Development and Validation of a Highly Sensitive e1a2 (p190) BCR-ABL Test to Determine Complete Molecular Response As a Primary Endpoint for Patients with Newly Diagnosed Philadelphia Chromosome Positive Acute Lymphoblastic Leukemia (Ph+ ALL)

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Introduction
In Ph+ ALL, the absence of detectable disease has shown prognostic value for a reduced risk of relapse and improved survival. However, as the level of undetectable disease is determined by the lower limit of detection of the test in use, standardization of such an endpoint for a drug regulatory submission is critical. To date, no tyrosine kinase inhibitor (TKI) patient population has been prospectively defined around Ph+ ALL undetectable disease resulting in precise and standard definitions.

Takeda (Millennium Pharmaceuticals, Inc.) is conducting a phase 3, randomized, open-label, multicenter efficacy and safety study comparing ponatinib versus imatinib, administered in combination with reduced-intensity chemotherapy, in patients with newly diagnosed Ph+ ALL (NCT01602262). The primary endpoint for this study is a minimal residual disease (MRD) negative complete remission (CR), whereas MRD-negativity is defined as a BCR-ABL raw ratio of ≤0.01% (M40.0) at the end of induction. Patients who achieve post-induction ponatinib or imatinib maintained MRD-negative CR could potentially delay or avoid stem cell transplantation.

For assessment of the e1a2 (p190) BCR-ABL transcript, we developed and validated a one-step reverse transcription, quantification polymerase chain reaction (RT-qPCR) test in order to accurately and precisely assess all clinical decision points and disease levels for this study. Because of the lack of available reference material, decision points in this study were determined as the nominal level at which there was 95% detection. Limit of Quantitation (LOQ) was determined as the nominal level which met the 95% detection level with a precision of less than 10% above the nominal level and a %CV of 10%. The LOQ and LOQ + was tested to ensure the assay is linear from MR0.5 to MR5.0 for donor PB or BMA and performing a serial dilution using the BCR-ABL negative donor RNA. Three operators tested each panel on three different instruments over three days for a total of 27 runs. Two additional runs were performed by a single operator for an inter-operator analysis (n=9 runs total) of Limit of Detection (LOD) accuracy. The LOD and LOQ + was 0.5µg input and demonstrated the LOD/LOQ + at 0.5µg input is also MR4.0 (see Figure 2). The Lq data was plotted against the nominal values (assumed perfect dilutions) and showed the assay is linear from MR0.5 to MR5.0 for both BMA (Figure 3) and PB (Figure 4).

Methods and Test Overview
The e1a2 (p190) BCR-ABL test is a quantitative real-time polymerase chain reaction test that provides sensitive and specific quantification of BCR-ABL e1a2 (p190) and ABL transcript levels in RNA extracted from PB and BMA samples collected from all patients. Total RNA serves as the template for RT-qPCR and 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 amplifiers are generated and detected in real-time using TaqMan® MGB probes. Quantitation is achieved using RNA calibration standards and linear regression analysis provided by the 510(k) cleared ABI 7500 Fast Dx PCR Instrument Software. BCR-ABL transcript levels are measured relative to the ABL transcript as a reference gene in an enogenous reference. The BCR-ABL/LMO2 ratio is calculated. Calibrators are analyzed for both BCR-ABL and ABL in every run simultaneously with patient samples. RNA controls provided at MR0.1, MR4.0, and MR0.0 allow for on-site verification of assay accuracy and productivity over the entire test range.

BCR-ABL negative donor PB or BMA were collected in PAXgene RNA tubes. For the limit of detection, precision, and accuracy study, SUP donor PB or BMA were collected in PAXgene RNA tubes. For the limit of detection, precision, and accuracy study, SUP donor PB or BMA to achieve 27 samples of varying BCR-ABL positive PB or BMA to achieve 27 samples of varying BCR-ABL positive.

Assay Metrics, Limit of Blank (LOB), Specificity
Forty runs were analyzed for calibration and control performance, and demonstrated excellent precision, linearity, and well-targeted control levels. The metrics for the calibration curve and the RNA controls are presented in Table 1, as well as the results for the linearity of blank and specificity.

A limit of blank (LOB) was determined by analyzing 20 known BCR-ABL negative cell lines in duplicate. Two wells out of eighty were detected with a calculated LOB of 70% (data not shown). Specificity was determined through bi-directional Sanger sequencing which showed at least 95% identity to the reference sequence (data not shown).

Assay Summary
We have developed an e1a2 RT-qPCR assay with an LOB and LOQ of at least MR4.0 for both BCR-ABL gene expression and BCR-ABL percentage. The assay is linear from MR0.5 to MR5.0 and shows excellent precision down to MR4.5. The assay is specific to the target sequence and has an LOB of MR7. The assay meets or exceeds all validation acceptance criteria. The highly sensitive, accurate, and precise test is critical for monitoring ALL patients undergoing treatment in Takeda’s Phase 3 clinical trial and may help establish validated surrogate endpoints for improved long-term outcomes in these patients.