



# Guiding the global evolution of cytogenetic testing for hematologic malignancies

Yasmine M. N. Akkari,<sup>1</sup> Linda B. Baughn,<sup>2</sup> Adrian M. Dubuc,<sup>3</sup> Adam C. Smith,<sup>4</sup> Mar Mallo,<sup>5</sup> Paola Dal Cin,<sup>3</sup> Maria Diez Campelo,<sup>6</sup> Marta S. Gallego,<sup>7</sup> Isabel Granada Font,<sup>8</sup> Detlef T. Haase,<sup>9</sup> Brigitte Schlegelberger,<sup>10</sup> Irma Slavutsky,<sup>11</sup> Cristina Mecucci,<sup>12</sup> Ross L. Levine,<sup>13</sup> Robert P. Hasserjian,<sup>14</sup> Francesc Solé,<sup>5</sup> Brynna Levy,<sup>15</sup> and Xinjie Xu<sup>2</sup>

<sup>1</sup>Departments of Cytogenetics and Molecular Pathology, Legacy Health, Portland, OR; <sup>2</sup>Division of Hematopathology, Department of Laboratory Medicine and Pathology, Mayo Clinic, Rochester, MN; <sup>3</sup>Department of Pathology, Brigham and Women's Hospital and Harvard Medical School, Boston, MA; <sup>4</sup>Laboratory Medicine Program, University Health Network and Department of Laboratory Medicine and Pathobiology, University of Toronto, Toronto, Canada; <sup>5</sup>MDS Group, Microarrays Unit, Josep Carreras Leukaemia Research Institute, Barcelona, Spain; <sup>6</sup>Hematology Department University Hospital of Salamanca, IBSAL, Salamanca, Spain; <sup>7</sup>Laboratory of Cytogenetics and Molecular Cytogenetics, Department of Clinical Pathology, Italian Hospital, Buenos Aires, Argentina; <sup>8</sup>Hematology Laboratory, Germans Trias i Pujol University Hospital-Catalan Institute of Oncology, Josep Carreras Leukemia Research Institute, Barcelona, Spain; <sup>9</sup>Clinics of Hematology and Medical Oncology, University Medical Center Göttingen, Göttingen, Germany; <sup>10</sup>Department of Human Genetics, Hannover Medical School, Hannover, Germany; <sup>11</sup>Laboratory Genetics of Lymphoid Malignancies, Institute of Experimental Medicine, Buenos Aires, Argentina; <sup>12</sup>Laboratory of Cytogenetics and Molecular Medicine, Hematology University of Perugia, Perugia, Italy; <sup>13</sup>Department of Medicine, Human Oncology and Pathogenesis Program, Memorial Sloan-Kettering Cancer Center, New York, NY; <sup>14</sup>Department of Pathology, Massachusetts General Hospital, Boston, MA; and <sup>15</sup>College of Physicians and Surgeons, Columbia University Medical Center and the New York Presbyterian Hospital, New York, NY

**Cytogenetics has long represented a critical component in the clinical evaluation of hematologic malignancies. Chromosome banding studies provide a simultaneous snapshot of genome-wide copy number and structural variation, which have been shown to drive tumorigenesis, define diseases, and guide treatment. Technological innovations in sequencing have ushered in our present-day clinical genomics era. With recent publications highlighting novel sequencing technologies as alternatives to conventional cytogenetic approaches, we, an international**

**consortium of laboratory geneticists, pathologists, and oncologists, describe herein the advantages and limitations of both conventional chromosome banding and novel sequencing technologies and share our considerations on crucial next steps to implement these novel technologies in the global clinical setting for a more accurate cytogenetic evaluation, which may provide improved diagnosis and treatment management. Considering the clinical, logistic, technical, and financial implications, we provide points to consider for the global evolution of cytogenetic testing.**

## Introduction

Genomic characterization has become an essential component in the clinical management of hematologic malignancies, supporting diagnosis and prognostication as well as informing therapeutic decisions.<sup>1-7</sup> Cytogenetics, the study of chromosome number and structure, represents a central facet of diagnostic genomic analysis with a longstanding history. For the past 50 years, chromosome banding has permitted single-cell and genome-wide analysis of chromosomal alterations, generating a foundational knowledge base of chromosome abnormalities and their clinical associations.<sup>7-13</sup> Presently, for most hematologic malignancies, chromosome banding is an essential component of the diagnostic workup that guides clinical care.<sup>1,2,14-16</sup> Chromosome banding has long remained the gold standard for cytogenetic studies globally, primarily for its high success rate in detecting critical copy number variation (CNV) and structural variation (SV) known to initiate and drive disease progression.<sup>7,17-21</sup>

However, an inherent limitation of chromosome banding is the dependency on cells dividing in vitro. In some hematologic malignancies, a sufficient number of analyzable metaphase cells

may not be obtained in culture to achieve a complete chromosome banding study.<sup>22</sup> Moreover, even when cells readily divide and render ample metaphases, chromosome banding has limited resolution with challenges in detecting alterations <10 Mb. Supplementary technologies, namely fluorescence in situ hybridization (FISH) and chromosomal microarray (CMA), were subsequently developed and overcame many of the inherent limitations of chromosome banding analysis.<sup>23</sup> These platforms do not necessarily require dividing cells and can detect abnormalities <10 Mb, effectively expanding the resolution from large chromosome bands down to gene-level imbalances. FISH is a targeted assay that can be used to detect CNVs and SVs in targeted regions. While widely adopted, there exists great variability in the clinical use of FISH testing, which is often predicated on a priori knowledge of a specific gene or region of interest.<sup>24</sup> Moreover, clinical laboratories are also largely reliant on the development of commercially available FISH probes.<sup>25</sup> CMA is a powerful whole-genome technology with the ability to detect CNV and copy-neutral loss-of-heterozygosity (CN-LOH) in the kilobase range using an unbiased approach.<sup>25-32</sup> However, as CMA cannot detect balanced chromosomal rearrangements, which are hallmarks of many hematologic malignancies, its utility

remains limited to diseases primarily driven by CNVs or unbalanced SVs. CMA adoption worldwide has been hampered by financial constraints and reimbursement practices. While several evidence-based reviews have highlighted the clinical utility of CMA for a diverse spectrum of hematologic malignancies, to date, CMA has not been universally incorporated into current clinical guidelines.<sup>32-38</sup> Given the rapid advancements of genomic technologies that have the potential to comprehensively detect clinically significant cytogenetic alterations, the international diagnostic community must carefully evaluate the implementation of these next-generation cytogenetic methodologies and their implications for clinical care by considering the following questions.

## Are our current traditional cytogenetic diagnostic assays sufficient, or could newer technologies improve clinical management?

For myeloid malignancies, chromosome banding and FISH can be used to detect the majority of the CNVs and SVs needed for appropriate clinical management.<sup>14,39-41</sup> The high success rate at identifying acute myeloid leukemia (AML)-specific abnormalities reflects the ability of myeloid cells to divide readily in culture.<sup>42,43</sup> Of 250 adult AML cases with concurrent chromosome banding and FISH testing, a successful karyotype result (defined as having  $\geq 20$  sufficiently analyzable metaphases) was attained in 88% of cases, 10% of cases had between 1 and 19 metaphases, and only 2% of cases had no metaphases for analysis.<sup>44</sup> Supplementation by AML-directed FISH yielded a 98% concordance between chromosome and FISH analysis (in cases with an adequate chromosome study).<sup>44</sup> Such evidence-based studies provided the foundation for the clinical guidelines supported by the European LeukemiaNet (ELN) ([www.leukemia-net.org/home](http://www.leukemia-net.org/home)), the National Comprehensive Cancer Network (NCCN) ([www.nccn.org](http://www.nccn.org)), and the Revised International Prognostic Scoring System (IPSS-R) ([www.mds-foundation.org](http://www.mds-foundation.org)).<sup>5,20,21,44-47</sup>

The success of chromosome analysis is, however, not uniform across all hematologic malignancies.<sup>48</sup> A successful chromosome analysis would provide  $\geq 20$  metaphases of sufficient resolution to identify chromosomal abnormalities representative of the hematologic disease being evaluated. In contrast to myeloid malignancies, the success rate of chromosome analysis in acute lymphoblastic leukemia (ALL) is approximately 70% to 75%; failures are primarily attributed to substandard chromosome morphology rendering the cells unanalyzable, and/or poor in vitro cell growth of the ALL clone with an inadequate number of analyzable metaphases.<sup>48</sup> When chromosome banding studies are successful, an ALL-specific clonal abnormality can be identified in about 60% to 80% of ALL cases.<sup>22,48</sup> Similar to ALL, the frequency of detecting an abnormal clone in chronic lymphocytic leukemia (CLL) using a CpG oligodeoxynucleotide-stimulated culture is 50% to 70%, as reported in multilaboratory studies.<sup>49,50</sup> In contrast, the frequency of identifying an abnormal plasma cell clone in the case of a plasma cell neoplasm (PCN) such as multiple myeloma is only 10% to 20%<sup>51</sup> since malignant plasma cells rarely undergo cell division, except in cases of advanced disease.<sup>52</sup>

Ongoing cancer research studies utilizing novel technologies continue to expose inherent limitations of current cytogenetic diagnostic testing. In hematologic malignancies, SVs involving enhancer hijackers can reposition promoter and/or enhancer elements of the genome (such as immunoglobulin enhancer sequences) and drive the overexpression of a nearby oncogene without producing in-frame chimeric gene fusions.<sup>53-55</sup> These types of abnormalities are relatively common in lymphoid malignancies and have been shown to lead to false-negative<sup>56-58</sup> or, in rare instances, false-positive FISH results.<sup>59</sup> For example, the MYC break-apart FISH probe demonstrates an approximately 4% false-negative rate in diffuse large B-cell lymphoma<sup>56</sup> and a 50% to 70% false-negative rate in PCNs.<sup>58,60</sup> Novel molecular approaches have also identified new aberrations that play a role in cancer initiation and progression, some of which can be targeted using novel therapeutics.<sup>61</sup> Understanding the limitations of current “gold standard” diagnostic tools and identifying technologies that may be more effective in addressing these vulnerabilities will be crucial to the advancement of clinical care.

Innovative molecular approaches designed to overcome present-day limitations include mate pair sequencing (MPseq) and optical genome mapping (OGM). MPseq is a variation of whole-genome sequencing (WGS) utilizing a specialized library preparation of long input DNA followed by circularization, fragmentation, and sequencing of smaller paired-end fragments.<sup>58,62</sup> OGM electrophoreses high molecular weight DNA into nanochannels, linearizes them for imaging, and creates a consensus genome map from processed images.<sup>63-65</sup> Both MPseq and OGM can identify CNVs and SVs, including balanced rearrangements that escape detection by CMA. Recent studies have highlighted the advantages of OGM as an unbiased whole-genome technology in hematologic malignancies, demonstrating concordance with chromosome banding while revealing additional abnormalities missed by routine workups and refining complex karyotypes such as derivative and marker chromosomes.<sup>64,66-68</sup> In addition, whole transcriptome sequencing (WTS) and targeted RNA-seq assays have been developed to enable the identification of expressed gene fusions. These approaches have shown promising results in pediatric ALL,<sup>69</sup> some lymphomas,<sup>70</sup> multiple myeloma,<sup>71,72</sup> T-cell lymphoblastic lymphoma,<sup>73</sup> and AML.<sup>74</sup> Generally, RNA-based assays detect transcribed genomic regions and highly expressed fusion products, which preclude their detection of SVs involving enhancer hijackers that do not result in chimeric gene fusions. In contrast, DNA-based WGS provides a comprehensive and unbiased characterization of gene rearrangements. Importantly, formalin-fixed, paraffin-embedded material, commonly available for extramedullary hematologic malignancies such as lymphoma, generally only allows DNA-based assays such as WGS for fusion detection in these malignancies. Although targeted RNA-seq assays are commonly used in the context of hematologic malignancies, WTS has not been widely adopted in the clinical setting.

The technologies described above provide viable alternatives to traditional cytogenetic approaches (chromosome banding, FISH, and CMA) with improved resolution. However, all the aforementioned techniques examine the genome through a singular lens, limited to cytogenetic aberrations. Today, diagnostic evaluation of hematologic disorders requires the evaluation of other types of genomic alterations, including single nucleotide variants (SNVs) (eg, *TP53*, *RUNX1*, *IDH*, *NPM1*, and *FLT3-ITD*).<sup>9,15,75-77</sup> Detection

**Table 1. Advantages, limitations, and clinical utility of currently available genomic technologies in hematologic malignancies**

Analyte	G-banding	FISH	CMA	WGS	Targeted sequencing panels		RT-PCR	MPSeq	WTS	OGM
					DNA	RNA				
Coverage	Chromosome in dividing cells	DNA in interphase nuclei and metaphase	DNA	DNA	DNA	RNA	RNA	DNA	RNA	DNA
Distinction of individual cell clones	Whole	Targeted	Whole	Whole	Targeted	Targeted	Targeted	Whole	Whole	Whole
Analysis bias	Yes	Yes	No	No	No	No	No	No	No	No
Turnaround time (d)	Yes (if cultured)	Yes (if cultured)	No	No	Yes	Yes	No	No	No	No
Unmapped region detection	3-7	4 h to 2 d	3-7	7-14	7-14	7-14	4 h to 5 d	7-14	14-21	7-10
Ability to multiplex	Yes	No	No	No	No	No	No	No	No	Some Alu and LINE elements
Analytical sensitivity (%)	Low	Low	High	High	High	High	High	High	High	Low to medium
SVs	1*-3 out of 20 metaphases	1-10	10-20	20-30	1-10	5-10	~0.01	10	1-10	5-20
CNVs	Yes	Yes	No	Yes (long-read or short-read deep sequencing)	No	Gene fusion	Limited	Yes	Yes	Yes
SNVs	Yes	Yes	Yes	Yes	Limited	Limited	Limited	Yes	Limited	Yes
Disease status	No	No	No	Yes	Yes	Yes	No	Limited	Yes	No
Well-established	Diagnosis, disease monitoring, relapse	Diagnosis, disease monitoring, relapse	Diagnosis, relapse	Diagnosis, relapse	Diagnosis, disease monitoring, relapse (if deep coverage)	Diagnosis, disease monitoring, relapse, MRD (if deep coverage)	Diagnosis, disease monitoring, MRD, relapse	Diagnosis, relapse	Diagnosis, relapse	Diagnosis, relapse
Cost	High	High	High	Low	High	High	High	Low	Low	Low
	++	++	+++	+++++	+++	++++	++	+++++	+++++	++++

\*Depending on the clinical situation, 1 metaphase with a recurring abnormality may be considered evidence for an abnormal clone.

of these alterations can be achieved using multiple molecular methods, including reverse transcription polymerase chain reaction (RT-PCR), Sanger sequencing, or next-generation sequencing (NGS) approaches. Thus, for many hematologic malignancies, detection of clinically relevant genomic variants requires a battery of individual assays. Therefore, a single, comprehensive, and affordable assay that captures SNVs, CNVs, and SVs is highly desirable. Please see Table 1 for the list of currently available genomic technologies, their advantages, and limitations.

## Can a single assay provide comprehensive genomic characterization?

The evolution of Sanger sequencing to NGS, coupled with the decreasing costs of DNA resequencing, ushered in an unprecedented genomics era.<sup>78,79</sup> In the current clinical diagnostics setting, NGS assays are frequently limited to targeted gene-specific panels that require regular updates as new clinically relevant genes are identified.<sup>80-82</sup> However, there is considerable variability in content, analysis, and interpretation between different genomics laboratories.

Whole exome sequencing (WES) covers all exonic sequences and has the potential to eliminate much of the variance, at least relating to content. While WES costs have dramatically decreased over the past 5 years, they nonetheless remain prohibitive in many parts of the world. With each evolutionary advance, we see a benefit but also recognize other deficiencies.<sup>83-86</sup> Improved bioinformatics algorithms have enabled WES to detect most CNVs, albeit with variable performance, depending on the quality and depth of the sequencing data and normalization parameters. In addition, since WES excludes intronic and regulatory genomic regions, it is unable to detect a large subset of SVs. This shortfall is significant, as detection of some intronic SVs is essential in the evaluation of certain hematologic malignancies. Thus, WES has the potential to rapidly adapt to new information stemming from translational research, while it also suffers from the inability to detect all pertinent genomic aberrations.<sup>84</sup>

WGS, particularly involving long-read or long-insert DNA fragments, is an attractive technology that can detect all 3 genomic variants (SNVs, CNVs, and SVs) in an unbiased manner.<sup>87-89</sup> Despite these advantages, WGS is currently not adopted for clinical use worldwide because of the complexity of WGS compared with other technologies in relation to the processes, workflows, and resources necessary for its implementation. At present, WGS is primarily used in large academic centers, mostly for translational research purposes. A recent publication by Duncavage et al proposed a paradigm shift in clinical laboratory genomic testing by suggesting WGS as an alternative to cytogenetic analysis in myeloid cancers.<sup>90</sup> The authors performed WGS in a cohort of 263 patients with myeloid malignancies and confirmed 40 recurrent translocations and 91 copy-number alterations previously identified by cytogenetic studies. In addition, WGS identified additional genomic events (CNVs and/or SVs) in 40 of 235 patients (17.0%). When used prospectively in 117 consecutive patients, WGS provided new genetic information in 29 (24.8%) and changed the risk category in 19 (16.2%). This publication was met with both enthusiasm and skepticism,<sup>5,91-95</sup> and subsequently sparked a discussion among clinical laboratories globally, dividing those who were actively promoting an

evolution of testing and clinical adoption of WGS vs those who debated the real world and current feasibility of this transition.

## Implementing new technologies in a clinical diagnostic laboratory

To justify the introduction of a new clinical genomic assay as a replacement for the current gold standard cytogenetic testing (ie, chromosome banding coupled with FISH), multiple factors should be carefully considered. These include the following interconnected considerations: clinical, logistic, technical, and financial.

### Clinical considerations

The utilization of new comprehensive molecular methodologies has the potential to improve clinical care by leading to a more accurate diagnosis and enhancing treatment selection based on higher resolution and more precise genomic data. In these instances, a genome-wide assessment may reveal uncommon but known entities that are not routinely surveyed. Some example scenarios include, but are not limited to:

1. Hematologic malignancies with a broad spectrum of genomic driver alterations of various classes that would require multiple assays to effectively evaluate, such as Ph-like B-ALL, PCN, CLL, and non-Hodgkin lymphomas.
2. Hematologic malignancies with well-documented enhancer hijacking alterations associated with risks of false-negative or false-positive findings by conventional diagnostic approaches (eg, *IGH* and/or *MYC* rearrangements).
3. Cases in which chromosome studies are not successful or are known to demonstrate an unacceptable failure rate (especially in follow-up studies of posttreatment specimens).
4. Atypical clinical presentations warranting the identification of genomic drivers for appropriate management (eg, mixed lineage leukemias).
5. Detection of cytogenetic abnormalities that require further workup to completely characterize their origin and genomic makeup (eg, derivative and marker chromosomes).
6. Investigation of possible false-negative findings when a normal karyotype/FISH result is obtained, but clinical presentation and pathology support the evidence of a neoplasm.

Technological advances have uncovered additional genomic aberrations and complexities often unappreciated using conventional diagnostic approaches. However, interpretation of these novel findings with unclear clinical significance remains a challenge and will likely require additional translational research initiatives to understand their clinical significance. In the interim, the question remains as to how to handle these new findings that are revealed when employing novel technologies. While most laboratories are likely to only report abnormalities of known clinical significance, consideration should be given to possibly reporting novel abnormalities to potentially facilitate future translational research studies involving large patient cohorts. Such studies would focus on correlating novel genomic abnormalities with patient outcomes and their response to therapies. Indeed, this would require a clear description/disclaimer of the unknown significance of the novel findings, which could potentially be reported under a special heading on the clinical report.

The value of this approach was recently demonstrated by Tyner et al.<sup>96</sup> In this study, the authors reported the integration of whole-exome and transcriptome sequencing in the analysis of 672 patients with AML while examining recurrent mutations (and combinations thereof) to determine drug sensitivity. This study summarized functional, genomic, and transcriptomic data from this large, clinically characterized AML cohort to date and paved the way for establishing the clinical utility of novel technologies in myeloid disorders. Similar reports have been published for other hematologic malignancies such as pediatric ALL and PCN. Nordlund et al used linked-read WGS to generate haplotype information and successfully detected CNVs and SVs in ALL patients, including cryptic and key abnormalities such as *DUX4*, *ZNF384*, and *PAX5* gene rearrangements.<sup>97</sup> Moreover, Hollein et al recently reported the use of WGS in combination with RNA-seq in 211 diagnostic PCN samples and observed 92% concordance of FISH and WGS results for CNVs and SVs evaluation.<sup>72</sup> However, some abnormalities, such as *MYC* translocations in PCN, are more reliably identified using WGS rather than FISH approaches.<sup>60</sup> Such translational studies afford the opportunity to correlate conventional diagnostic approaches with novel genomic studies. In this instance, a comparative nomogram study could correlate the clinical significance of low-level abnormal clones detected by WGS with the number of abnormal metaphase cells detected by chromosome banding. This would be critical to our future understanding of small clonal populations identified by WGS. As the clinical significance of most submicroscopic CNVs below the resolution of current cytogenetic profiling is not known, sufficiently powered studies are needed to delineate their clinical, prognostic, and therapeutic relevance. Publicly available databases are also critically needed to facilitate data sharing (both genomic and clinical information) to enhance our understanding of these genomic findings within the clinical setting. This is by no means an easily accomplished task, as it will require the interpretation and integration of large amounts of data for each patient while holding the promise of improved clinical management. To this end, population reference databases such as the Genome Aggregation Database<sup>98</sup> and Database of Genomic Variants,<sup>99</sup> among others,<sup>100,101</sup> have provided reference maps allowing improved interpretation of SNVs and CNVs, including some SVs. Recently, a population reference map of SVs has been made available,<sup>102</sup> providing a reference of SVs from nearly 15 000 genomes from a diverse population. This tool will be instrumental in the interpretation of incidental and benign SVs.

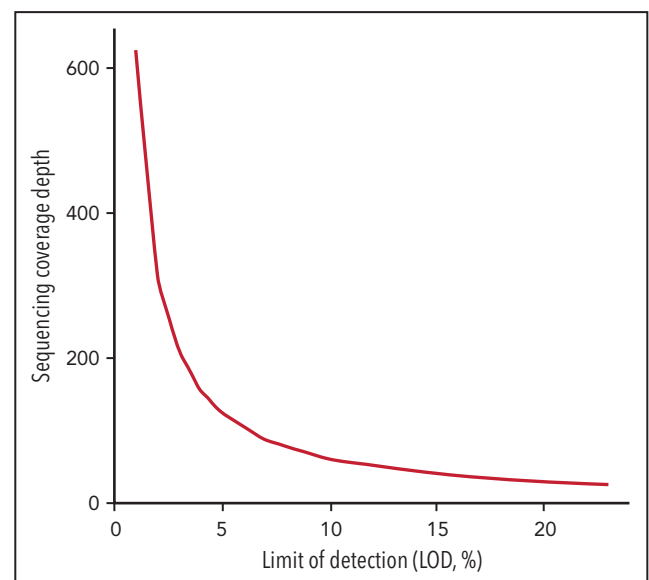
One commonly encountered clinical challenge in oncology testing is the identification of incidental findings of germline variants of clinical significance. To this end, the American College of Medical Genetics and Genomics has published guidance for the reporting of secondary findings in the context of clinical exome and genome sequencing.<sup>103</sup> The secondary findings maintenance working group has recommended a list of genes that should be evaluated in individuals undergoing clinical-grade sequencing. Based on these recommendations, these secondary findings should also be reported when encountered in the analysis of SVs and CNVs using NGS. To simplify workflows, however, filters can be applied to allow a focused analysis on clinically significant regions related to the reason for testing. Informed consent and genetic counseling should also be recommended.<sup>103</sup>

In some circumstances, particularly when the tumor represents nearly 100% of the tested tissue, it is unclear if the abnormality identified by NGS is germline or somatic. In such instances, similar to other clinical testing scenarios, a nontumor sample can be requested (blood or skin) to evaluate the germline status. This can be a desirable follow-up approach that minimizes the need to perform side-by-side germline and somatic testing. Currently, as there are no guidelines to address all the above possibilities in oncology testing, each laboratory should establish its own policies to address incidental findings, including the option to only analyze targeted regions.

We suggest that for early adopters of new technologies without a full understanding of many of the novel variants that will be encountered, a tiered reporting strategy is necessary to clearly delineate the well-established genomic alterations from other variants of unknown significance. Such a tiered approach has been recommended by numerous professional organizations in the analysis of CNVs, SNVs, and CN-LOH in cancer.<sup>26,82,104-106</sup> Moreover, as translational and clinical research studies continue to deliver evidence-based data supporting the significance of novel genomic findings, reanalysis of existing WGS data can be achieved to update the patient's diagnosis/prognosis/management if novel, clinically significant variants are identified, potentially minimizing additional expenses associated with repeat genomic testing.

### Logistic considerations

The impact of adopting new technologies such as WGS must be considered at a system level. On a global scale, the replacement of conventional testing may be impractical at this time. Multiple factors must be considered and carefully navigated, including the availability of sequencing equipment, trained personnel, infrastructure to support reagent transportation and distribution, impacts of turnaround time, complexity of analysis, development of an integrated workflow between the molecular and cytogenetics laboratories, computing and data storage infrastructure,



**Figure 1. Sequencing coverage depth needed for LOD in the range of 1% to 25% calculated based on confidence interval at 0.95 with threshold of minimum 3 unique sequencing reads according to the binomial distribution.**



and cost (including reimbursement).<sup>107</sup> Given that evaluation of novel technologies may require a significant upfront investment in the forms of capital equipment, bioinformatics means, data storage capabilities, and human resources, the transition to any disruptive technology must be well-planned in order to avoid disruption to patient care and confusion to the clinical stakeholders. The gradual expansion of testing should enable harmonization of laboratory processes, maintaining quality control and reporting guidelines, and facilitate the adoption of those technologies with the greatest improvements over present standards.

### Technical considerations

The early adoption of WGS as an alternative to conventional cytogenetic testing has brought about questions regarding the need to replace current cytogenetic approaches. Indeed, this “replacement” is much more likely to be an “evolution” with the gradual adoption of innovative technologies following the establishment of carefully considered disease lists and/or clinical scenarios where a given technology shows the greatest promise. Presently, many clinical situations optimally require the combinatorial use of multiple technologies to appropriately balance time, cost, and clinical needs. Targeted assays may be supplemented if new technologies cannot return critical results in a timely fashion, potentially delaying appropriate clinical management. In the context of AML, WGS will likely take a longer time to complete than traditional approaches. Therefore, coupling WGS with more rapid, targeted assays, such as FISH for *PML::RARA* or *BCR::ABL1* fusion, may permit results to be returned in a timely fashion to determine initial therapy at diagnosis/presentation. Subsequently, WGS results can be delivered before the end of the initial induction therapy for a more comprehensive genomic profiling that may provide novel but clinically important information for subsequent therapeutic/risk stratification decisions. This approach requires built-in redundancies in testing parameters until turnaround times can match present standards.

Despite the comprehensive nature of genome-wide assays such as WGS, there remain regions in the genome or critical aberrations that are not amenable to WGS. For example, a fraction of the genome, such as subtelomeric and centromeric regions, is refractory to sequencing.<sup>108</sup> Although some of these limitations could be overcome with the use of ultralong read sequencing,<sup>109</sup> this approach is not widely accessible. In addition, internal tandem repeats (eg, *FLT3-ITD*) may escape detection by amplicon-based WGS and will likely require orthogonal approaches to uncover these types of genomic lesions.<sup>110</sup>

Current published guidelines for clinical germline WGS specifically note that laboratories are not required to validate all classes of variation that include all potential iterations of genomic lesions but can instead adopt a phased approach to validations. However, laboratories must establish operational familiarity with all quality control parameters and identify any factors that would adversely affect the detection of certain variants (eg, PCR amplification for library preparation and CNV detection). In certain clinical situations, orthogonal confirmation of clinically actionable pathogenic calls may be warranted, although this additional effort would further increase the cost and turnaround time of clinical testing.<sup>105</sup>

Another challenge in the universal use of WGS is the inconsistency in the variant components of the bioinformatics pipelines used by each laboratory. These include alignment and variant calling in addition to the transfer of data to new human genomic builds. In addition, the depth of sequencing coverage would optimally be adapted according to the reason for testing (diagnosis, response to treatment, or minimal residual disease [MRD] assessment). In general, cancer NGS tests with the goal to guide targeted therapy have a limit of detection (LOD) of around 5%. However, in the context of MRD detection, cancer NGS tests may need to reliably detect alterations down to  $\leq 1\%$ .<sup>111,112</sup> The sensitivity of WGS is typically lower than other testing methodologies such as targeted RT-PCR. Thus, for MRD purposes, private and novel variants identified by WGS at diagnosis could be used to customize an RT-PCR assay that would allow higher sensitivity. While Duncavage and colleagues suggested that WGS could be a viable alternative to cytogenetic analysis in myeloid cancers with a target coverage of 60x for their entire cohort, this level of coverage is predicted to provide a LOD of 10% using 3 reads as the minimum number to call SNVs with 95% confidence interval based on binomial probability distribution. This would be insufficient for either guiding targeted therapy or tracking MRD.<sup>112-114</sup> In addition, a small subset (4.3%) of their cohort had less than 25x genome coverage, corresponding to a LOD of 23%. To reach a LOD of 5% and 1% for appropriate NGS tests to guide targeted therapy or to detect MRD, the sequence coverage needs to be at 124x and 625x, respectively, which would increase sequencing cost significantly (Figure 1). The LOD to detect CNV, on the other hand, varies based on the size and genomic location of these alterations. For CNVs that include junction sequences, split reads can support breakpoint detection and improve the sensitivity compared with CNV detected using read-depth alone.<sup>115</sup> In the Duncavage et al study, the following criteria were used to call CNVs >5Mb: (1) Having at least 2 paired and 2 split reads supporting the breakpoints, and (2) the ratio between coverage depth and normalized

**Table 2. Estimated cost for the adoption and validation of WGS in a clinical laboratory setting**

Item	Estimated cost range (\$)	Comments
Capital equipment	800 000-1 000 000	Ex: Illumina NovaSeq 6000
Bioinformatics pipeline	10 000-30 000	Ex: Illumina Dragon license
Test validation	39 000-100 000	30-50 samples
Staff salary	50 000-200 000 (varies per country)	Technologist and research and development scientist (3-6 mo), bioinformatician (3-6 mo), laboratory geneticist and/or pathologist (1-3 mo)
Total	899 000-1 330 000	

control being  $<0.8$  for deletion or  $>1.3$  for duplication. This entails that the tumor burden is  $\geq 20\%$  to call a deletion  $>5\text{Mb}$  or  $\geq 30\%$  to call a duplication  $>5\text{Mb}$ .

In general, LOD increases for smaller size CNVs, thus making it challenging to identify gene-level or exon-level CNVs  $<5\text{Mb}$  with 20% to 30% tumor burden using  $\sim 60\times$  WGS. The LOD for CN-LOH detection is still not well understood, and further studies are needed. It is therefore critical that such issues be thoroughly investigated so that laboratories can identify both advantages and limitations and ensure that alternative tests are available in the laboratory's testing menu if, and when, a diagnostic result cannot be achieved (eg, due to sequencing failures, gaps in coverage, complex repetitive sequences, appropriately tracking MRD, etc). Therefore, if WGS is not successful, the laboratory can use conventional chromosome studies from samples that have been already grown and stored as a fixed cell pellet. This illustrates the importance of maintaining some degree of redundancy, with competency in a more diversified testing portfolio to address the various testing deficiencies defined above. Put simply, FISH and chromosome banding may remain necessary in certain instances, particularly in instances of compromised WGS studies. All these regulatory processes are time-consuming and will slow down the speed of establishment of WGS for routine diagnostics.

### Financial considerations

The financial considerations for performing WGS as a clinical test include both the cost to adopt and validate WGS and the cost to routinely perform it in a clinical laboratory setting. The major cost to adopt WGS is capital equipment, personnel cost for staff technologist/scientist with experience in NGS and bioinformaticians to build and maintain the pipeline, reagents for test validation, data storage, database generation, and qualified laboratory geneticists or molecular pathologists for data interpretation and reporting. As shown in Table 2, the total cost is estimated in the \$0.9 to \$1.5 million range in each laboratory.

The total cost to run WGS as a clinical test with  $\sim 60\times$  coverage is estimated to be in the \$1300 to \$2500 range,<sup>90,116,117</sup> with the cost breakdown to routinely perform WGS in a clinical laboratory setting discussed in detail in Duncavage et al ( $\sim$ \$2 million for a laboratory running 1000 WGS tests per year). This is significantly higher compared with chromosome banding, which remains in the range of \$150 to \$750 (varies by country). Moreover, this cost is based on the estimate of batching  $\sim 10$  to 20 samples per sequencing flow cell, which may not be feasible for most laboratories, as batching will invariably interfere with maintaining an appropriate turn-around-time for hematologic malignancy testing. In addition, depending on the robustness of curated knowledge bases, the cost for employing scientists and laboratory geneticists and/or pathologists to interpret and report cases will vary and remain a significant financial consideration. Optimistically, for future adoption of WGS, laboratories must batch patient samples with different clinical indications, including hematologic malignancies, solid tumors, and congenital conditions, which could help maintain a clinically appropriate turn-around-time and decrease cost.<sup>118</sup>

Based on these cost considerations, currently, only large institutions in developed countries with substantial financial support can afford the implementation of WGS. The financial and other

logistical barriers remain prohibitive for smaller laboratories, especially those in low-income countries, to adopt WGS.

In the United States, reimbursement by private and public payers only occurs if a test has an associated current procedural terminology code (CPT; a medical code used to report medical, surgical, and diagnostic procedures and services to entities such as physicians, health insurance companies, and accreditation organizations) ([www.cms.gov](http://www.cms.gov)).<sup>119,120</sup> The CPT codes for a given test are linked to a specific technology (eg, chromosome banding/FISH/RT-PCR/CMA/WES/WGS); however, a technology-associated CPT code does not necessarily guarantee reimbursement. The newer the code, the more likely insurance companies are to deny payment, substantiating their actions by claiming the technology to be "experimental." US Food and Drug Administration (FDA) approval of a specific technology-associated test essentially converts an experimental test to one accepted for use in clinical diagnosis, thus facilitating reimbursement. However, considerable resources are needed to navigate the FDA approval process, making this pathway more likely to be initiated by commercial laboratories or large medical institutions. Reimbursement can be linked to FDA approval, particularly when a therapeutic drug is associated with a specific diagnosis supported using an FDA-approved test ([www.fda.gov](http://www.fda.gov)).<sup>121</sup> In such instances, access to therapy may be tied to the specific testing dictated by pharmaceutical companies. Therefore, even if a laboratory offers WGS, a specific targeted assay such as FISH may instead be required to assure reimbursement for the disease-associated therapy. In countries with public health systems (eg, Canada, Germany, Italy, Spain, and the United Kingdom), government bodies work with scientific associations or physician organizations to determine which tests will be financially reimbursed and often have fixed annual budgets that make adding expensive testing difficult.<sup>116</sup> In many instances, centralized clinical laboratories then provide these approved tests. The same holds true for many Latin American countries, such as Argentina, that have a mix of public and private health systems. Lagging reimbursement by public and private payers remains the primary obstacle to the widespread adoption of novel technologies that offer more sensitive and comprehensive testing modalities. Unfortunately, this factor often discourages the adoption of new tests despite a body of literature that clearly demonstrates their clinical utility and validity.

Financial considerations remain key factors for clinical laboratories to consider adopting clinical WGS. The cost to adopt and validate WGS, followed by routinely performing WGS while maintaining a reasonable turn-around-time, may not be currently applicable for most laboratories globally. In addition, without clear pathways for reimbursement, creating a business plan to justify adopting the new technologies may be impractical at this time.<sup>116</sup>

### Toward real-time changes in clinical guidelines

Chromosome banding is an integral part of current clinical guidelines and prognostic systems (NCCN, ELN, and IPSS-R). The value of novel technologies has been highlighted in this review, and the frequent lag in their adoption constitutes an obstacle to improved diagnostics and patient outcomes. This is especially significant as access to the newest therapies through enrollment in clinical trials relies on current guidelines. It is therefore important for our

laboratory and clinical communities to formulate a more dynamic response to the evidence emerging from novel technologies and more rapidly and continuously update the guidelines. We argue that clinical guidelines should adopt technology-agnostic standards so long as a laboratory appropriately validates a given technology. Constraining diagnostic genomic laboratories to a particular testing methodology is counterproductive and decreases patient access to local laboratories that could potentially offer the same test by another validated method in a more affordable manner.

## Alternative approaches to improving patient outcomes

As improvement in patient outcomes remains the ultimate objective of clinical genomic testing, it is important to consider alternative and innovative approaches that may reduce the necessity for comprehensive genome-wide testing strategies. Clinical management is often guided by the optimal therapy choice based on biomarker status: as more targeted therapies for specific biomarkers are developed, clinical management via personalized medicine becomes paramount and may eliminate reliance on WGS altogether. While we remain in the early days of single-cell sequencing, a deeper understanding of clonal heterogeneity and a change in therapeutic strategies based on these minor but potentially aggressive clones may become more widely used. In addition, the significance of novel therapies such as chimeric antigen receptor T-cell or tumor mRNA vaccines may be agnostic to tumor biomarkers and become a “magic bullet” for some types of cancer therapy.<sup>122</sup> Finally, it is unclear whether a combined omics approach (genomic, transcriptomic, proteomic, methylomic) may be a better and more comprehensive approach not only to understand but also to treat human disease.

## Concluding remarks

From a global health perspective, with the goal to provide equitable care for all patients worldwide, access to novel and sophisticated genomic testing tools may not be immediately feasible across all clinical laboratories. Presently, there are significant impediments to the immediate adoption of WGS by clinical laboratories worldwide. While the clinical utility of WGS is undeniable, chromosome banding and FISH with SNP array integration will likely continue to serve as the gold standard of cytogenetic testing for hematologic malignancies. We remain optimistic that by establishing data-generating initiatives through translational research, expediting the adoption of published guidelines (such as NCCN and ELN), validating novel technologies for clinical utility, and ensuring appropriate turnaround times and reimbursement policies, we can embrace the change and drive the evolution of genomic technologies. Looking ahead, and as our understanding of the genome and the molecular pathogenesis of disease continues to advance, we will undoubtedly find ourselves looking into (epi)genomic, transcriptomic, and proteomic tools for the full characterization of human diseases. While one

single test may not replace the totality of tests that currently exist, the active exploration and adoption of novel approaches in defined clinical settings may indeed allow for a continued and controlled evolution resulting in the successful clinical implementation of WGS in the near future.

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## Authorship

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ORCID profiles: Y.M.N.A., 0000-0002-3072-7388; L.B.B., 0000-0001-5229-4897; A.C.S., 0000-0001-9927-4914; M.M., 0000-0001-7741-498X; P.D.C., 0000-0002-7426-5293; M.D.C., 0000-0002-1467-6779; I.G.F., 0000-0002-4275-0104; B.S., 0000-0001-5256-1270; I.S., 0000-0002-0611-2367; C.M., 0000-0002-1623-0148; R.P.H., 0000-0002-7753-0697; F.S., 0000-0002-3251-2161; B.L., 0000-0002-4213-3480; X.X., 0000-0001-8373-1834.

Correspondence: Xinjie Xu, Division of Hematopathology, Division of Laboratory Genetics and Genomics, Department of Laboratory Medicine and Pathology, Mayo Clinic, 200 First Street SW, Rochester, MN 55905; e-mail: xu.xinjie@mayo.edu; Brynna Levy, College of Physicians and Surgeons, Columbia University Medical Center and the New York Presbyterian Hospital, 3959 Broadway CHC – Room 406b, New York, NY 10032; e-mail: bl2185@cumc.columbia.edu; and Francesc Solé, MDS Group, Institut de Recerca Contra la Leucèmia Josep Carreras, Edifici IJC, Campus ICO-Germans Trias i Pujol, Ctra de Can Ruti, Camí de les Escoles s/n, 08916 Badalona, Barcelona, Spain; e-mail: fsole@carrerasresearch.org.

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## REFERENCES

1. Taylor J, Xiao W, Abdel-Wahab O. Diagnosis and classification of hematologic malignancies on the basis of genetics. *Blood*. 2017;130(4):410-423.
2. Nangalia J, Campbell PJ. Genome sequencing during a patient's journey through cancer. *N Engl J Med*. 2019;381(22):2145-2156.
3. Granada I, Palomo L, Ruiz-Xivillé N, Mallo M, Solé F. Cytogenetics in the genomic era. *Best*



- Pract Res Clin Haematol.* 2020;33(3):101196.
4. Ribeiro IP, Melo JB, Carreira IM. Cytogenetics and cytogenomics evaluation in cancer. *Int J Mol Sci.* 2019;20(19):4711.
  5. Greenberg PL, Tuechler H, Schanz J, et al. Revised international prognostic scoring system for myelodysplastic syndromes. *Blood.* 2012;120(12):2454-2465.
  6. Bernard E, Nannya Y, Hasserjian RP, et al. Implications of TP53 allelic state for genome stability, clinical presentation and outcomes in myelodysplastic syndromes [published correction appears in *Nat Med.* 2021;27(5):927]. *Nat Med.* 2020;26(10):1549-1556.
  7. Mitelman F. Recurrent chromosome aberrations in cancer. *Mutat Res.* 2000;462(2-3):247-253.
  8. Mrózek K, Bloomfield CD. Chromosome aberrations, gene mutations and expression changes, and prognosis in adult acute myeloid leukemia. *Hematology Am Soc Hematol Educ Program.* 2006;2006:169-177.
  9. Ogawa S. Genetics of MDS. *Blood.* 2019;133(10):1049-1059.
  10. Shallis RM, Ahmad R, Zeidan AM. The genetic and molecular pathogenesis of myelodysplastic syndromes. *Eur J Haematol.* 2018;101(3):260-271.
  11. Solomon E, Borrow J, Goddard AD. Chromosome aberrations and cancer. *Science.* 1991;254(5035):1153-1160.
  12. Yang JJ, Park TS, Wan TS. Recurrent cytogenetic abnormalities in acute myeloid leukemia. *Methods Mol Biol.* 2017;1541:223-245.
  13. Mitelman F. Catalogue of chromosome aberrations in cancer. *Cytogenet Cell Genet.* 1983;36(1-2):1-515.
  14. Arber DA, Orazi A, Hasserjian R, et al. The 2016 revision to the World Health Organization classification of myeloid neoplasms and acute leukemia *Blood.* 2016;127(20):2391-2405.
  15. de Haas V, Ismaila N, Advani A, et al. Initial diagnostic work-up of acute leukemia: ASCO clinical practice guideline endorsement of the College of American Pathologists and American Society of Hematology Guideline [published correction appears in *J Clin Oncol.* 2019;37(3):239-253]. *J Clin Oncol.* 2019;37(7):612.
  16. Döhner H, Estey E, Grimwade D, et al. Diagnosis and management of AML in adults: 2017 ELN recommendations from an international expert panel. *Blood.* 2017;129(4):424-447.
  17. Heim S, Mitelman F. Cytogenetic analysis in the diagnosis of acute leukemia. *Cancer.* 1992;70(6 suppl):1701-1709.
  18. Heim S, Mitelman F. Molecular screening for new fusion genes in cancer. *Nat Genet.* 2008;40(6):685-686.
  19. Mitelman F, Heim S. Quantitative acute leukemia cytogenetics. *Genes Chromosomes Cancer.* 1992;5(1):57-66.
  20. Haase D, Germing U, Schanz J, et al. New insights into the prognostic impact of the karyotype in MDS and correlation with subtypes: evidence from a core dataset of 2124 patients. *Blood.* 2007;110(13):4385-4395.
  21. Schanz J, Tüchler H, Solé F, et al. New comprehensive cytogenetic scoring system for primary myelodysplastic syndromes (MDS) and oligoblastic acute myeloid leukemia after MDS derived from an international database merge. *J Clin Oncol.* 2012;30(8):820-829.
  22. Moorman AV, Harrison CJ, Buck GA, et al; Adult Leukaemia Working Party, Medical Research Council/National Cancer Research Institute. Karyotype is an independent prognostic factor in adult acute lymphoblastic leukemia (ALL): analysis of cytogenetic data from patients treated on the Medical Research Council (MRC) UKALLXII/Eastern Cooperative Oncology Group (ECOG) 2993 trial. *Blood.* 2007;109(8):3189-3197.
  23. Ronaghy A, Yang RK, Khoury JD, Kanagal-Shamanna R. Clinical applications of chromosomal microarray testing in myeloid malignancies. *Curr Hematol Malign Rep.* 2020;15(3):194-202.
  24. Wheeler FC, Kim AS, Mosse CA, Shaver AC, Yenamandra A, Seegmiller AC. Limited utility of fluorescence in situ hybridization for recurrent abnormalities in acute myeloid leukemia at diagnosis and follow-up. *Am J Clin Pathol.* 2018;149(5):418-424.
  25. Rack KA, van den Berg E, Haferlach C, et al. European recommendations and quality assurance for cytogenomic analysis of haematological neoplasms. *Leukemia.* 2019;33(8):1851-1867.
  26. Mikhail FM, Biegel JA, Cooley LD, et al. Technical laboratory standards for interpretation and reporting of acquired copy-number abnormalities and copy-neutral loss of heterozygosity in neoplastic disorders: a joint consensus recommendation from the American College of Medical Genetics and Genomics (ACMG) and the Cancer Genomics Consortium (CGC). *Genet Med.* 2019;21(9):1903-1916.
  27. Simons A, Sikkema-Raddatz B, de Leeuw N, Konrad NC, Hastings RJ, Schoumans J. Genome-wide arrays in routine diagnostics of hematological malignancies. *Hum Mutat.* 2012;33(6):941-948.
  28. Peterson JF, Aggarwal N, Smith CA, et al. Integration of microarray analysis into the clinical diagnosis of hematological malignancies: how much can we improve cytogenetic testing? *Oncotarget.* 2015;6(22):18845-18862.
  29. Maciejewski JP, Tiu RV, O'Keefe C. Application of array-based whole genome scanning technologies as a cytogenetic tool in haematological malignancies. *Br J Haematol.* 2009;146(5):479-488.
  30. O'Keefe C, McDevitt MA, Maciejewski JP. Copy neutral loss of heterozygosity: a novel chromosomal lesion in myeloid malignancies. *Blood.* 2010;115(14):2731-2739.
  31. Mohamedali A, Gäken J, Twine NA, et al. Prevalence and prognostic significance of allelic imbalance by single-nucleotide polymorphism analysis in low-risk myelodysplastic syndromes. *Blood.* 2007;110(9):3365-3373.
  32. Tiu RV, Gondek LP, O'Keefe CL, et al. Prognostic impact of SNP array karyotyping in myelodysplastic syndromes and related myeloid malignancies. *Blood.* 2011;117(17):4552-4560.
  33. Akkari YMN, Bruyere H, Hagelstrom RT, et al. Evidence-based review of genomic aberrations in B-lymphoblastic leukemia/lymphoma: Report from the cancer genomics consortium working group for lymphoblastic leukemia. *Cancer Genet.* 2020;243:52-72.
  34. Chun K, Wenger 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 (CGC) working group for chronic lymphocytic leukemia. *Cancer Genet.* 2018;228-229:236-250.
  35. Pugh TJ, Fink JM, Lu X, et al; Cancer Genomics Consortium Plasma Cell Disorders Working Group. Assessing genome-wide copy number aberrations and copy-neutral loss-of-heterozygosity as best practice: an evidence-based review from the Cancer Genomics Consortium working group for plasma cell disorders. *Cancer Genet.* 2018;228-229:184-196.
  36. Kanagal-Shamanna R, Hodge JC, Tucker T, et al. Assessing copy number aberrations and copy neutral loss of heterozygosity across the genome as best practice: an evidence based review of clinical utility from the cancer genomics consortium (CGC) working group for myelodysplastic syndrome, myelodysplastic/myeloproliferative and myeloproliferative neoplasms. *Cancer Genet.* 2018;228-229:197-217.
  37. Afable MG II, Wlodarski M, Makishima H, et al. SNP array-based karyotyping: differences and similarities between aplastic anemia and hypocellular myelodysplastic syndromes. *Blood.* 2011;117(25):6876-6884.
  38. Arenillas L, Mallo M, Ramos F, et al. Single nucleotide polymorphism array karyotyping: a diagnostic and prognostic tool in myelodysplastic syndromes with unsuccessful conventional cytogenetic testing. *Genes Chromosomes Cancer.* 2013;52(12):1167-1177.
  39. Ademà V, Hernández JM, Abáigar M, et al. Application of FISH 7q in MDS patients without monosomy 7 or 7q deletion by conventional G-banding cytogenetics: does -7/7q- detection by FISH have prognostic value? *Leuk Res.* 2013;37(4):416-421.

40. Mallo M, Arenillas L, Espinet B, et al. Fluorescence in situ hybridization improves the detection of 5q31 deletion in myelodysplastic syndromes without cytogenetic evidence of 5q-. *Haematologica*. 2008;93(7):1001-1008.
41. Haferlach T, Schmidts I. The power and potential of integrated diagnostics in acute myeloid leukaemia. *Br J Haematol*. 2020; 188(1):36-48.
42. Dexter TM, Allen TD, Lajtha LG. Conditions controlling the proliferation of haemopoietic stem cells in vitro. *J Cell Physiol*. 1977;91(3):335-344.
43. Michaeli J, Lerer I, Rachmilewitz EA, Fibach E. Stimulation of proliferation of human myeloid leukemia cells in culture: applications for cytogenetic analysis. *Blood*. 1986;68(3):790-793.
44. He R, Wiktor AE, Hanson CA, et al. Conventional karyotyping and fluorescence in situ hybridization: an effective utilization strategy in diagnostic adult acute myeloid leukemia [published correction appears in *Am J Clin Pathol*. 2015;144(1):175]. *Am J Clin Pathol*. 2015;143(6):873-878.
45. Coleman JF, Theil KS, Tubbs RR, Cook JR. Diagnostic yield of bone marrow and peripheral blood FISH panel testing in clinically suspected myelodysplastic syndromes and/or acute myeloid leukemia: a prospective analysis of 433 cases. *Am J Clin Pathol*. 2011;135(6):915-920.
46. He R, Wiktor AE, Dumick DK, et al. Bone marrow conventional karyotyping and fluorescence in situ hybridization: defining an effective utilization strategy for evaluation of myelodysplastic syndromes. *Am J Clin Pathol*. 2016;146(1):86-94.
47. Baliakas P, Jeromin S, Iskas M, et al; ERIC, the European Research Initiative on CLL. Cytogenetic complexity in chronic lymphocytic leukemia: definitions, associations, and clinical impact. *Blood*. 2019;133(11):1205-1216.
48. Mrózek K, Harper DP, Aplan PD. Cytogenetics and molecular genetics of acute lymphoblastic leukemia. *Hematol Oncol Clin North Am*. 2009;23(5):991-1010, v.
49. Heerema NA, Byrd JC, Dal Cin PS, et al; Chronic Lymphocytic Leukemia Research Consortium. Stimulation of chronic lymphocytic leukemia cells with CpG oligodeoxynucleotide gives consistent karyotypic results among laboratories: a CLL Research Consortium (CRC) Study. *Cancer Genet Cytogenet*. 2010;203(2): 134-140.
50. Put N, Konings P, Rack K, et al; Belgian Cytogenetic Group for Hemato-Oncology (BCGHO). 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(10): 843-853.
51. Mellors PW, Binder M, Ketterling RP, et al. Metaphase cytogenetics and plasma cell proliferation index for risk stratification in newly diagnosed multiple myeloma. *Blood Adv*. 2020;4(10):2236-2244.
52. Dewald GW, Kyle RA, Hicks GA, Greipp PR. The clinical significance of cytogenetic studies in 100 patients with multiple myeloma, plasma cell leukemia, or amyloidosis. *Blood*. 1985;66(2):380-390.
53. Rovirosa L, Ramos-Morales A, Javierre BM. The genome in a three-dimensional context: deciphering the contribution of non-coding mutations at enhancers to blood cancer. *Front Immunol*. 2020;11:592087.
54. Montefiori LE, Bendig S, Gu Z, et al. Enhancer hijacking drives oncogenic BCL11B expression in lineage ambiguous stem cell leukemia. *Cancer Discov*. 2021; 11(11):2846-2867.
55. Rustad EH, Yellapantula VD, Glodzik D, et al. Revealing the impact of structural variants in multiple myeloma. *Blood Cancer Discov*. 2020;1(3):258-273.
56. King RL, McPhail ED, Meyer RG, et al. False-negative rates for MYC fluorescence in situ hybridization probes in B-cell neoplasms. *Haematologica*. 2019;104(6): e248-e251.
57. Peterson JF, Pitel BA, Smoley SA, et al. Elucidating a false-negative MYC break-apart fluorescence in situ hybridization probe study by next-generation sequencing in a patient with high-grade B-cell lymphoma with IGH/MYC and IGH/BCL2 rearrangements. *Cold Spring Harb Mol Case Stud*. 2019;5(3):a004077.
58. Smadbeck J, Peterson JF, Pearce KE, et al. Mate pair sequencing outperforms fluorescence in situ hybridization in the genomic characterization of multiple myeloma. *Blood Cancer J*. 2019;9(12):103.
59. Lopes JL, Webley M, Pitel BA, et al. Characterizing false-positive fluorescence in situ hybridization results by mate-pair sequencing in a patient with chronic myeloid leukemia and progression to myeloid blast crisis. *Cancer Genet*. 2020;243:48-51.
60. Sharma N, Smadbeck JB, Abdallah N, et al. The prognostic role of MYC structural variants identified by NGS and FISH in multiple myeloma. *Clin Cancer Res*. 2021; 27(19):5430-5439.
61. Roberts KG, Li Y, Payne-Turner D, et al. Targetable kinase-activating lesions in Ph-like acute lymphoblastic leukemia. *N Engl J Med*. 2014;371(11):1005-1015.
62. Aypar U, Smoley SA, Pitel BA, et al. Mate pair sequencing improves detection of genomic abnormalities in acute myeloid leukemia. *Eur J Haematol*. 2019;102(1): 87-96.
63. Lestrinant V, Duployez N, Penther D, et al. Optical genome mapping, a promising alternative to gold standard cytogenetic approaches in a series of acute lymphoblastic leukemias. *Genes Chromosomes Cancer*. 2021;60(10): 657-667.
64. Neveling K, Mantere T, Vermeulen S, et al. Next-generation cytogenetics: comprehensive assessment of 52 hematological malignancy genomes by optical genome mapping. *Am J Hum Genet*. 2021;108(8):1423-1435.
65. Barseghyan H, Tang W, Wang RT, et al. Next-generation mapping: a novel approach for detection of pathogenic structural variants with a potential utility in clinical diagnosis. *Genome Med*. 2017; 9(1):90.
66. Levy B, Baughn LB, Chartrand S, et al. A national multicenter evaluation of the clinical utility of optical genome mapping for assessment of genomic aberrations in acute myeloid leukemia. *medRxiv*. 2020: 2020.2011.2007.20227728.
67. Gupta A, Place M, Goldstein S, et al. Single-molecule analysis reveals widespread structural variation in multiple myeloma. *Proc Natl Acad Sci USA*. 2015; 112(25):7689-7694.
68. Yang H, Garcia-Manero G, Rush D, et al. Application of optical genome mapping for comprehensive assessment of chromosomal structural variants for clinical evaluation of myelodysplastic syndromes. *medRxiv*. 2021:2021.2001.2013.21249611.
69. Brown LM, Lonsdale A, Zhu A, et al. The application of RNA sequencing for the diagnosis and genomic classification of pediatric acute lymphoblastic leukemia [published correction appears in *Blood Adv*. 2020;4(7):1217]. *Blood Adv*. 2020;4(5): 930-942.
70. Debackere K, Marcellis L, Demeyer S, et al. Fusion transcripts FYN-TRAF3IP2 and KHDRBS1-LCK hijack T cell receptor signaling in peripheral T-cell lymphoma, not otherwise specified. *Nat Commun*. 2021;12(1): 3705.
71. Foltz SM, Gao Q, Yoon CJ, et al. Evolution and structure of clinically relevant gene fusions in multiple myeloma. *Nat Commun*. 2020;11(1):2666.
72. Höllein A, Twardziok SO, Walter W, et al. The combination of WGS and RNA-Seq is superior to conventional diagnostic tests in multiple myeloma: ready for prime time? *Cancer Genet*. 2020;242:15-24.
73. López-Nieva P, Fernández-Navarro P, Graña-Castro O, et al. Detection of novel fusion-transcripts by RNA-Seq in T-cell lymphoblastic lymphoma. *Sci Rep*. 2019;9(1): 5179.
74. Docking TR, Parker JDK, Jädersten M, et al. A clinical transcriptome approach to patient stratification and therapy selection in acute myeloid leukemia. *Nat Commun*. 2021;12(1):2474.
75. Papaemmanuil E, Gerstung M, Bullinger L, et al. Genomic classification and prognosis in acute myeloid leukemia. *N Engl J Med*. 2016;374(23):2209-2221.
76. Haferlach T, Nagata Y, Grossmann V, et al. Landscape of genetic lesions in 944 patients with myelodysplastic syndromes. *Leukemia*. 2014;28(2):241-247.
77. Papaemmanuil E, Gerstung M, Malcovati L, et al; Chronic Myeloid Disorders Working Group of the International Cancer Genome

- Consortium. Clinical and biological implications of driver mutations in myelodysplastic syndromes. *Blood*. 2013;122(22):3616-3627, quiz 3699.
78. Goodwin S, McPherson JD, McCombie WR. Coming of age: ten years of next-generation sequencing technologies. *Nat Rev Genet*. 2016;17(6):333-351.
  79. Wetterstrand KA. DNA sequencing costs: data from the NHGRI genome sequencing program. GSP; 2020.
  80. Braggio E, Egan JB, Fonseca R, Stewart AK. Lessons from next-generation sequencing analysis in hematological malignancies. *Blood Cancer J*. 2013;3(7):e127.
  81. Bersanelli M, Travaglini E, Meggendorfer M, et al. Classification and personalized prognostic assessment on the basis of clinical and genomic features in myelodysplastic syndromes. *J Clin Oncol*. 2021;39(11):1223-1233.
  82. Palomo L, Ibáñez M, Abáigar M, et al; Spanish Group of MDS (GESMD). Spanish guidelines for the use of targeted deep sequencing in myelodysplastic syndromes and chronic myelomonocytic leukaemia. *Br J Haematol*. 2020;188(5):605-622.
  83. Bailey MH, Meyerson WU, Dursi LJ, et al; PCAWG Consortium. Retrospective evaluation of whole exome and genome mutation calls in 746 cancer samples [published correction appears in *Nat Commun*. 2020.11(1):4748]. *Nat Commun*. 2020;11(1):4748.
  84. Rusch M, Nakitandwe J, Shurtleff S, et al. Clinical cancer genomic profiling by three-platform sequencing of whole genome, whole exome and transcriptome. *Nat Commun*. 2018;9(1):3962.
  85. Trudsø LC, Andersen JD, Jacobsen SB, et al. A comparative study of single nucleotide variant detection performance using three massively parallel sequencing methods. *PLoS One*. 2020;15(9):e0239850.
  86. Zare F, Dow M, Monteleone N, Hosny A, Nabavi S. An evaluation of copy number variation detection tools for cancer using whole exome sequencing data. *BMC Bioinformatics*. 2017;18(1):286.
  87. Kim JC, Zuzarte PC, Murphy T, et al. Cryptic genomic lesions in adverse-risk acute myeloid leukemia identified by integrated whole genome and transcriptome sequencing. *Leukemia*. 2020;34(1):306-311.
  88. Roepman P, de Bruijn E, van Lieshout S, et al. Clinical validation of whole genome sequencing for cancer diagnostics. *J Mol Diagn*. 2021;23(7):816-833.
  89. Logsdon GA, Vollger MR, Eichler EE. Long-read human genome sequencing and its applications. *Nat Rev Genet*. 2020;21(10):597-614.
  90. Duncavage EJ, Schroeder MC, O'Laughlin M, et al. Genome sequencing as an alternative to cytogenetic analysis in myeloid cancers. *N Engl J Med*. 2021;384(10):924-935.
  91. Nelson AC, Yohe SL. Cancer whole-genome sequencing: the quest for comprehensive genomic profiling in routine oncology care. *J Mol Diagn*. 2021;23(7):784-787.
  92. Dolnik A, Schrezenmeier J, Bullinger L. Genome sequencing in myeloid cancers. *N Engl J Med*. 2021;384(25):e106.
  93. Haferlach T, Hutter S, Meggendorfer M. Genome sequencing in myeloid cancers. *N Engl J Med*. 2021;384(25):e106.
  94. Mack E, Kremer J, Neubauer A. Genome sequencing in myeloid cancers. *N Engl J Med*. 2021;384(25):e106.
  95. Larkin KTM, Byrd JC. Whole-genome sequencing for myeloid disease: one assay to stratify them all? *Nat Rev Clin Oncol*. 2021;18(9):543-544.
  96. Tyner JW, Tognon CE, Bottomly D, et al. Functional genomic landscape of acute myeloid leukaemia. *Nature*. 2018;562(7728):526-531.
  97. Nordlund J, Marincevic-Zuniga Y, Cavelier L, et al. Refined detection and phasing of structural aberrations in pediatric acute lymphoblastic leukemia by linked-read whole-genome sequencing. *Sci Rep*. 2020;10(1):2512.
  98. Karczewski KJ, Francioli LC, Tiao G, et al; Genome Aggregation Database Consortium. The mutational constraint spectrum quantified from variation in 141,456 humans [published correction appears in *Nature*. 2021;590(7846):E53]. *Nature*. 2020;581(7809):434-443.
  99. MacDonald JR, Ziman R, Yuen RK, Feuk L, Scherer SW. The Database of Genomic Variants: a curated collection of structural variation in the human genome. *Nucleic Acids Res*. 2014;42(Database issue):D986-D992.
  100. lafrate AJ, Feuk L, Rivera MN, et al. Detection of large-scale variation in the human genome. *Nat Genet*. 2004;36(9):949-951.
  101. Sebat J, Lakshmi B, Troge J, et al. Large-scale copy number polymorphism in the human genome. *Science*. 2004;305(5683):525-528.
  102. Collins RL, Brand H, Karczewski KJ, et al; Genome Aggregation Database Consortium. A structural variation reference for medical and population genetics [published correction appears in *Nature*. 2021;590(7846):E55]. *Nature*. 2020;581(7809):444-451.
  103. Miller DT, Lee K, Chung WK, et al; ACMG Secondary Findings Working Group. ACMG SF v3.0 list for reporting of secondary findings in clinical exome and genome sequencing: a policy statement of the American College of Medical Genetics and Genomics (ACMG) [published correction appears in *Genet Med*. 2021;23(8):1582-1584]. *Genet Med*. 2021;23(8):1381-1390.
  104. Schoumans J, Suela J, Hastings R, et al. Guidelines for genomic array analysis in acquired haematological neoplastic disorders. *Genes Chromosomes Cancer*. 2016;55(5):480-491.
  105. Li MM, Datto M, Duncavage EJ, et al. Standards and guidelines for the interpretation and reporting of sequence variants in cancer: a joint consensus recommendation of the Association for Molecular Pathology, American Society of Clinical Oncology, and College of American Pathologists. *J Mol Diagn*. 2017;19(1):4-23.
  106. Sirohi D, Schmidt RL, Aisner DL, et al. Multi-institutional evaluation of interrater agreement of variant classification based on the 2017 Association for Molecular Pathology, American Society of Clinical Oncology, and College of American Pathologists standards and guidelines for the interpretation and reporting of sequence variants in cancer. *J Mol Diagn*. 2020;22(2):284-293.
  107. Mardis ER. The \$1,000 genome, the \$100,000 analysis? *Genome Med*. 2010;2(11):84.
  108. Vergult S, Van Binsbergen E, Sante T, et al. Mate pair sequencing for the detection of chromosomal aberrations in patients with intellectual disability and congenital malformations. *Eur J Hum Genet*. 2014;22(5):652-659.
  109. Miga KH, Koren S, Rhie A, et al. Telomere-to-telomere assembly of a complete human X chromosome. *Nature*. 2020;585(7823):79-84.
  110. He R, Devine DJ, Tu ZJ, et al. Hybridization capture-based next generation sequencing reliably detects FLT3 mutations and classifies FLT3-internal tandem duplication allelic ratio in acute myeloid leukemia: a comparative study to standard fragment analysis [published correction appears in *Mod Pathol*. 2020;33(3):514]. *Mod Pathol*. 2020;33(3):334-343.
  111. Ben Lassoued A, Nivaggioni V, Gabert J. Minimal residual disease testing in hematologic malignancies and solid cancer. *Expert Rev Mol Diagn*. 2014;14(6):699-712.
  112. Jennings LJ, Arcila ME, Corless C, et al. Guidelines for validation of next-generation sequencing-based oncology panels: a joint consensus recommendation of the Association for Molecular Pathology and College of American Pathologists. *J Mol Diagn*. 2017;19(3):341-365.
  113. Petrackova A, Vasinek M, Sedlarikova L, et al. Standardization of sequencing coverage depth in NGS: recommendation for detection of clonal and subclonal mutations in cancer diagnostics. *Front Oncol*. 2019;9:851.
  114. Starks ER, Swanson L, Docking TR, et al. Assessing limit of detection in clinical sequencing. *J Mol Diagn*. 2021;23(4):455-466.
  115. Chen X, Schulz-Trieglaff O, Shaw R, et al. Manta: rapid detection of structural variants and indels for germline and cancer sequencing applications. *Bioinformatics*. 2016;32(8):1220-1222.

116. Gordon LG, White NM, Elliott TM, et al. Estimating the costs of genomic sequencing in cancer control. *BMC Health Serv Res*. 2020;20(1):492.
117. Schwarze K, Buchanan J, Taylor JC, Wordsworth S. Are whole-exome and whole-genome sequencing approaches cost-effective? A systematic review of the literature. *Genet Med*. 2018;20(10):1122-1130.
118. Souilmi Y, Lancaster AK, Jung JY, et al. Scalable and cost-effective NGS genotyping in the cloud. *BMC Med Genomics*. 2015;8(1):64.
119. Dotson P. CPT codes: what are they, why are they necessary, and how are they developed? *Adv Wound Care (New Rochelle)*. 2013;2(10):583-587.
120. Nusgart M. HCPCS coding: an integral part of your reimbursement strategy. *Adv Wound Care (New Rochelle)*. 2013;2(10):576-582.
121. Cohen JP, Felix AE. Personalized medicine's bottleneck: diagnostic test evidence and reimbursement. *J Pers Med*. 2014;4(2):163-175.
122. Singh AK, McGuirk JP. CAR T cells: continuation in a revolution of immunotherapy. *Lancet Oncol*. 2020;21(3):e168-e178.

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