Cytogenetics in Chronic Lymphocytic Leukemia: ERIC Perspectives and Recommendations

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ABSTRACT

Mounting evidence underscores the clinical value of cytogenetic analysis in chronic lymphocytic leukemia (CLL), particularly as it allows the identification of complex karyotype, that has recently emerged as a prognostic and potentially predictive biomarker. That said, explicit recommendations regarding the methodology and clinical interpretation of either chromosome banding analysis (CBA) or chromosome microarray analysis (CMA) are still lacking. We herein present the consensus of the Cytogenetic Steering Scientific Committee of ERIC, the European Research Initiative on CLL, regarding methodological issues as well as clinical interpretation of CBA/CMA and discuss their relevance in CLL. ERIC considers CBA standardized and feasible for CLL on the condition that standards are met, extending from the use of novel mitogens to the accurate interpretation of the findings. On the other hand, CMA, is also standardized, however, robust data on its clinical utility are still scarce. In conclusion, cytogenetic analysis is not yet mature enough to guide treatment choices in CLL. That notwithstanding, ERIC encourages the wide application of CBA, and potentially also CMA, in clinical trials in order to obtain robust evidence regarding the predictive value of specific cytogenetic profiles towards refining risk stratification and improving the management of patients with CLL.

CYTOGENETIC ANALYSIS IN CHRONIC LYMPHOCYTIC LEUKEMIA: A HISTORICAL OVERVIEW

The first reports on chromosome banding analysis (CBA) in chronic lymphocytic leukemia (CLL) were published in the 1980s. Metaphases were successfully obtained in 40% to 50% of cases, 40% to 50% of them harboring at least one cytogenetic aberration.1 In the ensuing years, improved cultivation, including stimulation with B-cell mitogens led to a success rate to obtain metaphases of 90% and an aberration incidence of ~60% to 70%.2 In contrast to acute leukemias and myelodysplastic syndromes, CBA was not introduced into routine diagnostics at that time. The reasons were the still low in vitro proliferative capacity of CLL cells resulting in missing the abnormal CLL clone in a substantial fraction of cases, as revealed by array comparative genomic hybridization (CGH) and interphase fluorescence in situ hybridization (FISH) data.3,4

The hallmark paper on interphase FISH in CLL was published in 2000.3 With the help of only 4 probes targeting deletions of the 13q14, 11q22 (ATM), 17p13 (TP53) regions, and trisomy 12, cytogenetic abnormalities were detected in the great majority of cases and the prognostic value of interphase FISH in CLL was demonstrated4: thus, interphase FISH became the cytogenetic technique of choice for the characterization of CLL. This was reflected in the guidelines for the diagnosis and treatment of CLL published by the International Workshop on Chronic Lymphocytic Leukemia (iwCLL) in 2008 which recommended to perform interphase FISH always in clinical trials and suggested to perform it also in clinical practice before every new line of treatment.4

In parallel, the application of specific culturing protocols, particularly with the usage of specific mitogens, namely CD40...
ligation (CD40L) or CpG-oligonucleotide DSP30 plus interleukin-2 (IL-2)17 reached rates of aberrant karyotypes comparable to those obtained by FISH.6,9 Importantly, the usage of CpG/IL-2 did not induce any clonal cytogenetic changes,8,10 hence, the detected abnormalities represent true CLL-associated aberrations.

Studies using both CBA and interphase FISH in parallel revealed that these techniques complement each other.11 In more detail, in cases with normal FISH, CBA detected abnormalities that were not covered by the panel of FISH probes used.10,12 On the other hand, FISH detected abnormalities that were not identified by CBA due to its inherently lower resolution. Indicatively, the majority of 13q deletions in CLL are too small to be visible by CBA but can be detected by interphase FISH. Moreover, aberrations with potential clinical significance such as deletion of 6q21, gain of 2p, recurrent translocations [e.g. t(14;19)] or complex karyotypes (CK) are visible with CBA but not detected with the standard CBA FISH analysis.12-15 Thus, performing CBA in combination with interphase FISH results in a more comprehensive genetic characterization of CLL.

Similarly to FISH, the application of chromosome microarray analysis (CMA) in CLL has been associated with increasing detection of cytogenetic abnormalities with potential clinical significance. Genomic array platforms such as array-based CGH (array-CGH) and single nucleotide polymorphism arrays (SNP-arrays) allow the entire genome to be screened for copy-number alterations (CNAs) in a single experiment. In contrast to CBA analyses, input of array platforms only includes DNA and obviates the need for fresh isolated tumor cells and in vitro mitogen stimulation.16-18

An overview of the main limitations/benefits of CBA, FISH and CMA in CLL19 is provided in Table 1.

Summary statement

Cytogenetic analysis in CLL is feasible and can be performed with various methodologies (FISH/CBA/CMA). Each one of them provides different but complementary information regarding the genomic background of the malignant clone.

**METHODOLOGICAL ISSUES FOR CBA**

**Samples**

For the great majority of patients with CLL, peripheral blood (PB) is the most appropriate source of tumor cells as it usually has a high CLL cell fraction. Bone marrow (BM) samples may have higher amounts of contaminating nonmalignant cells; on the other hand, they are especially suitable for cytogenetic cultures in cases with few circulating clonal cells. Finally, lymph node biopsies may be an alternative option to obtain tumor cells, that is, in small lymphocytic lymphoma.

A total of 5 to 15 mL of PB (or alternatively 1 to 2 mL of BM) should be collected in heparinized tubes. Ethylenediaminetetraacetic acid (EDTA) is not suitable for cytogenetic cultures. That said, based on our experience, if the sample arrives in EDTA and it has been in this anticoagulant for <12 hours, the sample can still be washed twice with sterile Roswell Park Memorial Institute (RPMI) medium or 10× phosphate-buffered saline (PBS) before setting up cultures, especially if there is no possibility of obtaining a new heparinized sample. As a general recommendation, optimal results are obtained within 24 hours after collecting the sample. The transport time is highly relevant for samples with high white blood cell (WBC) counts.10-22

**Methods**

Before establishing cell cultures, the WBC count from the sample must be determined. A total of 2 x 10⁶ leukocytes/mL medium will be added to culture tubes or flasks.20 As mentioned above, addition of mitogens to the media is essential. This is due to the fact that regulation of early cell cycle progression differs between CLL cells versus normal B cells; indeed, the former are mostly arrested in the G₁/early G₂ phase of the cell cycle and characterized by marked hyporesponsiveness towards a variety of polyclonal B-cell activators.

From a practical point of view, it is recommended to set up 2 parallel cultures with different cell mitogens for each patient, one with 12-O-tetradecanoyl-phorbol-13-acetate and the other with IL-2 plus DSP30. Following this strategy, more cases with abnormal karyotypes are identified, as 5% to 20% of cases are found carrying aberration(s) only with 1 of the 2 mitogens. CLL cells remain in culture for 72 hours and, after that, the antimitic colcemid is added to the media to obtain metaphases (Table 2).

After incubation, harvesting of the cultures is performed following standard cytogenetic procedures.21-22 This can be undertaken manually or using automated robots. Basically, after centrifugation and removal of the supernatant, the cellular sediment is resuspended in potassium chloride hypotonic solution and incubated for 10 minutes at 37°C. The cell suspension is centrifuged again, supernatant is discarded and cells are fixed with fresh prepared Carnoy’s solution (3 methanol/1 acetic acid). After centrifugation and removal of supernatant, the sediment is resuspended again with Carnoy’s solution. This process is repeated 2 to 3 times until the pellet is clean (yellow/white). Finally, a cell suspension is obtained, adjusted at an optimal cell concentration (according to turbidity of the sample), and slides are prepared with 1 to 2 drops. It is important to dry the slides correctly to obtain good quality banding and staining. Once the slides are prepared, an aging process is required. After that, banding and staining is carried out using Giemsa or Wright’s solution, after 2× saline-sodium citrate or trypsin incubation, to obtain chromosome banding. In most cases, the resolution will be of 200 to 400 bands per haploid karyotype.

**Evaluation of metaphases**

Metaphases should be screened at the microscope or captured using a metaphase finder. A minimum of 20 metaphases should be analyzed in cases with normal karyotype. Ten metaphases should be fully analyzed, with a further 10 analyzed or counted and scored for relevant structurally abnormal chromosomes.11

**Summary statement**

Standardized methodologies should be followed when performing CBA in CLL. These are summarized in Table 2.

**BIOLOGICAL CONSIDERATIONS AND CLINICAL IMPLICATIONS: FOCUS ON CYTOGENETIC COMPLEXITY**

Cytogenetic complexity in hematological malignancies

Cytogenetic complexity is found in many types of tumors and can arise through multiple mechanisms. The combination of cytogenetic complexity and underlying genomic instability results in altered expression potentially of large numbers of genes and in genomic diversity, thereby enabling tumors to respond rapidly to selective pressures.23

In hematological malignancies such as myelodysplastic syndromes, acute myeloid leukemia, and acute lymphoblastic leukemia, a CK has independent prognostic and/or predictive value and the number of abnormalities with the greatest prognostic significance varies between 3 and 5 per clone.24,25 This variability in the clinical significance of CK reflects its underlying biological heterogeneity such that a clinically relevant definition is only possible within the context of an individual disease and should also take into account other features that influence cellular phenotype, including specific chromosomal abnormalities, gene mutations, the cell of origin, and microenvironmental interactions as well as specific treatment modalities.
Cytogenetic complexity in CLL

In CLL, CK is classically defined as the presence of ≥3 clonal structural or numerical abnormalities. Although present in 8% of monoclonal B lymphocytosis,26 CK ≥3 is associated with advanced stage disease, cases harboring unmutated IGHV genes (U-CLL), del(11q), TP53 aberrations [del(17p) and/or TP53 mutation], and telomere dysfunction.26–28

There is both experimental and in vivo data to indicate that (at least) some of the aforementioned associations are causal.26,28 TP53 aberrations promote genomic instability through a variety of mechanisms and also enable cells to tolerate the proteomic, metabolic, and other cellular stresses which arise secondary to major structural abnormalities.29

That said, not all CKs arise in synergy with TP53 dysfunction. It has been recently reported that in almost 20% of patients with CK in CLL, even among those with more than 5 chromosomal aberrations (high CK), no TP53 aberration could be detected despite using high-sensitive detection methods, that is, targeted next generation sequencing.28 Whether CK in these cases is an independent biological phenomenon or closely related to a non-TP53 dependent, still undefined, pathophysiologic mechanism, needs to be further investigated.

Genomic aberrations have traditionally been considered as a clonal signature of malignant cells. The situation appears more complex in mature B cell malignancies, including CLL, where the landscape of genomic aberrations is heterogeneous and diverse even within the same case: hence, such aberrations most often define subclones rather than the clone in its entirety.31

Contrasting this intraclonal genomic heterogeneity, all CLL clonal cells express an identical B cell receptor immunoglobulin (BcR IG) whose unique features critically impact on the natural history of CLL.30

With this in mind, it is indeed remarkable that specific genomic aberrations are significantly enriched in CLL subgroups defined by the expression of BcR IG with distinct immunogenetic features, suggesting links between particular antigenic triggering and distinct pathways of genomic evolution.31 CK fits with this pattern, considering that more than 75% of high-CK cases concern U-CLL, likely reflecting their high proliferative capacity.26 At the other end of the immunogenetic spectrum, it is worth mentioning that ~25% of the (infrequent) CK cases with mutated IGHV genes (M-CLL) displaying CK are classified in a distinct subgroup carrying co-existing trisomies of chromosomes 12 and 19 along with other numerical (usually +18) and/or structural abnormalities (see below).

In CLL, intense cellular proliferation has been associated with extensive telomere shortening which can be detected even in early stage CLL and is a powerful independent marker of poor outcome.26,32 Telomere dysfunction leads to telomere fusion with both telomeric and non-telomeric loci, formation of dicentric chromosomes, initiation of breakage–fusion–bridge cycles resulting in a variety of structural abnormalities which include deletions, duplications, non-reciprocal translocations, and chromothripsis and may also drive genomic instability, facilitating selection of clones with del(17p) or del(11q).27,28

| Cyogenetic test | Advantage (strength) of the test | Disadvantage (weakness) of the test |
|----------------|---------------------------------|-----------------------------------|
| CBA            | Genome-wide scan                | Requires culturing of cells with B-cell mitogen (eg, IL2 + CpG) to increase sensitivity |
|                | Single cell analysis            | Resolution limit is 10–20 Mb       |
|                | Detection of balanced chromosome rearrangements (ie, translocations, inversions) | Cannot detect regions of homozygosity (CN-LOH) |
|                | Sensitivity is circa 10% to 15% in routine analysis: 2 (loss) or 3 (gain) aberrant metaphases found in 20 analyzed cells | Analysis is laborious and slow (1 case at a time) |
|                | Detection of clonal evolution   | Detects only abnormalities where the probe was designed for |
|                | Discovery of novel abnormalities (and complex karyotype) | Multiple FISH probes are required to look at diverse abnormalities (routinely in CLL a 4–5 probe panel) |
| FISH           | Resolution is circa 150–900 kb, depending on probe-size | Clonal evolution may be overlooked |
|                | Sensitivity for detection of low level clones is around 3%–5% (usually 100–200 interphase nuclei scored) | Cannot detect regions of homozygosity (CN-LOH) or genomic instability (chromothripsis) |
|                | Does not require cultured cells | Cannot detect balanced chromosome rearrangements |
|                | Batch cases                     | Detection of multiple clones is feasible but not evident |
|                |                                | Sensitivity is 10%–20% (platform-dependent) |
|                |                                | B-cell enrichment may be required if tumor burden is low |
| CMA            | Whole genome scan               | Requires culturing of cells with B-cell mitogen (eg, IL2 + CpG) to increase sensitivity |
|                | Resolution is 50 kb or less (depending on platform design) | Resolution limit is 10–20 Mb |
|                | Discovery of novel abnormalities (and genomic complexity) | Cannot detect regions of homozygosity (CN-LOH) or genomic instability (chromothripsis) |
|                | Detection of regions of homozygosity (CN-LOH) if SNP-based platform significantly automated (batch cases) | Cannot detect regions of homozygosity (CN-LOH) or genomic instability (chromothripsis) |
|                | Does not require cultured cells | Cannot detect regions of homozygosity (CN-LOH) or genomic instability (chromothripsis) |
|                | Discovery of novel unbalanced abnormalities with exact definition of the regions (and genes) involved (within the limit of resolution) | Cannot detect balanced chromosome rearrangements |
|                | Detection of (submicroscopic) regions with genomic instability/chromothripsis | Detection of multiple clones is feasible but not evident |

CBA = chromosome banding analysis; CLL = chronic lymphocytic leukemia; CMA = chromosome microarray analysis; CN-LOH = copy neutral loss of heterozygosity; FISH = fluorescence in situ hybridization.

Adapted from Cooley et al, Schoumans et al, and Chun et al.18,40,42

Heterogeneity within CLL cases with CK

i. Number of abnormalities: The prognostic significance of the number of chromosomal abnormalities in cases with CK ≥3 was first observed in studies of factors influencing time-to-first-treatment33 and outcome following allogeneic stem cell transplantation.34 In each study, CK ≥5 was associated with a poorer outcome. The recent retrospective ERIC study of 5290 patients managed during the era of chemo(immuno)therapy enabled a more refined analysis of the clinicobiological associations and clinical impact of CK in CLL. CK cases were subdivided into 3 subgroups based on whether they were carrying 3, 4, or ≥5 abnormalities (comprising 45%, 21%, and 34% of all CK cases, respectively). Patients with ≥5 abnormalities, defined as high-CK, had a very poor outcome (median overall survival [OS] of 3.1 years) independently of clinical stage, TP53 aberrations, and IGHV gene somatic hypermutation status. In contrast, CK cases with 3 or 4 aberrations (low-CK and intermediate-CK, respectively) had a shorter survival (median OS of 4.3 years) only when accompanied by TP53 aberrations.26 These findings appear relevant for
Table 2.
Technical Recommendations for Chromosome Banding Analysis in CLL

| Item                        | Recommendations | Remarks |
|-----------------------------|-----------------|---------|
| Materials                   |                 |         |
| Anticoagulants              | Heparin         | EDTA is not suitable for cytogenetic cultures. However, in such case, it is worth trying to wash the sample twice with sterile RPMI medium or 10× PBS and set up cultures (if there is no other option) |
| Cells/tissue                | Peripheral blood| Bone marrow or lymph node biopsies may be an option in those cases with few circulating clonal cells |
| Conditions                  | Set up cultures before 24 h sample obtaining | The transport time is highly relevant for samples with high WBC counts 20–22 |
| Methods\(^a\)\(^b\)\(^c\)\)  | Adjust cultures to 2 × 10^6 leucocytes/mL medium | Following this strategy, more cases with abnormal karyotypes are identified, as 5% to 20% of cases are found carrying aberration(s) only with 1 of the 2 mitogens\(^d\) |
| WBC count                   |                 |         |
| Mitogens                    | Set up 2 parallel cultures: Culture A: 50 µL TPA |         |
|                            | Culture B: 500 µL IL2 + 100 µL (10 nimol) DSP30 |         |
|                            | Culture A: incubate 72 h at 37°C, add 50 µL Colcemid, incubate 2 h, harvest |         |
|                            | Culture B: incubate 48h at 37°C, add 100 µL Colcemid, incubate 16–24 h, harvest |         |
| Analysis                    |                 |         |
| Number of metaphases        | 20, fully analyzed | Recommended to avoid overlooking subclonal aberrations and complexity underestimation\(^e\) |
| Interpretation-clinical significance |                 | HC (≥5 structural/numerical abnormalities in the same clone) is generally associated with unfavorable prognosis, excepting patients with a CK harboring +12, +19. The predictive significance of high-CK in the era of novel agents is still unclear |

\(^a\)Harvesting, slides preparation, aging, and staining are performed following standard cytogenetic procedures.\(^b\)DSP30: sequence: 5′-TcGgTsCgsCtgCsTcgcCtcTcgcCtcCtcTcgcCtcCtcTcgcCtcCtcTcgcCtcCcC.
\(^c\)Colcemid concentration 0.15g/mL.
\(^d\)OX = complex karyotype; CLL = chronic lymphocytic leukemia; EDTA = ethylenediaminetetraacetic acid; HC = high-complexity; PBS = phosphate-buffered saline; RPMI = Roswell Park Memorial Institute; WBC = white blood cell; TPA = 12-O-tetradecanoly-phorpol-13-acetate.

risk stratification of patients with CLL. However, caution is still warranted since the published evidence derives from retrospective studies mostly performed outside clinical trials with all the implicit caveats.

i. Type of abnormalities

Multiple trisomies: Approximately 10% of cases with trisomy 12 also have trisomy 19 and are characterized by a distinctive constellation of features, including mutated IGHV genes, biased expression of lambda light chains, exclusive expression of IgG-switched heavy chains, a low prevalence of TP53 aberrations and a more indolent clinical course than cases with isolated trisomy 12. The great majority (~70%) of +12, +19 cases also display additional trisomies, usually +18, and/or structural abnormalities, most frequently del(13q), thereby fulfilling the criteria for CK.\(^1\)\(^5\)\(^6\)\(^7\)\(^8\) Such cases comprise 10% of all CK ≥3 cases and are characterized by an extremely indolent course with prolonged time to first treatment (TTFT) and OS which is longer than either other CK cases or cases without CK, including other M-CKL.\(^9\)\(^6\)\(^3\)\)

Specific copy number aberrations: In patients with CK, especially those with CK ≥5, the distribution of structural abnormalities not detected by the standard FISH panel is non-random. Many studies employing CBA or genomic arrays have identified recurring gains of 2p, 3q, 8q, and losses of 3p, 4p, 6q, 8p, 9p, 15q, and 18p, many of which encompass genes known to be relevant to CLL biology. In univariate analyses, gains of 2p and 8q and losses of 9p and 18p in unselected cohorts, and gains of 8q and losses of 3p, 8p, and 9p in cases with del(17p) (especially if due to i(17q)) have been associated with poor outcome in the chemo/chemoimmunotherapy era,\(^9\)\(^13\)\(^36\) while resistance to ibrutinib has been associated with loss of 8p and 18p.\(^37\)

However, larger cohorts will be required to demonstrate independent prognostic significance of specific copy number aberrations in multivariable analyses which include CK ≥5.

iii. Presence of subclones: CKs may show considerable karyotypic heterogeneity. In one study, the median number of clonal aberrations was 7 (range 3 to 17) and clonal evolution or a composite karyotype (when clonal heterogeneity was too complex to allow enumeration of individual subclones) was found in 74% of patients.\(^3\)\(^2\) The potential clinical significance of measuring clonal heterogeneity is supported by preliminary results using a new method for inferring clonal heterogeneity from SNP array data. When applied to samples from 238 previously untreated CLL patients, 44% had >1 clone with a maximum of 3 clones. The presence of multiple clones was significantly associated with Rai stage, age at diagnosis, del(17p), and del(11q). There was a statistically significant independent association between the presence of multiple clones and OS even when accounting for CK ≥3 as a potential confounder.\(^3\)\(^8\) Of note, molecular methodologies such as short read whole genome sequencing (WGS) or CMA are unable to identify the different independent clones and therefore cannot provide information on the intraclonal cytogenetic heterogeneity.

Summary statement

The well-known heterogeneity of CLL extends even within patients with cytogenetic complexity, who should not be considered a priori equivalent.

Chromosome microarray analysis

CMA was initially developed as a genetic discovery tool in research laboratories in the 1990s. A decade later, this was moving rapidly into the clinical cytogenetic laboratories for the evaluation of constitutional chromosomal aberrations as well
as acquired genomic abnormalities in human cancers, including hematological malignancies such as CLL.

Nowadays, most cytogenetic laboratories apply Agilent, Affymetrix, or Illumina platforms for CMA in hematological malignancies. The Illumina and Affymetrix SNP-arrays effectively combine the detection of CNAs and copy-neutral events (CN-LOH) with very high resolution.

**Analysis and interpretation of microarray data in CLL**

Tools for analysis and criteria for interpretation and reporting of microarray-based genomic profiling have been previously provided. Cytogenetic laboratories have primarily used these guidelines in CLL diagnostics. In brief, interpretation criteria are set in such a way that non–tumor-related copy number changes are excluded. Only gross CNAs (≥5 Mb) and CN-LOH (≥10 Mb and extending to the telomeres) are considered as tumor-associated abnormalities. Focal CNAs (<5 Mb) are only reported when they involve known tumor-related genes (https://cancer.sanger.ac.uk/cosmic).

**Clinical use of chromosomal microarray analysis in CLL**

Microarrays are well suited for genome-wide analysis in CLL resulting in accurate detection of chromosome abnormalities with established prognostic value in CLL (trisomy 12, deletions of 13q14, deletions of 11q22, and deletions of 17p13) but also allowing for the discovery of genomic complexity (GC). That said, small clones may be overlooked. The presence of 3 or more genomic abnormalities is signified as GC and is associated with shorter survival and advanced disease. Many relapsed/refractory CLL cases display GC with additional CNAs next to similar profiles with Chun et al. Very similar to CBA analysis, provided GC in low-GC (0 to 2 CNAs), intermediate-GC (3 to 4 CNAs) retrospectively from real-world databases, exhibiting largely concordant results when compared to CBA studies. That said, high GC (≥5 CNAs) has been recently associated with dismal clinical outcome also in a clinical trial context (MURANO trial) in patients treated with both standard chemoimmunotherapy (bendamustine + R) and novel agents (BCL-2 inhibitor venetoclax + rituximab).

Presently, CBA is the only standardized methodology that is capable of providing information of the whole genome, while also allowing the overview of the clonal landscape and intraclonal hierarchy. Novel methodologies such as WGS appear promising. However, despite the advances regarding the technical standardization several issues remain open particularly regarding the harmonization of the interpretation of the WGS findings in the clinical setting and whether it can replace CBA without the need of validation. CBA offers the possibility to detect chromosomal aberrations not covered by the standard FISH panels/probes and also reveals the existence of CK which is not possible using standard FISH-based diagnostics. Still, it is not yet precisely known how to use CBA information in the clinical setting and open questions abound. Indicatively, should all CKs be considered as equivalent? Should chemoinmunotherapy regimen be avoided in patients with CK and, if yes, which should be the recommended treatment? Should the search for cytogenetic complexity by CBA be undertaken in all patients before treatment or only restricted to cases lacking TP53 aberrations? Besides the aberrations included in the standard FISH-analysis, are there additional recurrent cytogenetic abnormalities with prognostic or predictive significance in CLL, especially in the era of the novel agents? If access to a robust methodology is available, when should CBA be performed in CLL? How should CBA results be reported and interpreted in CLL? Could CBA be replaced by a less cumbersome method?

Starting from the final question, despite recent advances in next generation sequencing, the time is not yet right for giving up CBA as the routine diagnostic setting. WGS may appear as an attractive future alternative, however, several issues, including both high cost and the lack of methodological standardization and harmonization, preclude considering it as a realistic imminent option. As mentioned above, CMA could serve as a surrogate methodology, at least for the identification of cases with high GC. That said, even within this group of patients there is significant discordance between CBA and CMA.
Since the precise significance of CK in CLL is still debatable, ERIC holds the view that the most prudent policy is “watch and wait” until the evidence is substantial and robust enough for allowing safe recommendations. We therefore encourage performing CBA before treatment administration but dissuade from using only CBA findings for treatment decision making. Identification of CK seems highly relevant clinically but caution is needed to avoid overdiagnosis. In the absence of TP53 aberrations, low and intermediate CK (3 and 4 chromosomal aberrations, respectively) should not be considered by default as a synonym for aggressive disease. Moreover, in line with the notion that cut-offs in medicine are often arbitrary, we should refrain from considering all CKs as equivalent, a view amply exemplified by the case of CK with +12, +19 (Figure 1). Finally, we strongly recommend to always perform CBA within clinical studies and endorse the inclusion of patients with CK or other cytogenetic abnormalities of potential clinical significance in clinical trials with novel agents.

Regarding CMA, we definitely encourage its inclusion in the context of clinical trials, however not yet in routine clinical practice. More generally, we would also like to underscore the fact that, despite being capable to detect recurrent aberrations that dictate treatment choices, CMA is less appropriate than CBA for the detection of CK, particularly due to its inherent limitation to detect structural aberrations (ie, translocations), although, admittedly, it may be informative for the grade of GC.19 Evidently, therefore, more data are needed to reach definitive conclusions.

CONCLUSION

Mounting evidence suggests that CBA has the potential of becoming a useful diagnostic tool in CLL, especially as it is standardized end-to-end. However, the clinical significance of the CBA findings is not yet fully elucidated, especially regarding CK but also other cytogenetic abnormalities not included in the standard CLL FISH-panels. The same stands for CG detected by CMA. To obtain definitive insight into the applicability/relevance of CBA findings in the routine clinical setting, we recommend to systematically perform CBA as a standard assessment in the context of prospective clinical trials. If available, CMA could also be performed either as a complement to CBA or as a sole methodology when CBA is not feasible. On the other hand, we dissuade from using only CBA/CMA findings for treatment decision making since the predictive value of cytogenetic findings such as CK is still debatable, especially in the era of targeted treatments.

AUTHOR CONTRIBUTIONS

PB, BE, CM, APK, DO, CH, and KS conceived the idea, analyzed data, and wrote the manuscript. MJ, PG, and AA made a critical review of the document. All authors read and approved the final version of the manuscript.

REFERENCES

1. Han T, Ozer H, Sadamori N, et al. Prognostic importance of cytogenetic abnormalities in patients with chronic lymphocytic leukemia. N Engl J Med. 1984;310:288–292.
2. Oscier DG, Gardiner AC, Mould SJ, et al. Multivariate analysis of prognostic factors in CLL: clinical stage, IGVH gene mutational status, and loss or mutation of the p53 gene are independent prognostic factors. Blood. 2002;100:1177–1184.
3. Bentz M, Huck K, du Manoir S, et al. Comparative genomic hybridization in chronic B-cell leukemias shows a high incidence of chromosomal gains and losses. Blood. 1995;85:3610–3618.
4. Döhner H, Stilgenbauer S, Döhner K, et al. Chromosome aberrations in B-cell chronic lymphocytic leukemia: reassessment based on molecular cytogenetic analysis. J Mol Med (Berl). 1999;77:266–281.
5. Döhner H, Stilgenbauer S, Benner A, et al. Genomic aberrations and survival in chronic lymphocytic leukemia. N Engl J Med. 2000;343:1910–1916.
6. Hallék M, Cheson BD, Catovsky D, et al. Guidelines for the diagnosis and treatment of chronic lymphocytic leukemia: a report from the International Workshop on Chronic Lymphocytic Leukemia updating the National Cancer Institute-Working Group 1996 guidelines. Blood. 2008;111:5446–5456.
7. Hallaert DY, Jaspers A, van Noesel CJ, et al. c-Abl kinase inhibitors overcome CD40-mediated drug resistance in CLL: implications for therapeutic targeting of chemoresistant niches. Blood. 2008;112:5141–5149.
8. Put N, Konings P, Rack K, Belgian Cytogenetic Group for Hemato-Oncology (BCGHO), et al. Improved detection of chromosomal abnormalities in chronic lymphocytic leukemia by conventional cytogenetics using CpG oligonucleotide and interleukin-2 stimulation: a Belgian multicentric study. Genes Chromosomes Cancer. 2009;48:843–853.
9. Haferlach C, Dicker F, Schnittger S, et al. Comprehensive genetic characterization of CLL: a study on 506 cases analyzed with chromosome banding analysis, interphase FISH, IgV(H) status and immunophenotyping. Leukemia. 2007;21:2442–2451.
10. Dicker F, Schnittger S, Haferlach T, et al. Immunostimulatory oligonucleotide-induced metaphase cytogenetics detect chromosomal aberrations in 80% of CLL patients: a study of 132 CLL cases with correlation to FISH, IgVH status, and CD38 expression. Blood. 2006;108:3152–3160.
11. Rack KA, van den Berg E, Haferlach C, et al. European recommendations and quality assurance for cytogenomic analysis of haematological neoplasms. Leukemia. 2019;33:1851–1867.

12. Rigolín GM, Cibien F, Martellini S, et al. Chromosome aberrations detected by conventional karyotyping using novel mitogens in chronic lymphocytic leukemia with “normal” FISH: correlations with clinicobiologic parameters. Blood. 2012;119:2310–2313.

13. Cuneo A, Rigolín GM, Bigoni R, et al. Chronic lymphocytic leukemia with 6q− shows distinct hematological features and intermediate prognosis. Leukemia. 2004;18:476–483.

14. Kostopoulou F, Gabillard C, Chapiro E, French Innovative Leukemia Organization (FILO) group, et al. Gain of the short arm of chromosome 2 (2p gain) has a significant role in drug-resistant chronic lymphocytic leukemia. Cancer Med. 2019;8:3131–3141.

15. Chapiro E, Radford-Weiss I, Bastard C, et al. The most frequent t(14;19)(q32;q13)-positive B-cell malignancy corresponds to an aggressive subgroup of atypical chronic lymphocytic leukemia. Leukemia. 2008;22:2123–2127.

16. Sargent R, Jones D, Abruzzo LV, et al. Customized oligonucleotide array-based comparative genome hybridization as a clinical assay for genomic profiling of chronic lymphocytic leukemia. J Mol Diagn. 2009;11:25–34.

17. Simons A, Sikkema-Raddatz B, de Leeuw N, et al. Genome-wide arrays for repeated cytogenetic testing in acquired haematological neoplastic disorders. Genes Chromosomes Cancer. 2016;55:480–491.

18. Ramos-Campoy S, Puiggros A, Béa S, et al. Chromosome banding analysis and microarrays are both useful but not equivalent methods for genomic complexity risk stratification in chronic lymphocytic leukemia patients. Haematologica. 2022;107:593–603.

19. Baliakas P, Puiggros A, Xochelli A, et al. Additional trisomies amongst CLL study group (FILO). CHRONIC lymphocytic leukemia: definitions, associations, and clinical impact. Blood. 2019;133:1205–1216.

20. Haferlach C, Bacher U. Cytogenetic methods in chronic lymphocytic leukemia. Methods Mol Biol. 2011;730:119–130.

21. Bacher U, Haferlach T, Alpermann T, et al. Several lymphoma-specific genetic events in parallel can be found in mature B-cell neoplasms. Genes Chromosomes Cancer. 2011;50:43–50.

22. Miller CR, Heerema NA. Culture and harvest of CpG-stimulated peripheral blood or bone marrow in chronic lymphocytic leukemia. Methods Mol Biol. 2019;1881:27–34.

23. Ben-David U, Amon A. Context is everything: aneuploidy in cancer. Nat Rev Genet. 2020;21:44–62.

24. Döhner H, Estey E, Grimwade D, et al. Diagnosis and management of AML in adults: 2017 ELEN recommendations from an international expert panel. Blood. 2017;130:2444–2477.

25. Lazaryan A, Dolan M, Zhang MJ, et al. Impact of cytogenetic abnormalities on outcomes of adult Philadelphia-negative acute lymphoblastic leukemia after allogeneic hematopoietic stem cell transplantation: a study by the Acute Leukemia Working Committee of the Center for International Blood and Marrow Transplant Research. Haematologica. 2020;105:1329–1338.

26. Baliakas P, Jeromin S, Ikas M, et al. Cytogenetic complexity in chronic lymphocytic leukemia: increased chromosomal instability and breakpoint clustering in heterochromatic regions. Ann Hematol. 2017;96:1493–1500.

27. Baliakas P, Ikas M, Gardiner A, et al. Chromosomal translocations and karyotype complexity in chronic lymphocytic leukemia: a systematic reappraisal of classic cytogenetic data. Am J Hematol. 2014;89:249–255.

28. Jaglowski SM, Ruppert AS, Heerema NA, et al. Complex karyotype predicts for inferior outcomes following reduced-intensity conditioning allogeneic transplant for chronic lymphocytic leukemia. Br J Haematol. 2012;159:82–87.

29. Baliakas P, Puiggros A, Kochelli E, et al. Additional trisomies amongst patients with chronic lymphocytic leukemia carrying trisomy 12: the accompanying chromosome makes a difference. Haematologica. 2016;101:e299–e302.

30. Leekassa AC, Baliakas P, Moysiadis T, et al. Genomic arrays identify high-risk chronic lymphocytic leukemia with genomic complexity: a multi-center study. Haematologica. 2021;106:87–97.

31. Kadrí S, Lee J, Fitzpatrick C, et al. Clonal evolution underlying leukemia progression and Richter transformation in patients with ibrutinib-refractory CLL. Blood Adv. 2017;1:715–727.

32. Zucker MR, Abruzzo LV, Herling CD, et al. Inferring clonal heterogeneity in cancer using SNP arrays and whole genome sequencing. Bioinformatics. 2019;35:2924–2931.

33. Haraksingh RR, Abyzov A, Urban AE. Comprehensive performance comparison of high-resolution array platforms for genome-wide Copy Number Variation (CNV) analysis in humans. BMC Genomics. 2017;18:321.

34. Cooley LD, Lebo M, Li MM, et al. American College of Medical Genetics and Genomics technical standards and guidelines: microarray analysis for chromosome abnormalities in neoplastic disorders. Genet Med. 2013;15:484–494.

35. Gunnarsson R, Mansouri I, Isaksson A, et al. Array-based genomic screening at diagnosis and during follow-up in chronic lymphocytic leukemia. Haematologica. 2011;96:1161–1169.

36. Chun K, Wengler GD, Chaubey A, et al. Assessing copy number aberrations and copy-neutral loss-of-heterozygosity across the genome as best practice: an evidence-based review from the Cancer Genomics Consortium (CGG) working group for chronic lymphocytic leukemia. Cancer Genet. 2018;228:229–236+250.

37. Kater AP, Wu JQ, Kipp T, et al. Venetoclax plus rituximab in relapsed chronic lymphocytic leukemia: 4-year results and evaluation of impact of genomic complexity and gene mutations from the MURANO phase III study. J Clin Oncol. 2020;38:4042–4054.

38. Hallek M, Cheson BD, Catovsky D, et al. Guidelines for diagnosis, indications for treatment, response assessment and supportive management of chronic lymphocytic leukemia. Blood. 2018;131:2745–2760.

39. Herling CD, Klaumünzer M, Rocha CK, et al. Complex karyotypic and Kras and Poi1 mutations impact outcome in CLL after chimeric-bridged chemotherapy or chemoimmunotherapy. Blood. 2016;128:395–404.

40. Wendtner C-M, Dreger P, Eichhorst B, et al. Chronic Lymphatische Leukämie (CLL). 2020. Available at: https://www.onkopedia.guide/guide/index.html#guide-line/html/index.html. Last accessed March 22, 2022.

41. Quinqueløn A, Aurran-Schelinzit T, Clavert A, et al. Diagnosis and treatment of chronic lymphocytic leukaemia: recommendations of the French CLL study group (FILO). HemaSphere. 2020;4:e475.

42. Stilgenbauer S, Sander S, Bullinger L, et al. Clonal evolution in chronic lymphocytic leukaemia: acquisition of high-risk genomic aberrations associated with unmutated VH, resistance to therapy, and short survival. Haematologica. 2007;92:1242–1245.

43. Kreuzer KA, Furman RR, Stilgenbauer S, et al. The impact of complex karyotype on the overall survival of patients with relapsed chronic lymphocytic leukaemia treated with idelalisib plus rituximab. Leukemia. 2020;34:296–300.

44. Al-Sawaf O, Lilienweiss E, Bahlo J, et al. High efficacy of venetoclax plus obinutuzumab in patients with complex karyotype and chronic lymphocytic leukaemia. Blood. 2020;135:866–870.

45. O’Brien S, Furman RR, Coutre S, et al. Single-agent ibrutinib in treatment-naive and relapsed/refractory chronic lymphocytic leukaemia: a 5-year experience. Blood. 2018;131:1910–1919.