Indications and Use

Intended Use

This assay is intended for in vitro diagnostic analysis of FFPE prostate tumor biopsies for determination of the 10-year risk of both metastatic disease after single-mode definitive therapy and disease specific mortality if conservatively managed. The test is also intended to be used to identify whether a patient is appropriate for active surveillance, single modality treatment, or multimodality treatment.

Summary and Explanation

Approximately 248,580 men in the U.S. will be diagnosed with prostate cancer in 2021.1 Prostate screening allows for the early detection of cancer. However, many tumors detected through screening will be indolent, while other seemingly low-risk cancers can progress rapidly and become fatal. Most men diagnosed with prostate cancer will die of other causes. Current diagnostic tools are imperfect at distinguishing between indolent and aggressive tumors.

The majority of diagnosed individuals are older than 65 and disease often progresses slowly. While these individuals have the option to pursue active surveillance, many individuals with localized disease choose more aggressive clinical treatments, including radical prostatectomy and radiation treatment, which are associated with morbidity. Prognostic markers that can further stratify indolent versus aggressive prostate cancer can aid in the treatment decision-making process. The Prolaris® assay has been shown to be an independent predictor of prostate cancer-specific mortality risk for patients.

Description of Method

Acceptable sample types are limited to formalin-fixed paraffin-embedded (FFPE) tissue from blocks or slides of prostatic adenocarcinoma biopsies. Ideally, blocks should include at least 0.5 mm of linear tumor (with >75% tumor) on diagnostic H&E slides for sample processing and RNA extraction. In cases where blocks are not available, one 3-5 µm H&E slide followed by five consecutive 4-5 µm unstained slides may be acceptable. 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 a pathologist who determines the location and amount of tumor per slide. Using the H&E stained slides as a guide, tumor tissue is removed from the unstained slides and total RNA is extracted from the tissue.

The expression of 31 cell cycle genes, normalized by 15 housekeeper genes, is then measured in triplicate by quantitative RT-PCR to generate a Prolaris Score, also called the cell cycle progression (CCP) score, which is the molecular component of Prolaris. The CCP score is then combined with clinical components in the form of the CAPRA score, to produce a combined clinical risk (CCR) score, which is used to estimate the 10-year risk of both metastatic disease and prostate cancer-specific mortality. The clinical components used to calculate CAPRA are provided by the ordering healthcare provider on the test request form and the pathology report. Each biopsied site will be counted as one core based on NCCN guidelines (PRoS-2A footnote f). The CCR score encompassing both the CAPRA (linear) and CCP scores is calculated as CCR=0.39×CAPRA+0.57×CCP.6,8,9

Two thresholds have been validated10 to aid in refining risk assessment and the identification of potential treatment paths. Specific therapeutic decisions should consider all relevant clinical parameters including a patient’s age, overall health, etc.

Active Surveillance Threshold Validation Analysis

The Prolaris Score distribution was determined in a training cohort of men (N=505) who, based on NCCN-based clinical parameters, might be considered for active surveillance. A predefined combined clinical risk score was selected to define an AS threshold such that 90% of the men in the training cohort had scores below this AS threshold. An independent cohort of conservatively managed men (N=585) was evaluated and there were no observed prostate cancer deaths in patients below the threshold. This predefined CCR was associated with a 3.2% (95% CI: 2.0, 5.2) 10-year risk of prostate cancer-specific mortality in the combined cohort at the threshold.11,12,13

Multi-Modality Threshold Validation Analysis

A combined clinical risk (CCR) score threshold was predefined in a cohort (N=15,669) of NCCN unfavorable intermediate- and high-risk men with known CCR scores, such that the number of men who would be above the threshold would be similar to the number considered high-risk by NCCN clinical-pathological criteria. This predefined CCR-based threshold was evaluated in two cohorts of men with NCCN intermediate- or high-risk localized disease (N=7181 and N=7419) who received either single or multimodality therapy and had known outcomes. Multimodality therapy was defined as either radiation or surgery with androgen deprivation, or surgery with adjuvant radiation. Single modality therapy included surgery, external beam radiation therapy (with or without brachytherapy). Median follow-up in the combined cohort was 5.3 years. This predefined CCR threshold was associated with an 8.9% (95% CI: 5.3, 14.7) 10-year risk of metastasis with single modality therapy at the threshold.14,15,16

Performance Characteristics/Limitations

The Prolaris Score was trained on a pooled sample of 1,059 prostate cancer patients with complete clinical data.7 The Prolaris Score was then clinically validated on 1,106 FFPE prostate tumor biopsy samples from two British cohorts of conservatively managed (watchful waiting) patients.17 The distribution of Prolaris Scores in the U.S. population was estimated using 1,174 patients tested at Myriad and may be adjusted in the future to reflect additional observations (data on file).

Clinically Reportable Range

Prolaris Scores are calculated as previously described,14 and the score is then adjusted by +4 units in order to convert it to a more understandable range (approximately 0 to 10). The adjusted Prolaris Score is reported to the patient. The Interpretive Criteria in this document reference the adjusted Prolaris Score.

Based on analysis of 1,828 FFPE prostate tumor biopsy samples from two cohorts of conservatively managed patients and one cohort of treated patients,4,9,9 a clinically reportable adjusted Prolaris Score range of 1.8 to 8.7 was established. Scores outside of this range may be reported, but risk estimates of prostate cancer-specific mortality will not be provided.

Analytical Precision and Linearity of the Prolaris Score

A set of 13 biopsy samples and 2 radical prostatectomy samples was tested, with 3 biological replicates for each sample. The mixed sample set was representative of the distribution of sample types tested by MGL, and the standard deviation of the Prolaris Score was determined to be 0.22 score units (95% CI: 0.08-0.36).
In regards to RNA input linearity, the maximum RNA input concentration is 40 ng/µl (500 ng) and consistent results are obtained when samples are diluted until the average housekeeper gene Ct value exceeds 24.\(^6\) Samples with an average housekeeper value >24 are invalid and will lead to test cancellation.

**Dynamic Range of the Prolaris Score**

The dynamic range of the Prolaris Score component was determined to be from 1.0 to 11.0.\(^5\) In clinical validations, adjusted Prolaris Scores were observed from 1.8 to 8.7.\(^4\)\(^5\)\(^9\) Only Prolaris Scores within the clinically validated range will be reported with prostate cancer-specific mortality risk estimates.

**Quality Control Measures**

A minimum of one no-RNA control and one normal human RNA control with a previously determined Prolaris Score are analyzed within each sample run. Controls are analyzed to verify expected results.

**Interference**

Neoadjuvant hormonal therapy and radiation treatment can affect Prolaris Scores, potentially resulting in incorrect test interpretation. Patients receiving these treatments prior to biopsy are not suitable candidates for testing.

**Limitations**

Performance characteristics for the Prolaris assay have not been established for tissues other than human FFPE prostate tumor specimens. Thus, other tissue types will not be accepted for analysis. This test is not validated for the analysis of tumor samples from individuals with Total PSA levels >100 ng/ml. The FFPE tissue preservation process may cause RNA degradation resulting in insufficient RNA quality or quantity 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 prostatic adenocarcinoma (small cell carcinoma or sarcoma), samples that were previously frozen, samples from patients that received chemotherapeutics or radiation treatments prior to biopsy, or transurethral resection of the prostate (TURP) samples. Samples with insufficient clinical information provided may be canceled. Samples of insufficient tumor quantity (<0.5 mm linear tumor and/or <75% tumor), or insufficient quality may also be canceled. Insufficient quality may be due to damage during shipping or insufficient RNA yields. A test may also be canceled if the Prolaris Score is outside of the validated range of scores.

**Interpretive Criteria**

**Prolaris Scores between 1.8 and 8.7 (including 1.8 and 8.7)**

Prolaris Scores within this range are clinically validated and will be reported as the calculated Prolaris Score. Both the estimated prostate cancer-specific mortality risk and an estimated risk of metastatic disease for patients who underwent definitive treatment will be provided for the patient, based on a combination of their Prolaris and CAPRA Scores.

**Prolaris Scores less than 1.8 but greater than or equal to 1.0**

Linearity of Prolaris Scores within this range has been established. Thus, the calculated Prolaris Score will be reported. However, these scores lie outside of the clinically validated Prolaris Score range of 1.8 to 8.7. Neither an estimated prostate cancer-specific mortality risk nor a metastatic disease risk will be provided.

**Prolaris Scores less than 1.0 or greater than 11.0**

These scores may represent an artifact or technical error. Thus, these scores will not be reported and the test will be canceled.

**References**

5. NCCN Clinical Practice Guidelines in Oncology (NCCN Guidelines) for Prostate Cancer Version 4.2022.\(^2\) National Comprehensive Cancer Network, Inc. 2022. All rights reserved. Accessed [June 23, 2022]. To view the most recent and complete version of the guideline, go online to https://www.nccn.org/.