Abstract

Specific genome aberrations are recognised prognostic factors in CLL. FISH-based genomic risk classifications have been used in clinical decision making for over a decade. However, molecular karyotyping is gaining acceptance as an alternative, albeit cost and skill demanding, that not only overcomes the limitations of FISH but provides comprehensive whole genome scanning. Indeed, several recent studies (Bosiljak et al., 2011; Parker et al., 2011; Leukenia 2011; Querfeld et al., 2011) using different array platforms have revealed clinically relevant cryptic genome aberrations.

Here we compare FISH and molecular karyotyping data in diagnostic and follow-up samples from CLL patients using SureMap oligonucleotide genome arrays (Agilent). The array screening not only identified all clonal imbalances recognised by FISH when present above 15%, but provided novel information in 92% of the samples.

Samples and methods

Allgether 40 samples from 22 adults with CLL and available clinical, cytogenetic and FISH data were enrolled in the study. All patients have consented to participating in genome investigations. Two patient cohorts were selected from CLL samples entered into the database of the Molecular Cytogenetics Laboratory of Royal Free Hospital (ROSE). Cohort I (Table 1) comprised of 21 patients with a single sample analyzed at presentation (diagnosis). Cohort II had 17 patients with follow-up analysis and was subdivided into two groups. Group 1 contained 10 patients with 1-4 follow-up samples (minimal time after first FISH analysis was 2 months and maximum was 45 months) and group 2 had 7 post-treatment patients. The male to female ratio was 1.44 (13 males and 9 females) with a median age of 65.6 years (range 45-98).

Table Cohort 1 CLL presentation samples

<table>
<thead>
<tr>
<th>Sample</th>
<th>Patient</th>
<th>Sex</th>
<th>Age</th>
<th>Follow-up</th>
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<tbody>
<tr>
<td>CLL1</td>
<td>16 M</td>
<td>85</td>
<td>6 months</td>
<td></td>
</tr>
<tr>
<td>CLL2</td>
<td>47 F</td>
<td>85</td>
<td>12 months</td>
<td></td>
</tr>
<tr>
<td>CLL3</td>
<td>51 M</td>
<td>70</td>
<td>24 months</td>
<td></td>
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| ...    | ...     | ... | ...  | ...

Whole genome screening (aCGH) was performed as outlined below (Fig. 1) with whole genomic arrays (Agilent). From the microarray design of the SureMap 8x60K array (8x60K oligonucleotide arrays, Agilent), the probes were placed as an equidistant grid at a density of 5300 probes per Mb. Following manufacturer’s protocols and bioinformatics routines (Z score and ADM1/2 algorithms) aCGH was done at a cost neutral assay. A strategy by which array interrogation for chromosome imbalances with SurePrint G250K (Genomewide SNP and copy number microarray) and aCGH results are shown in fig 3.

Novel recurrent CNA: Loss at 4q21/22

A novel recurrent deletion in 4q21/22 was identified in 3 patients (Fig 5). In one patient who developed persistent CLL the deletion coexisted with a high-risk 11q23 deletion (class II/Type I). In the 2 other patients the 4q21/22 deletion was found in a low-risk patient (class I/Type 1), and in a patient with minimal follow-up (class I/Type 1). In the latter patient, the 4q21/22 deletion was found to be accompanied by 13q14 and ATM losses by FISH (b), and numerical changes by G banding (a) were shown to be related (c).

Conclusions

The application of SureMap genome arrays provides established and growing genomic risk clinically relevant information as a cost neutral assay. A strategy by which array interrogation is carried out at presentation, followed by therapy response monitoring using FISH on a selected marker in CLL, is a plausible alternative to routine practice. In cases where FISH has provided evidence for persistent disease, array screening will assess the genome damage, especially if carried out on PCD38 (CFK) derived, to assist treatment decisions.