
Chronic Lymphocytic Leukemia: Current Knowledge and Future Advances in Cytogenomic Testing

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Cite this chapter as: Wainman LM, Khan WA, Kaur P. Chronic Lymphocytic Leukemia: Current Knowledge and Future Advances in Cytogenomic Testing. In: Sergi CM, editor. *Advancements in Cancer Research*. Brisbane (AU): Exon Publications; Online first 08 Feb 2023. p. 93–106

Doi: <https://doi.org/10.36255/chronic-lymphocytic-leukemia>

Abstract: Chronic lymphocytic leukemia (CLL) is the most common leukemia in Western countries. CLL remains incurable despite improvements in clinical outcomes from the identification of prognostic markers and the introduction of targeted therapies. Recent studies have identified differences in the epigenetic and the regulatory landscape of CLL that may provide molecular targets for future therapies. Optical genome mapping (OGM) is a new method that may improve clinical testing and CLL patient care because it can provide greater sensitivity and resolution of structural variation (SV) that is currently detected by chromosome banding analysis (CBA). The practical issues around diagnosis, molecular cytogenetic prognostic markers, pathobiology, and targeted therapies are discussed with brief reference to OGM.

In: Sergi CM, editor. *Advancements in Cancer Research*. Exon Publications, Brisbane, Australia. ISBN: 978-0-6453320-9-4. Doi: <https://doi.org/10.36255/advancements-in-cancer-research>

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Keywords: B cell Receptor; bcl2 inhibitors; chronic lymphocytic leukemia; *IGHV* mutation; optical genome mapping

INTRODUCTION

Chronic lymphocytic leukemia (CLL) is a B-cell leukemia with an incidence of 4.2/100,000 people per year and a median age of diagnosis of 72 years (1). CLL affects older adults and only about 10% of patients have been reported to be younger than 55 years of age. At 80 years of age, the incidence rate increases to 30/100,000 people per year. Risk of developing CLL is about two-times higher for men than for women. Racial differences are seen; non-Hispanic whites have the highest incidence rates for CLL, followed by blacks (2). Individuals with a family history of CLL have ~5-8-fold increased risk of developing CLL (3, 4). Genome-wide association studies have identified over 40 single nucleotide polymorphisms in nearly 30 loci, such as *IRF4*, *LEF1*, *BCI2*, and *TERT* that are associated with familial CLL, suggesting the role of genetic variation in CLL (5). Exposure to agent Orange and the contaminating chemical 2,3,7,8-tetrachlorodibenzodioxin is another risk factor for CLL (6).

CLL is characterized by the accumulation of functionally incompetent B lymphocytes in the bone marrow, blood, spleen and lymph nodes. As per the 5th edition of World Health Organization, diagnosis of CLL requires the presence of at least $5 \times 10^9/L$ or more peripheral blood monoclonal B-cells for a duration of at least 3 months, with characteristic CLL morphology and phenotype (7). The CLL cells, by flow cytometry, typically demonstrate light chain restriction (dim expression), CD19⁺, CD5⁺, CD23⁺, CD20^{dim+}, CD43⁺, CD200⁺, CD11c⁺variable, ROR1⁺, IgM^{dim+}, IgD^{+/-} (IgG⁺ in ~10% cases), CD10⁻, CD79b⁻, FMC7⁻, CD25⁻, CD103⁻, CD81⁻. A major consensus identified CD19⁺, CD5⁺, CD23⁺, CD20^{dim}, surface light chain as markers essential for the diagnosis of CLL and the rest as additional useful markers (8). CLL cells are monomorphic small mature-appearing lymphocytes with dense nuclear chromatin, scant cytoplasm and no significant nucleoli (9). Prolymphocytes are larger than typical CLL cells, have less-condensed nuclei and a single prominent nucleolus and should be < 55% of all lymphocytes. Patients who do not fulfil the quantitative criteria are classified as having monoclonal B-cell lymphocytosis (MBL) with a CLL phenotype. Bone marrow is usually hypercellular, and show an interstitial, nodular, diffuse or a mixed pattern of infiltration. As per the International Workshop on CLL, CLL cells should account for > 30% of all cells at the time of diagnosis. The extent of marrow infiltration correlates with prognosis and stage and the diffuse pattern is typically associated with advanced disease (10, 11).

In a related category, small lymphocytic lymphoma (SLL) is tissue/lymph node-based lymphoma with CLL phenotype, without cytopenias due to bone marrow infiltration, and < $5 \times 10^9/L$ peripheral blood clonal B-cells (12). Lymph nodes are enlarged (>1.5cm), with architecture partially or completely effaced by a diffuse infiltration of small lymphoid cells, often with variably prominent pale-staining proliferation centers (PCs) containing larger cells (either prolymphocytes or paraimmunoblasts, which are larger cells with round to oval nuclei, dispersed chromatin, central nucleoli and pale cytoplasm). By immunohistochemistry, SLL,

in addition to CLL associated phenotypic markers, also stain for LEF1, and are negative for Cyclin d1 and SOX11.

For practical purposes, CLL and SLL are considered the same disease and clinically treated as such. CLL/SLL can transform to aggressive lymphomas such as diffuse large B-cell lymphoma (Richter transformation- most common; 10% of all cases), Hodgkin Lymphoma (rare), plasmablastic lymphoma (rare), B lymphoblastic leukemia/lymphoma (rare), and Prolymphocytic leukemia (rare) (13). SLL cases with very large, prominent/confluent PCs (>20x field) or with high proliferation indices (>2.4 mitoses/PC or >40% Ki67⁺ in PCs) are designated “histologically aggressive” CLL/SLL (7). CLL cases can present concurrently with additional neoplasms such as Hodgkin lymphoma and plasma cell neoplasms especially in bone marrow.

PROGNOSTIC MARKERS

The Binet and Rai staging systems are two well-known clinical staging systems for CLL (14, 15). These staging systems are based on clinical parameters such as lymphocytosis, organomegaly, and cytopenia (anemia and thrombocytopenia). Low stage CLL is better delineated with additional prognostic cytogenetic-molecular markers.

IGHV mutation status

Damle and Hamblin et al. described two subtypes of CLL based on the mutation status of the immunoglobulin heavy-chain variable region (*IGHV*), with greater than 2% deviation from the germline sequences considered as **mutated *IGHV* (M-CLL)** and others as **unmutated *IGHV* (U-CLL)** (16, 17). This is a strong prognostic indicator and currently recommended in NCCN guidelines to be measured directly by sequencing instead of the use of surrogate markers such as Zap-70 (18). U-CLL cells originate from naïve B-cells and are associated with aggressive disease as compared to M-CLL cells that arise from a post-germinal center B-cell, undergo somatic hypermutation and exhibit good prognosis with low-risk genetic alterations. Additionally, Damle et al. found that patients with U-CLL showed significantly shorter telomeres (mean, 2.45 kb; range, 0.9–3.4 kb) than those with M-CLL (mean, 4.39 kb; range, 0.9–9.7 kb) indicating a higher proliferation history of CLL cells in U-CLL patients and potentially explaining poor prognosis associated with U-CLL (19).

Immunophenotypic markers

Immunophenotypic markers CD38, ZAP-70 and CD49d are used when *IGHV* mutation status cannot be directly tested per the NCCN clinical practice guidelines (18). **CD38** is a cell surface glycoprotein. **CD38** expression has been used as a surrogate marker for *IGHV* unmutated status and is shown as an independent prognostic factor for aggressive disease (17, 20). Its use has been hampered by discordance in the cutoff value and its variable expression over time (21). **ZAP-70 (Zeta-chain-associated protein kinase 70)** is expressed in normal pro/pre

B-cells, but not in mature B-cells and its expression is surrogate for *IGHV* unmutated status (22). ZAP-70 expression level appears to be constant during the course of disease and its protein expression is an independent predictor of time to first treatment. **CD49d**, is the $\alpha 4$ integrin subunit complexed with CD29 (the $\beta 1$ subunit) and high levels of CD49d protein, assessed by flow cytometry, is significantly associated with shorter time to treatment (23).

Cytogenetic markers

Cytogenetic markers are used because acquired chromosomal abnormalities are observed in approximately 80% of individuals with CLL. Cytogenetic markers can be used to categorize patients into prognostic groups: deletion 13q (median survival 133 months); deletion 11q (median survival 79 months); trisomy 12 (median survival 114 months); normal cytogenetics (median survival 111 months); and deletion 17p (median survival 32 months) (24). Deletion **13q14.3** is the most common chromosomal abnormality detected by banding and FISH techniques in CLL occurring in 40-60% of patients. The 13q14 chromosomal locus can be inactivated by other mechanisms such as copy neutral loss of heterozygosity and epigenetic silencing (25, 26). This region contains several genes including *DLEU7*, *miR15a* and *miR16* which are now recognized as tumor suppressor genes (27). Deletions within the chromosome **17p13** locus have been reported in 4 to 16% of the cases of CLL and show poor survival due to advanced disease at diagnosis, short time to first treatment, and high risk of chemorefractoriness to alkylating agents and purine analogues (28). *TP53* mutations can be seen in the absence of deletion 17p13 in at least 20% of the cases (29). The region **11q22.3-q23.1** deleted in CLL patients contains the tumor suppressor gene ataxia telangiectasia mutated (*ATM*) and is involved in DNA damage control. Disruption of *ATM* in CLL can occur either due to deletion of 11q or due to the presence of variants in the *ATM* gene. Alterations to *ATM* may portend treatment failures after chemotherapeutic drugs such as chlorambucil and fludarabine, rendering CLL cells resistant to apoptosis (30, 31). **Trisomy 12** defines a subgroup of CLL with more frequent atypical morphology including prolymphocytes, strong surface immunoglobulin and *FMC7* expression, and intermediate to poor prognosis (32). 50% of CLL patients show a single chromosomal abnormality, 25% display two chromosomal abnormalities, and the remaining cases demonstrate complex chromosome changes (24, 33). Figure 1 is showing an example of FISH using probes for centromere 12 and probes targeting 13q14.3 and karyotyping of a CLL patient sample where both techniques are necessary to detect all somatic structural variants (SVs).

Novel molecular variants

Whole genome/exome sequencing has uncovered novel somatic variants in CLL that also contribute to prognostic information and cellular transformation. The most frequently mutated genes in CLL are *NOTCH1* (10–15%), *SF3B1* (10%), *TP53* (5–10%), *ATM* (10-15%), and *MYD88* (3–8%) (34, 35). All of these frequently mutated genes except *MYD88* are associated with U-CLL. Several low frequency (less than 5%) genetic alterations are observed in *BIRC3*, *XPO1*, *CHD2*, *POT1*,

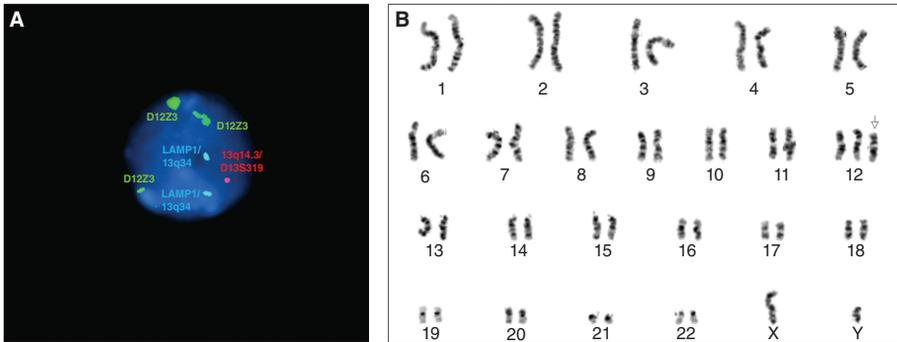


Figure 1. A representative FISH and karyotype analysis for CLL patient sample. **A:** Interphase FISH image using probes for centromere 12 (spectrum green) and locus specific probe targeting 13q14.3/ D13S319 (spectrum red) is shown. A control FISH probe mapping to LAMP1/13q34 locus is shown in spectrum aqua (Abbott Molecular, Inc.). A total of 72 out of 200 cells with chromosome 12 signal gain pattern (3 fluorescent signals of spectrum green) and 13q14.3 signal deletion patterns (one fluorescent signal of spectrum red) were observed from the FISH analysis. **B:** Findings in A were confirmed with the cytogenetic analysis of the B-cell mitogen-stimulated bone marrow preparation. Chromosome analysis revealed an abnormal male chromosome complement with three copies of chromosome 12 (arrowed) in nineteen out of 20 cells examined. Trisomy 12 is a commonly reported chromosomal aberration seen in B-cell CLL and is generally associated with an intermediate prognosis.

HIST1H1E, *NRAS*, *BCOR*, *ZMYM3*, *RIPK1*, *SAMHD1*, *KRAS*, *MED12*, *ITPKB*, *DDX3X2*, *EGR2*, *FBXW7*, *KLHL6*, *MAPK1*, and *RP1B* (36, 37). Activating mutations of *NOTCH1* are present in ~4–13% of CLL cases with one recurrent mutation; a 2-bp frameshift deletion [NM_017617.5: c.7544_7545del; p.(Glu2515ValfsTer3)] which accounts for approximately 80% of all *NOTCH1* alterations (38). *NOTCH1* alterations are more frequent in the U-CLL gene subtype of CLL (20.4%), fludarabine-refractory CLL disease, and 30% of patients with Richter's syndrome. *SF3B1* gene is located in the chromosome 2q33.1 and is a central component of the U2 spliceosome, which promotes excision of introns from pre-mRNA to form mature mRNA (39). *SF3B1* alterations are associated with faster disease progression, poor overall survival, and are observed more frequently in individuals with unmutated *IGHV* (36). *MYD88* gene mutations are seen in 3–10% of CLL cases. The recurrent *MYD88* variant (L265P) in CLL causes constitutive *MYD88*-*IRAK* signaling, resulting in constitutive NF- κ B activity. *MYD88* L265P alterations are associated with mutated *IGHV*, low levels of ZAP-70 and CD38 expression, and normal levels of β 2M portending favorable outcomes (40). *BIRC3* gene is a negative regulator of alternative NF- κ B signaling pathway. Alterations in *BIRC3* are noted in less than 5–8% of cases and lead to the activation of alternative NF- κ B pathway. Targeted sequencing of the *BIRC3* coding sequence in CLL showed that *BIRC3* inactivation is particularly common in fludarabine-refractory patients (24%) (41). *BIRC3* disruptions have been associated with unmutated *IGHV* gene configuration and 11q deletion with an inferior progression-free survival (42).

The CLL epigenome reflects cell of origin and is characterized by CLL-specific changes. Distinct DNA methylation signatures have been identified that largely correspond with *IGHV* mutation status and the associated prognoses, but an

intermediate DNA methylation profile has been identified in patient samples that corresponds to intermediate prognosis (43). Changes in the DNA methylation profiles of CLL patients are observed in progressive disease indicating that the CLL methylome can change during disease (44). CLL is characterized by an increase of open chromatin compared to normal B-cells (45–47). These regions of open chromatin contain 498 *de novo* active regions (including gained active genes and enhancers) and are enriched for NFAT family, FOX family, and TCF/LEF family binding motifs indicating transcription factors could serve as CLL-specific therapeutic targets (45). The chromatin landscape can distinguish CLL by *IGHV* mutation status with U-CLL associated with an increase in active chromatin compared to M-CLL (45). These findings indicate changes in the regulatory landscape may explain gene expression and prognosis differences between the two CLL subtypes. Furthermore, a genome-wide examination of CLL enhancers revealed CLL-specific enhancers were near genes important in CLL pathogenesis (*CXCR4*, *CD74*, *PAX5*, *CD5*, *KRAS*, and *BCL2*) (47). *PAX5* is identified as a key transcription factor regulating CLL enhancers and it is known to regulate genes associated with CLL pathology (*BCL2*, *CXCR4*, and *CD83*) making it a potential therapeutic target (47). Examination of the CLL epigenome has revealed that transcription factors and other molecular molecules that alter the epigenome could be therapeutic targets for future CLL treatments.

MicroRNAs (MiR)

MiR-16-1, miR-26a, miR-206, and miR-223, miR-155, miR-21, miR-150, miR-92 and miR-222, miR-181, miR-30d and let-7a are all differentially expressed in CLL cells compared to normal B-cells (48-50). The most promising MiR connection with CLL is the seminal finding that deletion of the 13q14 locus contains the *DLEU2* gene and the *miR-15a/miR-16-1* cluster (27). Cimmino et al. found that *miR-15a* and *miR-16-1* function as tumor suppressor genes by modulating the expression of *BCL2*, an anti-apoptotic protein that is highly expressed in CLL.

CLL PATHOGENESIS

Intratumoral heterogeneity in CLL is characterized by genetic, epigenetic, and transcriptional alterations that result in different clinical behaviors with a subset showing an aggressive clinical course (51). Traditionally, CLL has been defined as a disease of immunologically incompetent B-cells with presumed slow birth and death rates, but studies indicated that CLL cells are highly proliferative (52). Messmer et al. examined CLL cell proliferation by measuring CLL birth and death rates in vivo. The study demonstrated that the leukemic cells display proliferation rates between 0.10% and 0.81% per day of new leukemic cells compared to age matched healthy individuals with proliferation rates of 0.10% to 0.30% per day (53). In another study, Damle et al. demonstrated that CLL cells have shorter telomeres than normal age-matched B-cells suggesting that leukemic cells have extensive proliferative histories (19). Additionally, Damle et al. found that telomere

length corresponded with patient *IGHV* mutation status indicating a difference in proliferation history of CLL cells depending on mutation status. Gene expression profiling studies by Klein and Rosenwald et al. showed a common and characteristic gene signature of CLL cells with at least 32 genes overexpressed in CLL compared to normal B-cell subsets (naive, centroblasts, centrocytes, memory) and various non-Hodgkin lymphoma subtypes such as follicular lymphoma, diffuse large cell lymphoma, and Burkitt Lymphoma (54, 55). The cellular origin of CLL remains unclear, but the postulated normal counterpart for CLL cells is an antigen experienced mature CD5⁺ IgM/IgD B-cell with mutated or unmutated *IgHV* genes (56). The genetic evolution of CLL cells including cytogenetic abnormalities and gene mutations all impact CLL cell proliferation and pathogenesis.

CLL has been hypothesized to be a B cell receptor (BCR) signaling dependent malignancy because *IGHV* gene mutation status correlates with clinical outcomes. 30–35% CLL patients express a nearly identical BCR repertoire termed “stereotyped” receptors and BCR signaling is central to CLL cell proliferation in the lymph node microenvironment (57). BCR “stereotypy” refers to the highly restricted and sometimes identical variable HCDR3 sequences among different CLL patients that is observed and nearly two-thirds of these patients having unmutated *IGHV* (57). Analysis has identified 23 “major” subsets of BCR signaling profiles indicating that CLL ontogeny is related by common antigenic determinants. These BCR subsets correlate with shared somatic mutations, similar genetic and epigenetic profiles of clones, and similar clinical outcomes (57). BCR signaling and the microenvironment is important for CLL cell proliferation and CLL cell survival. CLL cells, like healthy normal B-cells become activated upon antigen ligation to the BCR, resulting in proliferation and differentiation. CLL cells and normal B-cells depend on external signals from the microenvironment, such as antigens, cytokines, and cell-cell interactions. Cytokines IL-4, IL-10, and interferon-gamma in the microenvironment can rescue CLL cells from programmed cell death by decreasing the expression of anti-apoptotic proto-oncogene *BCL2* (58). CLL cell growth takes place in PCs in lymph nodes where they interact with T-cells, mesenchymal stromal cells, and macrophages called “nurse-like cells”, that promote BCR signaling and provide a favorable environment for cell growth (59, 60).

Many of the genetic alterations observed in CLL determine patient prognosis because they affect many different pathways and cellular processes such as DNA damage response, cell cycle, RNA splicing, metabolism, B-cell transcription, chromatin modifiers and microenvironment-dependent signaling pathways. Current therapeutics are used to target pathways important in CLL pathogenesis independently or in combination with chemotherapy/immunotherapy depending on each patients’ prognostic markers (Table 1). Two of the three currently recommended targeted therapeutics aim to disrupt the BCR signaling pathway: Bruton’s tyrosine kinase (BTK) and PI3K inhibitors. BTK and PI3K inhibitors both target different kinases in the BCR signaling cascade to reduce CLL cell proliferation and survival. BTK inhibitors disrupt the proliferation of CLL cells in the lymphatic tissues and cause CLL cells to redistribute to the peripheral blood resolving lymphadenopathy by decreasing tissue burden and leading to CLL cell death (61). *BCL2* inhibitors decrease the expression of *BCL2* and sensitize CLL cells to apoptosis.

TABLE 1

Current targeted therapeutics for CLL treatment

Pathway	Treatments	Indication For Use	Comment
BTK inhibitors	Acalabrutinib Zanubrutinib Ibrutinib	With or without del(17p)/TP53 mutation	NCCN 2023 guidelines (18)
BCL2 inhibitors	Venetoclax	With or without del(17p)/TP53 mutation	NCCN 2023 guidelines
PI3K inhibitors	Duvelisib Idelalisib	With or without del(17p)/TP53 mutation	NCCN 2023 guidelines

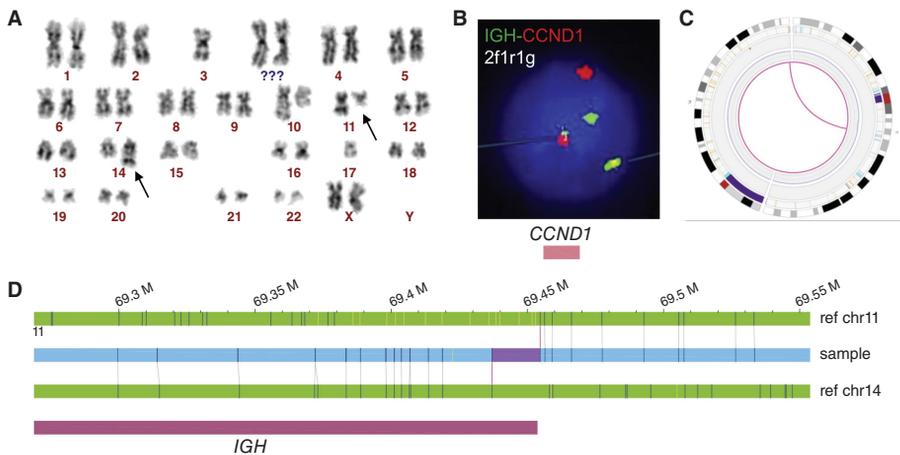
BTK = Bruton's tyrosine kinase

OPTICAL GENOME MAPPING ADVANCING CYTOGENOMIC TESTING

Novel advances in mapping the human genome are ushering in a new era of structural variant (SV) analysis. Recent commercialized efforts have developed multi-channel/massively-parallel platforms that permit high-throughput genome mapping of mega-base size DNA extracted from bone marrow, blood, fresh/frozen tissue, or tumor biopsy samples. An enzymatic reaction is used to place thousands of fluorescent labels throughout the genome at specific sequence motifs. The labeled DNA molecules are then linearized in nanochannel array on a chip and imaged in an automated manner on optical mapping instruments (e.g. Saphyr System; Bionano Genomics). Specific changes in patterning or spacing of the fluorescent labels are algorithmically processed and can be visualized to the nearest labeling site of the chromosomal aberration. This process is known as optical genome mapping (OGM). This technology allows for the accurate detection of SV (i.e., translocations, inversions, insertions, deletions, and tandem duplications of DNA), which shuffles genomic information that was previously unable to be resolved with a single assay.

The Bionano Saphyr System provides significantly enhanced sensitivity and specificity with >1000-fold improvement over karyotyping (62). Given these advances, it is becoming feasible to more comprehensively understand the spectrum of human genetic SVs and its role in disease processes and genome plasticity. With this approach SVs from 500 base pairs to 500 kilobases can be detected across the genome in an accurate and intuitive manner compared to standard of care technologies such as chromosome banding analysis (CBA), fluorescence in situ hybridization (FISH), and chromosomal microarray analysis (CMA) that have been in use for the last 40 years. This advanced technology can lead to therapeutic developments and provide data for clinical trials aiming to deploy targeted therapies specific to hematological malignancies and solid tumor pathology.

While CBA can provide a cost-effective manner to visualize the whole genome and information on balanced rearrangements is discernable, it does so at the cost of chromosomal SV resolution in the absence of critical gene-specific data. Further CBA requires chromosomal cell culture for CLL which involves the addition of specific adjuvants (e.g. CpG-oligodeoxynucleotide) to permit in vitro proliferation of the altered clones (63). Despite this special requirement, chromosome cell cultures may not be successful due to low number of mitoses; a pre-requisite for metaphase karyotype analysis. SVs in the form of copy number alterations can be defined at high resolution by CMA without having to culture samples, however balanced abnormalities remain covert by CMA and sub-clonal alterations (<20%) may be missed due to its lower sensitivity compared to single cell resolution approaches such as FISH. Finally, FISH is a targeted approach that provides single cell resolution and detection of low-level clones. It is, however, low-throughput, labor intensive, and lends itself to subjectivity in interpretation of complex fluorescent DNA probe rearrangement patterns (62). Conversely OGM is an *ab initio* genome wide approach that can consolidate these approaches into one technique and allow for higher sensitivity and specificity of SVs in a single assay (Figure 2). The improved resolution of SVs with OGM may also permit for the detection and understanding of complex karyotypes that can occur after chromothripsis, for example, which is observed in at least 2-3% of all cancers (64).



*CBA= chromosome banding analysis; OGM = optical genome mapping

Figure 2. Example of CLL CBA analysis compared to OGM to detect structural variants from a potential CLL vs. Mantle cell lymphoma differential diagnosis. **A:** Karyotype shows t(11;14) (q13;q32) as one of the pathogenic findings. **B:** Interphase FISH with *CCND1-IGH* dual color, dual fusion probes show a spectrum green (*IGH*), a spectrum red (*CCND1*), and two *CCND1-IGH* fusion signals. **C:** OGM circos plot shows chromosome 11 and 14 with a purple line in the middle of the plot connecting the translocated regions. **D:** Genome map view shows the translocation with the green bars depicting the reference map of chromosome 11 (upper bar) and chromosome 14 (lower bar). The blue bar represents the genome map of the test sample and the outside pink bars represent the genes of interest: *CCND1* and *IGH*. (From Mantere T, Neveling K, Pebrel-Richard C, et al. (65) PMID: 34237280; PMCID: PMC8387289; with permission)

Recent publications have demonstrated OGM's performance in the cytogenomic assessment of various hematological malignancies, with a focus on myeloid neoplasms such as acute myeloid leukemia, myelodysplastic syndrome and acute lymphoblastic leukemia. In these studies, OGM effectively detected clinically relevant abnormalities reported by standard of care approaches while providing, in some cases, new cytogenomic information (62, 66, 67). To this end, there has been a paucity of studies evaluating the utility of OGM in B-cell processes such as CLL (68). Puiggros et al. found 90.3% known alterations and identified additional structural information for aberrations in 55% of CLL patients showing OGM could be used for routine management of CLL patients and to ensure correct diagnosis of CLL using a single assay (Figure 2). CLL clinical testing includes identification of complex karyotypes and clonal evolution are typically associated with poor prognosis (69). Recent advances in genome mapping technology have demonstrated that OGM is able to provide the sensitivity to resolve simple as well as complex karyotypes and unravel chromothripsis in CLL patients.

CONCLUSION

Despite developments in understanding CLL pathology and improvements in CLL treatment, CLL remains a common hematological malignancy that is incurable in the majority of patients. Recent studies have identified changes in the epigenome and regulatory landscape between CLL cells and normal B-cells that may serve to better determine disease prognosis and provide targets for therapeutics to selectively target CLL cells. Advances in clinical testing and management of CLL patients using OGM is poised to improve the sensitivity of current testing methods and may lead to therapeutic developments. Overall, these advances in the understanding of CLL molecular landscape and application of new technologies in clinical testing can promote the development of personalized treatment for CLL patients.

Conflict of Interest: The authors declare no potential conflict of interest with respect to research, authorship and/or publication of this chapter.

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