PrismRA® Technical Specifications

Scipher Medicine Corporation

Indications and use

Intended use

The PrismRA test is a molecular signature test intended to be used by the treating physician for adults diagnosed with rheumatoid arthritis (RA) who are considering starting or changing targeted therapy. PrismRA identifies patients most likely to not respond to tumor necrosis factor-α inhibitors (TNFi) therapies. PrismRA evaluates RNA expression data from blood samples, demographic variables, clinical metrics, and anti-cyclic citrullinated protein (anti-CCP) to detect a molecular signal of non-response to TNFi therapies for patients with RA. This signal predicts the patient's likelihood of inadequately responding to all TNFi therapies. Response is defined as achieving ACR50 at 6 months. It is validated for use in adults diagnosed with RA.

Summary and explanation

PrismRA is a whole blood gene expression test used to measure inadequate response to TNFi therapies in patients with RA. The PrismRA test evaluates 19 gene expression features, sex, body mass index (BMI), patient global assessment, and anti-CCP.

The PrismRA result is an integer score that represents the likelihood of inadequate response to TNFi therapies in that patient. The 23 biomarkers are integrated into a single predictive model that generates a score on a scale of 1 to 25 that represents the likelihood of inadequate response to TNFi therapies.

Test methodology

PrismRA is performed as a laboratory service using RNA extracted from a PAXgene™ whole blood tube sample, and anti-CCP analyses from a serum separation tube. PAXgene specimens are processed using the MagMax™ for Stabilized Blood PAXgene Tubes RNA Isolation Kit. The extracted RNA samples are quantified with M200 absorbance. RNA integrity is determined per Agilent TapeStation or Bioanalyzer operating procedures. RNA samples (100-1000ng) are processed using the KAPA RNA HyperPrep Kit with RiboErase (HMR) Globin (KAPA/Roche). After library amplification, samples are quantified using Agilent D1000 reagents per the Agilent TapeStation or Bioanalyzer operating procedures. Samples are sequenced on a NovaSeq (Illumina).

Libraries are sequenced to high uniform depth (targeting > 7 million protein coding reads). Sequence data is then processed using a customized analysis pipeline designed to determine gene expression across the whole genome.

Interpretive criteria

The lower the test score, the less likely the patient will have an inadequate response to TNFi therapy. However, a low score does not ensure a positive response to TNFi therapies.

PrismRA scores <10.6

A patient with a PrismRA score <10.6 does not have a detectable molecular signal of non-response to TNFi therapies. In the PrismRA validation study, the observed response rate to TNFi therapies for such individuals was greater than that of the unstratified patient population.

PrismRA scores ≥10.6

A patient with a PrismRA score ≥10.6 has a high signal of inadequate response to TNFi therapies. This corresponds to an approximate 90% chance of inadequately responding to TNFi therapies. Thus, the patient has approximately 10% chance of responding to TNFi therapies.

PrismRA scores ≥18.5

A patient with a PrismRA score ≥18.5 has a very high signal of inadequate response to TNFi therapies. This corresponds to an approximate 95% chance of inadequately responding to TNFi therapies. Thus, this patient has an approximate 5% chance of responding to TNFi therapies.

Limitations

Contraindications

The PrismRA test is a blood draw, thus contraindications are those consistent with routine phlebotomy. The major contraindications for phlebotomy are skin conditions like cellulitis or abscesses that would cause direct seeding of infectious agents (like bacteria) into the blood. Other complications that should be taken into account are the presence of vascular access devices in hospitalized patients, vascular grafts, bleeding under the skin, and if the patient has palpable venous fibrosis.

Warnings and precautions

- For prescription use only. This test must be ordered by a qualified medical professional in accordance with clinical laboratory regulations.
- PrismRA is not intended for use in patients younger than 18 years of age.
- Decisions on patient care and treatment must be based on the independent medical judgment of the treating physician, taking into consideration all applicable information concerning the patient's condition, such as patient and family history, physical examinations, information from other diagnostic tests, and patient preferences in accordance with the standard of care in a given community.
- This test, and the interpretive content in the PrismRA report, were developed and the performance characteristics were determined by Scipher Medicine Corporation. The test and report have not been reviewed, cleared or approved by the Food and Drug Administration (FDA). The PrismRA test (including the interpretive content in the PrismRA report) is a Laboratory Developed Test that is not subject to FDA premarket review.

Performance characteristics

Accuracy

In a prospective, blinded validation cohort, the molecular signature response classifier identified likely inadequate responders with a positive predictive value of 86% (95% CI 73-95) and specificity of 70.7% (95% CI 54-84%) among patient samples naïve to targeted therapy and a positive predictive value of 92.0% (95% CI 75-99%) and specificity of 88.0% (95% CI 69-97%) among biologic-exposed patient samples. PrismRA predicts the likelihood of a patient failing to achieve an ACR50 response to TNFi therapies among targeted therapy naïve and biologic-exposed patient samples with an odds ratio of 5.1 (95% CI 2.8-10.3).

Precision and reproducibility

The precision and reproducibility data demonstrated a high level of concordance among the PrismRA results for inter-assay reproducibility (100%) and intra-assay reproducibility (92.6%). The overall precision was 96.8%. The analytical sensitivity of the PrismRA test is 93.1%.

Interference

Although rare, PrismRA test results and clinical interpretation may be impacted by other factors not addressed above.

Quality control measures

Minimum RNA sample quality control metrics:

- TapeStation RIN > 4
- Concentration ⩾ 10 ng/μL
- Library yield ⩾ 10 nM

Minimum RNA sequencing run quality control metrics:

- Perfect index: >85%
- Bases over Q30: >75%
- Mean quality score: >30

Minimum RNA sequencing quality control metrics required prior to PrismRA bioinformatics pipeline assessment:

- FASTQ files are properly formatted with appropriate header lines, separator lines and a uniform base pair length and quality score length for every read.
- Uniform base pair length and quality score length for every read
- Median Phred score >25 and a lower quartile >10 for all bases
- Mean Phred score for every tile <(Mean-2) Phred score for that base across all tiles
- The most frequent observed mean Phred score >27
- Protein coding reads >7 million

Sample rejection criteria

Inappropriate sample types can cause cancelation of the test. Inappropriate sample types include: samples from patients not diagnosed with RA, samples collected in expired tubes, unlabeled or incorrectly labeled samples, samples for which insufficient clinical information has been provided, samples of insufficient RNA quality (RNA integrity number <4), samples of insufficient RNA quantity (<7 million protein coding reads). Insufficient quantity or quality may be due to inadequate PAXgene tube inversions, damage occurring during shipping, an extended period of time between sample collection and receipt by the laboratory.

Sample Collection

Description of method

PrismRA is tested on blood samples; a PAXgene tube used for next-generation sequencing of RNA and a serum separation tube used to collect plasma for anti-CCP testing. All sample collection tubes are provided in a barcoded PrismRA kit. The blood samples must be collected following the applicable instructions of the manufacturer. The PAXgene tubes must be the last tubes drawn. Samples must be shipped to the Scipher Medicine Laboratory no later than 24 hours after the blood draw. PrismRA was developed and its performance characteristics were determined by Scipher Medicine.

Test kit contents

The PrismRA test kit includes a sample shipping kit that is sent to ordering laboratories. The shipping kit contains the following components:

- 23g 3⁄4-inch Safety-Lok™ Blood Collection Set (butterfly needle) with 12-inch tubing and holder
- One serum separation tube
- Two PAXgene™ Blood RNA tubes
- Absorbent sleeve
- 3 oz. gel wrap
- Test requisition form
- 5 labels for blood tubes (3 for blood sample tubes, 2 extra if needed)
- 1 label for the test requisition form
- Insulated foil envelope
- Biohazard zip poly bag
- Clinical Pak with pre-affixed return shipping label

All other reagents, materials and equipment needed to perform the assay are used exclusively in the Scipher Medicine Laboratory.

Test ordering

The PrismRA kit comes with a test requisition form, which must be fully completed and signed by the ordering physician or other authorized medical professional. In this form, information must be provided, including provider information, patient information, ICD-10 code(s), patient history, the test being requested, and billing information. A copy of the front and back of the policyholder's insurance card and a patient demographic face sheet must be attached as well. Furthermore, the phlebotomist must fill out a specific section of the requisition form. The completed form should be placed in the PrismRA collection kit to be returned to Scipher Medicine with the three blood tubes.

Sample collection

Blood will be drawn through standard manufacturer's instructions, using PAXgene tubes for RNA analyses and serum separation tubes for anti-CCP analyses.

Sample collection instructions are as follows:

1. The tubes must first be labeled before any sample collection. They must be labeled with the patient's name, date of birth, and the date of the blood draw on the three barcode stickers provided. Two PAXgene Blood RNA tubes and one serum separation tube must be labeled with these stickers.
2. The test requisition form must be completed, and all required fields must be filled out. The label should be affixed to the form.
3. The blood is then drawn through the attachment of the butterfly needle set. The serum separation tube must be drawn first, then the PAXgene tubes MUST be drawn last. If the vein fails and a redraw is needed, you must use another 23g butterfly needle and draw a discard tube prior to drawing the PAXgene tube. If a new tube(s) is needed, the contents from an additional PrismRA kit must be used. All tubes must be ensured to be labeled with the appropriate tube labels from the original kit. The sample tubes should NOT be shaken. The tube should be completely filled.
4. The tubes should be gently inverted 8-10 times immediately upon blood draw. The blood must be completely mixed with the PAXgene tube stabilization reagent, so the final color of the sample is uniform. The tubes are not to be shaken.
5. Store the tubes in a test tube rack at 18-25°C (ambient) for a minimum of 2 hours and for no more than 24 hours. The tubes should be immobile and upright for this period of time.
6. The tubes are packaged as follows:
    a. The three tubes are placed in an absorbent sleeve.
    b. The sleeve and 3 oz. gel wrap are placed in an insulated envelope.
    c. The envelope is placed in a biohazard zip poly bag.
    d. The bag is placed into the kit box.
    e. The completed test requisition form is added to the kit, and the box is closed.
    f. The kit box is placed in a pre-labeled FedEx Clinical Pak.
7. The FedEx Clinical Pak is shipped overnight to Scipher Medicine Laboratory. Samples must be shipped no longer than 24 hours after the blood draw, and samples must not be shipped on Saturdays.

Sample preparation

Total RNA is extracted from the blood sample and sequenced at Ambry Genetics utilizing validated methods. Serum anti-CCP measurements are performed at Scipher Medicine Laboratory using validated methods. Final algorithmic analysis is performed at Scipher Medicine Laboratory.

Sample stability

Blood will be drawn according to standard manufacturer's instructions, using PAXgene tubes for RNA analyses and serum separation tubes for anti-CCP analyses. PAXgene blood tubes contain a proprietary reagent for stabilization of intracellular RNA immediately upon collection. PAXgene blood RNA tubes can remain stabilized for up to 3 days at room temperature (18-25°C), up to 5 days at 2-8°C, and 11 years at -20°C or -70°C. Serum separation tubes contain a clot activator and a barrier polymer that has a density that causes it to move upward during centrifugation to the serum-clot interface. The serum separation tubes are stable up to 1 week when refrigerated. For prolonged storage, the separated serum should be kept frozen in a new tube at -20°C or lower.

Turnaround time

The turnaround time is approximately two weeks from the blood draw receipt to the report being delivered to the ordering physician.