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Research Article

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# Application of Optical Genome Mapping to the Risk Stratification and Treatment Optimization of Hematologic Diseases

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## Abstract

*Structural Variations (SVs) play a key role in the pathogenicity of hematological malignancies. Optical Genome Mapping (OGM) is an emerging technology that enables genome-wide detection of all classes of SVs at a high resolution and sensitivity. Identification of cryptic SVs leading to gene disruption or predicted novel gene fusions could be important drivers for cancer development and/or portend a prognostic relevance, which could be used to modify the treatment plan. A cohort of 106 consented cases that had a successful OGM analysis performed were included in the study. Demographic, clinical, laboratory and treatment data were collected. Routine diagnostic and prognostic testing were done on the peripheral blood and bone marrow aspirate as indicated. Additional samples of peripheral blood and/or bone marrow were sent for OGM testing. OGM led to a change in risk stratification in 17/66 (25.75%) of patients with hematological malignancies, the majority of these patients (15/66, 22.72%), having their risk stratification upgraded with a resulting change in treatment of 14 patients. This study highlights the ability of OGM to detect rare, cryptic and clinically relevant variants that potentially impact disease diagnosis, risk stratification and actionable treatment targets.*

**Keywords:** Structural Variation, Optical Genome Mapping, Cytogenetic, Hematological Disorders

## Abbreviations

**OGM**: Optical Genome Mapping,
**KT**: KaryoTyping,
**FISH**: Fluorescent In Situ Hybridization,
**CMA**: Chromosomal Microarray,
**NGS**: Next Generation Sequencing,
**AML**: Acute Myeloid Leukemia,
**ALL**: Acute Lymphoblastic Leukemia,
**MDS**: Myelodysplastic Syndrome,
**MPN**: Myeloproliferative Neoplasm,
**MPAL**: Mixed Phenotype Acute Leukemia
**CLL**: Chronic Lymphocytic Leukemia.

## Simple Summary

*Optical Genome Mapping (OGM) is an advanced technology for the detection of genome-wide structural variation. This*

*collaborative prospective study, focused on evaluating the utility of OGM for the diagnosis, risk stratification and management of hematologic disorders, was undertaken by multiple cancer centers participating in the International Hematology Consortium, based in Bengaluru, India. A total of 106 cases (Hematological Disorders) were included in this analysis, of which 94 were included for detailed risk stratification and prognostication. OGM led to a change in risk stratification in 17/66 (25.75%) of patients with hematological malignancies, the majority of these patients (15/66, 22.72%), having their risk stratification upgraded with a resulting change in treatment for 14 of these patients. This study emphasizes OGM as a valuable diagnostic tool, capable of detecting rare, cryptic, and clinically relevant variants, ultimately impacting disease diagnosis, risk stratification, and the management of hematological malignancies.*

## 1. Introduction

Detailed genetic analysis is an essential part of the management of hematological malignancies including for diagnosis, therapeutic decision-making, targeted therapy and prognostication [1,2]. Currently, conventional karyotyping, Fluorescent In Situ Hybridization (FISH) and, more recently Next Generation Sequencing (NGS), have been the principal diagnostic tools utilized across hematological malignancies [3-5]. For the past five decades, conventional karyotyping has been used to decipher the chromosome number and structure in various hematologic malignancies. However, it has a number of limitations. Firstly, the cells have to undergo culturing and therefore often there are an insufficient number of analyzable metaphase cells for the test to be informative. Even when successful, chromosome banding has limited resolution of approximately 10 Mb. There are samples (e.g. dry tap) and certain types of malignancies (e.g. B-ALL) where obtaining a successful and informative karyotype can be more challenging [6]. Other orthogonal techniques have been added to our clinical practice to aid in the detection of recurrent abnormalities, such as Fluorescence In Situ Hybridization (FISH), Chromosomal MicroArray (CMA), PCR assays, and more recently NGS-based technologies. However, based on cost, turnaround time, and raw genome-scale detection power for chromosomal changes- the karyotype has remained the front-line gold standard for many years. FISH does not require dividing cells and can detect abnormalities <10 Mb, effectively expanding the resolution from large chromosome bands down to gene-level imbalances, however, FISH is a targeted assay that is dependent on commercially available probes and thus is dependent on prior knowledge of the specific gene or region of interest [7-10].

Optical Genome Mapping (OGM) is an evolving technology for the detection of genome-wide structural variation using the Saphyr whole-genome imaging system (Bionano, San Diego). OGM provides two major benefits. First, a single test can unravel the underlying architecture of complex genomic rearrangements of multiple classes at high resolution (down to 500 bp). Second, OGM provides systematic genome-wide assessment of balanced and unbalanced rearrangements (translocations and inversions) [11-13]. These advantages provide the ability to identify recurrent as well as novel translocation, Copy Number Variants (CNVs), and other chromosomal anomalies [8-12].

Concordance to standard clinical testing methods was published for multiple hematological malignancies [11,14-30]. Levy and colleagues published a deep dive multisite study of Acute Myelogenous Leukemia (AML) where they revealed that OGM was 100% concordant with karyotyping and FISH and detected additional clinically relevant abnormalities missed by standard tests in 13% of cases [15]. They proceeded to show that these additional abnormalities resulted in a change in risk prognosis and made some of these cases eligible for clinical trials. A study of myelodysplastic syndrome was conducted on 101 samples where a 54-gene sequencing panel was combined with OGM, the authors reported that they could find at least one pathogenic variant in 97% of these cases [14]. They proceeded to show that 51% of SVs detected by OGM were cryptic to karyotyping and adding these additional SVs changed risk stratification in 21% of cases. In a study of 60 pediatric ALL cases, OGM was benchmarked against clinical karyotyping, FISH, and chromosomal microarray. OGM detected 95% of abnormalities detected with the combination of all three of these methods and went on to identify 19 recurrently altered regions never previously reported. This type of exciting finding may lead to the discovery of new biomarkers useful to provide better prognosis or treatment options [23].

Studies on the value of Whole Genome Sequencing (WGS) in AML have likewise been reported. Compared to OGM, WGS is much more complex and expensive, especially for generation of the higher coverage depths that are required for detection of structural variants with low variant allele fractions [31]. A key advantage of OGM is the relative ease to implement – it does not require specially trained lab technicians, is extremely robust, and the analysis software provides a simple graphical user-friendly interface. This manuscript describes the first prospective study of the utility of OGM for the diagnosis, risk stratification and management of hematologic disorders. The participating sites in this collaborative study are from the International Hematology Consortium, based in Bengaluru, India.

## 2. Materials and Methods

### 2.1. Clinical Data

Demographic, clinical, laboratory and treatment data were collected. Routine diagnostic and prognostic testing were done on the peripheral blood and bone marrow aspirate, where indicated. Conventional karyotype, FISH and NGS was performed in local laboratories, as per the preferences of the treating physicians. Additional samples of peripheral blood and/or bone marrow were collected for OGM. OGM testing has been available in India since January 1st, 2023. All the patients who had OGM reports available until May 1st, 2023, were included in the analysis. All procedures performed in the current study were approved by the institutional ethical committee in accordance with 1964 Helsinki declaration and later amendments.

### 2.2. Conventional Cytogenetics

For karyotyping, heparin BMA/peripheral blood samples were used and cultured for 48 hours (72 hrs for multiple myeloma) in RPMI1640 medium which is supplemented with 10% fetal calf serum and antibiotics. After hypotonic treatment with 0.075 M KCl and fixation in methanol/acetic acid (3:1), microscopic slides (GTG banding) were prepared. Chromosomes were G-banded with trypsin and Giemsa. At least 20 metaphases were analyzed in case of a normal karyotype and at least 10 in case of an abnormal karyotype. Karyotypes were reported according to ISCN 2020. The cases in which FISH was performed, the same chromosome preparations with commercially available probes were used and processed according to the manufacturer's protocol (MetaSystems). Details of probes used for FISH analysis have been provided as a supplement sheet.

### 2.3. Next Generation Sequencing

For NGS Blood/Bone Marrow collected in EDTA were used for nucleic acid extraction followed by library preparation using the commercially available Illumina® Ampliseq TM Myeloid panel consisting of 40 DNA alterations (hotspot mutations and whole exons of select genes) along with 29 fusion driver genes on RNA analysis for conditions such as AML, CML, MDS, MPN and MDS/MPN. Bioinformatics analysis was performed on the proprietary Strand OMS pipeline and limit of detection was >5% Variant Allele Frequency (VAF). Sequencing used for this study was short-read sequencing technology. Commercially available targeted gene panels utilizing short read technology were sequenced on Illumina Nextseq platform and analyzed using a proprietary bioinformatics pipeline.

### 2.4. OGM Analysis

Samples were sent for genomic structural variation analysis by OGM to Bionano Laboratories (Bionano, San Diego) via international courier. Briefly, ultra-high-molecular-weight DNA was isolated (bone marrow and peripheral blood), fluorescently labeled, and processed for analysis on the Bionano Genomics Saphyr platform following the manufacturer's protocols (Bionano, San Diego, CA); sequence-motif specific labelling of the DNA with DL-green fluorophore followed by electrophoretic linearization and flow through the Saphyr nanochannel arrays allowed capture of label patterns on the long, single DNA molecules. The overall expected DNA molecule data were targeted to achieve >400x effective coverage of the genome, >70% mapping rate, label density of 13 to 17 (per 100kb), and >230kb N50 (of molecules >150kb). Data analysis was performed utilizing the proprietary rare variant pipeline included in Bionano Access version 1.7.

Molecules passing quality metrics were directly aligned to human genome assembly version GRCh38 and evaluated for a broad range of structural variations (insertions, copy number variations, inversions and translocations) on the basis of the differences in the alignment of labels between sample and reference assembly. Additionally, a coverage-based algorithm enabled. Detection of large CNVs and whole chromosome aneuploidies Tier 1A/1B and Tier 2 mutations were considered for clinical decision making. Diagnoses were reported as per the WHO Classification of Hematolymphoid tumors, 4th ed. 2017. Prognostication was as per the European Leukemia Net (ELN) 2022 recommendations for AML, BFM 2002 for acute lymphoblastic leukemia (ALL), European Leukemia Net (ELN) 2020 recommendations for CML, IPSS-R criteria for MDS, International Prognostic Index (IPI) for CLL, R-IPI for lymphoma and the mSMART for multiple myeloma. Chi-square test was used for calculating statistical significance.

## 3. Results

The average transit time for the samples to reach the lab in San Diego, CA from various collection points in India, via international courier, was 6.6 days. OGM analysis was attempted on 106 samples, of which 6 (5.66%) failed to meet quality thresholds for reporting, giving a karyotype report with or without structural variants in 95.3%. A total of 106 cases with completed and reported OGM analysis were included in this analysis. The average age of patients was 47.7 years with a median age of 50.5. The cases included 42 females and 64 males. The details of the diagnosis are given in Table 1. The ratio of abnormal to normal OGM results was 6.6 to 3.4. Of the cases with abnormal OGM results, 46% had a complex genome and 54% had a simple genome. Acute Myeloid Leukemia (AML) constituted the largest diagnostic subset, (n=25, 23.58%) followed by ALL (n=17, 16.03%), MPN (n=15, 14.15%), Lymphoma (n=11, 10.37%), Non neoplastic conditions (n=12, 11.32%), multiple myeloma (n=8, 7.54%), MPAL (n=5, 4.71%), MDS (n=5, 4.71%), CLL (n=4, 3.77%) and MDS/MPN (n=4, 3.77%).

| Diagnosis | Number of Patients (%) |
|---|---|
| AML | 25 (23.58%) |
| ALL | 17 (16.03%) |
| MPN | 15 (14.15%) |
| Lymphoma | 11 (10.37%) |
| Multiple myeloma | 8 (7.54%) |
| MPAL | 5 (4.71%) |
| MDS | 5 (4.71%) |
| CLL | 4 (3.77%) |

| | |
|---|---|
| MDS/MPN | 4 (3.77%) |
| Non-Neoplastic | 12 (11.32%) |
| Total | 106 (100%) |

**Table 1: Distribution of Cases Based on Clinical Diagnosis N=106**

### 3.1. Comparison of Karyotype, FISH, NGS and OGM in Hematologic Disorders

As detailed in table 2, we compared the positivity of cytogenetics (KT + FISH), NGS and OGM. This analysis was done for all diseases. In AML samples, cytogenetics was positive in 9/19 cases (47.36%) whereas NGS was informative in 11/13 cases (84.61%) and OGM in 19/25 (76%) (p = 0.04). In ALL cases, cytogenetic, NGS and OGM showed positivity in 7/12 cases (58.33%), 4/5 cases (80%), 14/17 cases (82.35%) respectively (p=0.33). While in MPAL the cytogenetic, NGS and OGM positivity was in 3/5, 2/2 and 4/5 cases. In MPN cases positivity was 4/7, 2/4 and 12/15 respectively. In CLL, cytogenetic, NGS and OGM were informative in 2/3, 1/1 and 3/4 cases respectively, while in lymphomas cytogenetic helped in 3/3 of cases and OGM in 10/11 cases. In MDS, cytogenetics, NGS and OGM were helpful in 2/4, 3/3 and 3/5 cases respectively while in MDS/MPN they were informative in 3/3, 4/4 and 2/4 cases. In Multiple myeloma, OGM was useful in 3/8 cases while cytogenetic in 5/7 cases.

| Diagnosis | Cytogenetics Positive | NGS Positive | OGM Positive |
|---|---|---|---|
| AML (25) | 9/19 | 11/13 | 19/25 |
| ALL (17) | 7/12 | 4/5 | 14/17 |
| CLL (4) | 2/3 | 1/1 | 3/4 |
| MPN (15) | 4/7 | 2/4 | 12/15 |
| MDS (5) | 2/4 | 3/3 | 3/5 |
| MDS/MPN (4) | 3/3 | 4/4 | 2/4 |
| MPAL (5) | 3/5 | 2/2 | 4/5 |
| Multiple Myeloma (8) | 5/7 | 0 | 3/8 |
| Lymphoma (11) | 3/3 | 0 | 10/11 |
| Non-Neoplastic (12) | 5 | 0 | 2/12 |
| Total (106) | 43 | 27 | 72 |

**Table 2: Karyotype/FISH, NGS and OGM Positivity**

Cases with potentially non-neoplastic unclassifiable disorders included (n=12) reactive plasmacytosis (1), post chemotherapy myelosuppression (1), primary immunodeficiency syndrome (1), anemia under evaluation (1), congenital dyserythropoietic anemia (1), large granular lymphocytosis (1), hypereosinophilic syndrome (1), ITP (1), Aplastic anemia (1), Chediak Hegashi syndrome (1) and Treatment dependent anemia with dysplasia (1) and fanconi anemia(1).

Further analysis was performed on the samples of patients who had hematological malignancies (n=94) and other 12 non-neoplastic cases were excluded from further analysis. NGS was useful in 27/32 cases. However, NGS results were not discussed in detail as the main aim of our study was to compare the risk stratification and prognostication between conventional cytogenetics and OGM results. Considering Tier 1A/1B and Tier 2 mutations, in most of the patients, OGM detected more abnormalities than conventional cytogenetic analysis. (Table 3)

| Diagnosis | Mean Number of Abnormalities Detected by Cytogenetics | Mean Number of Abnormalities Detected by OGM |
|---|---|---|
| AML (25) | 1.1 | 4.6 |
| ALL (17) | 1.5 | 8.9 |
| CLL (4) | 1 | 3 |
| MPN (15) | 1 | 2.5 |
| MDS (5) | 3 | 11.3 |
| MDS/MPN (4) | 1 | 0.75 |
| MPAL (5) | 1.5 | 4.3 |

| | | |
|---|---|---|
| Multiple myeloma (8) | 2.4 | 11.3 |
| Lymphoma (11) | 1 | 9.7 |
| Total (94) | | |

**Table 3: Average Number of Structural Variants Detected by Cytogenetics Versus OGM**

### 3.2. OGM in Diagnosis

OGM played a diagnostic role in a total of 9 patients with MPN (1 primary myelofibrosis and 8 CML) and all CLL (4 cases). It confirmed/corroborated the diagnosis by identifying the diagnostic hallmark of CML t(9;22) in 8 out of 10 cases. OGM showed clonal evolution in the remaining 2 cases of CML. Another 1 of these 8, elucidated by OGM and not by cytogenetics. One case out of 8 showed additional abnormalities by OGM in the form of trisomy 8 and 17p11.2 deletion. OGM detected abnormalities assisted in diagnosis in all 4 CLL cases. Out of these, in 3 cases, cytogenetic analysis was performed. In one case, a complex karyotype was missed by FISH and it was detected by OGM. OGM analysis helped in determining both diagnosis and prognosis of these patients.

### 3.3. OGM in Prognostication

Conventional risk stratification was done using cytogenetics, in patients with hematologic malignancies, and the details of this are given in table 4. Patients were risk stratified into low/standard/good, intermediate, and high/adverse-risk categories using European Leukemia Net (ELN) criteria for AML (8%, 56%, 12% respectively), BFM UK MRC for ALL (52.94%, 0%, 17.64% respectively), mSMART for myeloma (25%, 0%, 62.5% respectively), IPSS-R criteria for MDS (40%, 20% and 20% respectively) and International Prognostic Index (IPI) for CLL (50%, 0%, 25% respectively). There were some in which risk stratification could not be performed (NA=not applicable). In AML, 6 cases (24%), 5 ALL cases (29.41%), 1 CLL case (25%), 1 MDS case (20%), 1 MDS/MPN case (25%) and one multiple myeloma case (12.5%) risk stratification was not performed because conventional karyotype/FISH was not available. Also, in MPN (N=15) and lymphoma (N=11) cases the risk stratification could not be performed as European LeukemiaNet (ELN) 2020 recommendations for CML and Revised International Prognostic Index (R-IPI) for lymphoma assign no role of cytogenetic aberrations for risk stratification.

| Diagnosis | Low/Standard | Intermediate | High | NA |
|---|---|---|---|---|
| AML (25) | 2 (8%) | 14 (56%) | 3 (12%) | 6 (24%) |
| ALL (17) | 9 (52.94%) | 0 | 3 (17.64%) | 5 (29.41%) |
| CLL (4) | 2 (50%) | 0 | 1 (25%) | 1 (25%) |
| MPN (15) | 0 | 0 | 0 | 15 (100%) |
| MDS (5) | 2 (40%) | 1 (20%) | 1 (20%) | 1(20%) |
| MDS/MPN (4) | 3 (75%) | 0 | 0 | 1 (25%) |
| MPAL (5) | 2 (40%) | 0 | 3 (60%) | 0 |
| Multiple Myeloma (8) | 2 (25%) | 0 | 5 (62.5%) | 1 (12.5%) |
| Lymphoma (11) | 0 | 0 | 0 | 11 (100%) |
| Total (94) | 21 | 15 | 17 | 41 |

**Table 4: Conventional Risk Stratification at the Time of Initial Diagnosis**

Additional prognostic information was obtained with OGM primarily in subsets of patients with AML, ALL, CLL and MDS. Overall, seven cases with AML, four with ALL, two with CLL, one with MDS and one with MDS/MPN, risks were restratified to high risk due to OGM. In 2 multiple myeloma cases, risk based on OGM was low although conventional karyotype based stratification was high risk, this may have been because karyotype was performed on CD138 enriched cells while OGM was performed on whole bone marrow. One case each of AML, CLL and MDS and 2 cases of ALL were not risk stratified by OGM as these samples failed. In one CLL case, OGM was only diagnostic. Consequently, these cases were categorized as Not Applicable (NA).

| Diagnosis | Low/Standard | Intermediate | High | NA |
|---|---|---|---|---|
| AML (25) | 3 (12%) | 11 (44%) | 10 (40%) | 1 (04%) |
| ALL (17) | 8 (44.44%) | 0 | 7 (41.17%) | 2(11.76%) |
| CLL (4) | 0 | 0 | 3 (75%) | 1 (25%) |
| MPN (15) | 0 | 0 | 0 | 15 (100%) |
| MDS (5) | 1(20%) | 1 (20%) | 2 (40%) | 1 (20%) |
| MDS/MPN (4) | 2 (50%) | 1 (25%) | 1(25%) | 0 |
| MPAL (5) | 2 (40%) | 0 | 3(60%) | 0 |
| Myeloma (8) | 5 (62.5%) | 0 | 3 (37.5) | 0 |
| Lymphoma (11) | 0 | 0 | 0 | 11(100%) |
| Total (94) | 22 | 13 | 28 | 31 |

**Table 5: Risk Stratification After Availability of OGM Results**

Risk stratification was changed in 17 cases [upgraded (15) and downgraded (2)]. Thus, due to OGM, the risk stratification of 15/66 (22.72%) patients were upgraded from low or intermediate risk to high risk. This was seen particularly in AML, ALL, CLL and MDS and MDS/MPN, where structural variants are known to be strong prognostic factors. In the upgraded AML cases, 3 had negative FISH/Karyotype and in 4, FISH/Karyotype was unavailable, while OGM showed complex karyotypes. Similarly, in 4 ALL cases, 2 cases were negative by FISH/KT and in 2 cases FISH/KT was unavailable while OGM showed complex karyotype. In 2 CLL cases, FISH/KT was negative while OGM showed complex karyotypes. In 1 MDS case, FISH/KT was not done while OGM detected monosomy 7. In 1 MDS/MPN, Conventional karyotype was normal while OGM detected monosomy 7. Risk stratification was lower compared to standard cytogenetics in 2/66 (3.03%), both multiple myelomas. In these two multiple myeloma cases the discordance between positive FISH and OGM results is likely due to the fact that FISH testing was performed on purified CD138 positive plasma cells whereas OGM performed on whole BMA. In two cases, 1 ALL and 1 CLL, standard cytogenetics was not done and OGM failed and was listed as not applicable (NA)

| Diagnosis | Upgraded | Downgraded | Unchanged | N/A |
|---|---|---|---|---|
| AML (25) | 7 (28%) | 0 | 18 (72%) | 0 |
| ALL (17) | 4 (23.52%) | 0 | 12 (70.58%) | 1 (5.88%) |
| CLL (4) | 2 (50%) | 0 | 1 (25%) | 1 (25%) |
| MPN (15) | 0 | 0 | 0 | 15 (100%) |
| MDS (5) | 1 (20%) | 0 | 4 (80%) | 0 |
| MDS/MPN (4) | 1(25%) | 0 | 3(75%) | 0 |
| MPAL (5) | 0 | 0 | 5(100%) | 0 |
| Multiple myeloma (8) | 0 | 2 (25%) | 6 (75%) | 0 |
| Lymphoma (11) | 0 | 0 | 0 | 11 (100%) |
| Total (94) | 15 | 4 | 47 | 28 |

**Table 6: Risk Stratification Outcomes Following OGM Analysis**

### 3.4. Change in Treatment Due to OGM

The 15 patients in whom risk stratification was upgraded due to OGM, treatment was changed in 14 (14/94= 14.89%) of the patients. Treatment was intensified in all of these patients (14 patients), by posting them for an allogeneic stem cell transplant. One patient with CLL was upgraded due to OGM but he was only on observation, so treatment modification was done. (Table 7)

| Diagnosis | No Change | Intensified | Downgraded |
|---|---|---|---|
| AML (25) | 18 (72%) | 7(28%) | 0 |
| ALL (17) | 13 (76.47%) | 4 (23.52%) | 0 |
| CLL (4) | 3 (75%) | 1 (25%) | 0 |
| MPN (15) | 15 (100%) | 0 | 0 |
| MDS (5) | 4 (80%) | 1 (20%) | 0 |
| MDS/MPN (4) | 3 (75%) | 1 (25%) | 0 |
| MPAL (5) | 5 (100%) | 0 | 0 |
| Multiple Myeloma (8) | 8 (100%) | 0 | 0 |
| Lymphoma (11) | 11 (100%) | 0 | 0 |
| Total (94) | 80 | 14 | 0 |

**Table 7: Effect of OGM on Treatment.**

## 4. Discussion

The examined cohort comprises consecutive patients who underwent OGM as part of the evaluation of their hematologic condition, with results compared to gold standard cytogenetic methods like chromosome karyotyping and FISH, as well as NGS, where available. Notably, the study not only demonstrated a change in risk stratification based on OGM results but also, for the first time, documents a tangible shift in clinical care, according to the additional information provided by OGM. This transformative shift included, for example, active preponement of Stem Cell Transplantation (SCT) in 7 AML patients, exemplifying the immediate and practical impact of OGM-derived information on therapeutic decisions, accentuating its real-world implications for patient care. This study represents a pioneering milestone as the first prospective study wherein treatment decisions were directly influenced by OGM findings. In contrast, prior retrospective studies explored the potential impact of OGM results on prognostic considerations and clinical care without implementing these insights in actual treatment protocols. However, we understand the potential risks associated with relying solely on OGM results for clinical decision-making. Caution must be exercised when considering its findings for clinical decision-making and need validation to ensure the reliability and accuracy of the findings in future research.

Several important workflow considerations were assessed. First, samples in this study were shipped from India to San Diego, USA for testing, shipping proceeded at ambient temperature with common international shipment practices and time in transit was assessed with a median transit time of 6.0 days (ranging from 3-17 days) for successful samples. There were some failures upon DNA isolation and analysis amounting to 5.66 % of samples and other samples required a second preparation attempt for success. Considering that this transit time is longer than recommended by the manufacturer (Bionano Genomics, Inc.), success rate is expected to be better when following recommendations more strictly. Even so, when compared to the failure rate for karyotype analysis 15%, in the local testing labs for the subset of the samples that received both tests, OGM far outperformed. This success rate underscores the robustness of the OGM methodology. This study also emphasizes the collaborative nature of the study, involving international partnerships. With the ongoing establishment of multiple OGM sites worldwide, each equipped with their own validated Laboratory Developed Tests (LDTs), the need to send samples exclusively to Bionano Labs is gradually diminishing. This growing network of OGM facilities signifies an expanding array of options for referral of samples within this network.

In previous studies, OGM was shown to be very close to 100% concordant with cytogenetics and higher sensitivity and resolution resulted in changes in prognostication and opportunity for different therapy options [14-16]. In agreement with previous publications, our analysis showed that the mean number of SVs detected by OGM was higher than those identified by conventional cytogenetics; the number of SVs per abnormal OGM result classified as Tier 1A, Tier 1B and Tier 2 were 2.35, 1.25 and 5.5, respectively. The magnitude varied by disease from approximately 2-4 fold greater number of clinically relevant SVs detected by OGM.

As a result of detection of more SVs compared to SOC, change in risk stratification was made for 17/66 (25.75%) of patients with majority of patients (15/17), having their risk stratification indicate more aggressive disease compared to SOC. This is in line with previous publications showing that higher resolution and sensitivity for detection of known recurrent structural variants results in more adverse risk stratification rate. Our study aims to underscore the diagnostic advantages of OGM while acknowledging certain limitations. Specifically, we concur in that OGM alone may not fully capture certain aberrations, notably Single Nucleotide Variants (SNVs) and small insertions/deletions. However, OGM is capable, as a single platform, of detecting all classes of structural variants in the genome, namely deletions, insertions, inversions, translocations, aneuploidy, triploidy, and Absence of Heterozygosity (AOH) segments. The study showcases OGM's potential for comprehensive genome analysis.

### 4.1. Limitations of OGM

While OGM offers comprehensive genome analysis by detecting

various classes of structural variants, it is not without limitations. Although positioned as an alternative to traditional cytogenetic techniques, including karyotyping and FISH, OGM is not sensitive to the identification of Single Nucleotide Variants (SNVs) and small indels (less than 500 bp), underlying the need to maintain complementary methodologies such as NGS for comprehensive analysis. Additionally, OGM faces challenges in identifying SVs within or overlapping regions with uninformative DLE1 label patterns, predominantly found in the centromeric regions of chromosomes. Moreover, distinguishing cases of hyper- and hypodiploidy can be complex, and OGM is unable to explicitly define clonal makeup in a similar way to karyotyping.

In summary, this prospective study is the first study from India which has provided insights into risk stratification, prognostication, and treatment optimization in patients with hematological malignancies by using OGM in comparison to conventional cytogenetic techniques. In our analysis, the risk stratification of 15 patients was upgraded from low or intermediate risk to high risk due to OGM. This was seen particularly in AML, ALL, CLL and MDS, where structural variants are known to be strong prognostic factors; treatment was changed in 14 of the patients.

Treatment was intensified in all these patients, by posting them for an allogeneic stem cell transplant. Risk stratification was downgraded from high to low or intermediate risk in 1 case each of ALL and MPAL along with two cases of multiple myeloma. This could be because of low yield due to lack of CD138 enrichment of cells. OGM did not contribute to alterations in risk stratification in MPN and lymphoma. However, the patient numbers in these categories were low, to draw any conclusion. In our study 2/66 cases were downgraded as a result of OGM. These two downgraded cases corresponded to two multiple myeloma cases which showed discrepancy between positive FISH results and normal OGM results. These discrepancies can be attributed to the difference in sample types used for testing, as FISH analysis was conducted on purified CD138-positive plasma cells, whereas OGM was performed on fresh BMA samples.

As this study was prospective in nature and yielded OGM results within approximately two weeks, the information provided in OGM-based reports could be included in the patient's workup and could be a part of informed treatment decisions. Notably, a total of 14 patients either underwent or were advised to undergo treatment intensification or expedited allogenic stem cell transplantation based on these findings. Based on these results, a larger prospective study should be conducted to understand the improvement in overall and disease-free survival in patients with hematologic malignancies, who undergo a change in treatment based on the additional genomic information provided by OGM. Protocols also need to be created to integrate OGM into the routine diagnostic and prognostic evaluation of hematologic malignancies.

## 5. Conclusion

OGM resulted in a more complete assessment of complex cytogenetic events refining the underlying genomic structure which had been reported by traditional cytogenetic methods and detecting additional clinically relevant variants. It thus helped in the diagnosis, prognosis, and risk stratification of several patients with hematological malignancies. Most notably, physicians were able to make a change in management of 14/94 (14.89 %) patients as a direct result of OGM data.


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## Ethics Approval and Consent to Participate

Informed consent was obtained from all subjects involved in the study. The study was conducted according to the guidelines of the Declaration of Helsinki.

## Author Contributions

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## Conflicts of Interest

Trilochan Sahoo, Jen Hauenstein, Jenna Finley, Anusha Mylavarapu, Beth Matthews, Stephen J. Wicks, Alex Hastie and

Alka Chaubey are full time employees of Bionano and hold equity in the company.

## References


1. Taylor, J., Xiao, W., & Abdel-Wahab, O. (2017). Diagnosis and classification of hematologic malignancies on the basis of genetics. *Blood, The Journal of the American Society of Hematology, 130*(4), 410-423.
2. Rack, K. A., van den Berg, E., Haferlach, C., Beverloo, H. B., Costa, D., Espinet, B., ... & Hastings, R. J. (2019). European recommendations and quality assurance for cytogenomic analysis of haematological neoplasms. *Leukemia, 33*(8), 1851-1867.
3. National Comprehensive Cancer Network. NCCN Clinical Practice Guidelines in Oncology NCCN Guidelines®. Myelodysplastic Syndromes. Version 3. 2021.
4. WHO Classification of Tumours of Haematopoietic and Lymphoid Tissues. WHO Classification of Tumours, Revised 4th Edition, Volume 2. Editors: Swerdlow SH, Campo E, Harris NL, Jaffe ES, Pileri SA, Stein H, Thiele J. 2017.
5. National Genomic Test Directory for Cancer, 20 September 2023: © NHS in England.2023:
6. Percival, M. E., Lai, C., Estey, E., & Hourigan, C. S. (2017). Bone marrow evaluation for diagnosis and monitoring of acute myeloid leukemia. *Blood reviews, 31*(4), 185-192.
7. Granada, I., Palomo, L., Ruiz-Xivillé, N., Mallo, M., & Solé, F. (2020). Cytogenetics in the genomic era. *Best Practice & Research Clinical Haematology, 33*(3), 101196.
8. Döhner, H., Estey, E., Grimwade, D., Amadori, S., Appelbaum, F. R., Büchner, T., ... & Bloomfield, C. D. (2017). Diagnosis and management of AML in adults: 2017 ELN recommendations from an international expert panel. *Blood, The Journal of the American Society of Hematology, 129*(4), 424-447.
9. Hallek, M., Cheson, B. D., Catovsky, D., Caligaris-Cappio, F., Dighiero, G., Döhner, H., ... & Kipps, T. J. (2018). iwCLL guidelines for diagnosis, indications for treatment, response assessment, and supportive management of CLL. *Blood, The Journal of the American Society of Hematology, 131*(25), 2745-2760.
10. Treatment of multiple myeloma with high-risk cytogenetics: a consensus of the International Myeloma Working Group.
11. Neveling, K., Mantere, T., Vermeulen, S., Oorsprong, M., van Beek, R., Kater-Baats, E., ... & Hoischen, A. (2021). Next-generation cytogenetics: Comprehensive assessment of 52 hematological malignancy genomes by optical genome mapping. *The American Journal of Human Genetics, 108*(8), 1423-1435.
12. Bionano Genomics:Bionano Solve Theory of Operation: Structural Variant Calling.
13. Mantere, T., Neveling, K., Pebrel-Richard, C., Benoist, M., van der Zande, G., Kater-Baats, E., ... & El Khattabi, L. (2021). Optical genome mapping enables constitutional chromosomal aberration detection. *The American Journal of Human Genetics, 108*(8), 1409-1422.
14. Yang, H., Garcia-Manero, G., Sasaki, K., Montalban-Bravo, G., Tang, Z., Wei, Y., ... & Kanagal-Shamanna, R. (2022). High-resolution structural variant profiling of myelodysplastic syndromes by optical genome mapping uncovers cryptic aberrations of prognostic and therapeutic significance. *Leukemia, 36*(9), 2306-2316.
15. Levy, B., Baughn, L. B., Akkari, Y., Chartrand, S., LaBarge, B., Claxton, D., ... & Broach, J. R. (2023). Optical genome mapping in acute myeloid leukemia: a multicenter evaluation. *Blood advances, 7*(7), 1297-1307.
16. Gerding, W. M., Tembrink, M., Nilius-Eliliwi, V., Mika, T., Dimopoulos, F., Ladigan-Badura, S., ... & Vangala, D. B. (2022). Optical genome mapping reveals additional prognostic information compared to conventional cytogenetics in AML/MDS patients. *International Journal of Cancer, 150*(12), 1998-2011.
17. Sahajpal, N. S., Mondal, A. K., Tvrdik, T., Hauenstein, J., Shi, H., Deeb, K. K., ... & Kolhe, R. (2022). Clinical validation and diagnostic utility of optical genome mapping for enhanced cytogenomic analysis of hematological neoplasms. *The Journal of Molecular Diagnostics, 24*(12), 1279-1291.
18. Smith, A. C., Neveling, K., & Kanagal-Shamanna, R. (2022). Optical genome mapping for structural variation analysis in hematologic malignancies. *American journal of hematology, 97*(7), 975-982.
19. Pang, A. W. C., Kosco, K., Sahajpal, N., Sridhar, A., Hauenstein, J., Clifford, B., ... & Chaubey, A. (2022). Clinical Validation of Optical Genome Mapping for the Detection of Structural Variations in Hematological Malignancies. *medRxiv*, 2022-12.
20. Vangala, D. B., Nilius-Eliliwi, V., Gerding, W. M., Schroers, R., & Nguyen, H. P. (2023). Optical Genome Mapping in MDS and AML as tool for structural variant profiling—comment and data update on Yang et al.:"High-resolution structural variant profiling of myelodysplastic syndromes by optical genome mapping uncovers cryptic aberrations of prognostic and therapeutic significance". *Leukemia, 37*(1), 248-249.
21. Balducci, E., Kaltenbach, S., Villarese, P., Duroyon, E., Zalmai, L., Friedrich, C., ... & Couronné, L. (2022). Optical genome mapping refines cytogenetic diagnostics, prognostic stratification and provides new molecular insights in adult MDS/AML patients. *Blood Cancer Journal, 12*(9), 126.
22. Rack, K., De Bie, J., Ameye, G., Gielen, O., Demeyer, S., Cools, J., ... & Dewaele, B. (2022). Optimizing the diagnostic workflow for acute lymphoblastic leukemia by optical genome mapping. *American journal of hematology, 97*(5), 548-561.
23. Lestringant, V., Duployez, N., Penther, D., Luquet, I., Derrieux, C., Lutun, A., ... & Ferret, Y. (2021). Optical genome mapping, a promising alternative to gold standard cytogenetic approaches in a series of acute lymphoblastic leukemias. *Genes, Chromosomes and Cancer, 60*(10), 657-667.
24. Brandes, D., Yasin, L., Nebral, K., Ebler, J., Schinnerl, D., Picard, D., ... & Wagener, R. (2023). Optical genome mapping identifies novel recurrent structural alterations in childhood ETV6:: RUNX1+ and high hyperdiploid acute lymphoblastic

leukemia. *HemaSphere, 7*(8), e925.
25. Vieler, L. M., Nilius-Eliliwi, V., Schroers, R., Vangala, D. B., Nguyen, H. P., & Gerding, W. M. (2023). Optical genome mapping reveals and characterizes recurrent aberrations and new fusion genes in adult ALL. *Genes, 14*(3), 686.
26. Puiggros, A., Ramos-Campoy, S., Kamaso, J., de la Rosa, M., Salido, M., Melero, C., ... & Espinet, B. (2022). Optical Genome Mapping: A promising new tool to assess genomic complexity in chronic lymphocytic leukemia (CLL). *Cancers, 14*(14), 3376.
27. Valkama, A., Vorimo, S., Kumpula, T. A., Räsänen, H., Savolainen, E. R., Pylkäs, K., & Mantere, T. (2023). Optical genome mapping as an alternative to FISH-based cytogenetic assessment in chronic lymphocytic leukemia. *Cancers, 15*(4), 1294.
28. Kriegova, E., Fillerova, R., Minarik, J., Savara, J., Manakova, J., Petrackova, A., ... & Papajik, T. (2021). Whole-genome optical mapping of bone-marrow myeloma cells reveals association of extramedullary multiple myeloma with chromosome 1 abnormalities. *Scientific reports, 11*(1), 14671.
29. Giguère, A., Raymond-Bouchard, I., Collin, V., Claveau, J. S., Hébert, J., & LeBlanc, R. (2023). Optical genome mapping reveals the complex genetic landscape of myeloma. *Cancers, 15*(19), 4687.
30. Stinnett, V., Jiang, L., & Haley, L. (2021). Adoption of optical genome mapping in clinical cancer cytogenetic laboratory: a stepwise approach. *J Clin Anat Pathol, 6*(2), 117.
31. Duncavage, E. J., Schroeder, M. C., O'Laughlin, M., Wilson, R., MacMillan, S., Bohannon, A., ... & Spencer, D. H. (2021). Genome sequencing as an alternative to cytogenetic analysis in myeloid cancers. *New England Journal of Medicine, 384*(10), 924-935.