H38. A Novel Nanofluidics-Based Approach for Simultaneous and Quantitative Detection of Multiple Recurrent Translocations in Hematologic Malignancies

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Introduction: Chromosomal translocations that result in chimeric transcripts are useful as molecular markers for a variety of hematologic malignancies. Current molecular approaches to interrogate these markers include quantitative (qPCR) assessment of each translocation individually or qualitative assessment of a predefined set of translocations as in Signature® LTx (LTx) Panel (Asuragen, Austin, TX). These approaches require 1-2 ug of RNA/sample. Alternatively, FISH can be used, but this approach also is limited by the evaluation of one translocation at a time and is both labor-intensive and costly. To overcome these limitations, we developed a novel nanofluidics-based approach for simultaneous and quantitative detection of multiple translocations using nanograms of RNA in nanoliter reaction volumes. **Methods:** We utilized Integrated Fluidic Circuit (IFC) arrays (Fluidigm®, San Francisco, CA) and Taqman probe-based qPCR to simultaneously detect 10 leukemia-associated fusion transcripts: b2a2/b3a2 and e1a2 forms of *BCR/ABL1* [t(9;22)(q34;q11.2], *RUNX1/RUNX1T1* [t(8;21)(q22;q22)], *E2A/PBX1* [t(1;19)(q23;p13.3)], *ETV6/RUNX1* [t(12;21)(p13;q22)], *PML/RARα* long and short forms [t(15;17)(q22;q12)], *CBFβ* A and D variants [inv(16)(p13.1q22)], and *MLL/AF4* [t(4;11)]. ABL was assessed as an internal control. A total of 112 peripheral blood or bone marrow aspirate specimens (AML 67, ALL 35, CML 7, other: 3), previously tested by qualitative LTx panel were analyzed by the nanofluidic 48X48 IFC array format that allows analysis of 48 samples and 48 targets/ sample. A total of 60 ng of RNA was used for each sample. Each of the targets was analyzed in triplicate. Plasmids containing known amounts of fusion sequences were utilized to generate standard curves for quantification. Results were also compared with available karyotype and FISH results. **Results:** IFC arrays correctly detected fusion transcripts with high specificity. Data output was easy to interpret and results were highly reproducible. FISH and IFC arrays were in concordance in all but two cases, where IFC arrays, but not FISH, detected a translocation. Overall concordance between nanofluidics and LTx screen was 99.7% across all translocations. Unlike the qualitative screen, IFC arrays do not require a second qPCR assay for quantification. **Conclusions:** Simultaneous and quantitative detection of multiple recurrent translocations using nanofluidics arrays provide an efficient and cost-effective tool for rapid screening and classification of newly diagnosed leukemias and, for quantitative monitoring. Since each transcript is analyzed in individual wells, additional targets can be easily added to the panel without altering existing assays. Similarly, more than one internal control can be tested when necessary.