Prolaris® Biopsy Technical Specifications
Myriad Genetic Laboratories, Inc.
Effective Date: Aug 15, 2023

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 prostate tumor biopsies for determination of the 10-year risk of both metastatic disease with 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 288,300 men in the U.S. will be diagnosed with prostate cancer in 2023. 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 in 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 molecular score, also called the cycle cell progression (CCP) score, which is the molecular component of Prolaris. The CCP score is then combined with clinical components in the form of the University of California, San Francisco, Cancer of the Prostate Risk Assessment (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 CCR was trained on a pooled sample of 1,059 prostate cancer patients. The CCR score combining the CAPRA and CCP scores is calculated as CCR = 0.39 x CAPRA + 0.57 x CCP.

The clinical components used to calculate CAPRA are provided by the ordering healthcare provider on the test request form and the pathology report. In cases where the percent of biopsy cores positive for cancer reported on the pathology report and the test request form do not align, the total number of cores/sites biopsied and the percent positive for cancer will be determined based on information provided on the pathology report. Each biopsied site will be counted as one core based on NCCN guidelines (PROS-2A footnote f).

The continuous CCR score and two thresholds have been validated to aid in refining risk assessment and the identification of potential treatment paths. The 10-year risk of prostate cancer-specific mortality with conservative management and 10-year metastasis risks with different treatment plans are provided. Specific therapeutic decisions should consider all relevant clinical parameters including a patient’s age, overall health, etc.

Risk of Prostate Cancer-Specific Mortality and the Active Surveillance (AS) Threshold

The active surveillance threshold was selected in a clinically tested cohort of patients that might be considered for active surveillance based on clinical parameters (Gleason score <3+4; PSA <10 ng/ml; <25% cores positive; and clinical stage <T2a; N = 505) such that 90% of patients would be below the threshold. Ten-year risk of prostate cancer-specific mortality with conservative management was then estimated in two, independent cohorts (N = 180, N = 585). The 10-year risk of prostate cancer-specific mortality at the active surveillance threshold in these combined cohorts is 3.2% (95% CI: 2.0, 5.2). Men with scores above the threshold were at significantly higher risk of prostate cancer specific mortality than those below the threshold. No prostate cancer-related deaths within 10 years of diagnosis were observed in these cohorts in men with scores at or below the threshold.

Risk of Metastasis and the Multi-Modal Threshold

The multi-modal threshold was selected such that the proportion of individuals in a large, commercially tested cohort (N = 15,669) who would be above the threshold would be similar to the proportion considered high-risk by NCCN clinicopathologic criteria. Ten-year risk of metastasis with single-modal therapy was then estimated in two, independent cohorts of patients treated with single-modal therapy (N = 561, N = 351). The 10-year risk of metastasis at the multi-modal therapy threshold in these combined cohorts is 8.9% (95% CI: 5.3, 14.7). Patients above the threshold who were treated with single-modal therapy were at significantly higher risk of developing metastasis than those below the threshold. Single-modal therapy was defined as either surgery or radiation therapy (RT). Multi-modal therapy was defined as either radiation therapy (RT) with androgen deprivation therapy (ADT), or surgery with intensified therapy per guideline recommendations.

Absolute Risk Reduction (ARR) Metrics

The 10-year risk of metastasis with ADT in addition to RT was estimated using a cohort of men treated with single-modal RT (N = 467) and assuming a 41% relative reduction in risk of distant metastasis from ADT added to RT, as estimated in a meta-analysis of ADT benefit. ARR is calculated by subtracting the 10-year risk of metastasis with RT+ADT from the 10-year risk of metastasis with RT alone. The number needed to treat (NNT) is calculated as the inverse of ARR.

Risk stratification details

The distribution of CCR scores in the U.S. population was estimated in each NCCN category using a total of 152,311 patients clinically tested at Myriad Genetics Laboratories, and may be adjusted in the future to reflect additional observations.

Performance Characteristics/Limitations

Clinically Reportable Range

Prolaris molecular score (CCP) is calculated as

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previously described, and the score is then adjusted by adding 4.0 units in order to convert it to a more understandable positive range (approximately 0 to 10). The adjusted Prolaris molecular score is reported to the patient. The Interpretive Criteria in this document reference the adjusted Prolaris molecular 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, a clinically reportable adjusted Prolaris molecular 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 Molecular 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 Myriad Genetics Laboratories, and the standard deviation of the Prolaris molecular score was determined to be 0.22 score units (95% CI, 0.16, 0.35). 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 24.10 Samples with an average housekeeper gene Ct value >24 are invalid and will lead to test cancelation.

Dynamic Range of the Prolaris Molecular Score
The dynamic range of the Prolaris molecular score component was determined to be from 1.0 to 11.0. In clinical validations, adjusted Prolaris molecular scores were observed from 1.8 to 8.7. Only Prolaris molecular scores within the clinically validated range will be reported with prostate cancer-specific mortality and metastasis risk estimates.

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

Interference
Neoadjuvant hormonal therapy and radiation treatment can affect Prolaris molecular 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 prostate 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 molecular score is outside of the validated range of scores.

Interpretive Criteria
Prolaris molecular scores between 1.8 and 8.7 (including 1.8 and 8.7) Prolaris molecular scores within this range are clinically validated and will be reported as the calculated Prolaris molecular score. Both the estimated prostate cancer-specific mortality risk and estimated risk of metastatic disease will be provided for the patient based on their CCR score (combination of their Prolaris molecular and CAPRA scores).

Prolaris molecular scores less than 1.8 but greater than or equal to 1.0
Linearity of Prolaris molecular scores within this range has been established. Thus, the calculated Prolaris molecular score will be reported. However, these scores lie outside of the clinically validated Prolaris molecular score range of 1.8 to 8.7. Neither estimated prostate cancer-specific mortality risk nor estimated metastatic disease risk will be provided.

Prolaris molecular scores greater than 8.7 but less than or equal to 11.0
Linearity of Prolaris molecular scores within this range has been established. Thus, the calculated Prolaris molecular score will be reported. However, these scores lie outside of the clinically validated Prolaris molecular score range of 1.8 to 8.7. Neither estimated prostate cancer-specific mortality risk nor estimated metastatic disease risk will be provided.

Prolaris molecular 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.

CCR scores > 3.690
Ten-year risk of metastasis with RT+ADT and ARR will not be provided for the top 1% of clinically tested patients (CCR scores >3.690 as determined in a set of 56,485 clinically tested patients). Tests with a CCR score >3.690 will instead report that the risk is estimated to be in the top 1% of tested patients. Risk of prostate cancer-specific mortality and risk of metastasis with single-mode therapy will be provided granted the test meets other interpretive criteria.

References


