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Clinical Application of Array-Based Comparative Genomic Hybridization for the Identification of Prognostically Important Genetic Alterations in Chronic Lymphocytic Leukemia

Russell A. Higgins,¹ Shelly R. Gunn^{1,2} and Ryan S. Robetorye¹

1 Department of Pathology, The University of Texas Health Science Center at San Antonio, San Antonio, Texas, USA

2 Combimatrix Molecular Diagnostics Inc., Irvine, California, USA

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Abstract

Genomic aberrations have increasingly gained attention as prognostic markers in B-cell chronic lymphocytic leukemia (CLL). Fluorescence *in situ* hybridization (FISH) has improved the detection rate of genomic alterations in CLL from approximately 50% using conventional cytogenetics to greater than 80%. More recently, array comparative genomic hybridization (CGH) has gained popularity as a clinical tool that can be applied to detect genomic gains and losses of prognostic importance in CLL. Array CGH and FISH are particularly useful in CLL because genomic gains and losses are key events with both biologic and prognostic significance, while balanced translocations have limited prognostic value. Although FISH has a higher technical sensitivity, it requires separate, targeted hybridizations for the detection of alterations at genomic loci of interest. Array CGH, on the other hand, has the ability to provide a genome-wide survey of genomic aberrations with a single hybridization reaction. Array CGH is expanding the known genomic regions of importance in CLL and allows these regions to be evaluated in the context of a genome-wide perspective. Ongoing clinical trials are evaluating the use of genomic aberrations as tools for risk-stratifying patients for therapy, thus increasing the need for reliable and high-yield methods to detect these genomic changes. In this review, we consider the use of array CGH as a clinical tool for the identification of genomic alterations with prognostic significance in CLL, and suggest ways to integrate this test into the clinical molecular diagnostic laboratory work flow.

B-cell chronic lymphocytic leukemia (CLL) is the most common adult leukemia in the US.^[1] The clinical behavior of CLL is widely variable and poses a considerable challenge for determining prognosis for individual patients. While many patients have

indolent disease with prolonged treatment-free survival, others exhibit rapid disease progression and require early therapeutic intervention. New clinical tools are required to predict these outcomes and to risk-stratify patients for treatment decisions.

Current staging systems, such as the widely used Rai and Binet systems, separate groups according to disease distribution and burden. Although these staging groups correlate relatively well with survival in symptomatic late-stage CLL, clinical outcomes in untreated early-stage CLL groups cannot be reliably assessed by these methods, with slightly more than half of these patients requiring treatment because of disease progression.^[2] In addition, these systems do not predict the likelihood of response to treatment in individuals with more advanced disease, so it is particularly important for clinicians to be able to predict outcomes for such patients. Well described prognostic tools such as lymphocyte doubling time, β -2 microglobulin, thymidine kinase, and soluble CD23, have been used to predict clinical behavior in CLL, but these indicators may actually reflect tumor burden rather than predicting the biological behavior of CLL.^[3] Over the last decade, determination of the mutational status of the variable region of the immunoglobulin heavy chain (*VH*) gene has become an important prognostic marker,^[4,5] but testing is technically demanding and not widely offered. Therefore, surrogate markers of *VH* gene mutation status such as CD38 and ZAP-70 have been utilized with variable success.

There is an emerging interest in the use of recurrent genomic aberrations as prognostic indicators in CLL.^[3,6,7] As early as 1990, genomic aberrations have been associated with prognosis in CLL using G-banding techniques.^[8] More recent studies using fluorescence *in situ* hybridization (FISH) have increased the ability to detect small genomic aberrations and confirmed their prognostic value for survival prediction in CLL.^[9,10] Recurrent genomic aberrations are common in CLL and can be detected in greater than 80% of cases when FISH analysis is performed. The prognostic value of 17p13 deletions that include the p53 tumor suppressor (*TP53*) gene locus and 11q23 deletions that include the ataxia telangiectasia mutated (*ATM*) gene has been effectively demonstrated using FISH probes targeting these regions. Deletions at these loci in CLL patients result in significantly decreased survival and shorter intervals to therapy after diagnosis. Furthermore, these deletions have retained their prognostic value in multivariate analyses that also include age and clinical stage.^[9]

The independent prognostic significance of 17p13 and 11q23 deletions has been further validated by additional studies that evaluated these deletions in patients with known *VH* gene mutational status.^[11,12] Additionally, 17p13 deletion and/or mutation on the opposite allele predicts poor response to therapy with purine analogs in patients with CLL.^[13-16] Similarly, CLL with *ATM* deletion and/or mutation also appears to respond poorly to chemotherapy agents.^[17] These findings may reflect a perturbation of the

role of the *TP53* and *ATM* genes in inducing apoptosis in cells damaged by chemotherapy. The early identification of 11q23 and 17p13 deletions in CLL patients may allow clinicians to use alternative therapies such as alemtuzemab^[18] or stem cell transplant.^[19] Ongoing clinical trials that stratify patients according to 17p13 and 11q23 deletions may result in changes in current strategies for the treatment of CLL.

Trisomy 12 was previously thought to be the most common genomic aberration in CLL when G-banding was the mainstay of chromosome analysis. However, subsequent FISH analyses have clearly demonstrated 13q14 deletions to be more common, present in approximately half of all patients with CLL.^[9] Although a specific candidate gene with prognostic significance has not been definitively demonstrated on chromosome 12, the mouse double minute 2 homolog (*MDM2*) gene, encoding a p53-binding protein, has been implicated and is overexpressed in many CLL patients. CLL with trisomy 12 has been correlated with atypical morphology^[17,20-23] and poor prognosis.^[11,20,21] Oscier et al.^[11] demonstrated trisomy 12 to be a significant prognostic factor in a univariate analysis; however, trisomy 12 did not have independent prognostic value in the context of other genomic aberrations and *VH* gene mutation status in a subsequent multivariate analysis. While trisomy 12 is not as predictive or prognostic as 17p13 or 11q23 abnormalities, there is potential for biologic significance that remains to be explored.

13q14 deletion is the most common genomic alteration in CLL and is correlated with a good prognosis. The retinoblastoma tumor suppressor (*RBI*) gene is near the commonly deleted region; however, genomic DNA telomeric to the *RBI* gene is more frequently deleted. Although the biologic role of this deletion is the subject of ongoing research, two recently identified microRNA genes located in the deleted region – *MIR15A* and *MIR16-1* – have been implicated in the pathogenesis of CLL.^[24] These two microRNAs appear to be responsible for negatively regulating *BCL2* protein expression and promoting apoptosis, so it is not surprising that these two genes are frequently targeted for deletion in CLL.

Although genomic copy number changes at 11q23, 13q14, and 17p13 and of whole chromosome 12 do not represent the full spectrum of genomic changes in CLL, they do have biologic and prognostic significance. Strategies to risk-stratify patients with CLL according to underlying genomic aberrations have the potential to optimize treatment decisions and to improve patient counseling. Evidence suggests that the genome of CLL is not stable and that there is an evolution of genomic aberrations that occurs during disease progression.^[25-28] Therefore, it may be necessary to moni-

tor the tumor genome at periodic intervals to reassess risk and therapeutic options. In addition, the study of recurrent genomic aberrations in CLL may identify new targets for novel therapeutic agents.

Commonly employed techniques for detecting genomic aberrations in CLL are conventional cytogenetics with G-banding and FISH. With ongoing clinical trials using genomic aberrations to risk-stratify patients for varying therapies, there will be an increasing need to obtain this information efficiently. Array comparative genomic hybridization (CGH) is a DNA-based test that is ideally suited for identification of recurrent genomic alterations in hematologic malignancies. In this paper, we discuss the advantages and limitations of array CGH and suggest ways to incorporate its use into clinical laboratories for the identification of prognostically important genomic alterations in CLL.

1. Techniques for Detecting Genomic Aberrations in Chronic Lymphocytic Leukemia (CLL)

While conventional G-banding provides a whole-genome perspective, the resolution of this technique is approximately 5 Mb,^[29] thereby limiting its ability to detect smaller deletions. Furthermore, conventional cytogenetic techniques require dividing tumor cells, so the diagnostic yield may be low in tumors with low mitotic activity. When conventional G-banding techniques are applied to the analysis of CLL, aberrations are identified in only about 50% of cases, with trisomy 12 being the most commonly identified aberration.^[8] Newer cytogenetics techniques involving stimulation with CD40 ligand or CpG-oligodeoxynucleotides have increased diagnostic yield in CLL, but these techniques are not practical for routine clinical laboratory testing and have not been widely adopted.^[30,31] In contrast to conventional G-banding, FISH analysis does not require dividing cells and may be performed on interphase nuclei with a resolution determined by the size of the probe (about 100 Kb). Targeted techniques using FISH have resulted in the identification of genomic aberrations in approximately 80% of CLLs, with 13q14 deletion (55% of cases) being the most commonly identified alteration, followed by 11q22-23 deletion (18%), trisomy 12 (16%), 17p13 deletion (7%), 6q21 deletion (6%), 8q trisomy (5%), and t(14q32) [4%].^[9] However, the information gained by FISH analysis is limited to the targets of the selected probes, in marked contrast to the genome-wide perspective provided by conventional G-banding.

Multiplex PCR has also been adopted to detect common genomic aberrations in CLL.^[32] This technique performs well when targeting common aberrations in CLL, but the genomic alterations

detected using this technique are limited by the selected primers. In addition, PCR does not provide a genome-wide perspective and cannot provide any information about the increasing number of recognized aberrant regions in CLL. However, because of these important limitations and the complexity of this test, most institutions do not perform multiplex PCR to identify genomic alterations in CLL.

CGH has also been used to detect genomic aberrations in CLL.^[33,34] This technique utilizes a normal metaphase spread as the template for hybridization to a mixture of differentially labeled patient and normal DNA samples. The relative fluorescence of the sample versus that of the normal control provides information about chromosomal gains and losses. This technique provides information about the genomic location of aberrations and structural changes at a resolution of approximately 5–10 Mb. The low resolution and technical challenges of conventional CGH have led to the recent movement of array-based CGH from research laboratories into the clinical arena.^[35] This technique utilizes numerous bacterial artificial chromosome (BAC) or oligonucleotide probes robotically spotted onto glass slides, forming arrays of DNA probes from known locations in the genome. Similar to CGH, differentially labeled patient and reference DNA samples are hybridized to the glass arrays. The arrays are scanned (figures 1a and 1b) and software is used to assimilate the fluorescence signals into usable data by signal normalization and subtraction of background noise. Software programs are used to display genomic gains and/or losses in relation to their position along a genomic map (figures 1c and 1d). The probes used in the arrays are usually selected to cover the genome at approximate intervals that define the array resolution (distance between probes on the array). In addition, arrays may be targeted to specific regions of the genome or to clinically significant genomic regions with the addition of extra probes in the regions of interest or of prognostic importance.

The most widely used arrays are constructed with large-insert clones such as BAC or P1-derived artificial chromosome (PAC) clones. The resolution of the arrays is dependent on the sizes of the clones as well as the distance between the clones in the genome. Oligonucleotide-based arrays are becoming increasingly more popular due to their higher resolution compared with BAC and PAC arrays. The higher resolution of oligonucleotide arrays is due to the utilization of more numerous probes of shorter length (25–60 nucleotides). While earlier efforts used reduced complexity DNA samples for oligonucleotide arrays because of lower signal-to-noise,^[36] many oligonucleotide array platforms have since demonstrated high performance using full-complexity DNA.^[37,38] Higher resolution arrays are more likely to detect small

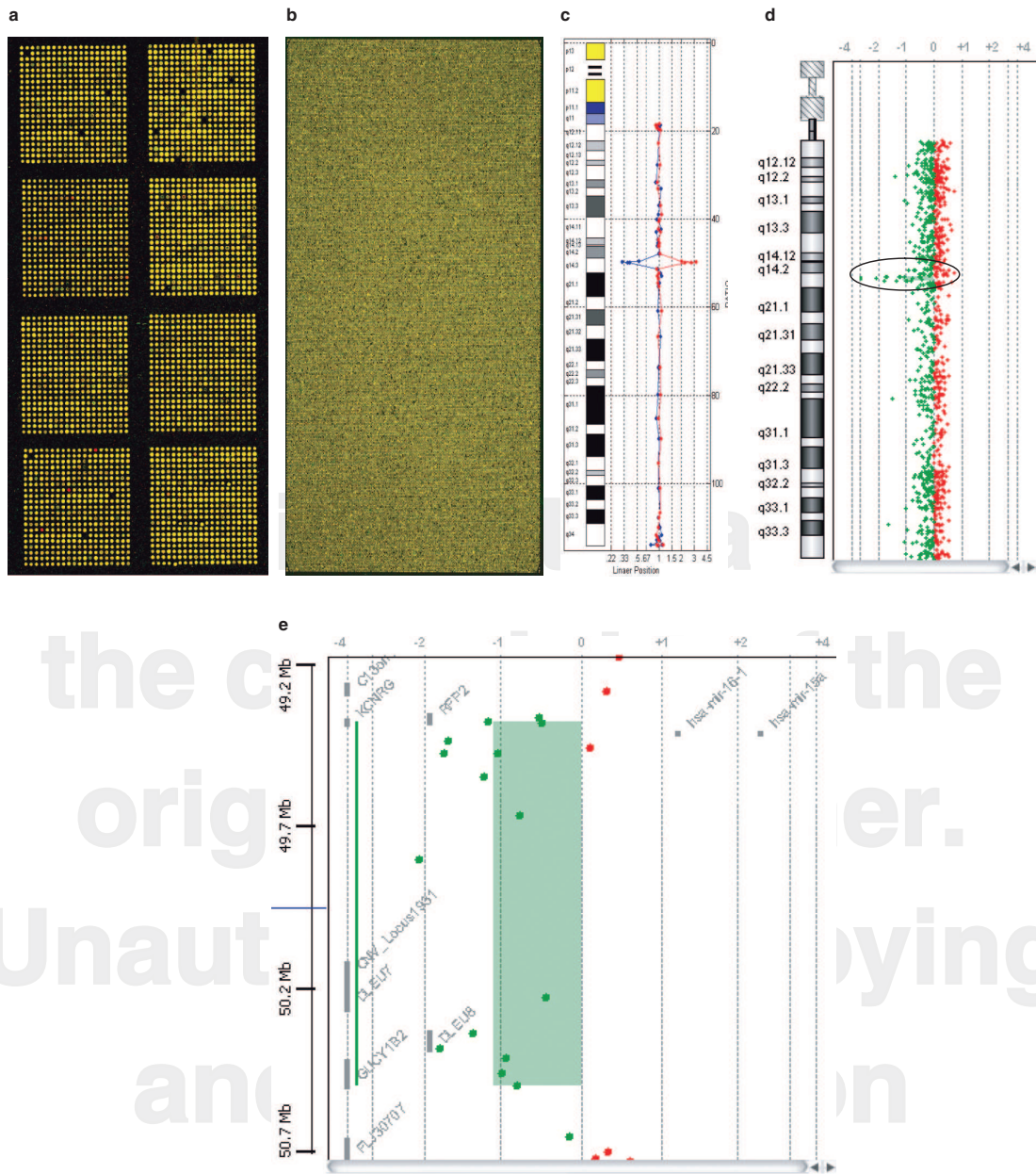


Fig. 1. Array-based comparative genomic hybridization (CGH) microarray scans and chromosome ideograms: (a) bacterial artificial chromosome (BAC)-based microarray scan; (b) high-density oligonucleotide-based microarray scan; and (c) chromosome 13 ideogram in a chronic lymphocytic leukemia (CLL) case exhibiting a small bi-allelic 13q14 deletion analyzed by BAC array. Losses in DNA copy number at particular loci are observed as the simultaneous deviation of the ratio plots from a modal value of 1.0, with a blue ratio plot representing a negative deviation (to the left) and a red ratio plot representing a positive deviation at the same locus (to the right). Conversely, DNA copy number gains show the opposite pattern. (d) Chromosome 13 ideogram generated from the same CLL case as in (c), analyzed using oligonucleotide-based array CGH. The green and red dots represent the fluorescence ratios of individual oligonucleotide probes on the microarray. Red dots represent probes with positive fluorescence ratios and green dots represent probes with negative fluorescence ratios. The small cluster of green probes significantly shifted to the left of zero at 13q14 (circled in the figure) represents a small deletion. (e) High-resolution gene view of the 13q14 deletion in the same CLL case as in (c) and (d), identifying the approximate breakpoints and the genes present in the deleted region. Breakpoint mapping of the endpoints of this deletion indicates that it includes approximately 1 Mb (1 046 757 bp) of genetic material. The vertical green bar at the left of the figure and the green-shaded rectangle represent the deleted region at 13q14 (determined to be statistically significant by the software used to analyze the array data).

genomic aberrations and are better able to approximate the breakpoints of the aberrations. However, the utilization of higher

resolution arrays to identify biologically and clinically significant genomic regions in CLL has also resulted in the detection of

additional genomic alterations of unknown clinical significance.^[39] Additional clinical correlation will be required to determine the possible prognostic value of these novel alterations in CLL.

2. Technical Limitations of Array Comparative Genomic Hybridization (CGH) Analysis of CLL

Array CGH requires intact, high molecular weight DNA for optimum sensitivity and accuracy of the analysis. However, we have not found this to be a problem for our laboratory as the vast majority of bone marrow aspirate and peripheral blood samples subjected to array CGH analysis are submitted in ethylene diamine tetra-acetic acid (EDTA) blood tubes, as these samples are also required to make flow cytometric diagnoses of CLL (see figure 2). DNA isolated from these tubes is highly stable and readily obtained using either manual or automated DNA isolation methods.

Array CGH is able to detect copy number changes in the genome; however, it does not detect structural rearrangements such as balanced translocations or inversions. Likewise, array CGH cannot differentiate disomic versus tandem copy number gains. Array CGH is well suited for the identification of genomic alterations of prognostic importance in CLL because the most common and important genomic aberrations are losses and gains, whereas translocations are rare. Translocations do occur in B-cell

CLL but their prognostic significance is currently controversial. A recent publication using standard B-cell mitogen-stimulated cytogenetics analysis showed a worse prognosis in CLL cases with translocations; however, this study was limited by low patient numbers ($n = 65$) and an over-representation of patients with advanced disease.^[40] Interestingly, many translocation breakpoints are associated with deletions at known regions of genomic aberration in CLL.^[30,40]

A major limitation of array CGH is its technical sensitivity. Array CGH can detect genomic aberrations when tumor cells constitute approximately 25–30% of the cells within a sample, but conventional cytogenetics and FISH can detect aberrations when clonal aberrations are present in as little as 2–3% of cells. This lower sensitivity may result from either dilution of the tumor sample with DNA originating from non-tumor cells (e.g. normal lymphocytes) or from tumor cells that exhibit clonal heterogeneity, with the presence of small subclones that comprise less than 25–30% of the total tumor cells. Therefore, samples from patients with newly diagnosed CLL or progressive disease with high percentages of circulating tumor cells are suitable for analysis by array CGH. On the other hand, samples obtained from patients closely following chemotherapy or a stem cell transplant may have low numbers of circulating tumor cells and are not suitable for analysis by array CGH unless prior cell enrichment methods are

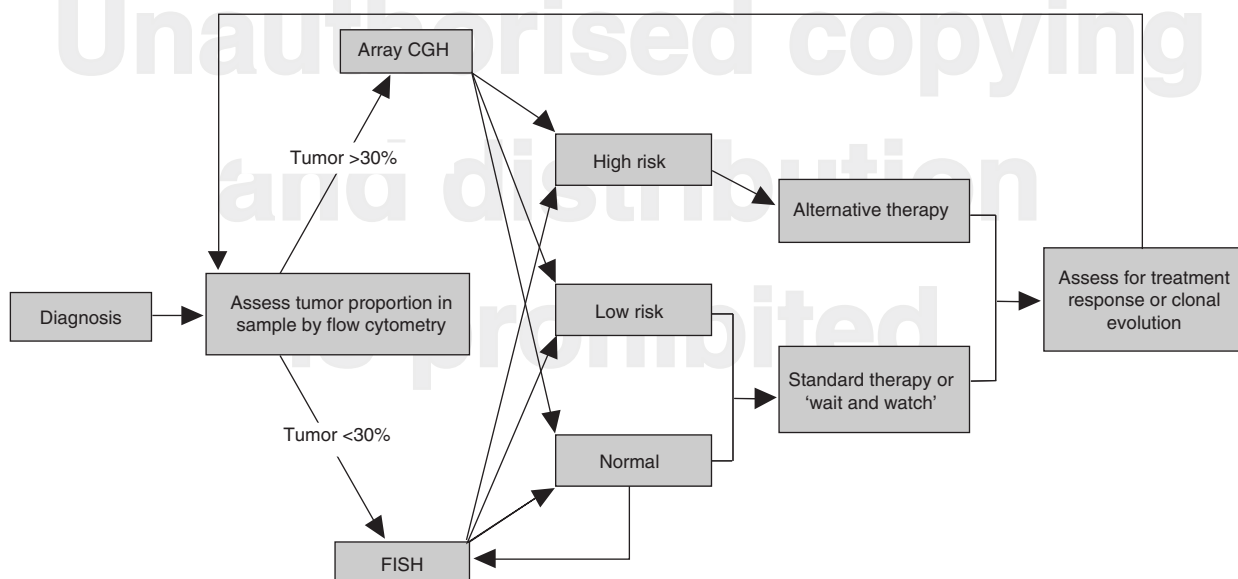


Fig. 2. Suggested algorithm for integration of array comparative genomic hybridization (CGH) into the clinical evaluation of chronic lymphocytic leukemia (CLL). Array CGH is ordered as a first-line test for all CLL peripheral blood samples containing more than 30% tumor cells as determined by flow cytometry. It is recommended that the patient's tumor genome be monitored periodically for clonal evolution and/or rescanned if there is a significant change in the clinical presentation. Cases with normal results by array CGH should be subjected to fluorescence *in situ* hybridization (FISH) analysis. This algorithm can be a cost-effective method for the use of FISH and array CGH as complementary tests in the clinical laboratory.

employed. Likewise, detection of evolving subclones within the tumor array CGH may be limited when the proportion of tumor cells carrying the genomic alteration is small. In CLL cases where the percentage of tumor cells is low, FISH or multiplex PCR are better suited for detection of genomic aberrations.

3. Array CGH Analysis as a Tool in the Clinical Laboratory

Experience with utilization of array CGH as a clinical tool began with analysis of patients with suspected congenital abnormalities^[41,42] but has grown to include other clinical applications such as tumor genome analysis. Schwaenen et al.^[43] first described their experience in 2004 with an automated array-based CGH method for the identification of important genomic alterations in CLL with clinical application in mind. In this study, BAC and PAC clones were selected for whole-genome coverage as well as to specifically interrogate 3q26, 6q21, 8p24, 11q22, 12q13, 13q14, 17p13, and 18q21 as important genomic regions in CLL. Blood, bone marrow aspirate, and lymph node specimens obtained from 106 patients with CLL were subjected to array CGH analysis and the results were compared with FISH studies. The sensitivity of the array analysis varied with the percentage of tumor carrying the genomic aberrations as assessed by FISH. Using predefined cutoff values, sensitivity was 100% for samples when >53% of the cells exhibited abnormal signals by FISH. However, the ability of array CGH to identify genomic alterations decreased when the percentage of cells with clonal aberrations was 33–53%.

Recently, a number of additional studies have been completed that clearly demonstrate the feasibility of using array CGH as a clinical tool to identify genomic alterations of prognostic importance in CLL.^[44–46] A paper by Patel et al.^[44] used a customized DNA microarray comprised of 220 BAC and PAC clones targeting genomic regions of importance in 31 cases of CLL. Copy number changes were detected in 87% of the samples with a sensitivity of 100% when clonal abnormalities were present in at least 23% of the cells (as determined by FISH). Interestingly, this study also identified nine cases in which genomic alterations were observed that were not detected by standard cytogenetic and/or FISH analyses. A paper by Sargent et al.^[45] used a customized oligonucleotide-based array platform to identify genomic aberrations in a total of 100 CLL cases and compared these results with those obtained by FISH analysis of the same cases. Array CGH results showed a high concordance with parallel FISH studies except when FISH aberrations were present in less than 25% of the

cells. In this study, no genomic aberrations were identified using array CGH that were not detected by multi-probe FISH. In a recent paper from our laboratory,^[46] we evaluated array CGH as a clinical tool to identify genomic aberrations with prognostic importance in 174 cases of CLL using a 1093 clone BAC microarray. In a comparison of array CGH results with those obtained from FISH analyzes, we found that we could correctly identify aberrations identified by FISH 96% of the time. The discordant results were due to clonal cell populations comprising less than 30% of the sample, and all involved CLL cases with small (~2 Mb) deletions of chromosome 13 interrogated by two FISH probes for the 13q14 region. Interestingly, two cryptic (~1 Mb) 13q14 deletions detected by the BAC array were missed by both of the 13q14 region FISH probes used in the study.

Each of these four recent studies^[43–46] has clearly demonstrated the ability of array CGH to identify alterations of prognostic significance in CLL that can also be detected by FISH. A common finding among them is that tumor cells must comprise a minimum proportion of the samples (approximately 25–30% in the most recent studies) in order for genomic aberrations to be detected by the technique. However, flow cytometry is routinely used for the diagnosis of CLL, and the percentage of tumor cells can also be readily determined from this analysis. We recommend a cost-effective algorithm in which array CGH is used as a first-line test for the identification of important genomic changes in cases of CLL with over 30% tumor cells as determined by flow cytometry, with utilization of FISH in cases with negative results by array CGH (figure 2). In our experience, array CGH analysis detects genomic aberrations with prognostic significance in approximately 80% of CLL cases. In the remaining 20% of cases found to be normal by array CGH, approximately half of these cases will contain clonal cell populations below the 25–30% detection limit of array CGH that can be identified by FISH analysis. Examples of important genomic aberrations identified in CLL by both BAC- and oligonucleotide-based array CGH analyses are illustrated in figure 3. In addition to the detection of genomic alterations of known prognostic significance, both array types can identify novel recurrent aberrations that may prove to have future prognostic importance. In contrast to BAC arrays, oligonucleotide-based arrays also have the ability to identify aberration breakpoints and the size of the alterations (figure 1e). This capability can facilitate the identification of specific genes affected by recurrent genomic alterations and may aid in the development of additional therapeutic options for CLL patients in the future.

4. Challenges in the Utilization of Array CGH in Clinical Molecular Diagnostics

As array CGH has become more widely used in clinical medicine, guidelines for its use have been recently released by the American College of Medical Genetics.^[47] Although these guidelines primarily address the use of this technique for the identification of genomic alterations in individuals with suspected inherited congenital anomalies, many of these guidelines are also directly applicable to the use of array CGH for the analysis of cancer genomes. For example, it is recommended that laboratories using array CGH should validate arrays for their intended use, define cut-off values for calling abnormalities, define specimen acceptability, participate in proficiency testing, monitor measures of quality, and report data in a meaningful manner. In addition, the International Standing Committee on Human Cytogenetic Nomenclature has developed nomenclature in order to standardize the reporting of array data;^[48] this nomenclature should be used when possible. Thus, by applying these somewhat universal laboratory practices, array CGH can be used confidently as a tool in the clinical laboratory.

The reporting of results from array CGH analyses is complicated by the enormous amount of information provided by the array. Arrays are designed for a wide variety of clinical purposes and may contain probes intended to interrogate genomic regions of known clinical significance, probes from other regions of unknown significance for discovery purposes, and probes used for normalization of the results and data analysis. Moreover, experience with array CGH has uncovered a large number of copy number variations (CNVs) in the genome that represent polymorphisms.^[49,50] Over-interpretation of regions of CNV with unknown clinical significance may lead to the need to perform additional costly and time-consuming confirmatory testing.^[51] CNVs are readily identified by both oligonucleotide and BAC array CGH at an average of 3–5 variants/genome, and are identified in virtually every case of CLL. With few exceptions, these changes clearly represent polymorphisms involving single-clone gains or losses overlapping previously reported regions of genomic variation (<http://projects.tcag.ca/variation/>).^[49] Detection of CNVs by array CGH is analogous to the finding of heteromorphisms by conventional cytogenetics and are an expected finding in all comprehensive genomic evaluations. More experience with CNV identification and a better understanding of the significance of recurring genomic aberrations in CLL will permit more accurate and efficient interpretation of genome-wide array CGH data.

While some laboratories will invest in the expensive capital equipment required to produce their own quality arrays, most diagnostic laboratories will choose to purchase arrays from commercial sources for array CGH testing. The quality of array CGH results is directly dependent on the quality of the probes that are spotted onto the array slides. While constructing a BAC array, Bejjani et al.^[51] found that 7% of their selected clones were mapped to the wrong loci, 16% cross-hybridized to other chromosomes, and 12% did not hybridize efficiently. These findings underscore the importance of quality control in developing the microarrays used for array CGH analysis. It is necessary for laboratory directors to rigorously verify the quality of any constructed or purchased arrays used in their clinical laboratories.

In the setting of ever-rising healthcare costs, the costs involved in performing array-based testing have become an important issue. Because of low yield and the inability of standard karyotyping to detect submicroscopic deletions (e.g. small 13q14 deletions in CLL), FISH is often ordered at an additional cost. Moreover, conventional cytogenetics has limited prognostic value in CLL (as discussed in section 1), making array CGH and/or FISH important ancillary tests for patient risk-stratification and clinical decision making. The cost of performing array CGH analysis at commercial laboratories is in the range of \$US1500–2500, which is actually not excessive considering the similar costs involved in utilization of the combination of conventional cytogenetics and applicable FISH panels. Smaller clinical laboratories can achieve substantial cost savings by purchasing commercially available arrays and performing the actual array CGH analyses in their own laboratories. Algorithms for the thoughtful application of array CGH and FISH allow for cost-effective utilization of these tests and can provide superior results to the currently employed methods.

5. Conclusions

We have discussed the feasibility of using array CGH as a clinical tool for the evaluation of recurrent genomic alterations with prognostic importance in CLL. A complete analysis of the CLL genome by array CGH can be completed with a reasonable turnaround time (as little as 36 hours per sample). Expected and novel genomic aberrations are quickly and accurately identified by this method and provide readily interpretable results that are suitable for clinical risk-stratification and treatment planning after the pertinent prognostic markers are identified. Compared with conventional cytogenetics for whole-genome analysis, array CGH is a DNA-based test with the ability to detect commonly assayed CLL prognostic markers at a level that exceeds the 80% rate

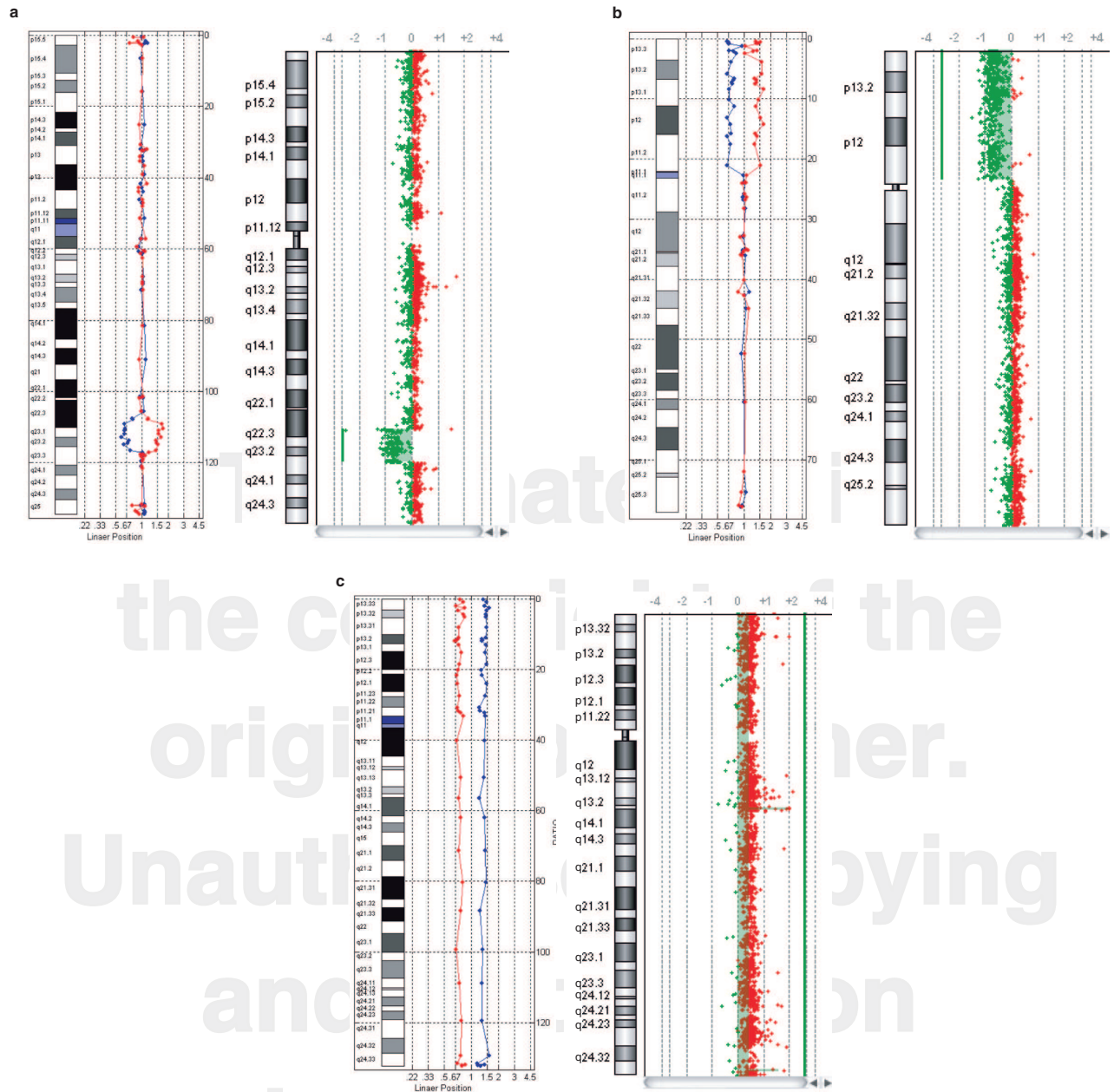


Fig. 3. Examples of important genomic aberrations of prognostic importance identified in chronic lymphocytic leukemia (CLL) by both bacterial artificial chromosome (BAC)-based and oligonucleotide-based array comparative genomic hybridization (CGH) analyses. **(a)** Chromosome 11q23 deletion (*ATM* gene) in a CLL case analyzed by BAC array (left) and oligonucleotide array (right). **(b)** Chromosome 17p13 deletion (*TP53* gene) in a CLL case analyzed by BAC array (left) and oligonucleotide array (right). **(c)** Chromosome 12 trisomy in a CLL case analyzed by BAC array (left) and oligonucleotide array (right). Losses in DNA copy number in BAC arrays at particular loci are observed as the simultaneous deviation of the ratio plots from a modal value of 1.0, with blue ratio plots representing a negative deviation (to the left) and red ratio plots representing a positive deviation at the same locus (to the right). DNA copy number gains show the opposite pattern. For oligonucleotide-based array analyses, the green and red dots represent the fluorescence ratios of individual oligonucleotide probes on the microarrays with red dots representing probes with positive fluorescence ratios and green dots representing probes with negative fluorescence ratios. Clusters of green probes significantly shifted to the left of zero represent losses, and clusters of red probes significantly shifted to the right of zero represent gains of genetic material. Vertical green bars and the green-shaded rectangles represent regions determined statistically significant by the software used to analyze the array data (regions of gain or loss of genetic material).

generally attributed to FISH. Array CGH is a robust, reliable, and cost-effective clinical tool that is suitable for inclusion into the

clinical laboratory test menu for the evaluation of prognostic markers in CLL within the context of the entire genome. The

clinical applications of array CGH for the analysis of tumor genomes will continue to grow as array platforms improve and become even more affordable.

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Dr Gunn serves as the Medical Director at Combimatrix Molecular Diagnostics, which develops and markets array comparative genomic hybridization (CGH)-based tests for hematological malignancies.

Drs Higgins and Robetorye declare no competing financial interests.

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Correspondence: Dr *Ryan S. Robetorye*, Department of Pathology, The University of Texas Health Science Center at San Antonio, Mail Code 7750, 7703 Floyd Curl Drive, San Antonio, TX 78229-3900, USA.
E-mail: robertorye@uthscsa.edu

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