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*, Weiyi 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 17p13 (TP53), 11q22 (ATM), 13q34 (IAP/15A/16.1), 12q24 (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) assessed by sequencing are valuable approaches. In recent years, genome-wide scanning technologies such as array-comparative genomic hybridization (aCGH) have revealed novel and refined known copy number alterations (CNAs) in the CLL genome. In order to evaluate the potential of array-CGH in prognosis in mature B-cell neoplasms, including CLL, a targeted oligonucleotide-based microarray (MatBA) was custom-designed.

Array-CGH Methods
Test DNA was extracted from cryopreserved mononuclear cell pellets and quality and quantity confirmed (ABDQ/A260 > 2.0, A260/A280 > 2.0). An equimolarity of male and female normal DNA (Promega) served as the reference DNA. Test and reference DNAs (1 ug) were digested with RsaI and AluI and hybridized to the array-CGH (Agilent Technologies). After washing, the slides were scanned and Feature Extraction (Agilent Technologies) was used for data extraction.

Clinical Datasets
Dataset 1: 119 IRB-approved QPCR
Dataset 2: 194 IRB-approved CLL DNAs

CNA Detection and Association with Time to First Treatment (TTFT) and Overall Survival (OS)
Method 1
- Aberrations were detected using Genomics Workbench Lite (Agilent Technologies) with the ADM2 algorithm (thresholds 4 and 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. 8q+ and 11q22 loci and sites of known CNVs were excluded from further analysis. CNAs associated with TTFT or OS with p < 0.05 were selected. All assessed aberrations were associated with short 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.

Results
- Recurrent regions were tested for association with TTFT and OS. Significant CNAs identified by Method 1 were confirmed plus four additional CNAs were found to associate with shorter TTFT and OS (p < 0.05). Only loss of 1p13 was found to be significant in both datasets.

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 1q deletions.
- Five new markers (11q22 > 1.5 Mbp and > 0.9 bp in size) of reduced overall survival were identified and validated in a second dataset.
- These data support the implementation of array-CGH into clinical practice in risk stratification of CLL patients for the detection of aberrations not routinely assessed by FISH.

Conflicts of Interest
- Jane Houldsworth (Employment, Equity Ownership), Asha Guttapalli (Employment), Charles Ma (Employment), Weiyi Chen (Employment)