NOs: 1 and 2, and/or HER2-specific primers having a length of 15 to 30 nucleotides and comprising at least 10 contiguous nucleotides of the sequences of SEQ ID NOs: 4 and 5, and/or Ki67-specific primers having a length of 15 to 30 nucleotides and comprising at least 10 contiguous nucleotides of the sequences of SEQ ID NOs: 7 and 8, and/or PGR-specific primers having a length of 15 to 30 nucleotides and comprising at least 10 contiguous nucleotides of the sequences of SEQ ID NOs: 10 and 11,

and/or RACGAP1-specific primers having a length of 15 to 30 nucleotides and comprising at least 10 contiguous nucleotides of the sequences of SEQ ID NOs: 13 and 14.

23. The method of any one of claims 1 to 22, comprising the use of an ESR1-specific probe having a length of 20 to 35 nucleotides and comprising at least 15 contiguous nucleotides of the sequence of SEQ ID NO: 3, and/or a HER2-specific probe having a length of 20 to 35 nucleotides and comprising at least 15 contiguous nucleotides of the sequence of SEQ ID NO: 6, and/or a Ki67-specific probe having a length of 20 to 35 nucleotides and comprising at least 15 contiguous nucleotides of the sequence of SEQ ID NO: 9, and/or a PGR-specific probe having a length of 20 to 35 nucleotides and comprising at least 15 contiguous nucleotides of the sequence of SEQ ID NO: 12, and/or a RACGAP1-specific probe having a length of 20 to 35 nucleotides and comprising at least 15 contiguous nucleotides of the sequence of SEQ ID NO: 15.

24. The method of any one of claims 1 to 23, wherein the expression level is normalized against the (mean) expression level of one or more reference genes in the sample of the tumor.

25. The method of claim 24, wherein the one or more reference genes are selected from the group comprising CALM2, B2M, RPL37A, GUSB, HPRT1 and GAPDH.

26. The method of any one of claims 1 to 25, wherein the adjuvant chemotherapy comprises administration of docetaxel.

27. Use of a kit for stratifying a breast cancer patient for adjuvant chemotherapy according to the method of any one of claims 1 to 26, said kit comprising a group of primer pairs and probes consisting of:

- at least one pair of HER2-specific primers and at least one HER2-specific probe;
- at least one pair of ESR1-specific primers and at least one ESR1-specific probe;
- at least one pair of PGR-specific primers and at least one PGR-specific probe; and
- at least one pair of Ki67-specific primers and at least one Ki67-specific probe; and, optionally,

- at least one pair of RACGAP1-specific primers and at least one RACGAP1-specific probe.

28. The use of claim 27, wherein the quantitative PCR is fluorescence-based quantitative real-time PCR.

29. The use of claim 27 or 28, wherein detection of the probe is based on amplification-mediated probe displacement.

30. The use of claim 29, wherein the probe is a dual-label probe comprising a fluorescence reporter moiety and a fluorescence quencher moiety.

31. The use of any one of claims 27 to 30, wherein the kit further comprises a reverse transcriptase and a DNA polymerase.

32. The use of claim 31, wherein the reverse transcriptase and the polymerase are provided in the form of an enzyme-mix which allows a one-step reverse transcription (RT) quantitative PCR.

33. The use of any one of claims 27 to 32, wherein the kit further comprises at least one pair of reference gene-specific primers and at least one reference gene-specific probe.

34. The use of claim 33, wherein the reference gene is one or more selected from the group comprising CALM2, B2M, RPL37A, GUSB, HPRT1 and GAPDH.

35. The use of any one of claims 27 to 34, wherein the kit further comprises at least one control RNA sample.

36. The use of any one of claims 27 to 35, wherein the primers provide an amplicon size of less than 120 bp.

37. The use of any one of claims 27 to 36, wherein the ESR1-specific primers have a length of 15 to 30 nucleotides and comprise at least 10 contiguous nucleotides of the sequences of SEQ ID NOs: 1 and 2, and/or wherein the HER2-specific primers have a length

of 15 to 30 nucleotides and comprise at least 10 contiguous nucleotides of the sequences of SEQ ID NOs: 4 and 5, and/or wherein the Ki67-specific primers have a length of 15 to 30 nucleotides and comprise at least 10 contiguous nucleotides of the sequences of SEQ ID NOs: 7 and 8, and/or wherein the PGR-specific primers have a length of 15 to 30 nucleotides and comprise at least 10 contiguous nucleotides of the sequences of SEQ ID NOs: 10 and 11, and/or wherein the RACGAP1-specific primers have a length of 15 to 30 nucleotides and comprise at least 10 contiguous nucleotides of the sequences of SEQ ID NOs: 13 and 14.

38. The use of any one of claims 27 to 37, wherein the ESR1-specific probe has a length of 20 to 35 nucleotides and comprises at least 15 contiguous nucleotides of the sequence of SEQ ID NO: 3, and/or wherein the HER2-specific probe has a length of 20 to 35 nucleotides and comprises at least 15 contiguous nucleotides of the sequence of SEQ ID NO: 6, and/or wherein the Ki67-specific probe has a length of 20 to 35 nucleotides and comprises at least 15 contiguous nucleotides of the sequence of SEQ ID NO: 9, and/or wherein the PGR-specific probe has a length of 20 to 35 nucleotides and comprises at least 15 contiguous nucleotides of the sequence of SEQ ID NO: 12, and/or wherein the RACGAP1-specific probe has a length of 20 to 35 nucleotides and comprises at least 15 contiguous nucleotides of the sequence of SEQ ID NO: 15.

39. The use of any one of claims 27 to 38, wherein the tumor is a solid tumor.