Myriad myPlan® Lung Cancer Technical Specifications
Myriad Genetic Laboratories, Inc. Effective Date: Oct, 2015

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

Indications and Use

Intended Use

This assay is intended for *in vitro* diagnostic analysis of FFPE Non-Small Cell Lung Cancer (NSCLC) adenocarcinoma stage I and II surgical specimens for determination of 5-year lung cancer mortality risk.

Summary and Explanation

Lung cancer is the most frequent cause of cancer-related death in the U.S., with more deaths than colon, breast, prostate and pancreatic cancers combined. NSCLC accounts for ~85% of all lung cancers.[1] The prognosis for a patient diagnosed with NSCLC remains poor. Even if diagnosed in early stages, 5-year overall survival rates are only 30%-60% and patients are at substantial risk for recurrence even after surgical resection.[2,3] Up to 40% of patients with stage I, 66% of stage II, and 75% of stage IIIA patients die within 5 years of resection. Use of adjuvant chemotherapy in NSCLC is based upon pathological stage and several studies have found that the absolute benefit of adjuvant therapy has been inconsistent and moderately low in early stage disease (approximately 3-5%).[4-7] Given the limited overall benefit of chemotherapy, the frequent co-morbidities in NSCLC patients, and the serious side effects of therapy, prognostic markers that further stratify patients can aid in the treatment decision-making process. The molecular component of the myPlan® Lung Cancer Prognostic Score has been shown to be an independent predictor of lung cancer mortality and adds significant prognostic information to pathological stage.[8-10]

Description of Method

Acceptable sample types are formalin-fixed paraffin-embedded (FFPE) tissue from blocks or slides of primary lung adenocarcinoma tumor resection specimens. Ideally, blocks should include at least 2x3 mm of tissue with at least 50% tumor on the diagnostic H&E slides for sample processing and RNA extraction. In cases where blocks are not available, one 2-5 µm H&E slide followed by five consecutive 4-5 µm unstained slides and a final H&E slide may be acceptable. Samples frozen prior to fixation are not appropriate for analysis. Blocks (or slides) 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 block (or slide). The H&E slides from each case are 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. A minimum of 2 ng/µL of RNA is required for testing. The expression of 31 cell cycle genes is then measured, normalized by the expression of 15 housekeeping genes. These data are used to generate a cell cycle progression (CCP) Score, which is combined with pathological stage to generate a myPlan™ Lung Cancer Prognostic Score.[9,10]

Performance Characteristics/Limitations

The Prognostic Score was trained in multiple datasets with a total of 495 lung adenocarcinoma patients.[9] A Cox proportional hazards model, stratified by cohort, was used to fit a linear combination of the CCP Score and pathological stage as a numerical variable (1=IA, 2=IB, 3=IIA, 4=IIB). The myPlan Lung Cancer Prognostic Score is calculated according to the resulting model as 20*(0.33*CCP Score + 0.52*stage) + 15.

In multiple pre-specified clinical validation studies, FFPE lung adenocarcinoma primary tumor surgical samples from 1,135 patients with stage I and II disease were analyzed and CCP Scores were calculated.[9,10] The CCP Score and the myPlan Lung Cancer Prognostic Score were each validated as significant predictors of five year lung cancer survival. The myPlan Lung Cancer Prognostic Score was a more significant predictor of five-year lung cancer survival than pathological stage alone.[9,10]

Clinically Reportable Range

Based on analysis of 650 FFPE lung adenocarcinoma primary tumor surgical samples, a clinically reportable Prognostic Score range of 7 to 72 was established.[9] Prognostic Scores outside of this range may be reported, but estimates of lung cancer mortality risk will not be provided (see Interpretive Criteria).

Analytical Precision, Linearity and Detection Limit

A set of 23 samples was tested, with 6 replicates for each sample, and the standard deviation of the CCP Score was determined to be 0.06 Score units (95% CI: 0.05, 0.09).[11] The linearity of the RNA input has been determined to be 1.5-780 ng of input RNA (RNA concentration, 0.12-62.4 ng/µL), and clinical samples are tested within from 25-500 ng of input RNA (RNA concentration, 2-40 ng/µL).[8-11]

Dynamic Range of the CCP Score

The dynamic range of the CCP Score was determined to be from CCP Scores of -13 through 14.[11] In recent clinical validations CCP Scores were observed to range from -3.0 to 2.8, which correlated to Prognostic Scores of 7 to 72[8-10]. Only samples with Prognostic Scores within this validated range will be reported with associated risk information (see Interpretive Criteria).

Quality Control Measures

A minimum of 1 no-RNA control and 1 normal human RNA control with a previously determined CCP Score are analyzed concurrently with each sample. Controls are analyzed to verify technical consistency.

Interference

Neoadjuvant chemotherapy and radiation treatment are known to affect CCP Scores, potentially resulting in incorrect test interpretation. Patients receiving these treatments prior to resection are not suitable candidates for myPlan Lung Cancer testing.

Limitations

Performance characteristics for the myPlan Lung Cancer assay have not been established for tissues other than human FFPE NSCLC adenocarcinoma stage I and II surgical specimens. Thus, other tissue types will not be accepted for analysis. The FFPE tissue preservation process may cause RNA degradation resulting in insufficient RNA yields for analysis. Results of this analysis should be used in conjunction with information available from clinical evaluation and other diagnostic procedures.

Sample Rejection Criteria

Inappropriate sample types can cause cancelation of the test. Inappropriate sample types include: tumors other than Stage I or II adenocarcinoma, biopsies, samples that were previously frozen, samples not fixed in neutral buffered formalin, or samples from patients that received

chemotherapeutics or radiation treatments prior to resection. Samples with insufficient clinical information provided may be canceled. Samples of insufficient quantity, or insufficient quality may also be canceled (which may be due to insufficient neoplastic cells within the sample, damage during shipping, or insufficient RNA yields). A test may also be canceled if the Score is outside of the validated range of Scores (see Interpretive Criteria).

Interpretive Criteria

Prognostic Scores between 7 and 72

Prognostic Scores within this range are clinically validated and will be reported. The estimated lung cancer mortality risk will be provided for patients based on the Prognostic Score.

Prognostic Scores less than 7 but greater than -14

Linearity of Prognostic Scores within this range has been established and will be reported. However, these scores lie outside of the clinically validated Prognostic Score range of 7 to 72. A Prognostic Score in this range will be reported, but an estimated lung cancer mortality risk will not be provided in these cases.

Prognostic Scores greater than 72 but less than 90

Linearity of Prognostic Scores within this range has been established and will be reported. However, these Scores lie outside of the clinically validated Prognostic Score range of 7 to 72. A Prognostic Score in this range will be reported, but an estimated lung cancer mortality risk will not be provided in these cases.

Prognostic Scores less than -14 or greater than 90

These Scores lie outside of the verified detection limits of this assay and may represent an artifact or technical error. Thus, neither a Prognostic Score, nor cancer mortality risks will be reported in these cases, and tests with these Scores will be canceled.

References

1. Siegel R, Ma J, Zou Z, Jemal A. Cancer statistics, 2014. *CA.* Jan-Feb 2014; 64(1):9-29.
2. Chansky K, Sculier JP, Crowley JJ, et al. The International Association for the Study of Lung Cancer Staging Project. *J Thorac Oncol.* 2009; 4(7):792-801.
3. Tsuboi M, Ohira T, Saji H, et al. The present status of postoperative adjuvant chemotherapy for completely resected non-small cell lung cancer. *Ann Thorac Cardiovasc Surg.* 2007; 13(2):73-77.
4. Crino L, Weder W, van Meerbeeck J, Felip E, Group EGW. Early stage and locally advanced (non-metastatic) non-small-cell lung cancer. *Ann Oncol.* 2010; 21 Suppl 5:v103-115.
5. Pignon JP, Tribodet H, Scagliotti GV, et al. Lung adjuvant cisplatin evaluation: a pooled analysis by the LACE Collaborative Group. *J Clin Oncol.* 2008; 26(21):3552-3559.
6. Pisters KM, Evans WK, Azzoli CG, et al. Cancer Care Ontario and American Society of Clinical Oncology adjuvant chemotherapy and adjuvant radiation therapy for stages I-IIIA resectable non small-cell lung cancer guideline. *J Clin Onco.* 2007; 25(34):5506-5518.
7. Strauss GM, Herndon JE, 2nd, Maddaus MA, et al. Adjuvant paclitaxel plus carboplatin compared with observation in stage IB non-small-cell lung cancer. *J Clin Oncol.* 2008; 26(31):5043-5051.
8. Wistuba, II, Behrens C, Lombardi F, et al. Validation of a proliferation-based expression signature as prognostic marker in early stage lung adenocarcinoma. *Clin Cancer Res.* 2013; 19(22):6261-6271.
9. Bueno R, Hughes E, Wagner S, et al. Validation of a molecular and pathological model for five-year mortality risk in patients with early stage lung adenocarcinoma. *J Thorac Oncol.* 2015; 10(1):67-73.
10. Rakha E, Pajares MJ, Ilie M, et al. Stratification of resectable lung adenocarcinoma by molecular and pathological risk esttimators. *Eur J Cancer*. 2015; 51(14):1897-1903.
11. Warf MB, Fosso PG, Hughes E, et al. Analytical validation of a proliferation-based molecular signature used as a prognostic marker in early stage lung adenocarcinoma. *Biomark Med.* 2015; 9(9):901-910.