Development and Validation of Highly Sensitive MRDx BCR-ABL Test for Monitoring Deep Molecular Response in Patients with Chronic Myeloid Leukemia

Julie A. Toplin, Kristy A. Draffal, Jonathan M. Ebl, Clark C. Fjeld, Thomas D. Yager, Staci A. McAdams, Kevin D. Hawkins, and Chad D. Galderisi, MolecularMD Corp., Portland, OR

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Introduction

Treatment of chronic myeloid leukemia (CML) patients with tyrosine kinase inhibitors (TKIs) has led to progressively lower levels of disease burden and higher rates of complete molecular responses. Very few works with histological assessments of if ≤10<sup>−1</sup> E13A2/B2A2 or ≤10<sup>−3</sup> E14A2/B3A2 have been published, and assay standardization at these levels has been problematic (ITMRC, 2005). In order to have two independent (A) standardization assays that can precisely and accurately detect ≤10<sup>−3</sup> log reduction in BCR-ABL ratios, the test system design must be tightly controlled to ensure this critical performance metric can be reproducible. The MolecularMD MRDx BCR-ABL test was developed and validated to be an accurate, reproducible, and highly sensitive standardization system to measure minimal residual disease (MRD) in CML patients using either peripheral blood or RNA isolated from the patient.

Test Overview

The MolecularMD MRDx BCR-ABL test is a quantitative and multiplex polymerase chain reaction (qPCR) test that provides for accurate and sensitive quantification of BCR-ABL transcripts in peripheral blood samples that have been collected from CML patient blood specimens (Figure 1). Total RNA is extracted from peripheral blood and serves as a template for qPCR amplification. The test system is performed using a one-step RT-qPCR protocol wherein the reverse transcription and quantitative real-time PCR reactions are performed in the same well. BCR-ABL and ABL murine leukemia virus (MLV) plasmid standards are used in the same format, and murine leukemia virus DNA is used as the standard reference. The BCR-ABL/ABL reference ratios are calculated and converted to the international scale. Calibrators are assayed for both MRDx BCR-ABL and ABL in every run alongside peripheral blood sample testing. qPCR controls provided a larger number of molecular responses (MRDx) and total molecular responses (MRDx) to allow for on-stream verification of test accuracy and reproducibility over the critical transcript range. The integrated conversion factor provides test results on the international scale.

Test Development

To achieve a sensitivity of ≤3 logs, several one-step qPCR systems were evaluated. The enzyme systems were tested in parallel and RNA calibrators with the same copy number to evaluate the efficiency of the reverse transcription (Figure 2). Of the systems evaluated, the MolecularMD qPCR SYBR Green detection system was the system with the highest reverse transcription efficiency and the best sensitivity and precision as determined by testing three independent calibration systems (Table 1). It is in addition, as order to better control for the variation of the reverse transcription, is in which transcribed RNA calibrators were developed to create a standard curve for each copy number determination rather than the more commonly used parallel calibrators that may directly affect the reverse transcriptase in the patient sample RNA.

Analytical Validation—Linearity

A series of samples were created by diluting total RNA from CML patients into RNA extracted from normal healthy donor blood sample (Table 2). The samples with the following dilutions were derived from both MolecularMD RNA isolated blood specimens and BCR-ABL blood isolates (10<sup>−1</sup>, 10<sup>−2</sup>, 10<sup>−3</sup>, 10<sup>−4</sup>, and 10<sup>−5</sup>). The molecular dilution factor is calculated by dividing the log reduction in the BCR-ABL ratio in the RNA, log R<sub>MRDx</sub>, by the log reduction in the BCR-ABL ratio in the RNA, log R<sub>MRDx</sub>. The mean BCR-ABL/ABL % IS ratio is 100% for BCR-ABL/ABL % IS ratios shown below for each dilution series. The results of the linear regression analysis done using the conversion of BCR-ABL/ABL % IS ratio are shown in Figure 2. The assay has been shown to be linear over at least 6 logs (10<sup>−4</sup>) for both MRDx extracted from blood collected in MolecularMD RNA isolated collections (ABL/ABL ratio) and BCR/ABL blood calibrator collection (ABL/ABL ratio). Figure 3. The range of the assay expressed in the BCR-ABL/ABL ratio is 0 to 95% for 0.955 logs. It has been shown to be 8% to 0.00095% for the BCR-ABL/ABL dilution series.

Analytical Validation—Alignment to the International Scale

The MolecularMD MRDx Test reports on the international scale with a conversion factor of 0.99 of use of the IMAGO International Standard for qPCR international reference (ITMRC, 2005). For quantification of BCR-ABL by RT-qPCR, secondary standards were created for locational monitoring of the assay (Tables 2 and 3). For the BCR/ABL assay, there are 5 different platforms, each a different MRDx primary diluent of a frozen/thawed sample that were standardized and corrected. The multiplexed-targeted qPCR Primary samples and the BCR/ABL IS ratio are standardized to the MolecularMD Test using equation RNA extracted using kit provided of impact. This study was repeated and the data combined (Figure 4). Calibration of the conversion factor is a small subset of samples was done by an independent laboratory using different test systems and molecular techniques (Figure 5).

Conclusions

Based on the validation data, the MolecularMD MRDx Test is an accurate, reproducible, and highly sensitive qPCR-standardized solution to the growing need for a reliable and robust quantitative BCR-ABL assay that can be used for the monitoring of minimal residual disease in CML patients.

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