

CLAIMS

What is claimed is:

1. A method for prognosing and/or predicting benefit from adjuvant radiotherapy in a subject having breast cancer, the method comprising:
 - a) obtaining or having obtained an expression level in a biological sample from a subject for a plurality of genes, wherein the plurality of genes are ~~selected from Table 2~~ NEDD4 binding protein 2 like 1 (N4BP2L1) and one or more optional genes selected from ATP binding cassette subfamily C member 8 (ABCC8), BTG anti-proliferation factor 3 (BTG3), cyclin B1 (CCNB1), centromere protein F (CENPF), creatine kinase mitochondrial 1B (CKMT1B), cornichon family AMPA receptor auxiliary protein 4 (CNIH4), cellular retinoic acid binding protein 2 (CRABP2), enoyl-CoA hydratase domain containing 2 (ECHDC2), eukaryotic elongation factor 2 kinase (EEF2K), eukaryotic translation initiation factor 3 subunit L (EIF3L), ectonucleoside triphosphate diphosphohydrolase 6 (putative) (ENTPD6), epoxide hydrolase 2 (EPHX2), H2A histone family member Z (H2AFZ), hydroxysteroid 17-beta dehydrogenase 4 (HSD17B4), karyopherin subunit alpha 2 (KPNA2), lymphoid enhancer binding factor 1 (LEF1), NIMA related kinase 10 (NEK10), prefoldin subunit 4 (PFDN4), pleckstrin and Sec7 domain containing 3 (PSD3), runt related transcription factor 1 (RUNX1), SEC14 like lipid binding 2 (SEC14L2), serine/threonine kinase 39 (STK39), TBC1 domain family member 8 (TBC1D8), thymosin beta 15a (TMSB15A), USO1 vesicle transport factor (USO1), and zinc finger and BTB domain containing 16 (ZBTB16); and
 - b) determining that the subject is at low risk or not at low risk of cancer recurrence based on the expression level, and/or likely to benefit from adjuvant radiotherapy based on the expression level, thereby prognosing and/or predicting benefit from adjuvant radiotherapy in the subject.
2. The method of claim 1, further comprising: ~~a) that the adjuvant radiotherapy therapy is to be administered if the subject is identified as likely to benefit from adjuvant radiotherapy, and recommending treatment intensification if the subject is not identified as likely to benefit from adjuvant radiotherapy; b) determining that the subject is at low risk of cancer recurrence based on the age of the subject, or determining that the subject is not at low risk of cancer recurrence based on the age of the subject; c) calculating a risk score for the subject, wherein adjuvant radiotherapy is to be administered to the subject if the subject is identified as being as likely to benefit from adjuvant radiotherapy based on both the risk score and the expression levels of the genes selected~~

from Table 2 in the biological sample, and recommending treatment intensification if the subject is not identified as being likely to benefit from adjuvant radiotherapy based on both the risk score and the expression levels of the genes selected from Table 2 in the biological sample; or d) that the subject is to be treated with mastectomy, radiation boost, or adjuvant systemic therapy.

3. The method of claim 1 or claim 2, wherein a) the cancer recurrence is local or locoregional recurrence or distant recurrence (metastasis); b) the plurality of genes are selected from the group consisting of ATP binding cassette subfamily C member 8 (ABCC8), BTG anti-proliferation factor 3 (BTG3), cyclin B1 (CCNB1), centromere protein F (CENPF), creatine kinase mitochondrial 1B (CKMT1B), cornichon family AMPA receptor auxiliary protein 4 (CNIH4), cellular retinoic acid binding protein 2 (CRABP2), enoyl-CoA hydratase domain containing 2 (ECHDC2), eukaryotic elongation factor 2 kinase (EEF2K), eukaryotic translation initiation factor 3 subunit L (EIF3L), ectonucleoside triphosphate diphosphohydrolase 6 (putative) (ENTPD6), epoxide hydrolase 2 (EPHX2), H2A histone family member Z (H2AFZ), hydroxysteroid 17-beta dehydrogenase 4 (HSD17B4), karyopherin subunit alpha 2 (KPNA2), lymphoid enhancer binding factor 1 (LEF1), NEDD4 binding protein 2 like 1 (N4BP2L1), NIMA related kinase 10 (NEK10), prefoldin subunit 4 (PFDN4), pleckstrin and Sec7 domain containing 3 (PSD3), runt related transcription factor 1 (RUNX1), SEC14 like lipid binding 2 (SEC14L2), serine/threonine kinase 39 (STK39), TBC1 domain family member 8 (TBC1D8), thymosin beta 15a (TMSB15A), USO1 vesicle transport factor (USO1), and zinc finger and BTB domain containing 16 (ZBTB16); and/or c) the biological sample is a biopsy or a tumor sample.
4. The method of any one of claims 1-3, wherein the method further comprises determining that the subject is at low risk of cancer recurrence based on the age of the subject, or determining that the subject is not at low risk of cancer recurrence based on the age of the subject.
5. The method of any one of claims 1-4, wherein the method further comprises calculating a risk score for the subject, wherein adjuvant radiotherapy is to be administered to the subject if the subject is identified as being as likely to benefit from adjuvant radiotherapy based on both the risk score and the expression levels of N4BP2L1 and the one or more optional genes in the biological sample, and recommending treatment intensification if the subject is not identified as being likely to benefit from adjuvant radiotherapy based on both the risk score and the expression levels of N4BP2L1 and the one or more optional genes in the biological sample.

6. The method of any one of claims 1-5, wherein the plurality of genes are NEDD4 binding protein 2 like 1 (N4BP2L1), ectonucleoside triphosphate diphosphohydrolase 6 (putative) (ENTPD6), and one or more optional genes selected from ATP binding cassette subfamily C member 8 (ABCC8), BTG anti-proliferation factor 3 (BTG3), cyclin B1 (CCNB1), centromere protein F (CENPF), creatine kinase mitochondrial 1B (CKMT1B), cornichon family AMPA receptor auxiliary protein 4 (CNIH4), cellular retinoic acid binding protein 2 (CRABP2), enoyl-CoA hydratase domain containing 2 (ECHDC2), eukaryotic elongation factor 2 kinase (EEF2K), eukaryotic translation initiation factor 3 subunit L (EIF3L), ectonucleoside triphosphate diphosphohydrolase 6 (putative) (ENTPD6), epoxide hydrolase 2 (EPHX2), H2A histone family member Z (H2AFZ), hydroxysteroid 17-beta dehydrogenase 4 (HSD17B4), karyopherin subunit alpha 2 (KPNA2), lymphoid enhancer binding factor 1 (LEF1), NIMA related kinase 10 (NEK10), prefoldin subunit 4 (PFDN4), pleckstrin and Sec7 domain containing 3 (PSD3), runt related transcription factor 1 (RUNX1), SEC14 like lipid binding 2 (SEC14L2), serine/threonine kinase 39 (STK39), TBC1 domain family member 8 (TBC1D8), thymosin beta 15a (TMSB15A), USO1 vesicle transport factor (USO1), and zinc finger and BTB domain containing 16 (ZBTB16).

7. The method of any one of claims 1-6, wherein the biological sample is a biopsy or a tumor sample.

4.8. The method of any one of claims 1-7, wherein the subject: a) is a human being; b) is to be treated with radiotherapy following breast conserving surgery (BCS); and/or c) has estrogen receptor positive (ER+) breast cancer, human epidermal growth factor receptor 2 negative (HER2-) breast cancer, Stage I-II breast cancer, or node negative breast cancer and/or is post-menopausal.

9. The method of any one of claims 1-8, wherein the method further comprises treating the subject with mastectomy, radiation boost, or adjuvant systemic therapy.

10. The method of any one of claims 1-9, wherein the subject is treated with radiotherapy following breast conserving surgery (BCS).

5.11. The method of any one of claims 1-10, wherein: a) the expression levels of N4BP2L1 and the one or more optional genes all of the genes selected from Table 2 are measured in the biological sample; b) the levels of expression of one or more of the genes selected from Table 2 are increased or reduced compared to a control; c) said measuring the levels of expression comprises performing in situ hybridization, a PCR-based method, an array-based method, an immunohistochemical method, an RNA assay

~~method, or an immunoassay method, optionally wherein said measuring the levels of expression comprises using a reagent selected from the group consisting of a nucleic acid probe, one or more nucleic acid primers, and an antibody; d) said measuring the level of expression comprises measuring the level of an RNA transcript; and/or e) the method is performed prior to treatment of the subject with adjuvant chemotherapy, endocrine therapy, or radiotherapy.~~

12. The method of any one of claims 1-11, wherein measuring the levels of expression comprises performing *in situ* hybridization, a PCR-based method, an array-based method, an immunohistochemical method, an RNA assay method, or an immunoassay method.
13. The method of any one of claims 1-12, wherein measuring the levels of expression comprises using a reagent selected from the group consisting of a nucleic acid probe, one or more nucleic acid primers, and an antibody.
14. The method of any one of claims 1-13, wherein measuring the level of expression comprises measuring the level of an RNA transcript.
15. The method of any one of claims 1-14, wherein the method is performed prior to treatment of the subject with adjuvant chemotherapy, endocrine therapy, or radiotherapy.
- 6-16. A kit for determining a prognosis of a subject having breast cancer and whether or not to treat the subject with adjuvant radiotherapy, the kit comprising reagents ~~agents~~ for measuring levels of expression of a plurality of genes, ~~selected from Table 2~~ wherein the plurality of genes are NEDD4 binding protein 2 like 1 (N4BP2L1) and one or more optional genes selected from ATP binding cassette subfamily C member 8 (ABCC8), BTG anti-proliferation factor 3 (BTG3), cyclin B1 (CCNB1), centromere protein F (CENPF), creatine kinase mitochondrial 1B (CKMT1B), cornichon family AMPA receptor auxiliary protein 4 (CNIH4), cellular retinoic acid binding protein 2 (CRABP2), enoyl-CoA hydratase domain containing 2 (ECHDC2), eukaryotic elongation factor 2 kinase (EEF2K), eukaryotic translation initiation factor 3 subunit L (EIF3L), ectonucleoside triphosphate diphosphohydrolase 6 (putative) (ENTPD6), epoxide hydrolase 2 (EPHX2), H2A histone family member Z (H2AFZ), hydroxysteroid 17-beta dehydrogenase 4 (HSD17B4), karyopherin subunit alpha 2 (KPNA2), lymphoid enhancer binding factor 1 (LEF1), NIMA related kinase 10 (NEK10), prefoldin subunit 4 (PFDN4), pleckstrin and Sec7 domain containing 3 (PSD3), runt related

transcription factor 1 (RUNX1), SEC14 like lipid binding 2 (SEC14L2), serine/threonine kinase 39 (STK39), TBC1 domain family member 8 (TBC1D8), thymosin beta 15a (TMSB15A), USO1 vesicle transport factor (USO1), and zinc finger and BTB domain containing 16 (ZBTB16).

~~7.17.~~ The kit of claim 16, wherein the kit comprises: a) agents for measuring the levels of expression of all of the genes listed in Table 2; b) at least one set of PCR primers capable of amplifying a nucleic acid comprising a sequence of a gene selected from Table 2 or its complement; c) at least one probe capable of hybridizing to a nucleic acid comprising a sequence of a gene selected from Table 2 or its complement said reagents comprise one or more of a microarray, a nucleic acid probe, a nucleic acid primer, or an antibody.

~~8.18.~~ The kit of claim 16 or claim 17, wherein said agents comprise a) reagents comprise reagents for performing in situ hybridization, a PCR-based method, an array-based method, an immunohistochemical method, an RNA assay method, or an immunoassay method; or b) one or more of a microarray, a nucleic acid probe, a nucleic acid primer, or an antibody.

19. The kit of any one of claims 16-18, wherein the kit comprises at least one set of PCR primers capable of amplifying a nucleic acid comprising a sequence of N4BP2L1 and the one or more optional genes or their complements.

20. The kit of any one of claims 16-19, wherein the kit comprises at least one probe capable of hybridizing to a nucleic acid comprising a sequence of N4BP2L1 and the one or more optional genes or their complements.

~~9.21.~~ The kit of any one of claims 16-20, further comprising: a) information, in electronic or paper form, comprising instructions on how to determine the prognosis of a subject having breast cancer and whether or not to treat the subject with adjuvant radiotherapy; or
b) one or more control reference samples.

22. The kit of any one of claims 16-21, further comprising one or more control reference samples.

~~10.23.~~ A probe set for determining a prognosis of a subject having breast cancer and whether or not to treat the subject with adjuvant radiotherapy, the probe set comprising a plurality of probes for detecting a plurality of target nucleic acids, wherein the plurality

of target nucleic acids comprises one or more gene sequences, or complements thereof, of NEDD4 binding protein 2 like 1 (N4BP2L1) and one or more optional genes selected from ATP binding cassette subfamily C member 8 (ABCC8), BTG anti-proliferation factor 3 (BTG3), cyclin B1 (CCNB1), centromere protein F (CENPF), creatine kinase mitochondrial 1B (CKMT1B), cornichon family AMPA receptor auxiliary protein 4 (CNIH4), cellular retinoic acid binding protein 2 (CRABP2), enoyl-CoA hydratase domain containing 2 (ECHDC2), eukaryotic elongation factor 2 kinase (EEF2K), eukaryotic translation initiation factor 3 subunit L (EIF3L), ectonucleoside triphosphate diphosphohydrolase 6 (putative) (ENTPD6), epoxide hydrolase 2 (EPHX2), H2A histone family member Z (H2AFZ), hydroxysteroid 17-beta dehydrogenase 4 (HSD17B4), karyopherin subunit alpha 2 (KPNA2), lymphoid enhancer binding factor 1 (LEF1), NIMA related kinase 10 (NEK10), prefoldin subunit 4 (PFDN4), pleckstrin and Sec7 domain containing 3 (PSD3), runt related transcription factor 1 (RUNX1), SEC14 like lipid binding 2 (SEC14L2), serine/threonine kinase 39 (STK39), TBC1 domain family member 8 (TBC1D8), thymosin beta 15a (TMSB15A), USO1 vesicle transport factor (USO1), and zinc finger and BTB domain containing 16 (ZBTB16) of genes selected from Table 2.

~~11.24.~~ The probe set of claim 2340, wherein the probe set comprises a plurality of probes for detecting a plurality of target nucleic acids comprising gene sequences, or complements thereof, of the genes selected from Table 2, optionally wherein at least one probe is detectably labeled.

~~12.25.~~ A kit for determining a prognosis of a subject having breast cancer and whether or not to treat the subject with adjuvant radiotherapy, chemotherapy, or endocrine therapy, the kit comprising the probe set of claim 2340.

~~13.26.~~ A system comprising:

- a) the probe set of claim 2340; and
- b) a computer model or algorithm for analyzing an expression level or expression profile of the plurality of target nucleic acids hybridized to the plurality of probes in a biological sample from the subject who has breast cancer and determining if the subject is at low risk of cancer recurrence based on the expression level or expression profile and predicting whether the subject will benefit from adjuvant radiotherapy.

~~14.27.~~ A kit comprising the system of claim 2643.

~~15-28.~~ The kit of claim ~~27~~4, further comprising a computer model or algorithm for a)
designating a treatment modality for the subject; or b) normalizing the expression level
or expression profile of the plurality of target nucleic acids.

29. The kit of claim 27 or claim 28, further comprising a computer model or algorithm for
normalizing the expression level or expression profile of the plurality of target nucleic acids.