Myriad EndoPredict® Technical Specifications
Myriad Genetic Laboratories, Inc. Effective Date: Feb 1, 2018

TEST RESULTS SHOULD BE USED ONLY AFTER REVIEW OF THE FOLLOWING SPECIFICATIONS:

Indications and Use

Intended Use

This assay is intended for *in vitro* analysis of FFPE resection tissue of primary female invasive breast cancer (estrogen receptor positive, HER2 negative), for the determination of the 10-year risk of distant recurrence (metastatic disease).

Summary and Explanation

Breast cancer is the most frequently diagnosed cancer in U.S. women (246,000 new cases annually), and is the second leading cause of cancer-related death.[1] Approximately 1 in 8 U.S. women, or 12%, will develop invasive breast cancer over the course of their lifetime.[1] Breast cancer is a heterogeneous disease and is often classified based on the status of the human epidermal growth factor receptor 2 (HER2) protein, as well as two hormone receptors (HR), which are the estrogen receptor (ER) and progesterone receptor. Based on the expression of these proteins, which can inform medical management, newly diagnosed patients with breast cancer are stratified into three groups: HR-positive/HER2-negative, HER2-positive, or triple negative (HR-negative and HER2-negative). Patients with HR-positive/HER2-negative invasive breast cancer have the most favorable 5-year outcomes, followed by patients with HER2-postive, and then triple negative disease.[2]

Patients specifically with ER-positive and HER2-negative (ER+/HER2-) invasive breast cancer respond well to endocrine therapy. Additional use of adjuvant chemotherapy can be considered for patients with high risk features; however, not all patients have the same potential for chemotherapy benefit.[3] EndoPredict® is a molecular test that identifies a large population of patients (55%-65% of patients tested in clinical trials) with excellent 10-year outcomes that can safely forgo adjuvant chemotherapy.[4-7] The EndoPredict Clinical (EPclin) Risk Score is generated from the combination of molecular and clinical data (described in more detail below) and has been shown to be a significant and independent predictor of breast cancer distant recurrence and adds significant prognostic information to clinical information alone.[4-7]

Description of Method

Acceptable sample types for testing are formalin-fixed paraffin-embedded (FFPE) tissue from blocks and/or slides of primary female invasive breast tumor resection specimens (ER+/HER2-). Blocks must have a portion of the lesion with ≥30% invasive tumor on the diagnostic H&E slide, with at least 40 µm of remaining unstained tissue for testing. In cases where blocks are not available, one 3-5 µm H&E slide followed by 4 consecutive 10 µm unstained slides may be acceptable. Samples frozen prior to fixation are not appropriate for analysis. Samples are shipped overnight with an ice pack to Myriad Genetic Laboratories, Inc. for analysis. Upon receipt, sample barcodes, which are scanned and tracked, are applied to each sample. Unused tissue is returned to the provider upon the completion of testing.

For testing, the H&E slide from each case is evaluated by an anatomic pathologist, who determines the location and amount of tumor per slide. Using the H&E stained slides as a guide, tumor tissue is macrodissected from the unstained sections and total RNA is extracted from the excised tissue. Quantitative RT-PCR is then used to measure, in triplicate, the expression of 8 signature genes, and 3 normalization genes, with 1 control gene to assess for DNA contamination. These molecular data are used to generate a 12-Gene Molecular Score. The molecular score is then combined with tumor size and lymph node status to generate an EPclin Risk Score and a 10-year risk of distant recurrence.[4-7]

Performance Characteristics/Limitations

The 12-Gene Molecular Score is calculated based upon the RNA expression of 8 target genes and 3 normalization genes, with 1 control gene used to assess for DNA contamination. The molecular score is combined with specific clinical data (tumor size and lymph node status) to genereate an EPclin Risk Score.[4] Both scores were trained in a large, multi-site cohort of 964 treatment-naive FFPE primary breast resection samples.[4] A Cox proportional hazards model was used to fit a linear combination of the 12-Gene Molecular Score, tumor stage and lymph node status, generating an EPclin Risk Score. The EPclin Risk Score is calculated, according to the model, as: EPclin Risk Score = (0.35 * tumor size) + (0.64 * lymph node status) + (0.28 * 12-Gene Molecular Score) [tumor size: 1 (T1ab, ≤1 cm), 2 (T1c, >1 but <2 cm), 3 (T2, >2 but < 5 cm), 4 (T3, >5 cm); lymph node status: 1 (no positive nodes), 2 (1-3 positive nodes, or micrometastases)]. Both the 12-Gene Molecular Score and EPclin Risk Score have been subsequently validated in multiple independent cohorts.[4-7]

Clinically Reportable Range

Based on the analysis of 1,702 treatment-naive FFPE primary breast resection tumor samples, a clinically reportable range was determined for the EPclin Risk Score from 1.1 through 6.2.[4,8] Scores out of the validated range will lead to test cancelation (see Interpretive Criteria).

Analytical Precision, Linearity and Detection Limit

A set of 12 samples was tested, with 3 replicates for each sample, and the standard deviation of the EPclin Risk Score was determined to be 0.06 score units.[8] The signature was originally validated to require a minimal input of RNA sufficient to generate a crossing threshold (C_t) value less than 40 for normalization and target genes.[4] In regards to RNA input linearity, the test reproduces valid results from a an average C_t of 19-27 for the housekeeper genes.[8] Samples with an average housekeeper value <19 or >27 are invalid and will lead to test cancelation.

Quality Control Measures

A minimum of one no-RNA control and one normal human RNA control (with a previously determined score) are analyzed concurrently with each sample. Additionally, one control that is positive for DNA contamination is measured with each sample. Controls are analyzed to verify technical consistency and samples are only reported when all controls tested with the sample perform within specifications.

Test Interference

Systemic treatment prior to resection of the primary breast tumor (*e.g.*, neoadjuvant chemotherapy, radiation treatment) may affect test performance, potentially resulting in inaccurate test results. Adjuvant chemotherapy after resection will affect test performance.[7] Therefore, patients receiving systemic treatments prior to resection, or adjuvant chemotherapy after resection, are not suitable candidates for testing.

Limitations and Sample Rejection Criteria

The reported 10-year distant recurrence risk is based on analysis of a cohort of post-menopausal women with resected ER+/HER2- invasive female breast cancer who have not been treated prior to resection with systemic neo-adjuvant therapy

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(*e.g.*, chemotherapy, radiation therapy, endocrine therapy) and who do not have a current or prior diagnosis of an additional cancer.[4] Risks may differ for individuals who do not meet the aforementioned clinical characteristics. This test result is invalid if the patient has already experienced a distant recurrence.

Performance characteristics for the test have not been established in non-invasive samples, tumors that are not ER+/HER2-, patients currently undergoing hormone replacement therapy, patients that received systemic treatment or localized radiation prior to resection, nor in male breast cancer tumors.[4] However, local radiation therapy after resection does not interfere with the test result. Patients that received hormone replacement therapy after menopause are suitable for testing, provided that the therapy was halted after diagnosis of breast cancer. Samples in non-validated tissues (*i.e.*, non-breast tissue) will not be accepted for testing. This test was validated in multiple cohorts of post-menopausal women,[4-5] and a mixed cohort of pre/post-menopausal women.[7]

Retrospective analysis of resection samples, up to 10 years after surgery, may be suitable, provided that the patient received uninterrupted endocrine therapy for 5 years (or until time of sample testing if it has been less than 5 years since the time of surgery) and has not received adjuvant chemotherapy subsequent to resection. The test result may not accurately reflect the patient's risk of recurrence if the patient received endocrine treatment for longer than 5 years, or had interruption in their 5 year endocrine treatment regimen. The test result has been demonstrated to not accurately reflect the patient's risk of recurrence if the patient has received adjuvant chemotherapy subsequent to resection.[7]

Two clinical parameters are required for calculation of the EPclin Risk Score, which are tumor size/stage and the number of positive lymph nodes. Only primary tumors that are T stage ≤3 are acceptable for testing. Tumors with grade G1-G3 or Gx are suitable for testing. Samples with 0-3 positive lymph nodes are currently accepted for testing. Samples lacking sufficient clinical information or samples with clinical variables that are unsuitable for testing may lead to test cancelation and/or follow-up with the referring healthcare provider. Samples with less than the 30% minimum invasive tumor may also lead to test cancelation.

Primary breast tumors with invasive ductal, invasive lobular, mixed histology (with both invasive ductal and invasive lobular characteristics), and primary invasive breast tumors with no specified histology are all acceptable for testing. Samples with micrometastatic disease in lymph nodes are also acceptable for testing. Samples that are recurrences of previous primary breast cancers or patients with synchronous cancer are not suitable for testing.

The FFPE tissue preservation process may cause RNA degradation resulting in insufficient quantity or quality of RNA for analysis. Samples with insufficient RNA quantity and/or quality may lead to test cancelation and/or follow-up with the referring healthcare provider.

Results of this analysis should be used in conjunction with information available from clinical evaluation and other diagnostic procedures.

Interpretive Criteria

The EPclin Risk Score will be reported, along with the 10-year risk for distant recurrence. The following criteria should be used to interpret the reported score.

EPclin Risk Scores from 1.1 through 3.3

Scores within this range are clinically validated and will be reported. Patients with scores in this range have a low 10-year risk for distant recurrence. The estimated 10-year risk of recurrence will be provided, based upon the EPclin Risk Score.

EPclin Risk Scores from 3.4 through 6.2

Scores within this range are clinically validated and will be reported. Patients with scores in this range have a high 10-year risk for distant recurrence. The estimated 10-year risk of recurrence will be provided, based upon the EPclin Risk Score.

EPclin Risk Scores less than 1.1 or greater than 6.2

Based upon 1,702 samples, a reportable range for EPclin Risk Scores was established from 1.1 through 6.2.[4] Scores outside of this range have not been validated and will lead to test cancelation.

References

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