Technical Specifications

Intended Use

Precise Molecular Residual Disease (Precise MRD) is a tumor-informed liquid biopsy test intended for use in patients with a confirmed diagnosis of a solid-tumor cancer. The test has been validated to detect the presence and amount of circulating tumor DNA (ctDNA) in the patient's blood at multiple timepoints. Precise MRD is ordered by qualified healthcare providers, and test results provide adjunctive information that may be considered in their clinical management decisions.

Description of Analysis

Precise MRD is a laboratory-developed tumor-informed test that detects the presence of circulating tumor DNA (ctDNA) and estimates the ctDNA fraction in plasma-derived cell-free DNA (cfDNA) from patients previously diagnosed with a solid-tumor cancer. The test utilizes a personalized panel tracking up to 1000 somatic variants identified by tumor profiling from matched tumor tissue and normal whole genome sequencing (WGS). Analysis of deep targeted sequencing of the personalized panel returns a qualitative result of "ctDNA DETECTED" or "ctDNA NOT DETECTED" that is determined by an empirically defined statistical threshold that supports ≥ 99.6% specificity and a quantitative result that estimates the ctDNA fraction in the plasma sample. ctDNA fraction describes the proportion of all cfDNA that comes from tumor cells, expressed as parts per million.

Description of Method

Creation of Personalized Panel

Tumor genomic DNA (gDNA) is extracted from formalin-fixed paraffin-embedded, sectioned, and macrodissected tumor tissue. Normal gDNA is extracted from buffy coat separated from whole blood. gDNA is prepared into libraries and undergoes WGS. Tumor and normal sequence data are analyzed together to call somatic variants—including single nucleotide variants (SNVs) and small insertions and deletions (INDELs)—identify copy number alterations, and estimate tumor cellularity of the sequenced tumor gDNA. A panel of 500 - 1000 targets is selected from the identified somatic variants based on their expected representation above background in plasma-derived cfDNA. Hybridization-capture probes that bind the panel targets are custom manufactured for each patient.

Determination of MRD Results

Plasma is separated from whole blood and extracted to obtain cfDNA. cfDNA is prepared into a library that undergoes hybridization-capture using the patient's personalized panel and sequencing at high depth. Sequencing data are error corrected and then analyzed using an algorithm that finds the most likely ctDNA fraction in the cfDNA sample based on a statistical model that accounts for the representation of variants from the panel in the sequence data, the expected genotype of the patient's tumor, and residual biological or

technical background error for the sample. The statistical likelihood of this most likely ctDNA fraction is compared with the likelihood that no ctDNA is present in the sample, resulting in a log likelihood ratio (LLR). When the LLR is greater than the detection threshold, a "ctDNA DETECTED" result is reported, along with the most likely ctDNA fraction when that ctDNA fraction is ≥ 1 ppm. When the LLR is less than or equal to the detection threshold, a "ctDNA NOT DETECTED" result is reported.

Performance Characteristics

Analytical Sensitivity and Specificity

The analytical specificity of the Precise MRD test is 99.62%. Analytical sensitivity varies based on biological and technical factors for each patient and plasma sample, e.g., ctDNA fraction, number of targets on a personalized panel, presence of copy number alterations in the patient's tumor, quantity of cfDNA obtained from the plasma sample, etc. To account for varied detection ability across a range of ctDNA amounts, analytical sensitivity is expressed as the ctDNA fraction at which ≥95% of plasma samples are reported as "ctDNA DETECTED", i.e., the Limit of Detection at 95% sensitivity (LoD_{95}). In a conservative assessment of typical conditions, the LoD_{95} of the Precise MRD test is 6 ppm. The LoD95 could be lower for samples with above-average cfDNA input and could be as high as 50 ppm for a sample tested with the minimum panel size and minimum cfDNA input. ctDNA may be detected and reported in a sample with ctDNA fraction below the LoD_{95}; however, the probability of calling such a sample positive (i.e., the sensitivity) may be less than 95%.

Quantitative MRD assays report ctDNA levels with different units, including VAF, MTM/ml, and ctDNA fraction, where ctDNA fraction can be quantified via different methods. Precise MRD estimates ctDNA fraction as the proportion of all cfDNA that comes from tumor cells, expressed as parts per million. This estimate is often approximately two-fold higher than ctDNA fractions calculated based on the proportion of cfDNA harboring somatic variants. Accordingly, the conservative Precise MRD LoD95 expressed in these latter variant-based units would be approximately 3 ppm rather than 6 ppm.

Reproducibility

Overall qualitative reproducibility of the Precise MRD test, assessed using 24 samples spanning a broad range of ctDNA fractions and tested with a minimum of 12 replicates each, is 99.9%. Intra-run repeatability and inter-run reproducibility, calculated as the percent of replicate tests with identical qualitative results, are both 99.8%.

Quantitative Precision

The linear range of ctDNA fractions obtained from Precise MRD test results is 1.5625 - 1,000,000 ppm, meaning that, over this range, the reported ctDNA fraction is directly proportional to the true ctDNA fraction of the plasma sample. The assay may have reduced quantitative precision at ctDNA fraction levels near the LoD_{95}.

Limitations of the Method

Performance of this test may be affected by the quality and quantity of DNA obtained from formalin-fixed paraffin-embedded tumor tissue or buffy coat, and cfDNA obtained from plasma, as well as biological factors specific to the patient's tumor, e.g., abundance of somatic variants and presence of copy number alterations. False positive and false negative results can occur, with possible sources including, but not limited to, sample mix-up, trace contamination, limited input, clonal hematopoiesis of indeterminate potential (CHIP), pregnancy, bone marrow transplantation, recent blood transfusion, chimerism or mosaicism, body-mass index (BMI), brain metastases and other biological or technical factors.

Precise MRD uses a personalized panel targeting somatic variants identified from one tumor submitted from a patient. ctDNA derived from an unrelated tumor, e.g., a second primary or multicentric tumor, may not be detected. Detection of and quantitation accuracy for ctDNA derived from heterogeneous tumors may be impaired.

This test was developed, and its performance characteristics determined, by Myriad Genetics, Inc. It has not been cleared or approved by the US Food and Drug Administration (FDA). The FDA does not require this test to go through premarket review. This test is used for clinical purposes. It should not be regarded as investigational or for research. This laboratory is certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA) as qualified to perform high-complexity clinical testing. These results are adjunctive to the ordering physician's evaluation.

Test Results and Interpretation

The Precise MRD test reports a qualitative ("ctDNA DETECTED" or "ctDNA NOT DETECTED") result for all samples. A "ctDNA DETECTED" result indicates that ctDNA was identified in the plasma sample, a finding associated with cancer. A "ctDNA NOT DETECTED" result does not definitively indicate the absence of cancer. A quantitative result (ctDNA fraction) is reported when ctDNA is detected and the ctDNA fraction is ≥ 1 ppm. Additional information on cancer burden may be gained through serial testing. Treatment decisions informed by this test should be made in consultation with a qualified, licensed healthcare provider with consideration for patient-specific clinical information.