Multi-Site Evaluation of a Multiplex Assay for the Rapid Detection of Leukemia-Associated Fusion Transcripts

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INTRODUCTION

Modern therapy for leukemia is based on the principle of risk stratification. Recurring genetic abnormalities commonly found in leukemia, including balanced chromosomal translocations, are often associated with either a deleterious or favorable prognosis enabling the use of more or less toxic interventions. Knowledge of the specific genetic abnormality can also facilitate the use of targeted therapies. At the molecular level, the chromosomal breakpoints can vary over a wide region within the genes involved, and it is often necessary to identify the specific fusion transcript variant expressed by leukemic cells for subsequent molecular measurement of patient response during treatment and for assessment of residual disease.

Accurate, rapid and sensitive molecular methods are therefore required to complement standard cytogenetic techniques and speed up the routine workup of leukemia. The main objective of this study was to evaluate in a clinical laboratory setting the potential utility of a multiplex molecular assay for the simultaneous detection of 12 leukemia fusion transcripts and to establish its preliminary performance relative to standard cytogenetic methods.

MATERIALS AND METHODS

Total RNA was isolated from peripheral blood or bone marrow specimens using laboratory-validated methods at site 2 (968) or 3 (WMRGL). Residual RNA samples were tested with the Signature® LTx v2.0 Kit (RUO)* at each site. Fifty independent specimens from site 2 were also tested at site 1 (Asuragen). Total RNA was reverse transcribed into cDNA and amplified by multiplex PCR using target-specific, biotin-modified primers. CAPDH transcripts were co-amplified in each sample and concurrently analyzed to serve as endogenous controls. The PCR products were then sorted on a liquid bead array containing oligonucleotide probes specific for each marker and detected using the luminex® 200 System. Qualitative calls (positive or negative for each target) were determined relative to a fixed cut-off signal at 350 MFI, above the distribution of negative signals (Figure 1). All archived specimens in this study were de-identified and no protected health information was released.

RESULTS

Figure 3. Study design and sample set. A total of 178 residual total RNA samples from study subjects previously evaluated by karyotyping and/or FISH and independent testing for site 2 were examined with the Signature® v2.0 Kit at 3 independent sites. Among site 1, 104 specimens were positive by cytogenetics for one of the 7 different chromosomal abnormalities included in the Signature assay design. Fourteen specimens were from follow-up samples, including 3 follow-up samples for K22 (20-21, 30-31, 10-11). The site 1 set was supplemented with 75 additional samples from a laboratory in the UK. The remaining 74 specimens were from centers in the USA or Canada, including 5 relapse cases at sites 2 and 3. Among the 96 specimens negative by cytogenetics, 2 were found low positive by Signature at site 2 (748 and 946 MFI for e14a2 or e1a2, respectively). This discrepancy was based on the difference in analytical sensitivity between the 2 methods (site 2 cases were FP at presentation and negative by FISH at follow up). Signature was found negative by both methods for 2 samples (one was low positive by cytogenetics), corresponding to an overall failure rate of 0.5% (1.4% at site 3).

Figure 4. Summary of results. The correct fusion transcript was detected with 96% accuracy rate. The Signature assay was 100% specific and sensitive for BCR-ABL1 e1a2, MLL-AFF1, PML-RARA bcr1, and TCF3-PBX1 e13a2. BCR-ABL1 e13a2, MLL-AFF1, PML-RARA bcr1, and TCF3-PBX1 e13a2, GAPDH is used as an endogenous control. The other assay contains various markers associated with unfavorable prognosis in AML (top panel). The assay detects RUNX1-RUNX1T1, BCR-ABL1 e1a2, MLL-AFF1, PML-RARA bcr1, and TCF3-PBX1 e13a2. The assay detects CBFB-MYH11 type E, PML-RARA bcr2 (or V form) and the 3 most common NPM1 mutations (A, B and D). A positive signal only on the probe specific for RARA exon 3 indicates detection of PML-RARA bcr2. For this assay, GAPDH is used as an endogenous control. The other assay contains various markers associated with less favorable prognosis in AML (bottom panel). The assay detects CBFB-MYH11 type E, PML-RARA bcr2 (or V form) and the 3 most common NPM1 mutations (A, B and D). A positive signal only on the probe specific for RARA exon 3 indicates detection of PML-RARA bcr2. For this assay, GAPDH is used as an endogenous control.

Figure 6. Analytical sensitivity. Preliminary sensitivity was assessed using total RNA isolated from cell lines expressing RUNX1-RUNX1T1, BCR-ABL1 (e14a2 or e1a2)-K562, MLL-AFF1 (e1e2 or e1e3)-K562, and TCF3-PBX1 (e13a2 or e13a2)-K562. Total RNA was tested either unedited (100%) or diluted at 10 or 1:10 in a background of total RNA isolated from the translocation- and mutation-negative HL60 cell line (Fig. 6A). The results of 178 samples from study subjects previously evaluated by karyotyping and/or FISH and independent testing for site 2 were examined with the Signature® v2.0 Kit at 3 independent sites. The assay was found positive for 12 leukemia fusion transcripts and to establish its preliminary performance relative to standard cytogenetic methods.

CONCLUSION

The Signature® LTx v2.0 Kit is compatible with representative RNA samples extracted from various lymphoid and myeloid malignancies, including AML, CML, ALL, MDS and MPN. The assay helped resolve complex cytogenetic cases and positively identified the expected fusion transcript in RNA samples from cases with low blast count or at relapse. The multiplex assay format and rapid time to results (about 2 hours from RNA isolation) make it suitable for clinical laboratory workflow. Additional analytical experiments confirmed assay specificity, established a preliminary sensitivity of 1%, and showed that the panel content can be expanded to include additional rare variants and other relevant biomarkers. Overall, the assay is a sensitive, specific and flexible molecular tool attuned to the clinical laboratory workflow that can complement current standard cytogenetic methods and help further improve and streamline the risk-based classification of leukemia."