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

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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) or 13q14 loss (Type I) or 17p13 (ATM) or 13q14 loss (Type II) are used for risk stratification using the ADM2 algorithm (thresholds 4 and 1.5 Mbp, respectively. Purified labeled DNAs were mixed and hybridized to reference DNAs (1 ng DNA (positive) and 1 ng DNA (negative) pellets and quality and quantity confirmed (A260/A280 = 1.6).

Array-CGH Methods
Test DNA was extracted from cryopreserved mononuclear cell pellets and quality and quantity confirmed (A260/A280 = 1.6, 2). A targeted oligonucleotide array detected aberrations in 87% of untreated specimens.

Clinical Dataset
119 IRB-approved cryopreserved mononuclear cells were tested for clinical association.

DNA from 30 specimens were assayed twice independently and all detected aberrations were reproducible between assays.

Accuracy/Precision
Aberrations detected in the following regions were confirmed by quantitative PCR (QPCR) as a second independent method of validation. Only one aberration could be copied for quantitative PCR (QPCR).

Recurrent aberrations within regions with a minimum size of 1.5 Mbp were tested for association with time to first treatment (TTFT) and overall survival (OS) by the log rank test and with IGHV mutation status using the Fisher’s two-sided exact test.

MatBA™
exARRAY®-designed Agilent oligonucleotide 4 x 44K 60 regions spanning in size from 0.3-2.1 Mbp regional resolution (diplicate) 1 Mbp backbone (duplicate) 5 x 20 replicates.

Table: Genes
<table>
<thead>
<tr>
<th>Gene</th>
<th>Aberration</th>
<th>Copy Number Alteration</th>
</tr>
</thead>
<tbody>
<tr>
<td>BCL2L1</td>
<td>Loss of 13q14</td>
<td>5.1 Mbp</td>
</tr>
<tr>
<td>TP53</td>
<td>Loss of 17p13</td>
<td>1.5 Mbp</td>
</tr>
<tr>
<td>ATM</td>
<td>Loss of 11q13</td>
<td>2.0 Mbp</td>
</tr>
<tr>
<td>JUN</td>
<td>Gain of 1p</td>
<td>3.0 Mbp</td>
</tr>
</tbody>
</table>

Copy Number Alterations

Recurrence associated with shorter time to first treatment (TTFT) and overall survival (OS) was observed. While loss of 13q14 was associated with a better overall outcome, no significant difference between Type I and Type II deletion with TTFT or OS was observed.

Recurrence CNA

Recurrent aberrations associated with TTFT or OS with p < 0.05 are listed. All aberrations exhibited association with a shorter TTFT or OS occurred at higher frequency in treated specimens.

Conclusions
- A targeted oligonucleotide array detected aberrations in 87% of CLL MNC specimens.
- Sensitivity and specificity were 95% and 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.
- Specimens with more than one aberration exhibited a shorter TTFT and OS.
- Four additional markers 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.
- CLANTRY® approved in November 2010/April 2011.

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