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Progressive but Previously Untreated CLL Patients with Greater Array CGH Complexity Exhibit a Less Durable Response to Chemoimmunotherapy

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Abstract

To better understand the implications of genomic instability and outcome in B-cell CLL, we sought to address genomic complexity as a predictor of chemosensitivity and ultimately clinical outcome in this disease. We employed array-based comparative genomic hybridization (aCGH), using a one-million probe array and identified gains and losses of genetic material in 48 patients treated on a chemoimmunotherapy (CIT) clinical trial. We identified chromosomal gain or loss in $\geq 6\%$ of the patients on chromosomes 3, 8, 9, 10, 11, 12, 13, 14 and 17. Higher genomic complexity, as a mechanism favoring clonal selection, was associated with shorter progression-free survival and predicted a poor response to treatment. Of interest, CLL cases with loss of p53 surveillance showed more complex genomic features and were found both in patients with a 17p13.1 deletion and in the more favorable genetic subtype characterized by the presence of 13q14.1 deletion. This aCGH study adds information on the association between inferior trial response and increasing genetic complexity as CLL progresses.

Keywords

CLL; aCGH; genotype; prognosis

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AUTHORSHIP

NEK, EB, DLVD, DFJ and JCB: designed the research

RCT, SVW and EB: performed the research

EB, TDS and JEP: analyzed the data

NEK, JEP, JCB, RF, TK, JCB: wrote the paper

CONFLICT OF INTEREST

The authors declare no conflict of interest in this analysis and study.

INTRODUCTION

Chronic lymphocytic leukemia (CLL) is an enigmatic disease in that it is often truly indolent and then it can become extremely aggressive for a given patient. Traditional clinical and basic laboratory factors such as Rai stage, lymphocyte doubling-time, pattern of infiltration in the marrow and beta-2 microglobulin can be useful in counseling CLL patients [1]. However, these factors do not stratify low-risk or early-stage patients with confidence, and interactions among these factors are complicated. More recently, a model that predicts 5- and 10-year overall survival and estimates median survival for previously untreated CLL patients was developed that incorporates easily obtainable features, i.e., age, sex, node status, Rai status, beta-2 microglobulin and absolute lymphocyte count [2]. In a study of newly diagnosed CLL patients seen at the Mayo Clinic, this model was effective for predicting time to treatment and discerning low-, intermediate-, and high-risk patients [3]. While these are promising models, they require collation of multiple clinical and laboratory parameters, it remains to be seen whether they will apply to typical CLL patients in the community. To seek what drives some of the dramatic clinical heterogeneity, we sought to understand genomic complexity of clonal cells as a possible explanation in clinical variability. When discussing prognostic implications for genetic aberrations in CLL, the unmutated IgHV status and high-risk FISH abnormalities (loss of 17p13.1 or 11q22.3) or reduced p53 expression are associated with more aggressive disease and a diminished response to effective therapies and/or low duration of response (DOR) [4–8]. The latter is of particular importance as a key regulator of genomic instability. We hypothesized that this genomic complexity would be associated with worsened outcome, and potentially do so as a consequence of functional p53 deficiency.

Because of the advantages afforded by simultaneous genome-wide and locus-specific assessment of leukemic CLL B-cells with a single assay, array-based comparative genomic hybridization (aCGH) is gaining acceptance as a clinical diagnostic tool to detect genomic copy number gains and losses of prognostic importance in CLL. Given the potential power of aCGH to both expand our understanding of the genetics of the leukemic B-cell and its potential to better predict clinical outcome, we conducted studies designed to generate a more global knowledge of genetic aberrations in CLL. To do this, we used a high-resolution aCGH method to detect *de novo* copy number variations in the leukemic clone in CLL patients who have progressed and were about to be enrolled onto a clinical trial.

METHODS

CLL patient

The blood samples were obtained from a previously described two-center, prospective, phase 2 clinical trial conducted at Ohio State University (Columbus, OH) and Mayo Clinic (Rochester, MN) [5]. All patients had untreated, progressive CLL as defined by NCI Working Group criteria [9]. Patients provided written informed consent for correlative studies according to the Declaration of Helsinki on an Institutional Review Board approved protocol for the collection and use of samples for research purposes from both participating institutions. Eligible patients received a regimen consisting of pentostatin [P] (2 mg/m²), cyclophosphamide [C] (600 mg/m²), and Rituximab [R] (375 mg/m²) [PCR] given intravenously on day 1 of a 21-day cycle for a maximum of 6 cycles. Responses were assessed by NCI Working Group criteria [9] and included a bone marrow evaluation and 2-color flow cytometry 2 months after completion of therapy.

Clinical responses for the CLL cohort

Responses were graded according to NCI Working Group criteria with bone marrows collected after 2 months completion of 6 cycles of treatment [9]. All responding patients were then followed-up at 3-month intervals for 5 years or until documented progression. For patients with CR, nodular PR, or PR, we evaluated CLL B-cell levels using 2-color (CD19, CD5) flow cytometry at the 2-month bone marrow collection time point.

Prognostic tests on the CLL patients entered onto the clinical trial

The following prognostic tests were performed on blood cells at the time of enrollment into the PCR trial, using previously published methods [10–12]: CD38 phenotype, fluorescence in situ hybridization (FISH) detectable defects (6q23.3-, 11q22.3-, +12, 13q14.3-, 17p13.1-), IgHV mutation status, and ZAP-70 levels.

Array CGH microarray experiments

All samples were collected at the time of enrollment into the PCR trial. Samples were run using the SurePrint G3 Human aCGH Microarray 1M (Agilent Technologies). The digestion, labeling and hybridization steps were performed as previously described with minor modifications [13]. Briefly, 1.2 µg of tumor and reference DNA were independently digested with Bovine DNaseI (Ambion; Austin, TX) for 12 minutes at room temperature. DNA samples from a pool of 9 human, female, lymphoblastoid cell lines (NA18517, NA19240, NA18555, NA18537, NA18980, NA18972, NA12878, NA12156 and NA15510, from Coriell repository) were used as the normal reference in the hybridization experiments. Tumor samples were labeled with Cy5 dye and the normal reference was labeled with Cy3 dye. Labeled reactions were cleaned-up and hybridized at 65°C for 40 hours. Microarrays were scanned and features were extracted with Feature Extraction software (Agilent Technologies). Log2 ratio data were imported and analyzed using DNA Analytics software v. 4.0.85 (Agilent Technologies).

Copy number abnormalities (CNA) were calculated using 3 probe and 0.2 log2 filters and aberration detection module (ADM)-2 algorithm [14] with a threshold of 5. The threshold setting is a subjective process that involves visual assessment of acceptable levels of overall aberration. We used the recommended default threshold for ADM-2 algorithm and the accuracy of aberration calls was confirmed based on known FISH aberrations. An interval-based text summary with all abnormalities was obtained and subsequently analyzed. For the purpose of this study, only copy number changes in genic and microRNA regions were considered, since the vast majority of intragenic CNA were located in copy number variant (CNV) regions. Physiological deletions of the IgH, IgL-k and IgL-l loci were also excluded from the analyses. To identify and eliminate common copy number variants (CNV) from the study, we created a CNV database including the most recent higher resolution studies available in the TCAG database [15–17] as well as our findings in 110 tumor samples run by Sureprint G3 arrays (CLL, mantle cell lymphoma, Waldenström's macroglobulinemia, multiple myeloma, T-cell lymphoma, Burkitt's lymphoma and cri-du-chat syndrome; unpublished data). All variant chromosomal regions that were present in HapMap samples or were recurrently identified in two or more tumor types were considered CNVs. HapMap and tumor CNV lists were merged with the subset of TCAG database previously mentioned, and a master CNV list was generated. Overlapping regions were merged in the same interval. In cases where discrepancies were identified in one of the breakpoints, the region was defined based on the bigger interval.

Statistical methods

The primary endpoint of interest was genomic complexity, which was defined in two ways for each patient. First, genomic complexity was defined as the total number of CNA detected using the aCGH platform. Second, genomic complexity was defined as defined as the sum of the genomic lengths of all detected gains and losses. When defining the sum of gain and loss size, we included all interstitial abnormalities with the exception of whole chromosome changes, thereby, excluding trisomy 12 in the analysis. When defining the sum of gain and loss size, we included all interstitial abnormalities in the analyses. On the other hand, whole chromosome changes were not considered in this part of the study, thereby, excluding trisomy 12 from the analysis. This was done since it was the only whole chromosomal loss observed and inclusion for only 2 patients could skew the results of our analysis. In addition, we tested the association between total aCGH deletion size and patient outcome on the clinical trial.

Unconditional logistic regression was used to evaluate the univariate association between genomic complexity and the following risk factors and clinical outcomes: ZAP70, CD38, IgHV status, FISH defects (11q22.3-, +12, 13q14.3-, 17p13.1-), Rai stage and responses as defined according to NCI Working Group criteria. Genomic complexity was evaluated both as a continuous variable and as a categorical variable. The Wald test was used to test for associations. The Kaplan-Meier method was used to obtain estimates of progression-free survival (PFS), duration of response (DOR), and time to first detectable response. DOR was defined as the time from first response until progression as defined by NCI Working Group criteria [9] and time to first detectable response was defined as the time from initiation of treatment until it was initially clear that patients were responding to the chemoimmunotherapy protocol. The log-rank test was used to test for differences across responses as defined by the NCI Working Group criteria [9] and between stratifications of complexity. Stratifications of complexity were defined based on the quantiles of CNA, total size of chromosomal gains and total size of chromosomal losses. Statistical significance was declared for p-values < 0.05.

RESULTS

Patient Characteristics

Of the original 64 patients on the trial, 52 had available mononuclear cells for DNA preparation. Four other patients were excluded due to technical, quality control, and eligibility reasons, leaving 48 patients for these analyses. Supplementary Table 1 lists the pertinent demographics of the current cohort, which are comparable to the original 64. The median age of the current cohort was 64.5 years, 79% were male, and 45 of the 48 patients responded to therapy. At the time of these analyses, 13 of the 48 patients have died and 14 have progressed. In the responding 45 patients, the estimated median DOR was 43.2 months (95% CI: 29.1 to not yet reached). DOR was significantly better for patients with complete response (CR) and those with nodular partial response (NPR) vs. partial response (PR) (data not shown). The median PFS of 31.2 months (95% CI: 20.3 to 47.4) for the 48 patients included in the current aCGH study is similar to that of the PCR trial cohort [5].

aCGH Results

Overall, 288 CNA were identified (median of 4 per patient; range 0–32) of which 215 were total deletions and 73 were total gains (Table 1). There was only one patient with no CNAs based on aCGH analysis and Table 1 highlights the distribution of CNAs using quantiles. As an example, 5% of patients had 0–1 CNA observed by aCGH. Of the 288 CNA, the average size was 11.35 Mb and 157 (54.5%) of the CNA were < 1 Mb. In addition to the known recurrent abnormalities scored by FISH, aCGH identified 6 chromosomal segments deleted

in more than 3 (> 6%) patients (Table 2). Except for 7 patients with biallelic deletions on 13q14.3, all observed deletions were monoallelic (Table 3).

Table 3 assesses high density aCGH detection of chromosome abnormalities targeted by a CLL FISH panel [10]. This aCGH method was able to pick up most FISH detectable defects in the 48 patients, with a complete concordance for 17p13.1- between aCGH and FISH. For discrepancies, aCGH detected 11q22.3- in one patient not seen by FISH, explained by an abnormal region that partially overlaps with the zone analyzed by FISH. Moreover, 7 chromosomal abnormalities were identified in 6 patients by FISH but not by aCGH (11q22.3- in 2 cases; trisomy 12 in 2 patients; and 13q-x1 in 3 patients). The likely cause is that for all but one of these patients, a very small percentage (4.5 – 12%) of abnormal nuclei observed by FISH (see Table 3 footnotes).

The association between gains and losses detected by aCGH and clinical and prognostic parameters was assessed. When defined as the total number of CNA detected, genomic complexity was inversely associated with high-risk ZAP70 status. The odds of being ZAP70+ decreased by 54% (95% CI: 32–90%; $p=0.018$) per single increase in the number of CNA. High-risk IgHV status tended toward an inverse associated with the number of CNA ($p=0.088$). Although ZAP70 and IgHV status were inversely associated with the number of CNA, a 17p13.1 deletion by FISH was positively associated with the number of CNA and total deletion size (Fig. 1 and Fig. S1 respectively). There was a clear correlation between 17p13.1- and genomic complexity. The odds of having an overall response decreased by 28% (95% CI: 5–55%; $p=0.015$) with each increase in the number of CNA for the 17p13.1- patients.

To evaluate the association between PFS and genomic complexity, we evaluated the extremes of genomic complexity. Although not statistically significant, Fig. 2a illustrates that the cohort with 0 or 1 CNA had a better PFS compared to patients with >1 CNA. We similarly evaluated patients with a large number of CNA and discovered that CLL patients with ≥ 15 CNA had a significantly worse PFS than patients with < 15 CNA (Fig. 2b; $p=0.0037$). The 5 patients with > 15 CNAs exhibited the following FISH patterns: +12/13q-x1/13q-x2, 13q-x1 ($n=2$), and 17p13.1 ($n=2$);

We also evaluated the association between DOR and genomic complexity. As for PFS, none of the patients with ≤ 1 CNA have progressed to date, thus, they had a longer DOR than those with > 1 CNA. Patients with ≥ 15 CNA appear to have a shorter DOR than those with < 15 CNA (Fig. 3 $p=0.0726$). The 3 patients with ≥ 15 CNA had the following FISH patterns: +12, 13q-x1 and 17p13.1.

We also assessed the association between genomic complexity and time from diagnosis to the start of the PCR trial. Patients with > 4 CNA had a significantly longer median time from diagnosis to start of PCR trial than patients with ≤ 4 CNA (Fig. 4 $p=0.0469$), 26.3 months (95% CI: 18.37–44.53) vs. 4.87 months (95% CI: 2.37–30.40), respectively. While this observation seems counter intuitive, we believe, it means that for certain CLL patients the longer they go untreated the more likely they are to have increased levels of CNA.

In addition to defining genomic complexity as the total number of CNA for each patient, we defined complexity as the total size across all interstitial chromosomal gains and losses. We focused the analysis on interstitial gains and losses and whole chromosome changes were excluded, more specifically trisomy 12 (identified in 2 patients). Table 1 provides the distribution of the total length of gains and losses across the 48 PCR trial patients, excluding the trisomies. The median total deletion size was 6.623 Mb (range: 0–307.573 Mb); 3 patients had no detectable deletion. The median total gain size was 0.055 Mb (range: 0–214.543 Mb); 14 patients had no detectable gain.

When defined as the total size of chromosomal gains, genomic complexity was significantly associated with deletion of 17p13.1 and worse overall response. Specifically, the odds of being 17p13.1- increased by 2.3% (95%CI: 0.4–4.1%; $p=0.016$) per Mb gained. Similarly, the odds of having an overall response decreased by 1.7% (95%CI: 0.1–3.4%; $p=0.038$) per Mb gained. We did not detect an association between total size of chromosomal gains and PFS, DOR, or time to first detectable response (data not shown).

When defined as the total deletion size, genomic complexity was again significantly associated with deletion of 17p13.1. Due to the complete separation of data points, it is not possible to obtain estimates of the odds ratio for 17p13.1-; however, it can be clearly seen that there is a strong correlation between 17p13.1- and genomic complexity (Fig S1). A total deletion size ≥ 66 Mb was significantly associated with worse PFS than was smaller total deletion size (Fig. 5a; $p=0.0062$). The FISH detectable defects in the 5 patients with a total deletion size ≥ 66 Mb were: 13q14.3-/17p13.1-; 17p13.1-($n=2$); 11q22.3-; and +12/13q14.3-. Patients with a total deletion size ≥ 66 Mb also had a shorter time to first response than patients with a total deletion size < 66 Mb (Fig. 5b; $p=0.070$). We did not detect an association between total size of chromosomal losses and DOR or time from diagnosis to start of PCR trial (data not shown).

DISCUSSION

This analysis utilized the global assessment of genomic abnormalities using a high-resolution aCGH platform for clinically progressive CLL patients who were about to be treated on a chemoimmunotherapy protocol. To the best of our knowledge, while SNP-based arrays have been used to evaluate genomic defects in CLL [18], this is the first report using a one-million oligonucleotide aCGH microarray to assess the relationship between genomic abnormalities and clinical outcome. aCGH provides information beyond the commonly detected genetic defects that are observed by FISH methods in at least 80% of CLL patients who have clinical progression [10,19]. Thus, in addition to finding 6 recurrent deletions not included in the standard FISH assay, we found at least one CNA in 45 of 48 (94%) patients and 75% of the patients had 7 or fewer CNA. We also found that 25% of progressive CLL patients had ≥ 15 CNA, indicating that some previously untreated CLL patients can have a complex set of genomic abnormalities by the time disease progression is observed. The patients with ≥ 15 CNA were not all 17p13.1- patients showing that complex GC can occur in patients with lower risk FISH status. In addition, several important relationships were observed between CNA and clinical outcome for this trial including trends to a worse PFS and DOR for patients with larger numbers of CNA. Finally, we found the high-risk prognostic factor, ZAP-70+, was inversely associated with increasing genomic complexity. This observation suggests that genetic complexity and ZAP-70 status are independent poor prognostic parameters.

A small caveat is that aCGH employs a reductionist approach creating an average value per probes representing the most common genomic status of a locus. While most FISH detectable genetic lesions were found by aCGH, there were 5 CNA that were not, and the most likely explanation is that the abnormalities were detected by FISH at a lower percent of cells than the threshold required to be detected by aCGH. (The aCGH employed here is generally able to identify deletions present with at least the presence of 20% leukemic cells.) Indeed, the percentage of cells with these abnormalities as identified by FISH confirms this suggestion. In the case with an abnormality identified by aCGH but not by FISH, the abnormality was only partially covered by the FISH probe used, which underscores the increased sensitivity of aCGH to identify very small copy number changes. These data support the use of aCGH to complement FISH in the assessment of genetic defects in CLL.

Prior work using SNP arrays found that a majority of CLL samples carried between 1 and 3 CNA abnormalities [18,20,21] corresponding closely to data in this report. In our study, we focused on a cohort of previously untreated but progressive CLL patients who met the NCI Working Group criteria [9] for need for therapy and who were treated with a common chemoimmunotherapy protocol. Here, we found that both the number of CNA and the total length of chromosome material lost were associated with a worse outcome for these patients. Patients having ≥ 15 CNA had worse PFS than patients with < 15 CNA. In addition, patients with total size of chromosome loss ≥ 66 Mb had worse PFS than patients with < 66 Mb. Thus, our study extends our understanding of the relationship of global genomic analysis using high-density microarray aCGH to predict individual patient outcome in terms of numbers of CNA and the deletion sizes. We recognize that this is a limited sample size and further work is needed on genomic complexity in clinical trial therapies for CLL patients. Larger sample size aCGH studies will also be able to test the clinical significance of individual recurrent copy number gains and losses.

The two prior studies looking at genomic complexity and clinical outcome found that the degree of CNA was increased in the 17p13.1- and 11q22.3- high-risk FISH groups [18]. Although we identified complex genomic defects (i.e., ≥ 15 CNAs) in patients with high risk FISH defects (i.e., trisomy 12 in association with 13q14.3- or in 13q14.3- with 17p13.1-, with 17p13.1- alone), we also observed complex defects in patients with only 13q deletion by FISH analysis. However, in univariate analysis, the higher the genomic complexity as determined by CNA the more likely the patient had a 17p13.1- defect. These findings also need to be contrasted with our finding that higher genomic complexity was inversely associated with the known adverse prognostic feature of ZAP-70+ status. The importance of finding complex genomic defects in so called “low risk” FISH such as patients with only 13 deletions is of interest. We believe this finding not only implies that unstable genomic features can be found in both low and high risk FISH cohorts but also may explain why some patients with only 13 q defects have more aggressive disease. Thus, progressive disease in CLL can be associated with the adverse feature of complex genomic changes even in patients who may be viewed as having a more favorable risk profile based on currently employed prognostic markers.

Chromosomal sites affected in more than 6% of our patients included 2 segments of chromosomes 3 and 13, and 1 each on chromosomes, 8, 9, 10, 11, 12, 14 and 17 (Table 2). Some of these were the sites of well recognized genetic defects on chromosomes 11, 12, 13 and 17. We also unexpectedly observed that there was a longer time to entry of the trial from diagnosis for CLL patients with higher levels of genomic complexity. This finding may be consistent with the more prolonged time that a patient may have to reach established criteria for therapy while still accumulating genomic defects. If these findings are verified in future studies, it may be appropriate to monitor even so called low-risk CLL patients for genomic complexity over the time course of their disease.

In summary, high-density aCGH analysis confirms the presence of variable but sometimes extensive genomic CNA in CLL patients. A higher number of CNA is associated with a diminished clinical response to even powerful approaches such as chemoimmunotherapy, and highlights the potential value of aCGH in stratification of CLL patients as they enter these trials. Most importantly, since genomic complexity appears to be positively associated with a poor response to even the most powerful combination approaches, new therapeutic strategies are warranted for these patients.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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FIG. 1a

Figure 1. Association between FISH defect 17p13.1- and total number of CNAs, $p=0.0057$ from Wilcoxon rank sum statistic.

FIG. 2a

FIG. 2b

Figure 2. Association between genomic complexity, defined as the total number of CNAs, and PFS (a) evaluating low complexity and (b) evaluating high complexity.

Figure 3. Association between genomic complexity, defined as the total number of CNAs, and DOR; DOR is defined as the time from first response until progression.

Figure 4. Association between genomic complexity, defined as the total number of CNAs, and time from diagnosis to start of trial stratifying by the median number of abnormalities.(n= 43; 5 subjects had missing diagnosis dates)

FIG. 5a

FIG. 5b

Figure 5.
 Association between total deletion size and (a) PFS and (b) time to first response.

Table 1

Distribution of CNA across the 48 CLL patients studied.

Quantile ^d	Number of CNA per patient ^a	Total size of losses (Mb) ^{a, b}	Total size of gains (Mb) ^{a, c}
0% (min) ^e	0	0	0
5%	1	0	0
10%	2	0.01	0
25%	3	0.48	0
50% (median)	4	6.62	0.055
75%	6.5	35.05	8.47
90%	15	66.08	132.20
95%	17	115.74	132.37
100% (max)	32	307.57	214.54
Mean	6	27.80	23.77
Standard deviation	5.8	53.31	51.40

^a Total size of gains and losses does not include trisomy 12, whereas the total number of CNAs does include trisomy 12.

^b 3 patients had no detectable loss of genetic material.

^c 14 patients had no detectable gain of genetic material, 2 of whom exhibited trisomy 12.

^d Percentage of condition (Number of CNA per patient/Total size of losses or gains) at or below the given point.

^e 1 patient had no detectable gains or losses.

Table 2

Regions that were recurrently affected in more than 6% (≥ 3 patients), including prevalence, minimal affected region and genes within the corresponding areas.

Chromosome	Abnormality	Start_bp ^a	Stop_bp ^b	Size (Mb)	Prevalence (%)	Number Genes/microRNA	Genes Included [*]
3p21.31	Loss	46927197	47059161	0.131964	8.3	3	CCDC12, NBEAL2, SETD2 SCAP, C3orf75, CSPG5, SMARCC1, DHX30, MAP4, CDC25A, CAMP, ZNF589, NME6, SPINK8, FBXW12, PLXNB1, CCDC51, CCDC72, ATRIP, TREX1, SHISA5, PFKFB4, UCN2, COL7A1, UQCRC1, TMEM89, SLC26A6, CELSR3, NCKIPSD, IP6K2, PRKAR2A, SLC25A20
3p21.31	Loss	47469018	48880788	1.41177	8.3	21	
8p11.21-p12	Loss	36392432	41892141	5.499709	6.25	58	
10q24.32	Loss	104151445	104187212	0.035767	6.25	5	NFKB2, PSD, FBXL15, CUEDC2, mir146b
11q22.3	Loss	107505926	108049450	0.543524	16.7	8	ACAT1, NPAT, ATM, C11orf65, KDELC2, EXPH5, DDX10
12q13.13-q24.3	Gain	49186782	132289090	83.102308	22.9	>100	
13q13.1	Loss	31224789	31311691	0.086902	6.25	1	RXFP2
13q14.3	Loss	49513749	49902773	0.389024	50	3	RXFP2 DLEU2, MIR16-1, MIR15a, DLEU1
14q24.1-32.33	Loss	68329278	105239424	36.910146	8.3	>100 ^c	
17p11.2-p13.3	Loss	47546	18063564	18.016018	6.25	>100	

* Gene names provided only if found in regions smaller than 5Mb

^aRepresents the start of the gain or loss site.

^bRepresents the end of the gain or loss site

^cThis region of loss did not include the IgVH gene locus.

Table 3

Comparison of FISH and Array CGH concordance.

FISH-Detected Abnormalities*	aCGH-Detected Abnormalities Concordant/Discordant with FISH	CD5/CD19
11q-	Concordant	94.7
13q-x2 ^a	Concordant	99.5
13q-x1	Discordant ^b	99.4
11q-	Discordant ^c	86.3
Normal	Concordant	99.8
13q-x1	Concordant	99.9
11q-/13q-x1	Concordant	80.4
+12	Concordant	99.7
13q-x1	Concordant	99.3
+12	Concordant	99.9
Normal	Concordant	99.7
+12	Concordant	99.7
13q-x1	Concordant	99.7
+12/13q-x1/13q-x2	Concordant	99.4
13q-x1	Concordant	99.9
+12/13q-x2	Concordant	99.1
11q-/12/13q-x1	Discordant ^d	99.5
Normal	Discordant ^e	58.7
11q-/13q-x1	Concordant	73.8
13q-x1	Concordant	99.6
Normal	Concordant	89.2
6q-	Concordant	99.9
13q-x1/13q-x2	Concordant	99.8
13q-x1	Concordant	99.4
+12	Concordant	99.0
13q-x1	Concordant	99.6
+12	Concordant	99.5
+12	Discordant ^f	98.5
+12/13q-x1	Discordant ^g	99.9
11q-	Concordant	99.7
17p-/13q-	Concordant	99.8
17p-	Concordant	95.4
13q-	Concordant	99.8
+12	Concordant	96.5
11q-/12/13q-x2	Concordant	99.8
13q-x1	Concordant	99.2
13q-x1/13q-x2	Concordant	99.9
+MDM2	Concordant	94.1

FISH-Detected Abnormalities*	aCGH-Detected Abnormalities Concordant/Discordant with FISH	CD5/CD19
+12	Concordant	99.4
13q-x1	Discordant ^h	99.5
13q-x1	Concordant	76.9
Normal	Concordant	96.3
13q-x1	Concordant	98.1
13q-x1	Concordant	99.7
11q-/13q-x1	Concordant	49.8
13q-x1/13q-x2	Concordant	99.5
13q-x1/17p-	Concordant	98.4
13q-x1	Concordant	99.9

^a Monoallelic 13q14.3 is designated as 13q-x1 while biallelic is designated as 13q-x2

^b 13q14.3 deletion by FISH (7.5% of nuclei) but not detected by aCGH

^c 11q22.3 deletion by FISH (4.5% of nuclei) but not detected by aCGH

^d 11q22.3 and 13q14.3 deletions by FISH (12% and 21.5% of nuclei, respectively) but not detected by aCGH

^e 11q- (0.54Mb deletion) by aCGH, including the entire ATM locus, but not detected by FISH because part of the region covered by the FISH probe was not deleted.

^f Trisomy 12 by FISH (4.5% of nuclei) but not detected by aCGH

^g Trisomy 12 by FISH (4.5% of nuclei) but not detected by aCGH

^h 13q- by FISH (9.0% of nuclei) but not detected by aCGH

* Each row represents one of the 48 CLL patients.