Review

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Cytogenetics and the Revolution of Optical Genome Mapping in the Diagnosis of Diseases

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The study of chromosomal shape, characteristics, and behavior in somatic cell division (mitosis) during growth and development and in germ cell division (meiosis) during reproduction is known as cytogenetics. Many techniques can be used for cytogenetics, including fluorescent *in situ* hybridization (FISH), spectral karyotyping (SKY), multicolor FISH (M-FISH), microarray, and optical genome mapping (OGM). OGM is a novel genome-wide method that can identify structural variants (SVs) and copy number variants (CNVs) with only one test. Genomic structural information that is difficult to obtain with DNA sequencing can be promptly obtained with OGM, in which large molecule lengths can be mapped at a reasonable cost. OGM is increasingly being used to investigate chromosome abnormalities in genetic disorders and human cancer, but it was first utilized in genome assembly and research. According to recent research, OGM is capable of identifying every clinically significant variation seen in trials using conventional care. OGM is being utilized to identify genomic abnormalities in patients with malignancies and constitutional illnesses. It is regarded as a revolution in the field of cytogenetics. Rather than sequencing DNA, OGM relies on DNA labeling. Currently, the OGM technique with the Saphyr system from Bionano Genomics is a widely utilized platform for cytogenetic analysis. In conclusion, OGM can now be considered a highly reliable method for the identification of chromosomal abnormalities in the diagnosis of tumors and hematological diseases.

Keywords: cytogenetics; molecular cytogenetics; optical genome mapping

Introduction to Cytogenetics

Cytogenetics is a branch of genetics, cell biology and cytology focused on the study of chromosomes and their aberrations during mitosis and meiosis, including missing, rearranged, or extra chromosomes [1]. The first observation of human chromosomes occurred in 1882 when Walther Flemming described the mitosis process [2]. In 1888, the term "chromosome" was coined by Wilhelm von Waldeyer [3,4]. The ordered arrangement of chromosomes was subsequently termed the "karyotype" by Levitsky. The karyotype is the general appearance of the complete set of chromosomes in the cells, including the phenotypic appearance of the somatic chromosomes, such as their sizes, numbers, and shapes [5–7].

Since the identification and determination of 46 chromosomes in humans, many studies have reported relationships between congenital disorders and chromosomal abnormalities. Constitutional cytogenetics (chromosomal aberrations) have been used to determine that some congenital disorders arise from nondisjunction, additions or deletions of entire chromosomes, such as Patau syndrome [8], Edwards syndrome [9], Down syndrome [10], Turner syndrome [11] and Klinefelter syndrome [12]. Acquired cytogenetics (the cytogenetics of cancer) has been used to show that chromosomal aberrations and balanced translocations in cancers, especially hematological malignancies, play critical roles in the oncogenic process. For example, an unusual rearrangement between chromosome 9 and chromosome 22 and a fusion gene called *BCR-ABL1* were identified in the Philadelphia chromosome in patients with chronic myelogenous leukemia (CML) [13]. Over more than a century, many techniques have been developed to study human chromosomes. In this review, we focused on molecular cytogenetics and the revolution of optical genome mapping (OGM) in the diagnosis of various diseases.

Molecular Cytogenetics

Fluorescence in Situ Hybridization

The fluorescent *in situ* hybridization (FISH) technique is a laboratory molecular cytogenetic technique used to detect specific DNA sequences on chromosomes, identify the positions of genes, and map genes on human chromosomes. The principle of the FISH technique is based on the ability of single-strand DNA to anneal complementarily to nuclear DNA during interphase or metaphase. In this technique, DNA or RNA sequences tagged with a fluorescent dye are

used as fluorescent probes to identify or quantify specific DNA sequences in a biological sample. Currently, FISH is used for clinical diagnosis [14,15]. However, while the FISH technique is widely used in the clinical diagnosis of various chromosomal abnormalities, such as deletions, duplications, and translocations, in preventive and reproductive medicine and oncology, some limitations of the conventional FISH technique in cytogenetics have become apparent and reported. The FISH technique can detect only known genetic aberrations for which specific probes are available. In addition, the FISH technique cannot serve as a screening test for chromosomal rearrangements such as duplications, deletions or inversions [16,17].

Spectral Karyotyping

Spectral karyotyping (SKY) is a new technique for chromosome analysis that is based on the FISH technique. The SKY technique was developed to help scientists unambiguously detect chromosomal abnormalities and identify all 24 human chromosomes simultaneously. SKY uses a series of 24 chromosome-specific painting probes to label the complete set of 24 human chromosomes. The SKY technique combines the basic principles of the FISH technique, chromosome painting, and multicolor fluorescence, which draws an entire image of a specific chromosome by using fluorescent signals. SKY provides the advantage of easy visual interpretation when analyzing results; however, it cannot be used to distinguish intrachromosomal rearrangements such as duplications, deletions or inversions because these rearrangements display the same color [18,19].

Chromosome Microarray

Chromosome microarray (CMA) is a powerful molecular karyotype diagnostic tool. CMA can identify genetic alterations in the chromosome that are too small to be identified by other traditional techniques such as FISH and conventional karyotypes [20]. CMA is commonly used to diagnose infants and children with unexplained health concerns such as congenital anomalies, developmental disabilities, autism spectrum disorder or intellectual disabilities [21]. Two CMA platforms can be employed in prenatal testing, including single-nucleotide polymorphism (SNP) arrays and array comparative genomic hybridization (aCGH) [22,23]. Although CMA is recommended as a first-tier test for prenatal diagnosis for detecting chromosomal abnormalities, challenges with interpreting the CMA results have been reported, resulting in variability in interpretations. Limitations of CMA include its inability to detect molecularly balanced chromosomal rearrangements as well as limitations in the detection of low-level mosaicism [24].

Copy Number Variation Sequencing

Copy number variation sequencing (CNV-Seq) is a new method developed and used to detect copy number variants (CNVs) based on next-generation sequencing (NGS) or whole-exome sequencing (WES). CNV-Seq seems to be a useful tool in the clinic for diagnosing prenatal and chromosomal abnormalities that are not detectable by karyotyping. The use of CNV-Seq can reduce the financial and time costs of CMA. Many bioinformatics tools have been developed to detect CNVs [25–29]. Some limitations have been reported for CNV-Seq, such as its inability to detect balanced chromosomal rearrangements; inability to detect polyploidies and regions of homozygosity (ROHs); and lower stability, reproducibility, and accuracy [24].

Optical Genome Mapping

OGM is a novel genome-wide technique that assesses each DNA molecule's fluorescence labeling pattern to provide an objective evaluation of genome-wide structural variations as small as 500 base pairs (bp) [30,31]. Currently, OGM is used for cytogenetic analysis to identify copy number variants (CNVs) and structural variants (SVs) in the same test. OGM is increasingly being used to investigate chromosome abnormalities in genetic disorders and human cancer, but it was first utilized in genome assembly and research [32,33]. Recent research has demonstrated that OGM can detect all clinically relevant variants seen in standard-of-care trials, even in germline contexts where CNV identification is largely carried out via CMA. Furthermore, because of the repetitive nature of the sequence and the short read length of traditional NGS technologies, whole-genome sequencing missed this ~2.8 kb insertion within SMARCB1 intron 2. This insertion suggested a novel mechanism of inactivation leading to cancer predisposition, especially in hematological cancers where chromosomal rearrangements are common [34]. OGM is used to visualize exceedingly long linear single-DNA molecules that have been tagged at specific places (median size >250 kb) (Table 1) [35]. OGM has been updated and commercialized by Bionano Genomics as an optical mapping method for structural variation analysis via whole-genome imaging through the combination of automated image analysis, high-resolution microscopy, and microfluidics to enable high-throughput whole-genome imaging and *de novo* assembly [33,36].

Revolution of OGM in the Diagnosis of Diseases

OGM is used by diagnostic laboratories to identify genetic abnormalities associated with malignancies and constitutional illnesses. OGM is thought of as a bridging approach between methods based on whole-chromosome analysis and those based on sequencing. A revised definition of next-generation cytogenetics has been proposed in light of this perspective. OGM has emerged as a very promising technique for identifying widespread structural variations in human genomes in recent years. Long molecules are used in OGM because they make it easier to

Table 1. Comparison of classical cytogenetics and OGM methodologies for structural variant detection.

Variant class	Karyotyping	FISH	CMA	OGM
Deletion	>5–10 Mbp	Targeted	Applicable	Applicable
Duplication	>5–10 Mbp	Targeted	Applicable	Applicable
Inversion	>5–10 Mbp	Targeted	Not applicable	Applicable
Translocation	>5–10 Mbp	Targeted	Not applicable	Applicable
Aneuploidy	Applicable	Targeted	Applicable	Applicable
Repeat expansion	Not applicable	Not applicable	Not applicable	Applicable
SNV	Not applicable	Not applicable	Not applicable	Not applicable
LOH	Not applicable	Not applicable	Applicable	Applicable

FISH, fluorescent *in situ* hybridization; CMA, chromosome microarray; OGM, optical genome mapping; SNV, single-nucleotide variation; LOH, loss of heterozygosity.

span repetitive regions and other difficult-to-map regions than short molecules do. This capability results in maps that can span an entire chromosome's arm and still depict insertions and deletions as small as 500 bp. The current standard procedure for implementing OGM entails fluorescently marking a certain short genome sequence pattern on the DNA [37,38].

The Saphyr system from Bionano Genomics (San Diego, CA, USA) is now the most widely utilized platform for OGM analysis [33,39,40]. Direct labeling and staining (DLS), a novel technique recently unveiled by Bionano, prevents double-strand breaks and nicks by inserting fluorophores directly into targeted DNA patterns. The labeling contiguity of molecules via this novel method is 50 times better than that achieved by the direct label enzyme enzyme-based technique. To find structural variants, labeled DNA is placed onto a chip that linearizes the molecules. The resulting genome-wide fluorescence pattern is then scanned and compared with a reference genome [41].

Two bioinformatics systems can be employed for analysis: a *de novo* assembly or a rare variant pipeline (RVP). The RVP bioinformatics system is intended to identify both SVs and CNVs that are common in heterogeneous populations, including cancer samples and samples with genetic mosaicism, by comparing target genomic molecules to the reference genome. There is a possibility to identify novel DNA fusions with a variant allele frequency (VAF) threshold of approximately 5% and SVs ranging in length from at least 5 kbp to tens of Mbp (often with a cutoff >100 kbp). Nonetheless, with a VAF of at least 10%, the CNV algorithm mostly detects significant aberrations (from 500 kbp up to aneuploidies), such as terminal deletions and partial aneuploidies that SV calling misses [38,42].

On the other hand, the *de novo* assembly bioinformatics system can detect smaller SVs of approximately 500 bp and achieves low sensitivity for rare events (VAF of at least 15–25%). This is because the system arranges all of the labeled molecules in a *de novo* genome assembly for each chromosome. Users of Bionano analysis software can examine and work with maps and SVs through a graphical user interface that does not require specialized knowledge in bioinformatics. The following data visualization techniques are used by the software: whole-genome plot, genome browser view, and Circos plot. The identified variations are summarized in the Circos figure, including cytobands, chromosomal number, variant allele fraction profile, and, if available, gene locations. The genome browser view is an interactive visualization tool for analyzing chromosomal variations. In three distinct plots, the whole-genome view displays the genomic locations of every chromosome together with the copy number, absence of heterozygosity (AOH)/loss of heterozygosity (LOH), and VAF [33,38,42].

OGM was compared with traditional molecular cytogenetic techniques for the identification of chromosomal abnormalities, including structural or numerical chromosomal variants, deletions, duplications, inversions, trisomy, and translocations, in many studies, which are the focus of the following sections.

OGM for the Diagnosis of Hematological Malignancies

Hematological disorders such as acute leukemia or multiple myeloma (MM), where complicated karyotypes are common and traditional cytogenetic analysis is insufficient on its own and requires additional confirmation via ancillary techniques, are among the most beneficial applications of OGM [38]. An RVP pipeline is the most useful for OGM analysis in patients with hematological malignancies. *De novo* assembly can also be used in some situations, such as B-cell acute lymphoblastic leukemia with immunoglobulin heavy chain locus-cytokine receptor-like factor 2 (*IGH-CRLF2*) fusion and the participation of X- and Y-chromosome pseudoautosomal regions, to identify which sex chromosome is rearranged [42]. Podvin *et al.* [43] (2022) conducted a brief investigation of two individuals who had hypereosinophilia linked to Platelet Derived Growth Factor Receptor Beta (*PDGFRB*) rearrangements and myeloid neoplasms. Pericentriolar material 1 (*PCM1*) and Golgin A4 (*GOLGA4*) are unusual partner genes that OGM identified as being involved in (*PDGFRB*) fusions. These genes map to 8p22 and 3p21, respectively. A tar-

geted NGS gene panel was used to corroborate the breakpoint localization, and OGM provided a clear characterization of the complicated genesis of chromosomal rearrangements containing these (*PDGFRB*) fusions [43].

Recently, the performance of OGM was defined in two studies that conducted clinical validation of OGM in the detection of cytogenetic abnormalities in hematological malignancies, in which OGM showed high sensitivity, specificity, and accuracy. In addition, OGM can identify novel clinically significant SVs in examined patients that other methods are unable to detect. This capability enables improved patient classification, prognostic stratification, and therapeutic decisions. As a result, OGM is currently used as a very trustworthy initial cytogenomic test for the detection of hematological malignancies, myelodysplastic syndrome (MDS), acute myeloid leukemia (AML), acute lymphoblastic leukemia (ALL), chronic lymphocytic leukemia (CLL), and MM [44–46].

Acute Myeloid Leukemia

AML and MDS are common hematological malignancies in adults. To compare OGM results with data from the typical diagnostic genomic workup and assess whether OGM can provide extra information that could alter treatment, several authors have examined the use of OGM in cohorts of patients with MDS/AML. [30]. Furthermore, according to OGM analysis, a subset of selected myeloid genes including lysine methyltransferase 2A-partial tandem duplication (*KMT2A-PTD*), ETS variant transcription factor 6 (*ETV6*), janus kinase 2 (*JAK2*), and runt-related transcription factor 1 (*RUNX1*) of potential clinical importance had unusual SV and CNV maps nearby. Moreover, OGM analysis provided a thorough characterization of complex rearrangements and revealed recurrent occurrences involving chromosomes 12 and 21 in several patients with complex karyotypes. Two of the disadvantages of the OGM approach are the detection of clones at low frequency and SVs in poorly covered locations, such as the centromeric and telomeric regions. These limitations explain the small percentage of cases in which OGM fails to detect chromosomal abnormalities that are identified by traditional cytogenetics [47].

Acute Lymphoblastic Leukemia

The hallmark of ALL, a heterogeneous hematopoietic malignancy, is an 85% increase in B-lineage lymphoid progenitor cells in the bone marrow and blood or, less frequently, a 15% increase [48,49]. New technologies that help speed up prognostic classification and diagnosis in ALL are desperately needed. Numerous groups have investigated the idea of replacing the current standard approaches for characterizing individuals with ALL upon diagnosis with OGM analysis, which focuses on their genome and molecular makeup. By eliminating the need for all of the standard procedures usually used at diagnosis to perform the molecular and cytogenetic characterization of patients with ALL, a study has shown that the use of OGM reduces turnaround times and improves the detection resolution of genomic rearrangements [50]. Lestringant *et al*. [50] released the first preliminary OGM data in ALL. According to a study by Lühmann *et al*. [51], OGM simplifies the diagnostic workflow by enabling the detection of all structural and quantitative genomic changes found via conventional diagnostic assays in a single session. The authors discovered that OGM enabled the identification of all genomic abnormalities—translocations, aneuploidies, and copy number variation—found in both prospective and retrospective research samples. This method can also be used to identify new structural differences that are useful for better defining prognosis and therapy alternatives. In twelve pediatric patients with ALL, this work used whole-genome molecular markers (OGM) to confirm all genomic abnormalities, including translocations, aneuploidies, and copy number variations that were discovered via traditional diagnostic techniques. Additionally, a complex three-way translocation, t(2;12;21)(p22.1;p13.2;q22.12), resulting in ETV6::RUNX1 fusion, and a new rearrangement, t(9;11)(24.1;q22.1), creating a fusion combining the genes *JAK2* and *NPAT*, were improved upon by OGM. Furthermore, OGM showed increased sensitivity in detecting copy number variations [51].

Chronic Lymphocytic Leukemia

Clonal proliferation of mature CD5+ B cells in the blood and lymphoid tissues causes chronic lymphocytic leukemia (CLL), a common lymphoproliferative disease. Puiggros *et al*. [52] contrasted the efficacy of OGM in describing the genomic modifications of CLL patients with information obtained from traditional cytogenetic procedures to determine genomic complexity and use it as a prognostic factor. A total of 42 CLL patients with prior results from FISH, CMA, and chromosome banding analysis (CBA) tests were included in this investigation. OGM confirmed 90% of the previously reported alterations but missed 30 genomic anomalies out of 309, mostly because of breakpoint localizations in the centromeric/telomeric region or because, in subclonal incidence cases, when a cutoff of >10 aberrations in relation to the concept of genomic complexity was used, a complex OGM group was identified, exhibiting a high incidence of tumor protein p53 (*TP53*) abnormalities and a notably shorter time to first treatment. Overall, this novel method has enhanced the definition of SV extension and genomic breakpoint localization [52]. OGM is a dependable, useful method for prognostic stratification or therapy choices and more precisely defines CLL diagnosis, according to the authors' conclusion. Recently, Ramos-Campoy *et al*. [53] investigated nine cases of CLL via OGM, including chromothripsis. While 1–3% of CLL patients exhibit chromothripsis, its mechanism is still unknown, and it is not detectable with CBA due to poor res-

olution. In conclusion, OGM is a state-of-the-art method that provides a more comprehensive description of these complex genomic processes than microarray analysis does, perhaps contributing to the identification of the underlying mechanisms [53,54].

Multiple Myeloma

Whole-genome optical mapping was utilized in the Bionano Genomics OGM to evaluate a number of patients. MM is a heterogeneous hematological malignancy caused by plasma cell clonal proliferation. Two stages of tumor growth and patient response to treatment were investigated for structural variations in the primary MM genome [54]. Combining data from DNA genomic large-scale sequencing with optical mapping results revealed substantial structural abnormalities and an increase in mutational burden with tumor progression in the MM patient under examination. The genomic architecture of CD138+ cells isolated from a tiny Saphyr system was recently studied in a pilot study in relation to extramedullary myeloma (EMM), a serious illness in which cancer cells invade other organ systems. Every MM patient in the study had at least two additional translocations, which frequently involved chromosomes 2, 3, 6, and 8. OGM confirmed that high-risk 14q32 translocation is a common primary event in MM. All of the analyzed MM patients also had a high incidence of interchromosomal translocations. Many of these translocations caused genes linked to cancer to be rearranged. Moreover, breakpoints in the same genomic regions are often associated with interchromosomal translocations and intrachromosomal rearrangements [55,56].

OGM for the Diagnosis of Solid Tumors

Genomic instability, which frequently impacts genes regulating cell division and genome integrity, is one of the distinguishing features of cancer. The resulting changes include point mutations known as single-nucleotide variations (SNVs) and SVs, which are caused by chromosomal perturbations such as deletions, insertions, duplications, inversions, and translocations that affect longer DNA segments. Because alignment- or assembly-based techniques are used, short-read sequencing is less effective at detecting SVs than it is at detecting SNVs. Large genomes (>500 bp) of SVs can now be analyzed via OGM [57–61].

OGM has been demonstrated to be valuable in solid tumor analysis in recent feasibility studies. OGM can provide a comprehensive picture of the structural variations present in a tumor and the cancer-related genes they impact, enabling the agnostic identification of the impacted genes without the preexisting prejudices that gene panels impose. Research on lung squamous cell carcinoma and metastatic prostate carcinoma, among other solid tissue tumors, has shown the value of Bionano DNA isolation procedures. These investigations broadened the viability of this analysis for hitherto unutilized human tissue and demonstrated the value of the DNA isolation process and SV analysis in a wide range of solid tissue types [62–65].

In a previous investigation of 20 solid tumors, the sample chosen with a high molecular weight contained only oncogenes that might be triggered by duplication or gene fusion and only tumor suppressor genes that might be inactivated by deletion, insertion, or fusion. Every tumor sample had at least one of these mutations in the cancer genes, and the majority had several hits. For example, cyclin dependent kinase 6 (*CDK6*) and erb-b2 receptor tyrosine kinase 2 (*ERBB2*) target oncogenes directly, whereas BRAF and cyclin-dependent kinase inhibitor 2A (*CDKN2A*) target the pathway downstream of the afflicted gene. A number of these genes present the possibility of targeted therapy. Owing to probable genomic instability, other impacted genes, such as MutS Homolog 2 (*MSH2)*, RAD51 paralog B (*RAD51B)*, RAD21 cohesin complex component (*RAD21)*, and RAD18 E3 ubiquitin protein ligase (*RAD18)*, may be targets of therapy, such as in PARP inhibitors or immunotherapy. Targeted gene panels, which are often utilized for the clinical evaluation of tumor genomes, are not able to quickly detect many of these mutations. Furthermore, the cancer genes whose SVs changed were frequently unrelated to the type of cancer that we observed. For example, CDK6 is a fusion of CDK6 in tongue cancers, although CDK6 was previously linked primarily to ALL. Similarly, because low-density lipoprotein receptor-related protein 1B (*LRP1B*) is frequently deleted in lung tumors, it is also frequently inactivated in ovarian cancer and CLL. Somatic structural variant identification by OGM may therefore offer valuable clinical insights that are not easily obtained through focused panels or conventional next-generation sequencing [65].

OGM for the Diagnosis of Soft-Tissue and Bone Tumors

A class of uncommon neoplasms known as soft-tissue and bone tumors typically arise from mesenchymal tissue and are still difficult to diagnose. In a diagnostic context, the identification of SVs or copy number alterations (CNAs) involving tumor suppressor genes or oncogenes is particularly difficult and frequently necessitates the combination of multiple cytogenetic techniques, such as FISH, karyotyping, and arrays. Interestingly, OGM can identify more SVs and CNAs and clarify the intricacy underlying diagnostic aberrations, such as chromothripsis and chromoplexy. In 5 out of 25 patients, OGM revealed that the diagnostic translocation was caused by chained rearrangements. OGM revealed numerous additional SVs and CNAs (500 bp to 5 Mb) in all the samples in addition to diagnostic abnormalities [66].

While second-generation sequencing can detect gene mutations, it is still difficult to identify SVs and CNAs using this approach. Several cytogenetic techniques, such as

karyotyping, fluorescence *in situ* hybridization, and arrays, are used to identify these changes, each of which has significant limitations. SVs and CNAs can be detected with high resolution via OGM, a non-sequencing-based method that was used in a retrospective collection of diagnostic soft-tissue and bone tumor samples. In this study, 38 out of 53 patients had successful sample preparation; nonadipocytic soft-tissue tumors had the highest success rate (24 out of 27 patients). OGM detected aberrations in 32 out of 35 instances with a diagnostic SV or CNA. These examples included a translocation t(1;5)(p22;p15) in a myxoinflammatory fibroblastic sarcoma and a POU2AF3::EWSR1 fusion in a round-cell sarcoma. Interestingly, OGM illuminated the intricacy of the genome that underlies the different abnormalities. OGM demonstrated that the diagnostic fusion was produced by a series of rearrangements in five samples, three of which involved chromoplexy. Furthermore, in nine cases, the creation of large marker/ring/double-minute chromosomes was directly caused by chromothripsis. Finally, OGM identified additional abnormalities not seen with standard-of-care cytogenetics, necessitating more research into their possible clinical significance [66].

Advantages and Limitations of OGM

This article highlights the value of OGM in the realm of medical research and disease diagnosis. OGM is highly valuable and has many advantages and some limitations. Chief among the advantages is the ability of OGM to detect large-scale genomic structures and CNVs. OGM is superior to DNA sequencing in several ways. First, it can produce extremely long genome maps up to megabase sizes. Second, it can potentially detect low quantities of target DNA with extremely high sensitivity, thereby avoiding the need for a cascade of conventional diagnostic methods. Third, compared with other molecular cytogenetic methods, OGM costs less and takes less time. However, the OGM approach has several limitations, particularly when data must be backed by CBA or CMA to identify and characterize ploidy changes, copy number neutral loss of heterozygosity (CN-LOH) and SNVs. In addition, some false-negative results and false-positive rearrangements have been reported [37,38,67]. OGM has other limitations as well. For OGM to extract ultralong DNA molecules, frozen tissues must be available, which is not usually the case in diagnostic facilities. OGM cannot detect breakpoint-containing translocations in highly repetitive areas such as the telomeres, the centromere, and the short arm of acrocentromeric chromosomes [68].

Conclusions

OGM is a useful strategy for overcoming some of the drawbacks of conventional techniques applied to clinical patients. OGM has been shown to be an effective means of validating SVs and CNAs that were first identified via alternative diagnostic techniques. The confirmation by OGM in nearly all of the instances covered here came with the bonus of a substantial increase in important genetic data. The SVs of interest in the situations discussed were all known ahead of time, allowing appropriate tailoring of the analysis. When this is not the case, however, a more thorough analysis of the OGM data is needed. The OGM can reliably identify genetic mutation hallmarks, such as rearrangements, including translocations, gene fusions, and copy number changes. By comparison, filtering against a matched-pair sample or the Bionano control sample database, somatic SVs can be ascertained. These SVs are very useful in the diagnosis and therapy of cancer and have considerable functional value. In conclusion, OGM can now be considered a highly reliable method for identifying genetic mutations, such as rearrangements, including translocations, gene fusions, and copy number changes.

Summary and Outlook

OGM can detect more SVs and CNAs than conventional standard-of-care testing due to its high-resolution capacity, which can detect aberrations as small as 500 bp. As a result, OGM can be used to detect hematological malignancies, solid tumors, bone and soft tissue tumors, and genetic diseases. However, the limitations of OGM include its requirement for a high molecular weight yield (>500 bp). OGM also clarifies the complexity underlying diagnostic aberrations, such as chromothripsis and chromoplexy. By using OGM widely across a range of cancer types and comparing the SVs found in that analysis with clinical results, new genetic markers for prognosis and treatment selection may be developed. OGM is an extremely exciting new technology that has shown promise in the cytogenetic diagnostic workup of genetic and malignant disorders. It also paves the path for the discovery of new important actors in other diseases. The use of current methods alone is frequently not sufficient to produce a genome assembly of desired reference-grade quality, especially in eukaryotic organisms. However, current advances in DNA sequencing and other cytogenetics methods have improved genomic research by producing more complete genome assemblies and detecting larger SVs. To accomplish chromosome-level genome assembly for species with large and complex genomes, the use of other genomic techniques, such as optical mapping, typically helps to overcome the gaps in sequencing data. In general, optical mapping has been shown to be quite valuable for genetic research. Optical mapping is anticipated to advance genomic studies by facilitating the construction of more complete genomes and the detection of novel variants owing to its increased application and development activities. It is also becoming more feasible to use optical mapping to determine the relationships between genotype and phenotype. It is anticipated that improved optical-mapping solutions will be created soon to significantly enhance ge-

netic research, despite several drawbacks with the current technology. OGM technology is regarded as a revolution in the fields of cellular genetics and illness detection, and we anticipate its use for larger and more comprehensive applications.

Availability of Data and Materials

Not applicable.

Author Contributions

OMA and YK made substantial contributions to conception and design of this review. Both authors were involved in drafting the manuscript and revising it critically for important intellectual content. Both authors gave final approval of the version to be published. Both authors agreed to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

Ethics Approval and Consent to Participate

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Conflict of Interest

The authors declare no conflict of interest.

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