Targeted Oligonucleotide Array Assessment of Genomic Copy Number Alterations for Risk Stratification in Chronic Lymphocytic Leukemia

Jane Houldsworth PhD, Asha Guttapalli MS*, Xiao Jie Yan MD PhD, Charles Ma PhD, Weiyl Chen PhD, Sujata Patil PhD, Kanti Rai MD, Nicholas Chiorazzi MD.

* Cancer Genetics, Inc.
† The Feinstein Institute for Medical Research
‡ Memorial Sloan-Kettering Cancer Center.

Introduction
Risk stratification in chronic lymphocytic leukemia (CLL) is highly desirable and should comprise clinical features and molecular prognostic markers. Currently, genomic abnormalities including loss of 13q14 (TP53), 11q22 (ATM), 13q22-24 (WRN/ERCC1), 1q22 (MYB), and gain of chromosome 12 are assessed by fluorescence in situ hybridization (FISH) and the mutation status of the variable region of the IGH gene (IGHV). Assaying for multiple copy number alterations (CNAs) in the CLL genome. In order to validate and improve the risk stratification of CLL patients, a novel targeted oligonucleotide array was custom designed.

Array-CGH Methods
Test DNA was extracted from cryopreserved mononuclear cell pellets and quality and quantity confirmed (AB法) using 180(A260/A280 > 1.90, A260/A230 > 2.0). An equimolar of male and female normal DNA (Promega) served as the reference DNA. Test and reference DNAs (1 μg) were digested with RsaI and AluI and differentially enzymatically-labeled with Cy5 and Cy3-dUTP respectively. Purified labeled DNAs were mixed and hybridized to MbaI-essentially as described by the manufacturer (Agilent Technologies). After washing, the slides were scanned and Feature Extraction (Agilent Technologies) was used for data extraction.

Analytical Sensitivity/Specificity
Cell line Dilution Studies: 30-40% FISH 20-25% Detection of 11q, 13q, and 17p loss, and 12 gain by FISH at 25% Dataset 1: sensitivity = 95%, specificity = 99% (n<23) Dataset 2: sensitivity = 100%, specificity = 92% (n=28)

Reproducibility
Dataset 1: DNA from 30 specimens were assayed twice or thrice independently and all detected aberrations in regions confirmed by quantitative PCR (qPCR) were reproducible between assays. Dataset 2: 67 CNAs were detected twice or thrice independently and all aberrations in the same regions were reproducible except for two that were < 0.5 Mbp in size.

Accuracy/Precision
Aberrations detected in the following regions were confirmed by qPCR in Dataset 1, as a second independent method of validation. Only one aberration could not be confirmed.

Clinical Datasets
Dataset 1: 119 IHR-approved qPCR cryopreserved mononuclear cells Dataset 2: 104 IHR-approved CLL DNA

CNA Detection and Association with Time to First Treatment (TTFT) and Overall Survival (OS)

Method 1
• Aberrations were detected using Genetics Workbench Lite (Agilent Technologies) with the ADAM algorithm (thresholds 4 and 2, log ratio ≥ 0.2 for gain and ≤ -0.2 for loss). Recurrent aberrations within regions with a minimum size of 1.5 Mbp were tested for association with TTFT and OS by the log rank test. X2 and IGL loci and sites of known CNVs were excluded from further analysis. CNAs associated with TTFT or OS with p < 0.05 were listed. All extractions associated with shorter TTFT or OS, and mostly occurred at higher frequency in treated specimens.

Method 2
GISTIC was used to detect commonly altered regions in both datasets based on frequency and amplitude.

Conclusions
• A targeted oligonucleotide array was designed to detect aberrations commonly altered in mature B-cell neoplasms, including CLL.
• Analytical sensitivity and specificity were 95-100% and 92-98% respectively based on 25% detection by FISH.
• No difference in TTFT or OS was found between specimens with Type I versus Type II 13q14 deletions.
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