

Optical genome mapping: technical basis and applications in hematological malignancies

Mapeo óptico del genoma: fundamentos tecnológicos y aplicaciones en neoplasias hematológicas

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Abstract

Cytogenomic analyses of hematological malignancies require the use of different techniques, whether combined or on isolation, for the detection of chromosomal abnormalities. Optical genomic mapping (OGM) is a new technology based on the analysis of long DNA molecules fluorescently labelled in specific sequences that create a unique pattern, allowing the simultaneous detection of numerical and structural abnormalities with higher sensitivity (up to 5%) and precision than karyotyping. The aim of this review is to describe the methodological and analytical basis of OGM, present the most relevant findings published to date, and raise future challenges regarding its implementation in diagnostic laboratories. Multiple studies have shown that OGM will potentially allow the cytogenetic approach of patients in a single test offering higher resolution (5 Kb vs 5-10 Mb) and better characterization of abnormalities than conventional techniques. This technique also presents some limitations such as a sensitivity limited to 20% for copy number alterations, or the inability to detect rearrangements affecting centromeric and/or telomeric regions. Its implementation in the routine diagnosis practice will require standardization of analysis criteria, validation of many of the new abnormalities identified through the technique, and comparative prospective studies to define in which entities and situations it can complement and/or replace the current methods.

Keywords: Optical genome mapping. Cytogenetics. Fluorescence in situ hybridization. Genomic microarrays. Hematological malignancies.

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Resumen

El estudio citogenómico de las neoplasias hematológicas requiere la aplicación de diferentes técnicas para la detección de alteraciones cromosómicas. El mapeo óptico del genoma (OGM) es una nueva tecnología basada en el análisis de moléculas largas de ADN marcadas fluorescentemente en secuencias específicas que generan un patrón único, permitiendo la detección simultánea de alteraciones numéricas y estructurales con una mayor sensibilidad (5%) y precisión que el cariotipo. El objetivo de esta revisión es describir las bases metodológicas y analíticas del OGM, presentar los hallazgos más relevantes publicados y plantear los retos futuros para su implementación en los laboratorios de diagnóstico. Numerosos estudios han demostrado que el OGM potencialmente permitirá realizar el abordaje citogenético de los pacientes en un único test, ofreciendo una mayor resolución (5 Kb vs. 5-10 Mb) y una mejor caracterización de las alteraciones que las técnicas convencionales. Esta técnica no está exenta de limitaciones, como la sensibilidad limitada al 20% para las alteraciones del número de copias y la incapacidad de detectar reordenamientos centroméricos o teloméricos. Su implementación requerirá una estandarización de los criterios de análisis y la validación de nuevas alteraciones, así como estudios prospectivos para definir en qué afecciones y situaciones podrá complementar o reemplazar a los métodos actuales.

Palabras clave: Mapeo óptico del genoma. Citogenética. Hibridación in situ fluorescente. Microarrays genómicos. Citogenómica. Neoplasias hematológicas.

Introduction

The cytogenomic study of hematologic neoplasms is based on the use of different techniques, either in isolation or combination, for the detection of chromosomal abnormalities with diagnostic, prognostic, or predictive value of treatment response. Karyotyping remains the standard technique in many hematologic neoplasms, because it provides an overall view of the genome and detects numerical (aneuploidies) and structural abnormalities¹. However, the limitations of karyotyping require additional studies with techniques such as fluorescent in situ hybridization (FISH) or genomic microarrays (SNP-array or CGH-array). However, these two are also limited, such as partial genome analysis in the former, or the inability to detect balanced rearrangements and low sensitivity in the latter².

Optical genome mapping (OGM) is a technology based on obtaining images of high molecular weight DNA molecules that are labeled using fluorescence at specific sequences, generating a unique labeling pattern in each region of the genome. Therefore, the comparison of the labeling pattern of each DNA molecule with a reference genome allows the simultaneous detection of numerical and structural abnormalities (both balanced and unbalanced) with greater sensitivity and precision than karyotyping (Table 1). The optical genome mapping technology was initially developed by Schwartz et al.³ at the University of Wisconsin-Madison (Madison, WI, United States) back in 1993 and has been adapted by Bionano Genomics® in its current version who has developed both the chemistry and imaging capture technology to achieve the current levels of resolution and productivity⁴. We should mention

that OGM is not a sequencing technique and does not identify changes to the DNA sequence (such as single nucleotide variants, SNVs, and small insertions or deletions [Indels]).

OGM technology has been successfully tested in the cytogenomic analysis of various hematologic neoplasms⁵⁻¹³. Numerous validation studies have shown that, with a minimum coverage of 400X, OGM can achieve a maximum sensitivity rate of 1%, 100% specificity, and positive predictive values > 80%. Consequently, OGM is poised as a technology with the potential to complement or replace classical cytogenetic tests in the near future¹⁴.

The aim of this review is to provide a practical description of the methodological and analytical foundations of OGM, present the most relevant findings published to this date regarding different hematologic neoplasms, and outline future challenges for its implementation in hematologic diagnostic laboratories. This review has been agreed with members of the OGM working group established within the Spanish Cooperative Group of Hematologic Cytogenetics of the Spanish Society of Hematology and Hemotherapy.

Technical considerations for genome optical mapping studies

Pre-analytical requirements

The primary requirement of the OGM technique is the extraction of ultra-high molecular weight (UHMW) DNA, which requires careful preservation and initial sample processing to prevent DNA fragmentation. Overall, to maximize cell viability, it is essential to

Table 1. Maximum sensitivity and minimum detectable size based on the abnormality found

Type of change	Maximum sensitivity	Minimum detectable size	
		Germline DNA testing	Tumor sample DNA testing
Insertions	1%	> 500 bp	> 5 Kb
Deletions	1%	> 700 bp	> 7 Kb
Duplications	3%	> 30 Kb	> 150 Kb
Inversions	1%	> 30 Kb	> 70 Kb
Balanced and unbalanced translocations	5%	> 70 Kb	> 70 Kb
Copy number variations (> 500 Kb)*	3%	> 500 Kb	> 500 Kb

*Includes aneuploidies.

process samples as quickly as possible. This is particularly relevant for bone marrow samples, where processing within the first 24 hours after bone marrow aspiration is of paramount importance. While DNA extraction from fresh samples is possible, in practice, most centers prefer to freeze (at -80°C) or cryopreserve samples until processing. When freezing the sample at -80°C , certain sample types (eg, bone marrow or heparinized peripheral blood) require the addition of a DNA stabilizing reagent. An important limitation of OGM is that DNA previously extracted through other means cannot be used, and DNA extraction from frozen cell pellets without prior addition of the stabilizing reagent is ill-advised.

Regarding the characteristics of the sample, having, at least, 1.5 million cells is essential, preferably calculated using an automated cell counter. Additionally, when starting from cryopreserved cells, evaluating the viability of the sample is important, with viabilities > 50% being advised to minimize the presence of degraded DNA from dead cells. Another factor that should be taken into consideration is the technique's sensitivity to detect mosaicism (around 5% for structural abnormalities and 15% to 20% for copy number variations (CNVs)). Therefore, the use of samples with infiltration rates above these percentages is advised or, whenever possible, to enrich the sample by selecting the leukemic population.

Technical protocol of genome optical mapping

The OGM technical protocol is categorized into 3 stages: 1) extraction of ultra-high molecular weight DNA; 2) enzymatic labeling of DNA; and 3) image capturing of labeled DNA (Fig. 1). Each stage is described in detail below.

EXTRACTION OF ULTRA-HIGH MOLECULAR WEIGHT DNA

This is the most critical step of the technique. The extraction protocol includes common steps in genomic DNA extraction (cell lysis, DNA retrieval, washes, and elution). However, it uses paramagnetic nanodiscs that prevent fragmentation and enable DNA extraction above 150 Kb. The resulting solution exhibits high viscosity due to the presence of these long-chain DNA molecules, requiring careful pipetting throughout the entire process to avoid fragmentation. Because of the high fragility of the DNA extracted, avoiding transportation is advised. Occasionally, it may be necessary to leave the DNA at room temperature for 3 days to ensure homogenization.

ENZYMATIC LABELING OF DNA

This step involves the use of a fluorophore, using restriction enzymes that recognize a specific 6-bp sequence (CTTAAG) repeated nearly 15 times every 100 Kb in the human genome (the theoretical resolution is every 6.6 Kb on average). This labeling generates a unique pattern of labels, akin to chromosomal banding, allowing for the unequivocal identification of the genomic location of each marked molecule. After a series of washes, counterstaining of DNA molecules with an additional fluorophore is performed, enabling their visualization during scanning. While the high viscosity of the working solutions can be a good indicator, the current protocol does not include any quality control to guarantee the length of molecules prior to sample scanning.

IMAGE CAPTURING OF LABELED DNA

The final stage of the process involves introducing the labeled DNA samples into a Saphyr® chip for scanning

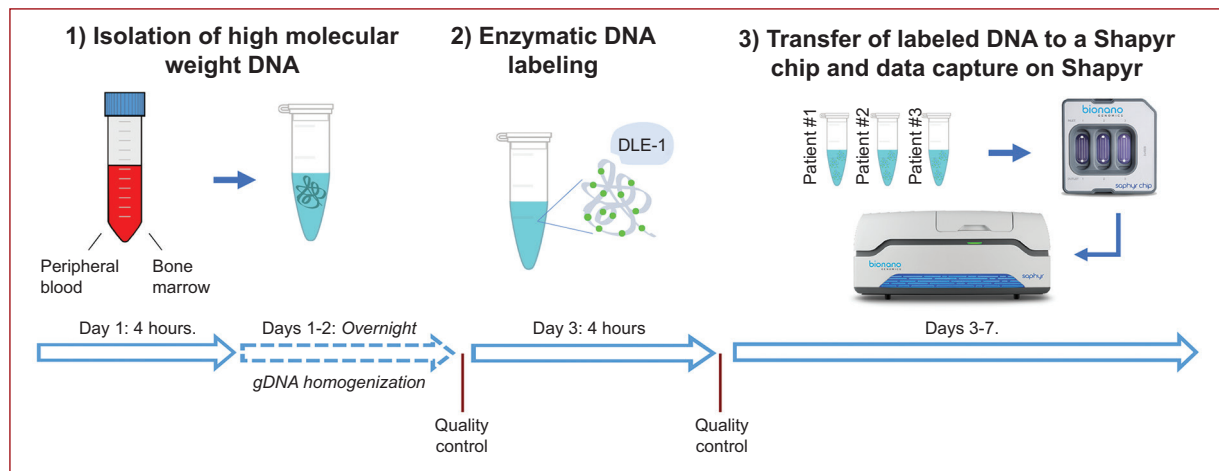


Figure 1. Schematic of the technical process for optical genome mapping.

using the Saphyr® system (Bionano Genomics). Each chip contains 3 wells (flow cells), one for each sample, that contain nanochannels that allow the passage of a single linearized DNA molecule through a process of electrophoresis. This is how the system acquires images from the labeled molecules to build up a precise genome map. For each experiment, the amount of data to be captured (in Gb of total DNA) is programmed based on the coverage wanted for each sample. Overall, the recommended target for hematological samples is the collection of 1500 Gb of data to find abnormalities with a variant allele frequency (VAF) of up to 5%.

Currently, sample preparation for optical genome mapping is performed manually. However, the automation of some parts of the protocol is already in the pipeline. Due to the fragility of long-chain DNA, processing > 3-6 samples in each of the extraction and labeling stages is ill-advised, with an approximate processing time of 3 to 7 days. Regarding image acquisition, the Saphyr® system allows us to load up to 2 chips (6 samples) that are scanned consecutively in a run. The scanning time required for each sample depends on both the quality and labeling of the DNA extracted, ranging from a few hours (approximately 8 hours) to a maximum of 65 hours.

Analysis of optical genome mapping results

Quality controls of the technique

At the end of the technical procedure, the Bionano Access software (Bionano Genomics) compares the images captured from the Saphyr system with a reference genome to generate a file of digital molecules. During this

process, various quality parameters are obtained that should be reviewed to make sure that the data acquired are optimal for analysis (Table 2). While it is possible to proceed with the analysis of the sample even if some values are out of range, it is the responsibility of each laboratory to establish an acceptable range for these values, considering their impact on sensitivity or specificity.

Analysis algorithms of results

Analysis can be performed using two bioinformatics algorithms that, with different strategies, establish the presence of structural abnormalities based on differences between the pattern of digital molecules of the sample and the reference genome. The algorithm recommended for the analysis of hematological neoplasms is the RVAS (rare-variant association study) that has been specifically designed to detect mosaic abnormalities. There is a different type of analysis capable of identifying smaller structural variants and regions with loss of heterozygosity (LOH), called de novo assembly. However, it has been designed to study of constitutional abnormalities, has limited sensitivity (15% to 20%), and its use should be limited to specific cases. Additionally, both algorithms allow us to deduce the presence of CNVs, or aneuploidies based on the oscillation in the coverage achieved for each genomic interval, up to a sensitivity of 15% to 20% in both cases.

Variant filtering, analysis, and interpretation

The analysis and further classification of abnormalities identified by bioinformatics algorithms are

Table 2. Quality parameters and recommended values for genome mapping analysis in hematologic neoplasm samples

Parameter	Definition	Values recommended
Total DNA (≥ 150 kbp)	Total amount of molecules ≥ 150 Kb mapped	≥ 1300 Gb
Map rate	Percentage of molecules ≥ 150 Kb mapped against the reference genome	$> 70\%$
Effective coverage of reference (X)	Effective coverage (total aligned DNA divided by the reference genome size multiplied by map rate)	> 300 X
N50 (≥ 150 kbp)	Weighted mean length of molecules ≥ 150 Kb	> 230 Kb
N50 (≥ 20 kbp)	Weighted mean length of molecules ≥ 20 Kb	> 150 Kb
Average label density (≥ 150 kbp) (/100 kbp)	Mean number of labels for every 100 Kb in molecules ≥ 150 Kb	14-17
Negative label variance (NLV)	Percentage of reference genome labels missing in mapped molecules	0-15%
Positive label variance (PLV)	Percentage of labels identified in mapped molecules missing in the reference genome	0-10%

conducted using the Bionano Access program. The first step in the analysis involves removing polymorphic variants or technique-specific artifacts (of low reliability). The program uses its own embedded multi-ethnic healthy population database or databases with polymorphic variants described in healthy populations (such as the DGV, Database of Genomic Variants), along with the confidence scores estimated for each variant identified, and the maximum values recommended according to the manufacturer's instructions for use. Additionally, abnormalities can also be filtered easily based on their overlapping with regions containing genes or that could be relevant to the study pathology using BED (Browser Extensible Data) files including such regions. This filtering is both automatic and dynamic, meaning it can be modified during the analysis of each sample. In a second phase, visual review of the abnormalities to rule out potential false positives, eliminate duplicates, and merge fragmented CNV changes is required (Fig. 2).

Regarding the information obtained from each abnormality, aside from the quality parameters obtained from OGM and the chromosomal position, the program also shows the genes that overlap with each abnormality, and the potential associated fusion genes. We should mention that some rearrangements, such as those involving immunoglobulin genes (IGH [14q32], IGL [22q11], and IGK [2p12]), T-cell receptor genes, or genes such as MYC (8q24), BCL2 (18q21), and BCL6 (3q27) are characterized by having complex

breakpoint architectures both upstream and downstream of the gene of interest. In these cases, the genes identified at the breakpoint by OGM are not always the ones actually impacting the neoplastic process. We should consider that the genes or regulatory regions involved in an abnormality can be located up to 1 Mb from the breakpoint^{15,16}.

Due to the recent addition of this methodology to diagnostic laboratories, international consensus guidelines for the analysis and interpretation of results are still in development. However, same as it happens with other cytogenomic and molecular techniques, the reports of OGM results should include clinically relevant variants (pathogenic or likely pathogenic abnormalities) in each hematological neoplasm. These would be abnormalities with recognized diagnostic, prognostic, or predictive value in current classifications or guidelines. On the other hand, regarding additional abnormalities (variants of uncertain significance), it is important for each laboratory to establish their own criteria to decide which ones should be included in the final report. Similarly, we should mention that, currently, retrospective OGM data is limited. As this technology becomes more widely implemented in laboratories, the creation of databases gathering detailed information on the abnormalities detected will be of paramount importance. This will allow for the identification of structural cytogenomic abnormalities that represent population polymorphisms not previously identified as such with the reference techniques used up to this point.

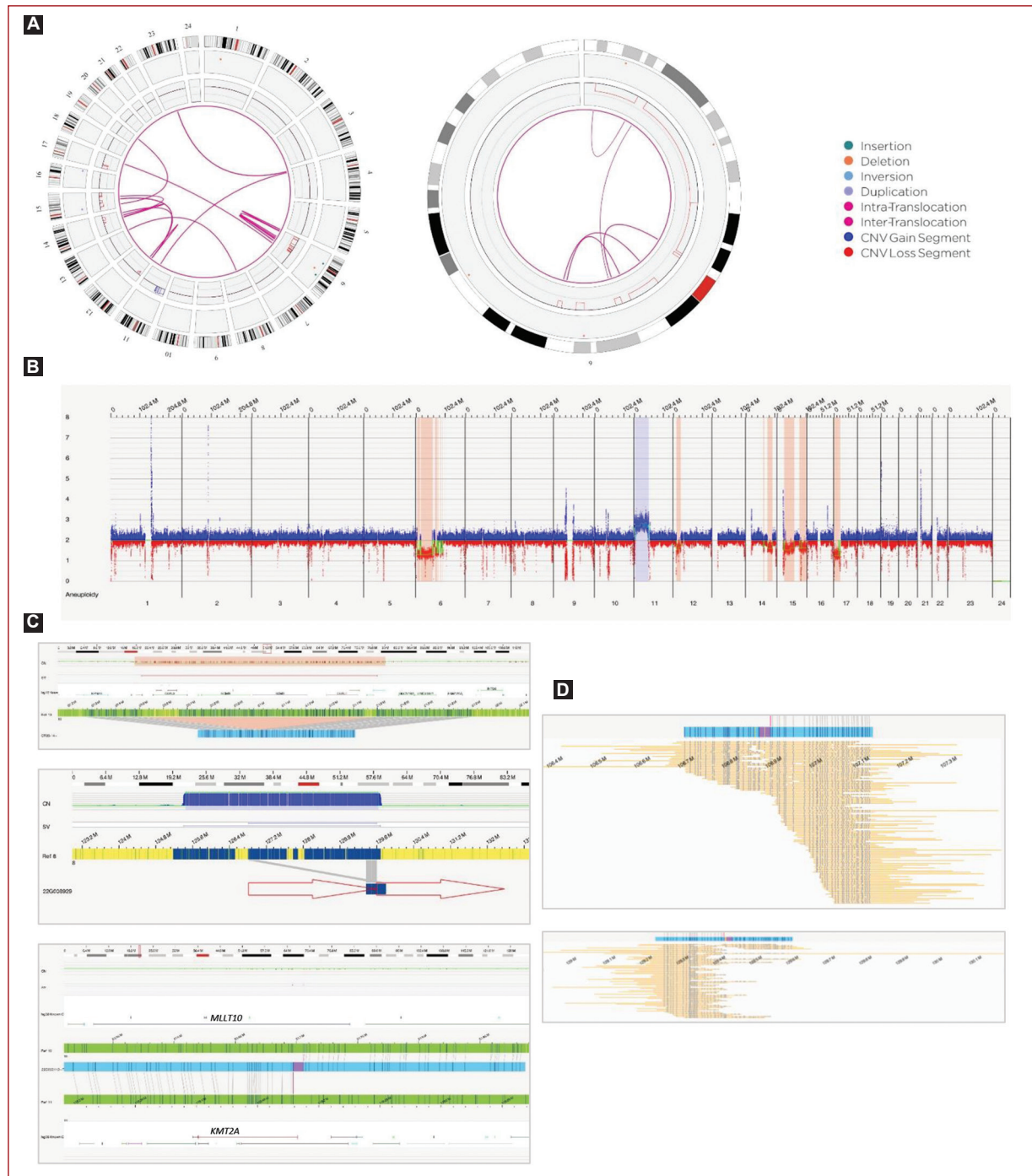


Figure 2. Examples of optical genome mapping result visualization using the Bionano Access software. **A:** circo plot showing abnormalities detected in the entire genome or a selected chromosome. Moving from outer to inner circles, it depicts chromosomal bands, the location of identified structural variants (color-coded dots for each), CNV (losses in red and gains in blue), and purple lines connecting breakpoints of detected translocations. **B:** genome-wide view for CNV visualization (losses in red and gains in blue). **C:** detail of abnormalities identified in specific regions. From top to bottom: CNV loss/deletion, gain/duplication, and interchromosomal translocation. Each region shows 2 tracks displaying CNVs or the structural abnormalities identified, along with the labeling pattern in the reference genome (green) and the sample mapped molecules (blue). **D:** visualization of individual molecules used to define abnormalities. The number of molecules and the number of labels mapped against the reference genome allow us to distinguish between highly reliable translocations (top) or potential false positives (bottom).

Current situation of genome optical mapping to study cytogenomic abnormalities with established clinical value

Various recent publications have demonstrated that OGM is a viable alternative as a first-line test to detect chromosomal abnormalities in different hematological neoplasms^{7,11,14}, primarily in acute myeloid leukemia (AML)^{8,12}, myelodysplastic syndromes (MDS)^{8,17}, acute lymphoblastic leukemia (ALL)^{5,6,10}, and chronic lymphocytic leukemia (CLL)^{9,13} (Table 3). However, international guidelines specifying when to prioritize this technology over conventional methods (karyotyping, FISH, genomic microarrays) and in which conditions the use of OGM would enhance patient management have not yet been published.

We discuss below the potential of using OGM in different hematological neoplasms, considering the results published, clinically relevant variants, and the authors' own experiences.

Myeloid neoplasms

In MDS the detection of recurrent genetic abnormalities is crucial to establish diagnosis, prognosis, and treatment strategies for the patients^{18,19}. Alongside determining clonality in patients with cytopenias in peripheral blood, karyotype analysis is important regarding risk stratification according to the revised International Prognostic Scoring System (IPSS-R)^{20,21}. Similarly, certain structural abnormalities, such as deletion in 5q or the biallelic inactivation of *TP53*, define diagnostic categories consistent with newer classifications^{18,19}. However, nearly 50% of MDS cases exhibit a normal karyotype, and some cases without metaphases assessed in cytogenetic culture require additional FISH or genomic microarray analyses. The OGM studies published so far have demonstrated that its use not only increases the percentage of patients with informative cytogenetic results but also provides better characterizations of certain abnormalities impacting the patients' prognostic stratification¹⁷. Regarding AML, currently, diagnosis is based not only on bone marrow or peripheral blood blast counts but also on the type of genomic abnormalities found^{18,19}. Therefore, cytogenetic analysis is essential to diagnose conditions included in AML with defining genetic abnormalities such as the following *PML rearrangements::RARA*, *RUNX1::RUNX1T1*, *CBFB::MYH11*, *DEK::NUP214*, *RBM15::MRTFA*, *BCR::ABL1*, or those affecting *KMT2A*, *MECOM*, and *NUP98* genes.

We should also identify recurrent abnormalities in MDS (such as deletion 5q, chromosome 7 abnormalities [-7/7q], deletions 11q, 12p, and 17p, or complex karyotypes, among others) to diagnose AML with abnormalities related to myelodysplasia. The OGM studies published so far on patients with AML have demonstrated that the technique is not only useful to identify known abnormalities but also to detect cryptic abnormalities in the karyotype (like *NUP98* rearrangements) and characterize gene rearrangements pairs like *KMT2A* that can impact patient prognosis and be valuable for disease monitoring^{8,12,22}.

Regarding myeloproliferative neoplasms, although the experience published to this date is limited¹¹, OGM holds high potential in the study of primary myelofibrosis because the presence of bone marrow fibrosis that is typical of this disease results in up to 50% of bone marrow karyotypes being uninformative²³. A study evaluated the utility of OGM analysis on peripheral blood samples and demonstrated the technique's reproducibility in this type of samples compared to bone marrow. Additionally, it resolved uninformative karyotypes, providing, eventually, complete cytogenetic information for the use of prognostic scales such as the DIPSS-PLUS, GIPSS, and MIPSS70v2, thereby enhancing patient management²⁴. Regarding patients with hypereosinophilia, OGM could improve differential diagnosis by distinguishing non-clonal benign proliferations (secondary eosinophilias) from clonal neoplastic proliferations (primary eosinophilias)^{25,26}. Currently, this process requires the analysis of multiple FISH probes or karyotyping. On the other hand, OGM could identify or exclude, in a single assay, myeloid/lymphoid neoplasms with eosinophilia associated with tyrosine kinase gene fusion that could benefit from targeted therapies with tyrosine kinase inhibitors *PDGFRA* rearrangements (4q12), *PDGFRB* (5q32), *ETV6::ABL1* [t(9;12)(q34.1;p13.2)], *FGFR* inhibitors [FGFR1 rearrangements (8p11.2)], *JAK2* inhibitors [JAK2 rearrangements (9p24.1)], and *FLT3* inhibitors [FLT3 rearrangements (13q12.2)]^{18,19}.

Lymphoid neoplasms

B-cell ALL (B-ALL) is a complex disease to analyze due to its genetic diversity and how challenging it is to find the primary abnormality or driver, both in pediatric and adult cases. Currently, karyotyping can detect frequently observed abnormalities, such as translocations t(9;22)(q34;q11.2) [*BCR::ABL1*], t(4;11)(q21;q23) [*KMT2A::AFF1*], and t(1;19)(q23;p13) [*TCF3::PBX1*]. However, in many cases, karyotyping is not enough,

Table 3. Summary of landmark studies published on genome mapping in hematologic neoplasms

Reference	Disease studied	n	Concordance with reference techniques	New abnormalities identified by OGM	New prognostic information
Lestringant et al. ⁶ , 2021	ALL	10 (6, B-cell ALL; 4, T-cell ALL)	90%	Detection of 12 additional abnormalities (7 confirmed through conventional techniques), including 3 rearrangements <i>IGH::CEBPB</i> , <i>MYC::TRB</i> , and complex translocations of <i>LMO2::TRA</i> and <i>TLX1::TRA</i>	
Lühmann et al. ⁵ , 2021	ALL	12	100%	Detection of multiple abnormalities, including redefinition of known SVs and new abnormalities of unknown significance. Detection of new fusion gene (<i>JAK2::NPAT</i>)	
Kriegova et al. ⁵⁵ , 2021	MM	11	98%	Detection of additional abnormalities in all patients	
Puiggros et al. ⁹ , 2022	CLL and MBL	42 (39, CLL, and 3 MBL)	90%	OGM provided additional information in 55% of the cases, cohort enriched with complex karyotypes. Detection of higher complexity compared to conventional techniques	Detection of higher complexity on the OGM (≥ 10 abnormalities) associated with worse prognosis
Valkama et al. ¹³ , 2023	CLL	18	100%	OGM provided additional information on 8/16 abnormalities previously identified by FISH, detection of abnormalities not covered by the CLL FISH panel in 78% of the patients	
Balducci et al. ²² , 2022	MDS and AML	68 (27, MDS, and 41, AML)	85%	Detection of clinically relevant abnormalities in 33% and 53% of the patients with MDS and AML, respectively	Change of risk category according to IPSS-R in 22% of the cases of MDS and according to the 2017 ELN scale in 5% of patients with AML
Gerding et al. ⁸ , 2022	MDS and AML	27 (6, MDS, and 21, AML)	93%	Detection of additional abnormalities clarifying karyotype in 67% of the cases	Change of therapeutic strategy in 1 patient and prognostic risk in another patient
Levy et al. ¹² , 2023	AML	100	98%	Detection of clinically relevant abnormalities in 13% of the cases	Change of risk category according to the 2022 ELN scale in 5 patients (5%)
Suttorp et al. ⁵⁶ , 2022	AML (pediatric)	24 (22, AML, and 2 mixed-phenotype AML)	63%	Detection of additional abnormalities in 63% of the cases	Change of risk category in 1 patient (5%) according to the AML-BFM criteria
Yang et al. ¹⁷ , 2022	MDS	101	97%	Detection of 383 cryptic abnormalities through conventional methods, clinically relevant in 34% of the patients	Change of risk category according to the CCSS and IPSS-R in 21% and 17% of the patients, respectively

(Continues)

Table 3. Summary of landmark studies published on genome mapping in hematologic neoplasms (*continued*)

Reference	Disease studied	n	Concordance with reference techniques	New abnormalities identified by OGM	New prognostic information
Gao et al. ³³ , 2022	B-cell ALL (pediatric)	46 (41, B-cell ALL; and 5 pre B-cell ALL)	100%	Description of 2 new fusion genes (<i>LMNB1::PPP2R2B</i> and <i>TMEM272::KDM4B</i>)	
Rack et al. ¹⁰ , 2022	ALL (pediatric and adult)	41 (29, B-cell ALL [12 adults and 17 children]; and 12 T-cell ALL [3 adults and 9 children])	97%	Detection of 10 additional abnormalities (cases with normal or uninformative karyotype). Identification of TRA/D, TRB, <i>TLX3</i> rearrangements and their pair (3/7 not identified by FISH)	Allocation of cytogenetic risk category to 30% more patients (from 23 up to 32 patients)
Soler et al. ⁵⁷ , 2023	ALL and AML	29 (2 B-cell ALL, 3 T-cell ALL, and 24 AML)	73%	Detection of clinically relevant abnormalities in 5 patients (17%)	The OGM findings did not trigger abnormalities to the cytogenetic risk according to the WHO or ELN in any of the patients
Vielor et al. ⁵⁸ , 2023	ALL	11 (9 B-cell ALL, and 2 T-cell ALL)	100%	The OGM provided additional information in 64% of the cases (additional abnormalities in 7 patients and karyotype redefinition in 2)	

ALL: acute lymphoid leukemia; AML-BFM: acute myeloid leukemia-Berlin-Frankfurt-Münster; AML: acute myeloid leukemia; CCSS: comprehensive cytogenetic scoring system; CLL: chronic lymphocytic leukemia; ELN: European leukemia net; FISH: fluorescence in situ hybridization; MBL: monoclonal B lymphocytosis; MDS: myelodysplastic syndromes; MM: multiple myeloma; OGM: optical genome mapping; R-IPSS: Revised International Prognostic Scoring System; SV: structural variant; WHO: World Health Organization.

and additional techniques are necessary: FISH can identify t(12;21)(p13;q22) [*ETV6::RUNX1*], iAMP21 (amplification of *RUNX1*), and other cryptic abnormalities (rearrangements of *CRLF2*, *PAX5*, *ZNF384*, *DUX4*, or *MEF2D*, among others) that can be representative of nearly 30% of the cases^{27,28}. The SNP-array technique can detect secondary microdeletions with poor prognosis (such as *IKZF1*, *CDKN2A/B*, *PAX5*, *BTG1*, *ETV6*, *EBF1*, *RB1*, and the *PAR1* region), some of which impact the therapeutic decisions. Also, it can identify subgroups of B-ALL with aneuploidies with established prognoses accurately, such as the near-haploidy/near-diploidy (23-29 chromosomes/50-58 chromosomes), low-hypodiploidy/near-triploidy (30-39 chromosomes/60-78 chromosomes), and high-hyperdiploidy (51-67 chromosomes) groups^{18,29-32}. For all this, OGM is an ideal technology to detect all types of abnormalities described in this disease through a single test^{5,6,10,33}.

T-cell acute lymphoblastic leukemia (T-ALL), like B-ALL also exhibits high genetic heterogeneity³⁴. Conventional cytogenetics often shows a high percentage of cases with uninformative karyotypes (30%) or normal results (30%) that could contain cryptic abnormalities³⁵. Although few studies have used OGM in this disease, they all

confirm that it provides much more information than conventional cytogenomic techniques. Similarly, these studies have identified fusion genes such as the TRA::LMO2, TRB::MYC, IGH::TAL1, TCF7::SPI1, TRA::NKX2-1, BCL11B::TLX3, TRA::ZEB2, TRB::HIBADH, TRB::TLX1, with great clinical potential^{6,10}.

CLL is a clinically heterogeneous lymphoproliferative neoplasm, in part due to genetic factors. The guidelines of the International Workshop on Chronic Lymphocytic Leukemia recommend analyzing those of del(11q), +12, del(13q), and del(17p) using FISH before starting any treatment^{10,36}. Additionally, the prognostic value of complex karyotype has been demonstrated in recent years, becoming very popular in clinical treatment guidelines such as those from the European Society for Medical Oncology, the National Comprehensive Cancer Network, or the German CLL Study Group. However, the true role of genomic complexity in CLL, whether analyzed through conventional cytogenetics or microarray techniques is still limited by methodological aspects³⁷. In this context, the use of OGM in CLL allows us not only to identify anomalies currently studied by FISH but also to provide a better definition of cytogenetic abnormalities. This includes accurate descriptions of structural

abnormalities, such as marker chromosomes or additional material of unknown origin; the identification of cryptic rearrangements and the precise detection of breakpoints (eg, IGH::BCL3, a group with particularly poor prognosis); and the identification of 2 patterns of chromothripsis (defined as the presence of CNVs with an alternating pattern of, at least, 7 to 10 segments, on one arm or the entire chromosome), depending on inter- or intrachromosomal involvement. OGM also provides a better definition of genomic complexity and proves to be a useful technique in patient prognostic stratification^{9,13,38}.

Multiple myeloma (MM) is a hematologic neoplasm that could greatly benefit from the use of OGM. MM is an extremely heterogeneous condition at cytogenetic level characterized by the presence of chromosomal abnormalities in 90% of the patients. The current international guidelines on the staging of MM, such as the Revised International Staging System (R-ISS) and the Second Revision of the International Staging System (R2-ISS)^{39,40}, include cytogenetic abnormalities associated with grim prognoses, including del(17p) (*TP53*), t(4;14)(p16;q32) [IGH::FGFR3], t(14;16)(q32;q23) [IGH::MAF], and gains or amplifications of 1q (3 copies or ≥ 4 copies). Because of how difficult it is to obtain metaphases from plasma cells, the reference technique to detect high-risk abnormalities is FISH in isolated CD138+ cells that can detect both losses or gains and rearrangements, requiring multiple targeted assessments. This approach involves not knowing the entire genetic heterogeneity of MM, which means being unable to study the contribution of other chromosomal abnormalities to prognosis or patient outcomes. Therefore, OGM is a very promising technique in the management of MM, although it is in the process of standardization in selected CD138+ cells¹¹. With the use of OGM, it will be possible to identify all relocations of 14q32 (IGH) and their pairs, primarily genes from the CYCLIN D (*11q13-CYCLIN D1*; *12p13-CYCLIN D2*; *6p25-CYCLIN D3*) and MAF (*16q23-MAF*; *20q12-MAFB*; *8q24.3-MAFA*), *4p16-MMSET/FGFR3*) families, as well as aneuploidies or rearrangements involving other chromosomal regions of interest, such as *8q24.21 (MYC)*, *2p11.2 (IGK)*, or *22q11.2 (IGL)*.

New genetic information provided by optical genome mapping

The ability to detect new abnormalities or redefine complex rearrangements using the OGM technique has been documented in most studies on hematologic neoplasms^{7,9-12,14,17}. Below, we describe some considerations on the additional information obtained using this technology.

Characterization and reinterpretation of known abnormalities

The OGM technique stands out for its capacity to define breakpoints and genes involved in rearrangements. This is particularly relevant in the characterization of those affecting genes with multiple pairs of rearrangements such as *KMT2A*, IGH, *MYC*, and *BCL6*, among others, for which specific FISH probes are not available. Also, the higher resolution of OGM enables the detection of clinically relevant rearrangements and deletions that could be cryptic through standard methods (eg, *MECOM* (3q26) rearrangements, *NUP98* (11p15) rearrangements, *KMT2A-PTD*, or deletions of *TET2*, *ETV6*, and *TP53*), as well as the characterization of complex abnormalities involving genes essential in leukemogenesis, such as *JAK2* and *RUNX1*^{5,8}. Additionally, OGM has redefined certain cytogenetic abnormalities that would have been misinterpreted using conventional techniques. Among these cases, the phenomenon known as “mimicking,” where some cytogenetic abnormalities defined by G-banding might not hold at molecular level when analyzed in detail. Examples described in the medical literature include t(9;22)(q34;q11.2), which does not involve *BCR::ABL1*, or t(4;12)(q12;p13) with an alleged *ETV6::PDGFRA* rearrangement that actually does not affect *PDGFRA*. In these instances, OGM allowed for higher-resolution identification of these abnormalities and explained the unexpected clinical behavior of patients who remained unresponsive to tyrosine kinase inhibitor treatment⁴¹.

Patient reclassification and identification of new therapeutic targets

We should mention that the improved definition of abnormalities through OGM has a direct impact on the clinical management of patients, allowing patient reclassification into new diagnostic categories and classification into different prognostic or predictive response groups to treatments. In this regard, Yang et al.'s study¹⁷ is particularly interesting. They analyzed a series of 101 newly diagnosed patients with MDS using OGM. The authors demonstrated that OGM allowed risk change based on the cytogenetic risk category and IPSS-R in 17% of the patients. In most cases, the change involved reclassification into a higher-risk group, although cases were also described where the change was seen in the opposite direction due to the characterization of patients with uninformative karyotypes or the identification of very favorable prognostic abnormalities (such as 11q deletion)

undetected using conventional techniques¹⁷. These results are consistent with the re-stratification described in studies of smaller cohorts, such as Balducci et al.'s study²², who reported a change of IPSS-R in 22% of MDSs followed by a change of prognostic stratification according to the 2022 European Leukemia Net (ELN) in 5% of the cases of AML²², or the increased allocation of patients with ALL to established cytogenetic risk groups (from 23 to 32) as described in Rack et al. study¹⁰. On the other hand, the high number of newly identified structural variants expands what we already know on the potential therapeutic targets for developing new targeted therapies. Similarly, the identification of cryptic rearrangements through conventional techniques facilitates the inclusion of patients in clinical trials with drugs they might not otherwise have access to. For instance, in the characterization of MDS through OGM, rearrangements involving *MECOM*, *NUP98*, and *KMT2A*, and the *KMT2A*-PTD translocation have been identified, enabling the selection of patients for targeted clinical trials in AML¹⁷.

Definition of complex karyotype using OGM

The concept of a complex karyotype (CK) is directly associated with a grim prognosis, which is why the risk associated with genomic complexity needs to be defined for each type of hematologic neoplasm. Back in 1981, Berger et al.⁴² defined CK in AML as the presence of ≥ 3 different cytogenetic abnormalities in the tumor clone. However, in recent years, new proposals have been suggested, and the number of abnormalities to consider a CK has varied depending on the diseases and study groups, with cut-off values from 3 to 5 abnormalities⁴³. For example, in AML, the ELN considers CK as ≥ 3 ⁴⁴, while the UK Medical Research Council sets the cut-off value at ≥ 4 abnormalities⁴⁵. Similarly, in CLL, a patient with CK is said to have ≥ 3 cytogenetic abnormalities, being high-risk CK defined as ≥ 5 abnormalities⁴⁶. In the case of T-ALL, the traditional cut-off value for CK has been the same as in B-ALL: ≥ 5 abnormalities⁴⁷. However, the analysis of a large series of adult patients with T-ALL treated homogeneously according to the PETHEMA protocols demonstrated that a CK of ≥ 3 abnormalities identified patients with worse survival and a higher relapse rate³⁵. The OGM technique allows for the detection of more cytogenomic abnormalities, both structural variants (SV) and CNV, which is why the number of abnormalities required to categorize a case as CK using this technique will need to be re-evaluated. In most reports including patients

studied with OGM, cases with CK are identified. The number of abnormalities required to consider a case as CK varies among them: ≥ 3 ^{12,17}, ≥ 4 ¹¹, and ≥ 5 ⁷. However, most do not discuss these relatively arbitrary criteria, perhaps due to the limited number of patients included and the lack of correlation with clinical data^{7,10-12,17}. In this context, Puiggros et al.'s study⁹ on the genomic complexity of CLL is worth mentioning here. They demonstrate that OGM allows patient categorization into different risk groups. The authors propose a cut-off value of ≥ 10 abnormalities to consider cases with high complexity, although they emphasize that the number is arbitrary and that studies with larger and unselected cohorts are needed to define standard criteria of genomic complexity using OGM.

Characterization and clinical implications of the chromoanagenesis phenomenon

The chromoanagenesis phenomenon includes multiple types of catastrophic events, through which multiple genetic abnormalities and chromosomal rearrangements are triggered. Historically, these events have been associated with the development of different types of neoplasms. Among them, chromothripsis, chromoanagenesis, and chromoplexy. Chromothripsis is defined as the fragmentation and localized rearrangement of one or more chromosomal segments during a single-step catastrophic event, with incomplete repair of double-strand breaks through non-homologous end joining. Chromoanagenesis occurs after erroneous DNA replication, with replication fork-stalling and abnormalities to the template strand used during the process, resulting in multiple copy number gains (duplications and triplications) combined with deletions. Lastly, chromoplexy is a phenomenon characterized by the presence of multiple chained and complex inter- and intrachromosomal translocations involving up to 8 different chromosomes, presumably occurring simultaneously and resulting in few or no abnormalities to the copy numbers⁴⁸⁻⁵¹. Up until now, these phenomena could only be identified using whole genome sequencing techniques or genomic microarrays, which provided biased information by only providing data on copy number abnormalities. With the OGM technique, we can obtain much more comprehensive and detailed information on these phenomena. Currently, few studies have been published on the clinical impact of these phenomena due to the recent and gradual implementation of OGM^{17,38}. These phenomena represent the genomic complexity that laboratories will need to consider in the future.

Limitations of genome optical mapping in hematologic malignancy studies

To date, the studies published have highlighted the high performance of OGM in cytogenomic studies of hematologic neoplasms. However, this technology also has some limitations as discussed below.

Sensitivity and clonality

Although one of the advantages of OGM is its high sensitivity that allows the detection of most structural abnormalities in clones with as little as 5% infiltration of the sample, this percentage goes up to 15%-20% for the detection of CNVs. This limitation can be relevant in patients with CLL and minor clones carrying TP53 deletion, which is associated with a grim prognosis and currently identified by FISH. In this regard, while OGM is a useful technique in CLL, it has proven unable to replace FISH for the analysis of this biomarker^{9,13}. Similarly, OGM underperforms in the detection of CNVs in total bone marrow samples from patients with plasma cell neoplasms, due to variability in the infiltration of tumor plasma cells in the samples obtained⁵². Therefore, similar to current recommendations for FISH analysis, the implementation of OGM in cytogenetic characterization should be optimized in selected CD138+ cell samples. Also, it has been demonstrated that, compared to karyotyping, the rate of detection of OGM abnormalities is compromised in peripheral B-cell lymphomas with flow cytometry infiltration < 20%⁵³. In these cases, enriching the sample through mononuclear cell selection via density gradient or immunomagnetic separation of CD19+ cells would improve OGM results.

Finally, we should mention that according to the standard protocols, OGM analyzes the total DNA of the sample but is unable to provide information on the patient's clonal architecture. Although the results include information on the allelic frequency of each abnormality identified, it is impossible to distinguish clonal evolution from the presence of independent clones carrying different abnormalities.

Abnormalities in repetitive regions

Repetitive regions, such as (peri)centromeric, telomeric regions, short arms of acrocentric chromosomes, or heterochromatin segments, are not well covered by OGM technology due to a lack of reference mapping in these regions. Therefore, OGM can't detect abnormalities with breakpoints within these regions. This limitation

means that some abnormalities can only be indirectly inferred through OGM, deducing their presence from associated CNVs. Examples include isochromosomes or dicentric chromosomes affecting 17p13 (*TP53*) in CLL or unbalanced translocations of chromosome 1q in myeloid neoplasms, being der(1;7)(q10;p10) the most common of all. Nonetheless, the pathogenic implication of the abnormality can still be detected by OGM due to the loss of *TP53* or the deletion of 7q, respectively. Similar considerations apply to the subtelomeric regions of sex chromosomes (Xp22.3/Yp11.3, PAR1 region) where the *CRLF2* gene is located. *CRLF2* rearrangements [such as the microdeletion inducing the *P2RY8::CRLF2* rearrangement or the *IGH::CRLF2* rearrangement associated with t(X;14)(p22;q32) or t(Y;14)(p11;q32)] are abnormalities included in the initial study of B-cell ALL, because patients with these abnormalities have a grim prognosis that may benefit from intensified treatment. Therefore, it is advised to inspect manually the molecules mapped in this region or apply the de novo assembly algorithm to detect them^{10,33}.

Allocation of ploidy

Despite OGM's capability to correctly identify aneuploidies (monosomies and trisomies), it has been demonstrated that the allocation of ploidy in the sample is not always accurate^{6,10}. This is particularly relevant in the analysis of patients with B-cell ALL, since the World Health Organization and the International Consensus Classification of Myeloid Neoplasms and Acute Leukemias^{18,30} consider groups with variable prognosis based on ploidy.

In the case of plasma cell neoplasms, abnormalities affecting ploidy are common although they're not included in the prognostic indices recommended^{39,40}. The chromosomes most commonly gained or lost in each of these conditions have been extensively described. Therefore, it is important to interpret the results of OGM correctly based on the chromosomes damaged. Thus, upon suspicion of endoreduplicated haploidy (eg, in MM with trisomy 18 and hypotriploidy), the implementation of the de novo assembly algorithm is advised to analyze regions with LOH and identify this phenomenon manually.

Identification of regions with LOH

The importance of regions with LOH and their implication in the prognosis of hematologic neoplasms has been growing. In fact, the new molecular categorization of MDS includes the combination of *TP53* mutation and LOH in the same gene as an adverse prognostic factor^{18,19}.

Table 4. Advantages and limitations of optical genome mapping

Advantages	Limitations
Enables analysis of the entire genome	Requires specialized equipment, reagents, and has a higher per-test cost compared to conventional techniques
Does not dependent on cell division of tumor cells	Requires starting with very long strand DNA (> 150 Kb). Unsuitable for DNA extracted through traditional methods, formaldehyde-fixed tissue, or paraffin-embedded tissue
Detects different abnormalities, both numerical and structural, balanced, or unbalanced	Does not distinguish individual clones when analyzing DNA collectively
Offers high detection resolution > 5 Kb (variable depending on the type of change)	Does not analyze abnormalities present in repetitive genome regions like centromeres and subtelomeric regions, or very small abnormalities like SNVs or indels
Variable sensitivity based on the type of abnormality analyzed (structural, 1% to 5%; copy number, 15% to 20%)	LOH cannot be determined using the tumor sample analysis (rare-variant association study)

Indels: insertions-deletions; LOH: loss of heterozygosity; SNV: single nucleotide variants.

Similarly, in a genomic classification of myeloproliferative neoplasms, the presence of LOH on chromosomes 9 or 17 had an impact on disease progression⁵⁴. Currently, the detection of LOH is mainly conducted using SNP microarrays and is not included in the OGM analysis algorithm advised for the study of hematologic neoplasms (RVAS). It has been demonstrated that by implementing the de novo assembly algorithm we can identify LOH in some cases. However, we should mention that this algorithm is optimized for the analysis of constitutional abnormalities and has limited sensitivity. Therefore, while the detection of LOH is technically feasible, the analysis algorithm for tumor samples is still under development.

Conclusions

Currently, the detection of chromosomal abnormalities with diagnostic, prognostic, or predictive value in hematologic neoplasms relies on karyotype analysis, FISH, or genomic microarrays. Additionally, the study of mutational profiles through next-generation sequencing (NGS) techniques has become very popular in recent classifications.

OGM is a novel technique with the potential to enable the cytogenetic assessment of patients through a single test. Also, it offers higher resolution compared to conventional techniques, thus improving the characterization of known abnormalities and allowing the identification of new cryptic ones. However, studies conducted so far have shown that this technique is no stranger to limitations, such as the inability to detect LOH regions, distinguish individual clones. Also, it exhibits limited sensitivity (15% to 20%) regarding gain and loss abnormalities (Table 4).

In conclusion, OGM is a highly promising technique for the cytogenomic analysis of hematologic neoplasms that may potentially be added as an additional tool to the techniques currently available. However, its implementation in routine diagnostics will require standardization of analysis criteria, result validation, and prospective comparative studies to determine under which conditions and situations it can complement or replace the cytogenomic techniques currently available. Additionally, it is of paramount importance that health professionals interpreting OGM results have knowledge on cytogenetics to accurately interpret the data and provide robust and validated information to health care professionals involved in integrated diagnosis and patient management. It is anticipated that the combo OGM + NGS will facilitate diagnosis and therapeutic decision-making in these patients.

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Conflicts of interest

None.

Ethical disclosures

Protection of human and animal subjects. The authors declare that no experiments were performed on humans or animals for this study.

Confidentiality of data. The authors declare that no patient data appear in this article.

Right to privacy and informed consent. The authors declare that no patient data appear in this article.

Use of artificial intelligence for text generation. The authors declare that they have not used any type of generative artificial intelligence for the writing of this manuscript, nor for the creation of images, graphics, tables, or their corresponding captions.

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