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# Optical genome mapping in acute myeloid leukemia: a multicenter evaluation

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**Key Points**

- OGM achieves diagnostic outcomes equivalent to, and in 13% of cases better than, standard-of-care technologies in a real-world setting.
- OGM may uncover findings that would alter recommended clinical care or render cases eligible for clinical trials.

Detection of hallmark genomic aberrations in acute myeloid leukemia (AML) is essential for diagnostic subtyping, prognosis, and patient management. However, cytogenetic/cytogenomic techniques used to identify those aberrations, such as karyotyping, fluorescence in situ hybridization (FISH), or chromosomal microarray analysis (CMA), are limited by the need for skilled personnel as well as significant time, cost, and labor. Optical genome mapping (OGM) provides a single, cost-effective assay with a significantly higher resolution than karyotyping and with a comprehensive genome-wide analysis comparable with CMA and the added unique ability to detect balanced structural variants (SVs). Here, we report in a real-world setting the performance of OGM in a cohort of 100 AML cases that were previously characterized by karyotype alone or karyotype and FISH or CMA. OGM identified all clinically relevant SVs and copy number variants (CNVs) reported by these standard cytogenetic methods when representative clones were present in >5% allelic fraction. Importantly, OGM identified clinically relevant information in 13% of cases that had been missed by the routine methods. Three cases reported with normal karyotypes were shown to have cryptic translocations involving gene fusions. In 4% of cases, OGM findings would have altered recommended clinical management, and in an additional 8% of cases, OGM would have rendered the cases potentially eligible for clinical trials. The results from this multi-institutional study indicate that OGM effectively recovers clinically relevant SVs and CNVs found by standard-of-care methods and reveals additional SVs that are not reported. Furthermore, OGM minimizes the need for labor-intensive multiple cytogenetic tests while concomitantly maximizing diagnostic detection through a standardized workflow.

## Introduction

Acute myeloid leukemia (AML), characterized by rapid abnormal proliferation and differentiation of a clonal population of myeloid stem cells, affects 3 to 5 individuals per 100 000 per year.[1,2] Most AML

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The full-text version of this article contains a data supplement.

cases carry somatic variants, more than half of which are large genomic rearrangements or structural variants (SVs) and copy number variants (CNVs).[3,4]

Given the consistent correlation of clinical outcomes with specific genomic aberration classes, the World Health Organization, National Comprehensive Cancer Network, and the European LeukemiaNet (ELN) developed recommendations for the diagnosis and management of AML in adults based on the spectrum of CNVs, SVs, and somatic single nucleotide variants (SNVs).[5-7] Although SNVs and small SVs can be identified using next-generation sequencing (NGS),[8] karyotyping and fluorescence in situ hybridization (FISH) are the standard-of-care technologies for the detection of chromosomal translocations, insertions, deletions, and inversions.[1,4,8] However, classical karyotyping techniques have a resolution limit of only 5 to 10 Mb, and some SVs are cryptic and cannot be identified regardless of resolution. FISH has demonstrated higher sensitivity than karyotyping but has the limited use of only evaluating specific targeted regions, thus requiring large FISH panels for a comprehensive evaluation of all clinically significant abnormalities in AML. Chromosomal microarray analysis (CMA) has proven to be useful for detecting CNVs beyond the resolution of karyotyping but is ineffective for the detection of balanced SV events. SVs frequently generate gene-fusion products, knowledge of which often informs prognosis and guides potential therapeutic regimens.[9,10] Moreover, classical cytogenetic techniques are operationally complex and costly, requiring skilled technical labor not only to culture tissue samples for procuring dividing cells but also to analyze metaphase cells with complex abnormalities.

Considering the individual limitations of karyotyping, FISH, and CMA, a single automated diagnostic assay that identifies all clinically significant SVs and CNVs is desirable. Recently, several methods have been developed for the clinical diagnostics field, including NGS,[11-14] mate-pair sequencing (MPseq)[15] and anchored multiplex polymerase chain reaction (PCR) (AMP).[16] However, NGS and MPseq suffer from their inability to identify SVs associated with repetitive elements or unmappable regions and require expensive instrumentation, complex bioinformatics pipelines, and qualified health care personnel to interpret the data. AMP requires a priori information and does not provide genome-wide analysis, thereby missing untargeted genomic aberrations.

Optical genome mapping (OGM) directly visualizes ultralong DNA molecules in their native state, allowing for rapid and efficient large-scale structural and copy number evaluation of the genome.[17,18] OGM has demonstrable advantages in the identification of all classes of SVs and CNVs in a single assay.[19-24] Specifically, OGM has greater sensitivity and resolution compared with karyotyping, can interrogate the whole genome in contrast to FISH, and can detect balanced events missed by CMA.[17-25] OGM is currently used in translational research studies to detect constitutional and oncological abnormalities.[19-30] Accordingly, we sought to assess whether OGM provides a facile alternative to conventional technologies for the detection of pathognomonic findings in patients with AML. Our study was designed as a feasibility evaluation and represents a real-world scenario. In such a setting, complete and uniform genetic testing is not always available for all patients because of either a failure to obtain enough metaphases for a complete chromosome study or variability in ordering practices among different institutions. Our results indicate that OGM achieves diagnostic outcomes equivalent to, and in some cases better than, standard-of-care technologies in this real-world setting.

# Methods

## Samples

A total of 98 diagnostic peripheral blood (PB) or bone marrow (BM) samples were obtained from patients with AML and subsequently treated using standard chemotherapy regimens. Two patient samples (cases 65 and 96) were obtained after relapse. Patients were randomly selected based solely on the availability of residual samples, and the overall patient cohort reflected real-life clinical testing ordering practices (Table 1). All patients had ≥20% blasts in BM or PB. All samples underwent karyotype analysis at a CLIA/CAP-certified laboratory, and in some cases, FISH (19 out of 100) (on interphase cells) and/or CMA (3 out of 100) were performed (supplemental Table 1). Patients were recruited under institutional review board protocols approved at each institution.

## Optical genome mapping

Ultrahigh molecular weight DNA was extracted from PB or BM aspirates, fluorescently labeled, and loaded on Bionano's Saphyr for imaging, as previously described.[19] Effective genome coverage of ~300× was achieved for all samples. Detailed methods are provided in the supplemental Material.

## Assessment of clinical utility

The clinical utility was assessed as follows: (1) concordance with clinically significant SVs/CNVs reported by routine cytogenomic testing and (2) identification of additional clinically significant SVs/CNVs not identified by routine testing. We focused exclusively on SVs and CNVs of potential clinical significance by filtering all additional OGM findings for overlap with genes (supplemental Table 2) and FISH probe loci (supplemental Table 3) associated with AML. We only considered variants with sizes >0.5 Kb for SVs and >5 Mb for CNVs, which are absent in the Bionano Solve 3.6 database of human control samples. Alternative algorithms for curating AML-specific genes enabled CNV detection as low as 360 Kb.

**Table 1. Clinical characteristics of the AML study cohort**

| | |
|---|---|
| No of patients | 100 |
| Median age in y (range) | 58 (22-84) |
| Male | 47 |
| WBC, ($\times 10^9$/L) median (range) | 41 (1.3-365) |
| Newly diagnosed AML | 98 |
| Relapsed AML | 2 |
| De novo/secondary | 87/13 |
| Therapy-related - number | 3 |
| ELN cytogenetic risk group | |
| Favorable | 14 |
| Intermediate | 62 |
| Adverse | 24 |

WBC, white blood cell.

### Curation of additional clinically significant SVs/CNVs not identified by routine testing

An expert review panel (L.B.B., R.K.-S., N.S., M.S., M.F.) compiled the final curated list of additional SVs and CNVs based on 2 tiers of clinical significance: (1) aberrations used in the ELN-2022 risk-stratification system (ELN-2022) and (2) abnormalities for which sufficient published data demonstrate clinical significance,[5] irrespective of their listing in ELN-2022.

### Concordance of samples at 5% or less allelic fraction

To emulate the practical coverage that a clinical laboratory would likely attain in routine testing, we generated ~300× effective coverage for each sample, which is predicted to identify SVs with an allele fraction of ≥5% (Bionano Genomics Theory of Operation: SV Calling 2020), the equivalent of 1 SV/CNV present in 2 cells out of 20 metaphases, similar to the clonality requirements of routine cytogenetics.[31] Therefore, SVs and CNVs identified by karyotype with an allelic fraction of <5%, but not by OGM, were not considered discordant (supplemental Table 1).

### Confirmation of additional SVs and CNVs

Additional SVs and CNVs identified by OGM and deemed to be of potential clinical significance were confirmed using CMA and/or long-range PCR, as appropriate.

### OGM nomenclature

We modeled the OGM nomenclature on the microarray nomenclature described by an international system for human cytogenomic nomenclature 2020.[32] We have chosen "ogm" as the 3-letter prefix. Because microarray technology cannot detect balanced rearrangements, we have proposed a unique nomenclature using the criteria of the international system for human cytogenomic nomenclature 2020.

## Results

### Sensitivity, specificity, and additional findings

OGM identified almost all the SVs that were reported by cytogenomics (supplemental Table 1), only failing to identify some of those at low levels in the sample (≤5% abnormal clone). Moreover, in samples with complex karyotypes, OGM identified chromoplexy with multiple translocations and deletions and clarified many of the ambiguities evident in the cytogenomic analysis. Importantly, OGM identified clinically relevant genomic abnormalities identified by routine cytogenomic analysis, demonstrating 98.4% concordance for all karyotypically observed sentinel events (Figure 1; supplemental Table 1) when clones were present in ≥5% of cells (yellow bars, Figure 1). The single-sentinel event missed [t(9;22) in case 50] was present in 2 of the 20 metaphases with a calculated allelic fraction of 4.2% for the p210 protein by quantitative PCR. Of note, this patient had a previous diagnosis of chronic myelogenous leukemia, and the AML in this patient is more likely to be derived from the Philadelphia negative clone. In 4% of cases (4 out of 100), OGM missed sentinel events that were present in <5% allelic fraction (red bars, Figure 1). The sensitivity of OGM vs karyotyping was found to be 98.4% for sentinel events (ELN 2022) at allelic fractions ≥5%, the specificity was 100%, and the accuracy for all results (true positive and true negative) was 99.6% (Table 2). In 6.0% (6 out of 100) of cases, OGM identified additional clinically relevant SVs/CNVs (supplemental Table 4A) and in 7.0% (7 out of 100), OGM identified additional clinically relevant SVs/CNVs as well as resolved/refined the karyotype (supplemental Table 4B). In total, OGM identified additional clinically significant SVs and/or CNVs in 13.0% of cases (13 out of 100; supplemental Table 4A-B), all confirmed by orthogonal methods (supplemental Table 1).

In 6.3% of cases (3 out of 48) with normal karyotypes reported, OGM uncovered cryptic translocations involving clinically significant gene fusions. These included 2 cases (cases 22 and 48, supplemental Table 4A) with a t(5;11)(q35.3;p15.4) leading to a *NUP98*::*NSD1* fusion and 1 case (case 23, supplemental Table 4B) with a t(3;12)(q26.2;p13.2) resulting in a *ETV6*::*MECOM* fusion. Although the t(3;12)(q26.2;p13.2) case was initially reported as 46,XY, subsequent FISH studies identified a *MECOM* rearrangement using a MECOM break-apart FISH probe (without a defined fusion partner by FISH). Similarly, of all reported abnormal karyotype cases, OGM revealed additional clinically significant events in 15.4% (supplemental Table 4A-B) of cases (10 out of 52) and characterized the genomic aberrations in 40.4% of cases (20 out of 52) by refining the break points and identifying unknown cytogenetic elements (supplemental Table 1). In case 93 (supplemental Table 1), OGM resolved the cytogenetic unknowns by revealing the complex genomic architecture, which highlighted a chromoplexy event involving chromosome 20 and its repackaging into chromosomes 17 and 22 (Figure 2A).

Detection of classic AML rearrangements, known to affect critical genes or yield gene fusions, relies on the karyotype and/or FISH for definitive identification. All 36 such cases were identified in a single step by OGM (supplemental Table 1), and examples of SVs and CNVs detected by OGM are shown in Figure 2. Routine cytogenetic testing missed a critical inv(16)(p13.11q22.1) in case 61 (supplemental Table 4A) and failed to reveal the complexities of a seemingly conventional t(8;21)(q22;q22) in case 75 (supplemental Table 4B; Figure 2B). In the latter, FISH was not performed, and a typical *RUNX1*::*RUNX1T1* fusion was assumed based on the karyotype. Although this assumption is accurate in most instances, the partial deletion of *RUNX1T1* accompanying this specific translocation/fusion was missed and could potentially have yielded a false-negative result if only FISH was performed. In addition, a cryptic but large unbalanced translocation, resulting in the loss of 5q and replacement of that region by an identically sized portion of 4q with a similar banding pattern, was uncovered by OGM (Figure 2B).

Although our investigation of only preexisting clinical cases precluded a full comparison of OGM with FISH, we had both OGM and FISH results for 19 of the 100 patients included in our study. Of these 19 cases, 5 had a normal FISH result, which the OGM results confirmed over the FISH probe locations. Of the 14 cases that were found to be abnormal by FISH, 24 individual FISH probes were performed. Of them, 16 (67%) showed concordant FISH and OGM results. In these cases, the percent abnormal for the FISH assays ranged from 20% to 95%. Eight of 24 (33%) showed discordance between FISH and OGM, mostly due to low-level clones. In these cases, the percent of cells deemed abnormal by FISH ranged from 3.0% to 20% (corresponding to 1.5% to 10% allele frequency). These abnormalities included a 3q25 gain in 3% of images, a *KMT2A* rearrangement

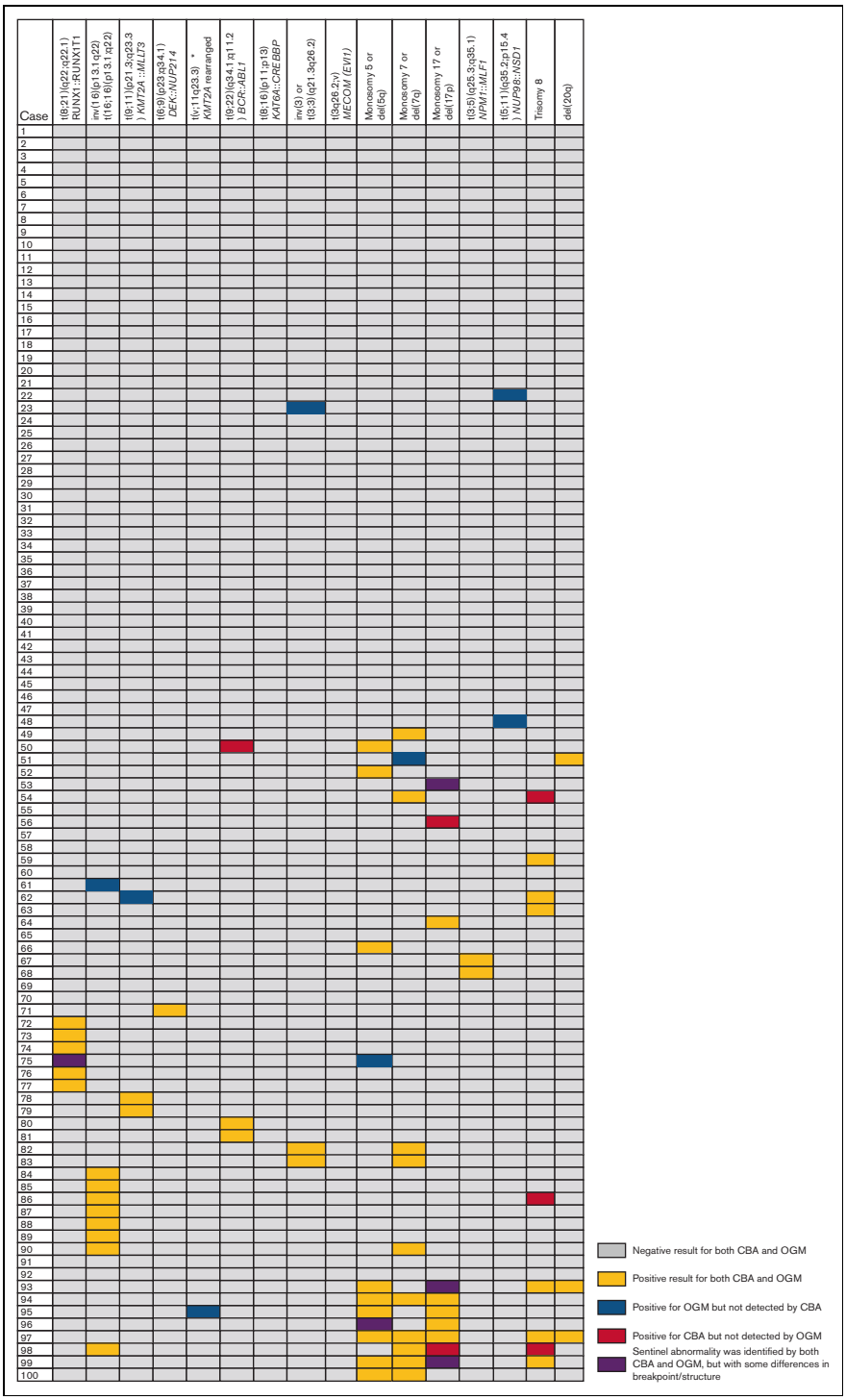


**Figure 1. Concordance of sentinel events between CBA and OGM.** *does not include t(9;11). CBA, chromosomal banding analysis.

in 4% of images, a *RUNX1* deletion in 7.5% of images, a 17p deletion in 12.5% of images, a 17q deletion in an unspecified fraction of images, and an 11q gain in 20% of images. This last SV was observed neither by OGM nor karyotyping. These observations suggest that OGM has the potential to miss finding genetic abnormalities below 20% (10% allele frequency) and may not be a suitable assay for low-level clones or for minimal residual disease detection.

## Change in risk stratification based on OGM findings

We evaluated the consequences of using OGM as a substitute for routine karyotyping to determine the risk stratification of patients in this study. This interpretation was based solely on the SVs determined by OGM and did not consider point mutations because OGM does not return SNV information and such data were not available for every patient, reflecting the real-world nature of clinical

**Table 2. Sensitivity and specificity of OGM vs karyotyping at allelic fractions above and below 5%**

| | Sentinel chromosomal abnormalities | |
|---|---|---|
| OGM vs karyotyping | <5% allelic fraction, % | ≥5% allelic fraction, % |
| Sensitivity/positive percentage agreement = TP/ (TP + FN) | 90.1 | 98.4 |
| Specificity/negative percentage agreement = TN/ (TN + FP) | 100.0 | 100.0 |
| Positive predictive value = TP/(TP + FP) | 100.0 | 100.0 |
| Negative predictive value = TN/(TN + FN) | 99.6 | 99.9 |
| Accuracy = TP + TN/all results | 99.6 | 99.6 |

FN, false negative; FP, false positive; TN, true negative; TP, true positive.

ordering practices reported in our study. OGM resulted in a change in ELN-2022 in 5 cases (Table 3). In case 51, cytogenetic studies revealed a simple karyotype, resulting in an intermediate risk stratification, whereas OGM identified additional chromosomal aberrations, including a 7q deletion, meeting the criteria for a complex karyotype, thereby upgrading the ELN-2022 from intermediate to adverse. The finding of a cryptic ETV6::MECOM fusion that was not evident by karyotyping also led to an upgrade of the ELN risk from intermediate to adverse in case 23. In case 61, OGM resulted in downgrading of the risk category from intermediate to favorable because of the detection of inv(16), which was not apparent by karyotyping. In case 75 (also described above), a cryptic unbalanced der(5)t(4;5)(q26;q21.3) and del(8)(q21.3q22.1) was uncovered by OGM in addition to the classic t(8;21)(q22;q22) translocation leading to *RUNX1::RUNX1T1* (Figure 2B). This finding was particularly relevant as the ELN-2022 categorization of favorable is made in the context of an assumed typical t(8;21), which is not the case here as exons 1 to 6 of *RUNX1T1* are deleted, thus generating a novel *RUNX1::RUNX1T1* fusion.

### Identifying opportunities for enrollment in clinical trials based on findings uncovered by OGM

In 8% of cases (8 out of 100), OGM identified additional genomic events that rendered those cases eligible for enrollment in clinical trials (Table 3). The discovery of 2 cases with cryptic translocations involving *NUP98::NSD1* (cases 22 and 48) offers potential eligibility in clinical trials for those genetic markers (ie, NCT03164057). Similarly, in case 95 (supplemental Table 1), the detection by OGM of the cytogenetically cryptic t(5;11)(p13;q23.3), involving the unfavorable *KMT2A,* may provide eligibility for at least 1 clinical trial (NCT03724084). In case 62, a complex karyotype was reported (adverse risk). Although OGM identified a t(9;11)(p21.3;q23.3), which by strict ELN-2022 would downgrade the risk to intermediate, some clinicians would still classify the case as adverse because of the complex clone. Nonetheless, knowledge of t(9;11)(p21.3;q23.3) offers the opportunity for participation in an ongoing clinical trial (NCT03513484). In case 53, a simple karyotype was suspected to have a *TP53* deletion (adverse risk), given an abnormal banding pattern on 17p. OGM changed the designation from simple to complex (>3 abnormalities, adverse risk) by revealing a deleted 12p involving ETV6 and resolving the abnormal 17p as a balanced t(1;17) without loss of *TP53.* Although this does not change the risk category, it would inform a clinician that the patient's adverse risk was independent of *TP53* and allow for potential enrollment in multiple trials that target complex karyotypes in patients with AML (eg, NCT03080766 and NCT03013998). OGM results for cases 56 and 93 also provided similar information regarding *TP53.*

## Discussion

Karyotyping has been the standard of care for patients with AML for decades, providing the basis for current management and risk stratification. This multicenter study demonstrates the performance of OGM in clinical AML samples containing a wide representation of hallmark chromosomal abnormalities. We recognize that our real-world setting represented a limitation to our study because we did not have complete conventional chromosome, karyotyping, FISH, and CMA studies available for all patients. This limitation prevented us from comparing the results of each genetic testing modality to OGM. Notably, though, the study highlights the overall variability in the genetic workup of patients with AML. For example, in case 61, inv(16) was not detected by karyotyping, and FISH was not ordered. Although it is apparent that FISH studies are necessary to definitively detect inv(16), they are not always performed in every laboratory and will inevitably be missed on some occasions. Indeed, Hernandez and colleagues reported that chromosome studies were able to identify the inv(16) in 9 out of 10 clinical samples, highlighting that in real-world practice, karyotyping failed to identify the inv(16) in a single case in their series.[33]

We have shown that OGM matches the diagnostic scope of current standard-of-care methods and adds new important clinical information in 13% of cases. Indeed, in 27% of cases, OGM assembled a more accurate and complete karyotype by refining cytogenetic break points, resolving unknown cytogenomic elements, and detecting additional clinically significant SVs and CNVs. This indicates that gold standard cytogenetic techniques do not provide the entire picture of genomic events, and prospective studies are required to assess patient outcome in the context of a more complete molecular cytogenomic OGM profile. As an example, the case with an apparently simple karyotype with t(8;21) revealed an atypical *RUNX1::RUNX1T1* fusion with an additional unbalanced translocation resulting in a loss of 5q. Although the presence of a typical t(8;21) would confer a favorable outcome, the uncertainty of a novel fusion coupled with the adverse outcome generally associated with a 5q deletion would prudently warrant increased surveillance of the patient.[34] Follow-up of such cases may shed light on why some patients with favorable aberrations relapse, potentially explaining the variability associated with survival differences.[35] We also note that multiple novel, uncurated SVs and CNVs were identified in this study and may serve as potential candidates for future research.

OGM shows no obvious deficiencies in performance compared with karyotyping, surpasses the combination of multiple costly tests, and presents a more refined and simplified workflow with additional cost benefits (Table 4). Several advantages are also apparent; OGM affords better statistical estimation of the clonal fraction than the karyotype as it uses single molecules derived from a minimum starting number of 1 million cells, making it more accurate than the analysis of 20 G-banded metaphases.[17-19] Moreover, because OGM does not require cell culture, the actual subclone frequency is more accurately reflected and not biased by

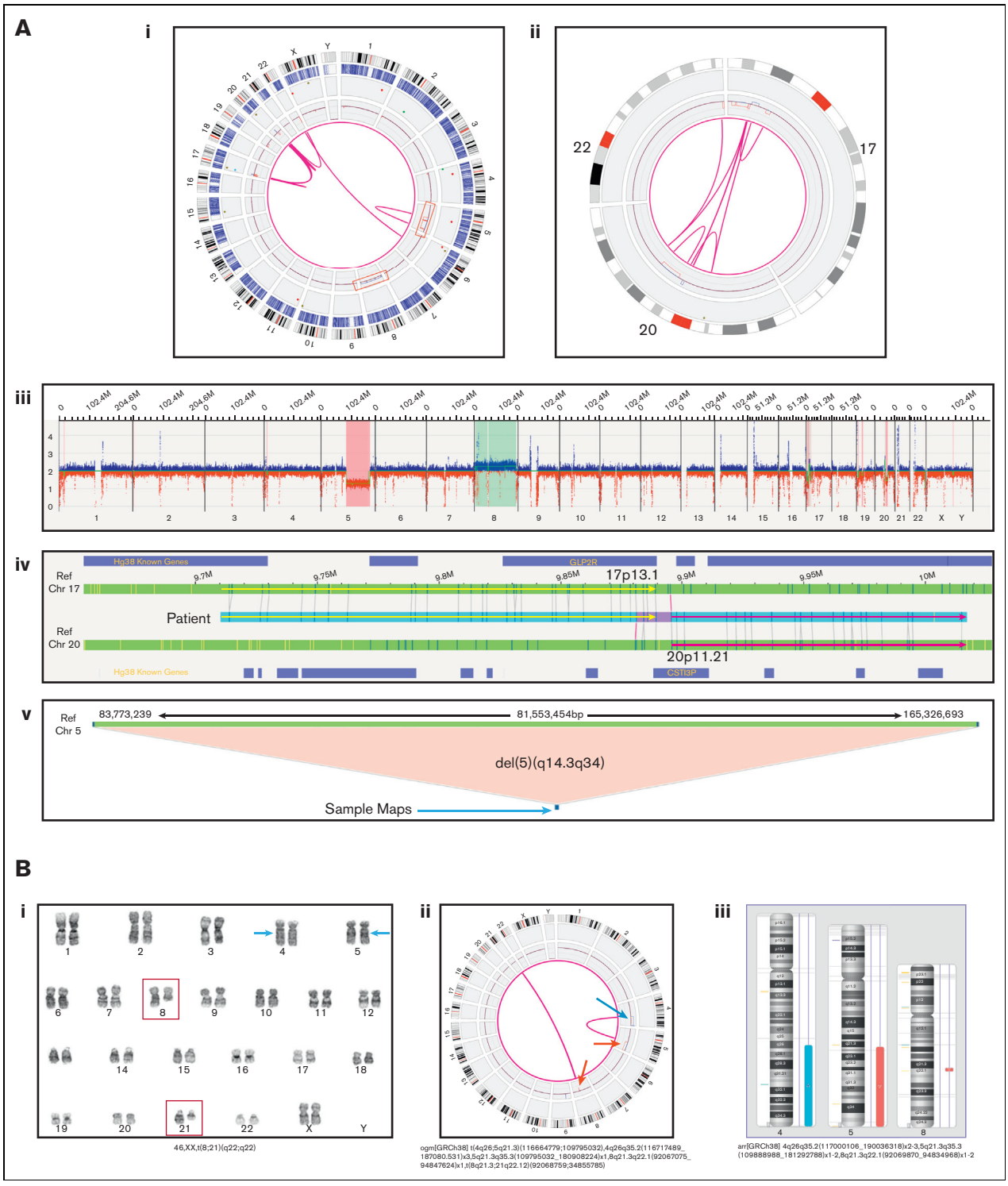


**Figure 2. Illustrative examples of SVs, CNVs, and unknown cytogenetic elements resolved by OGM.** (A) Case 93. (i) Circos plot showing a whole genome view of the multiple genomic rearrangements (pink lines) and copy number profiles (inner circle blue boxes indicate gains and red boxes indicate deletions). (ii) Circos plot showing a selected chromosome view of the complex genomic rearrangements (pink lines) and copy number profiles (inner circle blue boxes indicate gains and red boxes indicate deletions) between chromosomes 17, 20, and 22. (iii) Whole genome CNV profile showing an interstitial deletion on 5q as well a gain of chromosome 8. (iv) Single molecule view of patient DNA (blue) mapping to both reference chromosomes 17 and 20 with break points in *GLP2R* and *CSTI3P*, respectively. (v) Fine mapping of the chromosome 5 deletion indicates a large 81.5Mb deletion on the q arm of chromosome 5 between genomic coordinates 83 773 239 and 165 326 693 (human genome build GRCh38). (B) Case 75. (i) Karyotype showing the reported t(8;21)(q22;q22) (red boxes) and break points (blue arrows) of the cryptic der(5)t(4;5)(q26;q21.3). (ii) Circos plot showing the t(8;21) (q22;q22) and der(5) t(4;5)(q26;q21.3) (pink lines), deletions (red arrows) at 8q21.3q22.1 and gain (blue arrow) of 4q26q35.2. (iii) CMA profile of deletions and losses shown in panel Bii.

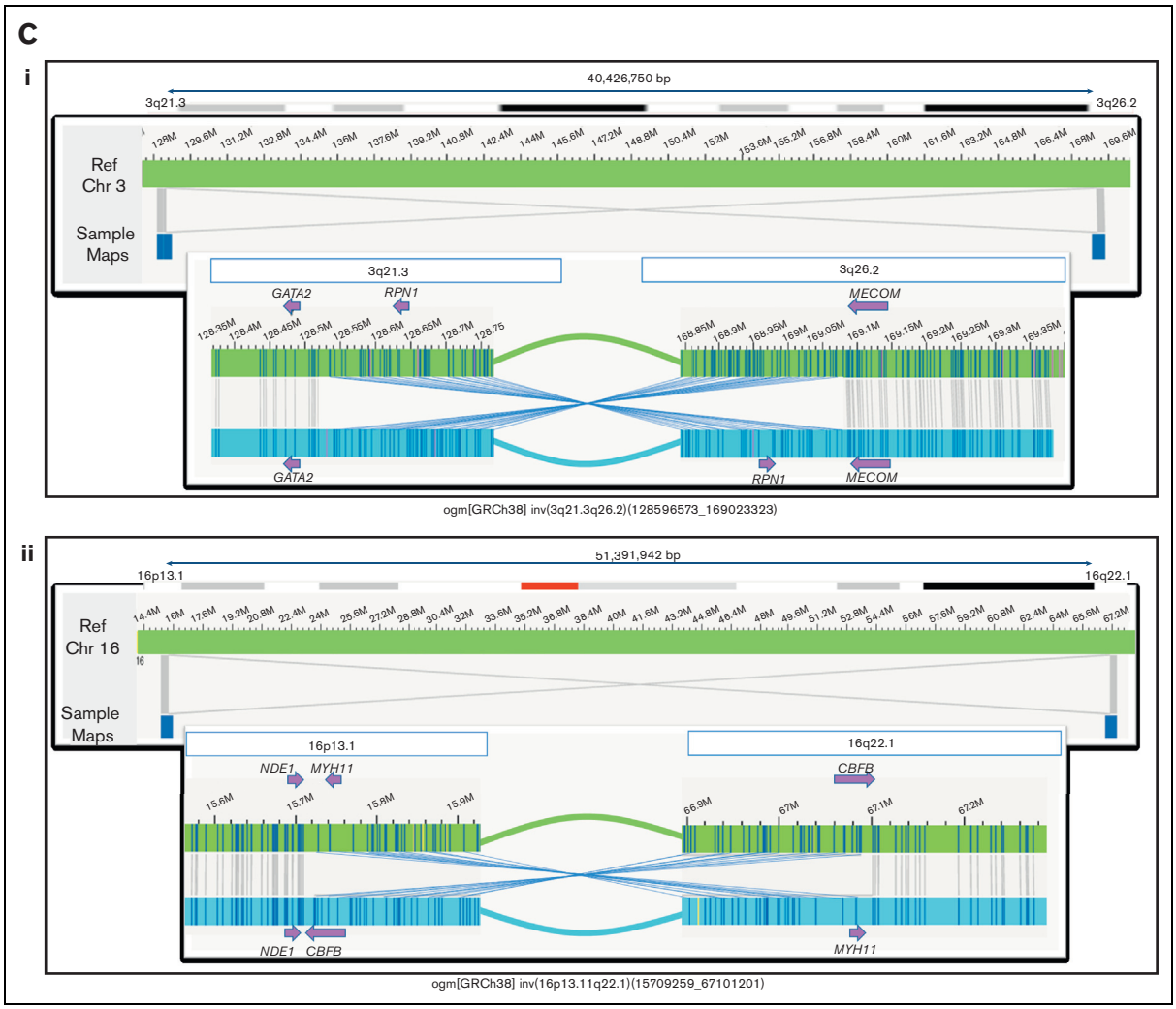


**Figure 2 (continued)** (C) Classic AML rearrangements. GRCh38 reference chromosomes with OGM label patterns are shown in green. Assembled sample maps with label patterns are shown in light blue. Label alignments between 2 maps are shown in gray strings. Overlapping genes are shown in purple. (i) Case 82 with inv(3)(q21.3q26.3). (ii) Case 84 with inv(16)(p13.11q22.1).

**Table 3. Cases identified with a change in ELN risk stratification and/or clinical trial opportunity**

| Case | Significant cytogenetic findings from chromosomes | ELN risk from chromosomes | Significant cytogenetic findings from OGM | ELN risk from OGM | Change in ELN | Clinical trial opportunity |
|---|---|---|---|---|---|---|
| 75 | t(8;21) | Favorable | Atypical *RUNX1*::*RUNX1T1*, del(5) | Adverse | 1 | |
| 61 | Simple karyotype | Intermediate | *CBFB*::*MYH11* | Favorable | 1 | |
| 23 | Normal karyotype | Intermediate | *ETV6*::*MECOM* | Adverse | 1 | |
| 51 | Simple karyotype | Intermediate | 7q deletion, *RUNX1* deletion | Adverse | 1 | |
| 68 | Simple karyotype with t(3;50) | Intermediate | t(3;5) with *RUNX1* deletion | Adverse | 1 | |
| 22 | Normal karyotype | Intermediate | *NUP98*::*NSD1* | Intermediate | | 1 |
| 48 | Normal karyotype | Intermediate | *NUP98*::*NSD1* | Intermediate | | 1 |
| 62 | Simple karyotype | Intermediate | *KMT2A*::*MLLT3* | Intermediate | | 1 |
| 53 | del(17p) | Adverse | Complex | Adverse | | 1 |
| 56 | Mono 17, monosomal karyotype | Adverse | Complex | Adverse | | 1 |
| 93 | del(5q), del(17p) | Adverse | del(5q) | Adverse | | 1 |
| 95 | del(5q), del(17p) | Adverse | del(5q), del(17p), *KMT2A r* | Adverse | | 1 |
| 99 | del(5q), mono 7, del(17p) | Adverse | del(5q), mono 7 | Adverse | | 1 |

**Table 4. Comparison of cytogenomic testing modalities**

| | Karyotype | FISH panels | CMA | NGS | AMP | MPseq | OGM |
|---|---|---|---|---|---|---|---|
| Genome coverage | Whole | Targeted | Whole | Whole (WGS) or targeted (WES, custom capture) | Targeted | Whole | Whole |
| Resolution | 5-10 Mb | 100-200 kb | 250-500 kb | 100-150 bp | 100-1000 bp | 100-1000 bp | >500 bp |
| Technical analysis bias | Yes | Yes | No | No | No | No | No |
| Sample requirements | Dividing cells | Interphase nuclei | High-quality DNA | High-quality DNA | High-quality DNA | High-quality DNA | Very high-quality DNA. Specialized extraction |
| FFPE acceptable | No | Yes | Yes | Yes* | Yes | No | No |
| Reporting time | ≥ 3 d | 4 h to >1 d | ≥3 d | 7-14 d | ≦3 d | 7-14 d | >3 d |
| Cost | ++ | +++ | +++ | ++++ | +++ | ++++ | ++ |
| Assay complexity | +++ | + | ++ | +++ | +++ | +++ | + |
| Copy neutral loss of heterozygosity | ND | ND | Detectable | Detectable | ND | In development | In development† |
| Detection of unmappable regions | Yes | No | No | No | No | No | Some Alu and Line elements |
| Analytical sensitivity | | | | | | | |
| Genome-wide Assessment | | | | | | | |
| Balanced SVs | 2 of 20 metaphases | ≥0.6% interphase nuclei‡ | ND | WGS only >20% sample | 50 copies of RNA fusion transcript | >10% sample | >10% sample |
| CNVs | 2 of 20 metaphases | 2.5%-9.5% interphase nuclei‡ | 20%-25% sample | >20% sample | ND | >25% sample | >10% sample |
| SNVs | ND | ND | ND | Detectable | ND | Some detectable with deeper sequencing | ND |
| Current reimbursement | Likely | Likely | Likely | Likely | Likely | Some | Unknown |
| Data storage | Low | Low | Medium | High | Low | High | Medium |
| Software availability | Commercially available | Commercially available | Commercially available | In-house software or commercially available software | Commercially available | In-house software | Commercially available |

+ refers to expense with + being least expensive and ++++ being the most expensive.
ND, not detectable; FFPE, formalin-fixed paraffin-embedded.
*Data derived from Aypar et al,[15] Zheng et al,[16] Murphy et al,[37] and Akkari et al.[8].
†cnLOH is currently detectable for constitutional samples but is in development for cancer samples.
‡Cutoffs for SVs/CNVs are dependent on the probe applied.

cultural artifacts. This becomes particularly relevant when addressing the lower limit of detection (LOD). Here, the LOD appeared to be around a 5% allele fraction, which is consistent with the LOD reported by Sahajpal et al,[36] who empirically demonstrated the LOD for several classes of SVs from 25% down to 5% allele fraction.[36] In cases 24 and 25, nonsentinel karyotype abnormalities were present at 10% and 11%, respectively. This translates to an allele fraction of 5% and 5.5%, respectively, which is right at the LOD threshold of OGM (5%). Because OGM is performed without culturing cells, and given the significantly higher number of molecules analyzed, the true variant allele frequency in the extracted bulk DNA used for OGM (preculture) in cases 24 and 25 may have been below 5%. Discrepancies in variant detection between karyotyping and OGM at the 5% allele fraction boundary have also been reported by other groups.[22,36] Because empirical experiments using OGM detect SVs down to a 5% allele fraction, the karyotype events missed by OGM most likely represent culture bias of allele fractions that were initially below 5% but artificially inflated during the cell culture process.

Another advantage is the precise assignment of SV and CNV break points, indicating gene fusions, uncovering cryptic translocations, and identifying CNVs below the resolution of standard G-banded karyotyping (<10 Mb). Although these smaller CNVs can be detected by CMA or targeted FISH, CMA is not yet universally performed, and only a limited number of FISH probes targeting specific AML gene fusions and/or hallmark abnormalities are used. Importantly, each additional FISH probe adds extra health care costs, and clinical laboratories vary greatly in the FISH panels offered, with the choice of panel following laboratory- or clinician-specific algorithms. In fact, we noted that genomic testing practices for AML differed widely even among the authors' respective institutions, highlighting the lack of standardization of the genetic workup for AML across laboratories.

Recent NGS-based approaches have been proposed and tested for replacement of traditional cytogenomics methods. All critical variant types important in AML appear detectable by using NGS strategies with the range of variant types detectable being dependent on the specific NGS assay and depth of sequencing.[8,12-14,17-19,38,39] Although whole genome sequencing (WGS) has been reported to provide clinical uses for the workup of AML, the challenges of adopting this technology worldwide and for all AML cases remain to be addressed. Many factors need to be considered to ensure that WGS is effective in the workup of AML cases.[8]

Adaptations of NGS have been applied to clinical cancer genomes, especially in the form of gene panels. AMP can detect point mutations as well as some fusions, but depends on the knowledge of 1 fusion partner with high precision.[16] MPseq is a powerful technique for detecting genomic fusions in a wholegenome approach and obviates some of the ambiguity associated with short-read sequencing.[40] Our study was not directed at a comparison of OGM with any of the NGS-based approaches, but other studies have shown that OGM and WGS identify different, albeit overlapping, sets of SVs, suggesting potential synergy between the 2 methods.[39,41-43] However, all NGS-based approaches share several major hurdles, including equipment price, complexity of the sequence library preparation, and data analysis intricacy.

OGM has a short turnaround time, requiring approximately 9 hours for DNA isolation and library preparation, with 2 hours of hands-on time, and 24 hours for automated data collection. As with other technologies, additional time is required for data analysis and interpretation by trained laboratory specialists, which, like CMA, is proportional to the complexity of the genomic aberrations. The assay can be conducted without significant specialized training by a laboratory technologist who has experience with general molecular biology techniques. The current average cost of OGM per sample is around $500, which makes this technology comparable to karyotyping alone, cheaper than FISH panels or CMA, and significantly cheaper than WGS and whole exome sequencing.

There are some drawbacks to the OGM technique. Firstly, OGM requires high-molecular-weight DNA isolated by specialized kits, which precludes its use on most archival DNA banks and formalin-fixed paraffin-embedded tissue. OGM is not a sequencing-based assay and therefore cannot identify SNVs. Moreover, OGM currently fails to detect SVs located exclusively in centromeric or telomeric regions. In addition, as noted in "Methods," the OGM coverage used in this study (300×) does not routinely detect variants present at 5% or less. This limitation may in rare cases affect treatment decisions but will be obviated soon with the introduction of routine OGM coverage of greater than 1000×. Finally, the throughput of the current OGM equipment is relatively low, which makes implementation in a high-volume laboratory challenging. However, since the beginning of the current study, throughput has quadrupled. Considering that abnormalities requiring 3 independent assays for identification can instead be detected at once with OGM, the current OGM workflow represents a significant reduction in time and costs for the clinical AML workup. In addition, because OGM analytics are automated, standardized care across different testing laboratories can be achieved, effectively minimizing health care disparities.

Our study demonstrates that OGM has the potential to be the standard-of-care methodology for cytogenomic evaluation of patients with AML. By identifying previously unrecognized SVs and CNVs, OGM could play a significant role in the identification of targetable genomic aberrations for novel breakthrough therapeutic options. This feasibility study can pave the way for similar studies on other neoplasms that rely on cytogenomic workup for risk stratification. Finally, this technology can be readily accommodated in countries lacking specialized cytogenetic technologists with limited resources and thereby expands the possibility of providing uniform diagnostic care and performance across the globe.

## Authorship

Contribution: B. Levy conceptualized the project, administrated the project, performed formal analysis, supervised, wrote the original draft, reviewed and edited the manuscript, and provided resources; L.B.B. and Y.A. performed formal analysis and wrote, reviewed, and edited the manuscript; S.C. conducted investigation; B. LaBarge was responsible for methodology; D.C. wrote, reviewed, and edited the manuscript, acquired funding, and provided resources; P.A.L. conducted investigation and wrote, reviewed, and edited the manuscript; C.C. conducted investigation and provided resources; R.K. conducted investigation, provided resources, and wrote, reviewed, and edited the manuscript; K.K. and B.P. conducted investigation; N.S. conducted investigation, performed analysis, and wrote the manuscript; M.S. and G.V. conducted investigation; L.Z. was responsible for the software and formal analysis; M.F. performed formal analysis, was project administrator, supervised,

and wrote, reviewed, and edited the manuscript; R.K.-S. performed formal analysis and wrote, reviewed, and edited the manuscript; and J.R.B. conceptualized the project, performed formal analysis, supervised, and wrote, reviewed, and edited the manuscript.

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