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Decoding Hematological Malignancies: an End-to-End Optical Genome Mapping Workflow

Unlock Structural Variants at Scale with a Sample-to-Report Solution

Introduction

Hematological malignancies (***Figure 1***), a complex and diverse group of cancers, have high mortality and morbidity rates. Chromosomal aberrations are a significant cause of these malignancies, providing insights into tumor pathogenesis. Therefore, understanding their genomic etiology can improve outcomes by identifying the underlying genomic abnormalities unique to each patient. Yet, current cytogenetic methods such as karyotyping, FISH, and microarrays often miss these due to their technical limitations, leaving many critical variants undetected. This gap in technology often leaves key chromosomal aberrations undiscovered, hindering our understanding and treatment of these complex diseases that significantly contribute to cancer mortality and morbidity.

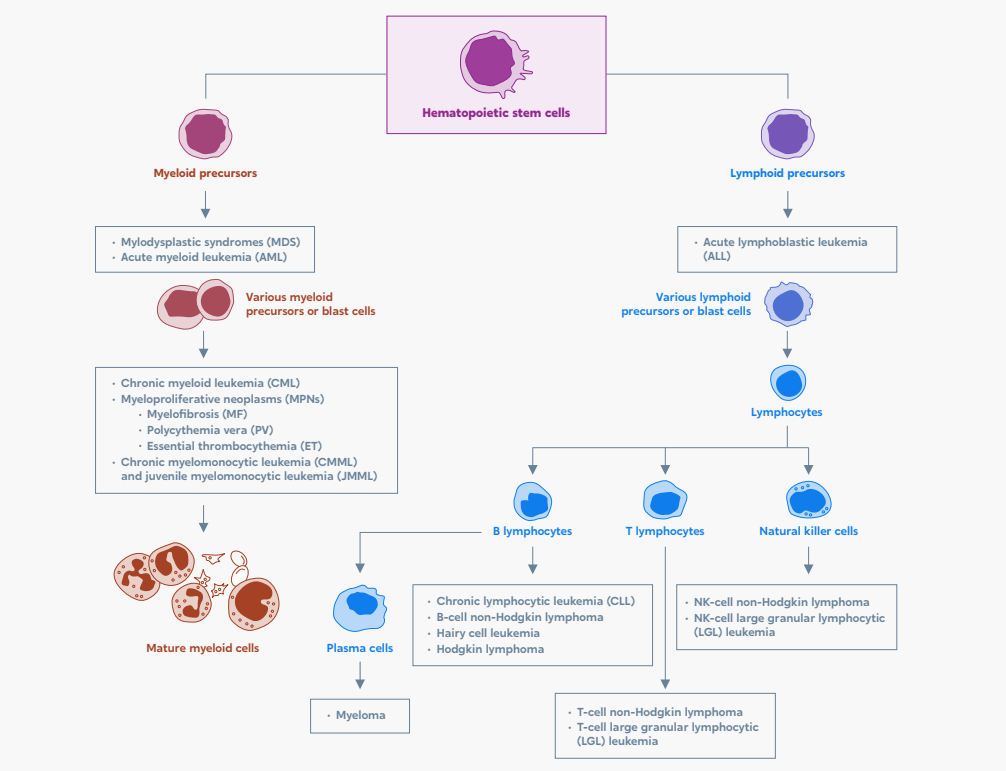

Figure 1. Adapted from the Leukemia Lymphoma Society. Five (5) Hematological malignancies can arise from a variety of different stem and progenitor cells.

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Addressing this challenge requires innovative tools and methodologies. Methodologies such as Optical Genome Mapping (OGM) offer promising solutions. Capable of detecting all types of structural variants (SVs), OGM has been shown in a variety of publications to be able to replace existing methods. Its streamlined workflow could expedite results, potentially advancing clinical research in hematological malignancies.

Bionano offers an end-to-end OGM workflow—including sample prep solutions, mapping systems, and software—aimed at enhancing the study of these malignancies, leading toward more detection of structural variants, which could lead to future personalized treatment options and the development of new therapeutics.

The Optical Genome Mapping (OGM) Workflow

OGM delivers genome-wide, high-resolution detection and automated analysis of all classes of structural variants (***Figure 2***). It captures all classes of structural and copy-number variants in a single assay—detection that usually takes multiple cytogenetic techniques together. Now labs can generate more meaningful information simply and efficiently.

The OGM genome-wide platform brings unbiased, digital precision to solve unresolved cases across hematological malignancies.

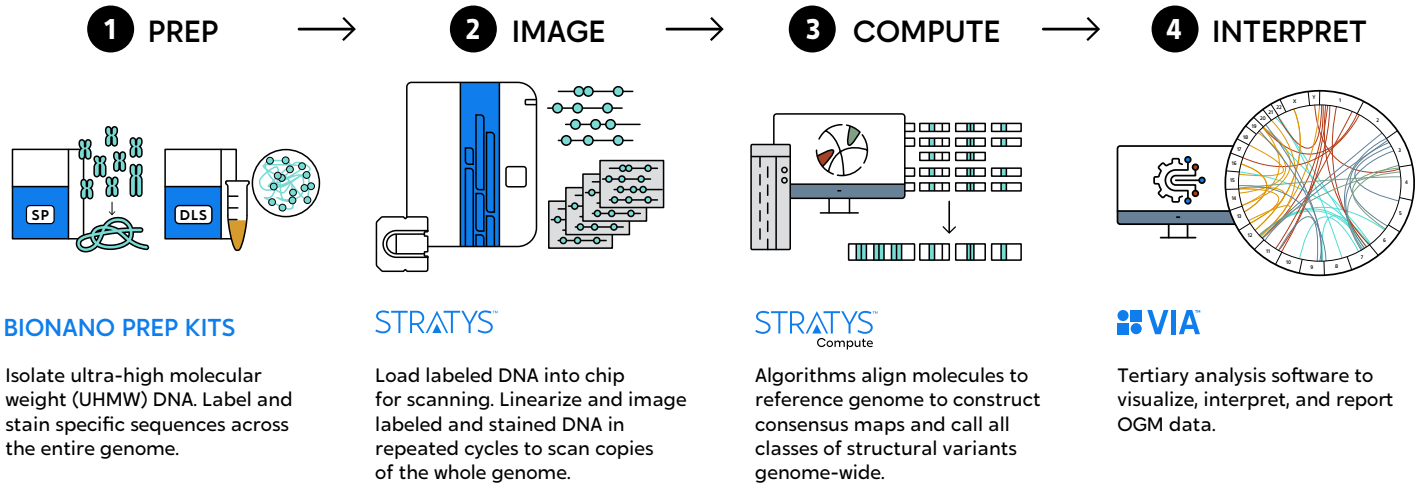

Figure 2. Optical Genome Mapping (OGM) Workflow.

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Achieve Robust Isolation and Labeling of UHMW DNA with Sample Prep Kits

Optical genome mapping (OGM) requires labeled ultra-high molecular weight (UHMW) DNA to identify structural variants. Bionano provides a suite of DNA sample prep kits for simple and robust isolation of UHMW DNA from a range of important sample types. Once DNA has been isolated, our Direct Label and Stain (DLS) kits can be used to label DNA for use with the Saphyr system. Bionano Sample Preparation Kits provide the reagents and protocols needed to extract and label UHMW DNA and are optimized for performing optical genome mapping applications on a variety of sample types.

Fresh/Frozen Blood

Fresh/Frozen Bone Marrow Aspirate (BMA)

Fresh/Frozen Cell Lines

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Elevate your OGM Workflow for Hematological Malignancies with the Stratys™ System

The Stratys System, which includes the Stratys Instrument with Instrument Controller, Bionano Access® Server and Stratys Compute Server, enables rapid, high-throughput optical genome mapping, allowing for sensitive and accurate detection of genome-wide structural variation. Additionally, it provides an integrated software solution for the visualization, interpretation, and reporting of results with the Variant Intelligence Applications™ (VIA™) software, which comes pre-installed with the solution.

The scalable throughput and unprecedented flexibility of the Stratys system allows users to uncover critical insights and significantly impact research progress.

High Sample Capacity

The Stratys instrument provides capacity for 12 single-sample, random-access chips. Load and unload chips as they complete–no need to wait for another sample to finish.

Ultimate Flexibility

No batching required. Optimize cost and efficiency in your lab based on level of demand, loading from one to 12 samples in any run.

"Jump the Queue" for Priority Samples

With reserved space for three samples so you can perform on-demand runs without disrupting the analysis of other samples on the system.

Reduced Turnaround Time

The Stratys system's simplified workflow and scalable sample capacity enables you to deliver results faster while managing lab resources more effectively.

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Stratys Chips and Applications

Stratys chips provide a nanofluidic environment in which DNA molecules linearize across hundreds of thousands of nanochannels where they can be imaged to reveal the underlying genomic structure and structural variation.

		Stratys Core Chip			Stratys Plus Chip			
Target Effective Coverage*		100x	200x	400x	100x	200x	400x	1200x
Recommended Applications								
FSHD		●			●			
Oncology	Hematological Malignancies			●			●	●
	Solid Tumors			●			●	●

*Target Effective Coverage calculated based on 80% map rate achieved.

Typical Metrics for Stratys Molecule Quality Report (MWR) for an AML Sample

Total amount of DNA from molecules that are 150 kbp or longer	**1,537 kbp**
N50 of DNA molecules that are 150 kbp or longer	**327 kbp**
N50 of the molecules that are 20 kbp or longer	**270 kbp**
Average label density for the molecules that are 150 kbp or longer	**16/100 kbp**
Effective Coverage	**471**
Map Rate	**94%**
Positive Label Variance	**6%**
Negative Label Variance	**8%**

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Harness the Full Potential of Your Data Using VIA™ Software

VIA software simplifies your sample-to-report workflow. It consolidates various data types into one view and automates variant calling, interpretation, and annotations. This reduces turnaround times and optimizes process steps.

Streamlining Analysis of Hematological Malignancies with OGM and VIA Software

The latest versions of Bionano™ Solve, Bionano Access™, and VIA software together deliver a comprehensive and automated workflow for analyzing, interpreting, and reporting SVs and copy number variants (CNVs) detected by OGM related to hematological malignancies.

This end-to-end workflow leverages intelligent software automation to reduce the time to result for hematological malignancy sample data from OGM. These oncology workflows are configurable to site-specific preferences.

Analysis Workflow for Hematological Malignancies

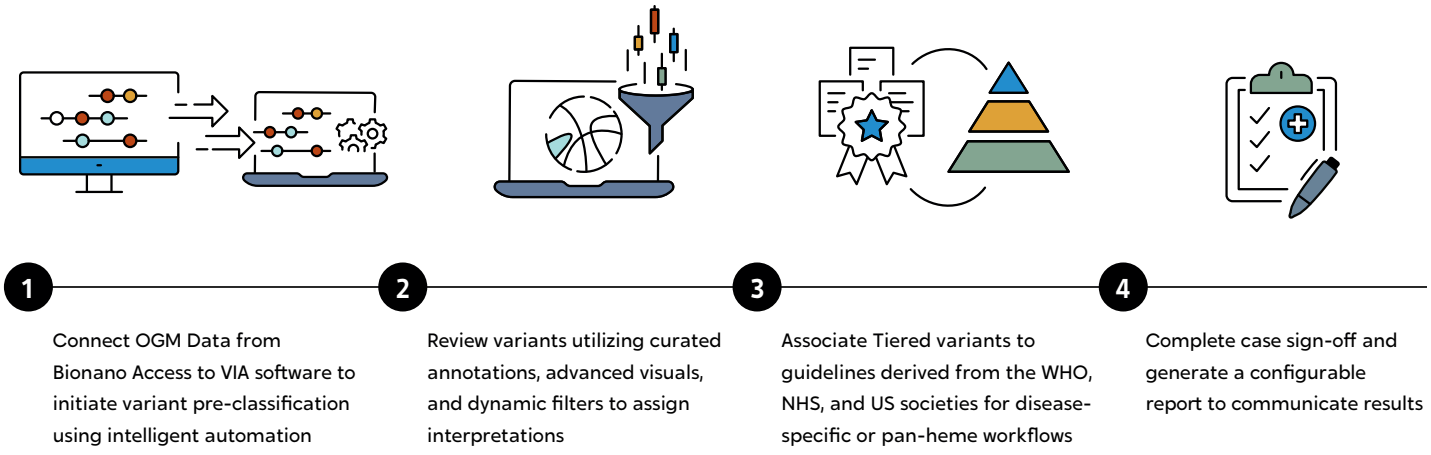

Figure 3. Heme OGM Data Analysis Workflow Schematic.

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Variant Detection

For hematological malignancies, a dedicated pipeline is used for the unbiased detection of SVs as small as 3 Kbp, down to 5% variant allele fraction, across the genome with high precision. Upon job completion, data and results are accessible through Bionano Access, which can be synced with VIA software to automatically, initiating the heme reporting workflow.

VIA software includes algorithms to detect CNVs and Absence of Heterozygosity (AOH) for OGM data. This innovative approach delivers an ability to detect CNVs as small as 500 kb through whole chromosome aneusomies with high sensitivity and precision. VIA also employs a unique approach to detect regions of AOH from OGM data by assessing B-Allele frequency at label sites overlapping known high minor allele frequency SNPs. Analyzing OGM data in VIA enables robust detection of all classes of structural and numerical variants present in the malignancy and automated interpretation tools to aid identifying the key variants of interest.

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Curated Guideline Resources from Global Societies

Bionano provides curated resources of global society guidelines within VIA software. The guideline targets are provided to serve multiple purposes: as a resource to aid interpretation, to inform automated variant tiering pre-classification, to serve as filter to focus on variants of interest, and as a list of selectable guidelines for indicating detection status. Resources are provided as aggregate summaries of guidelines from the U.S., National Health Services (NHS), and World Health Organization (WHO) societies for the assessment of the heme conditions in ***Table 1***.

Table 1. Disease conditions with curated resources provided by Bionano.

Acute lymphocytic leukemia (ALL)
Acute myeloid leukemia (AML)
Chronic lymphocytic leukemia (CLL)
Lymphoma
Myelodysplastic syndromes (MDS)
Multiple myeloma (MM)
Myeloproliferative neoplasms (MPN)
Pan Heme (all conditions)

In VIA software, the Oncology Heme Guideline Target files are available for display in the Tracks view and Detailed Variant Table with detailed descriptions for enhanced visual representation of the resource. The guideline target resources are monitored at a regular cadence for updates, which are distributed through the annotation and tracks resource updates provided in the VIA software.

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Automated Variant Tiering Pre-Classification

VIA software accelerates variant triaging using intelligent automation to pre-classify events related to the disease of interest and help increase the efficiency of the sample review process and the variants (***Table 2***). A preconfigured decision tree (DT) is provided to simplify getting started with analysis in VIA software, constructed to preclassify SV and CNV events based on overlap to Heme-guideline target lists curated by Bionano. Site-specific customization of the decision tree is possible to modify the criteria, resources and/or classification state to account for site-specific preference and is enabled by the site administrators, as necessary.

Table 2. Workflow Pre-classification.

Classification	Definition	Pre-classification Criteria
Tier 1A	Variants of strong disease significance included in professional guidelines	Tier 1A: Any variant overlaps disease-specific guideline overlap. Tier 1 Review: Translocations requiring manual review of orientation
Tier 1B	Variants of strong disease significance with supportive evidence	Tier 1B: Manually assigned
Tier 2	Variant of potential significance; guideline for different tumor type	Pan-Heme Overlap: Any variant that overlaps pan-heme guidelines CIViC Gene: Any variant that overlaps annotated CIViC genes
Tier 3	Variants of unknown significance	Tier 3: CNVs like previous events in case database
Tier 4	Benign or Likely Benign Variants	Tier 4: CNVs like DGV and previous events in case database Artifact: CNVs like Solve CNV mask and previous events in case databas

J Mol Diagn 2017, 19: 4-23; https://dx.doi.org/ 10.1016/j.jmoldx.2016.10.002

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Visualization of Results

VIA software includes powerful means to visualize genomic data to extract deep insights and meaningful context from genomic variants. Informed interpretations require comprehensive genomic annotations, which VIA displays in tracks, tables, and a unique variant dashboard that offers succinct summaries of pertinent information within a single screen. An example display of the Example displays of the Chromosome and Whole Genome browsers are provided in ***Figure 4*** and the Variant Details dashboard, which clearly displays the genes involved in a structural variant along with key variant information and fields to capture analyst notes and interpretations, in ***Figure 5***.

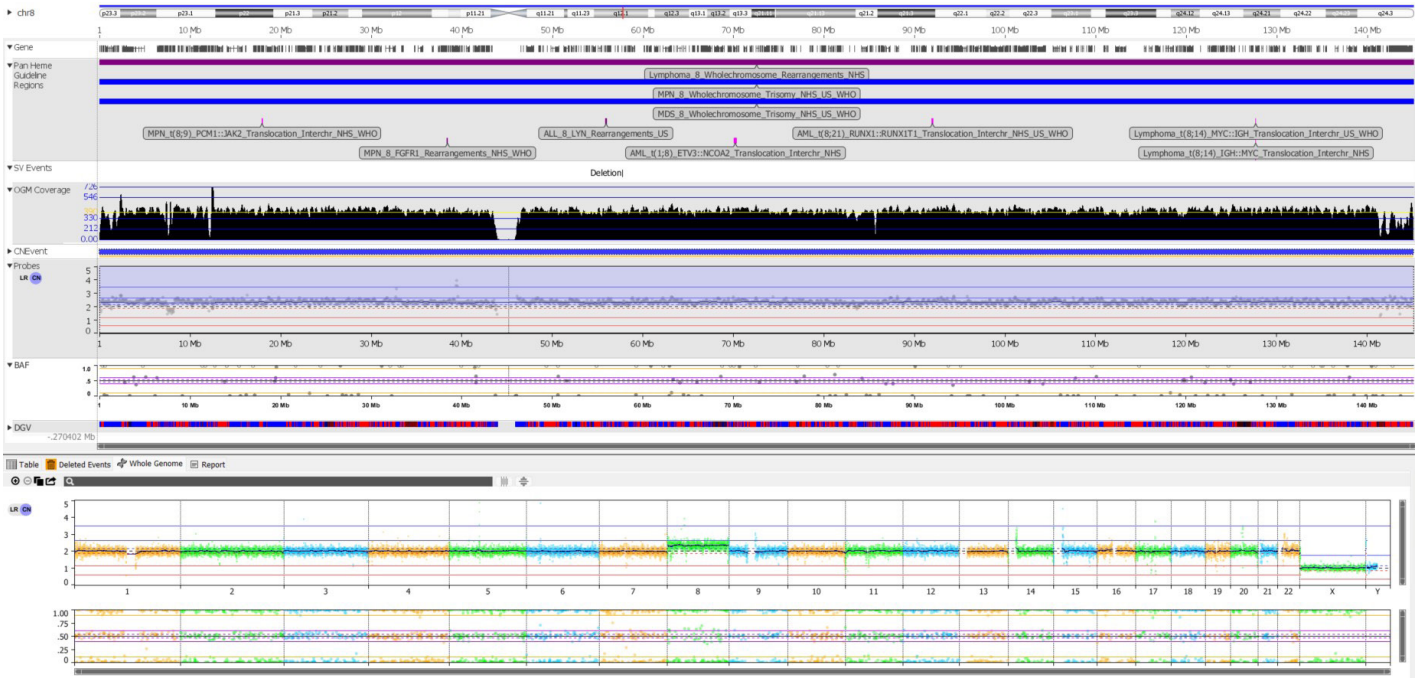

Figure 4. VIA Whole Genome Browser and Chromosome Browser views displaying trisomy of chromosome 8 with example supportive resource tracks.

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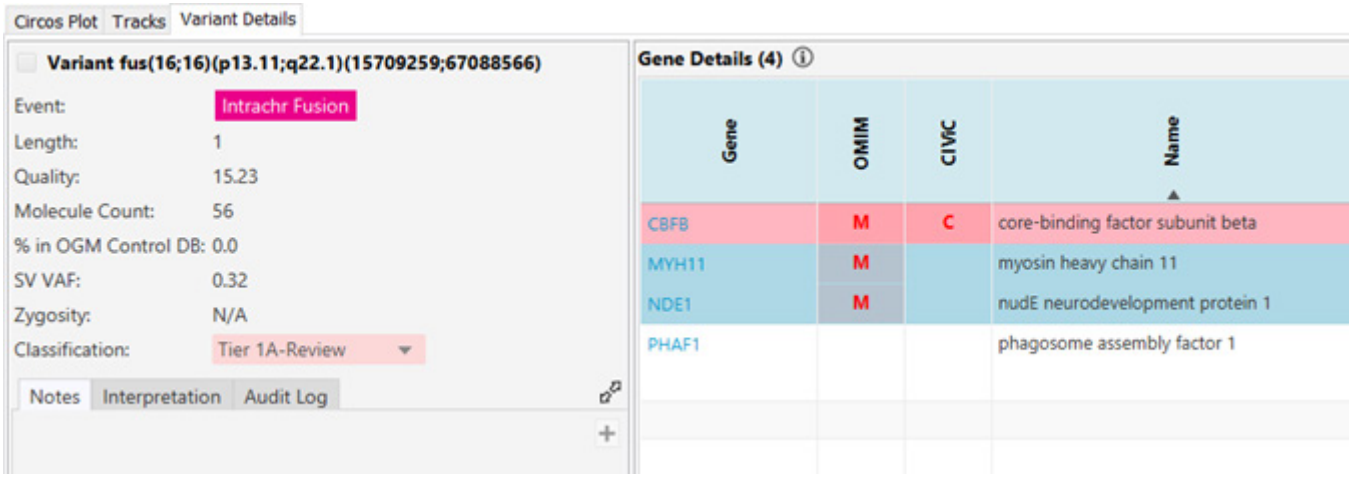

Figure 5. Variant Details view with comprehensive gene annotations for a structural variant in VIA.

Dynamic Variant Filtering

A major obstacle for any unbiased genomic analysis is the ability to extract the important variants from the thousands of detected variants that are related to the disease of interest. VIA offers a comprehensive set of variant filters for each class of variant that are dynamically applied so that you can efficiently and effectively sift through variants based on their characteristics. Multiple filter settings can be saved to allow users to apply the same filtering settings quickly and consistently across samples.

Users have the ability of setting custom panels of genes or regions to apply as a filter panel. VIA software is preconfigured with multiple panels for each disease type. The Bionano curated heme resources are separated into small and large variants based on the guideline size to allow for more effective filtering of variants. A Bionano curated pan-heme cancer gene panel is also provided for comprehensive assessment of genes known to be a factor with the progression of hematological diseases.

Disease-Specific Guideline Target Lists

Associate tiered events to a list of guideline targets within VIA software. A Guidelines list can be created from the Bionano provided curated society guideline resource or a custom list of targeted variants to concisely record the presence of absence of an findings for the disease condition. As illustrated in ***Figure 6***, the Guidelines panel allows the user to indicate whether each guideline is Detected or Not Detected based on the visual display of tiered variants. The completed table is saved with the sample data and can be include with the sample's report. Associating tiered variants to a guideline target provides additional insights on the relevance of findings to the hematological condition being interrogated.

AML Guideline Targets

Guideline Variants	Detected	Not Detected
Chr1 LMNA::NTRK1 translocation	○	○
Chr1 TPR::NTRK1 translocation	○	○
Chr1 NTRK1 rearrangements	○	○
Chr2 EML4::ALK translocation	○	○
Chr2 ALK rearrangements	○	○
Chr3 MECOM rearrangements	○	○
Chr3 GATA2::MECOM inversion/translocation	○	○
5q31.2 deletion	○	○
Chr5 monosomy	○	○
5q whole arm deletion	○	○
Chr7 monosomy	○	○
7q whole arm deletion	○	○
7q31.2 deletion	○	○
Chr7 BRAF rearrangements	○	○
Chr11 NUP98 rearrangements	○	○
Chr11 KMT2A rearrangements	○	○
Chr12 monosomy	○	○
12p whole arm deletion	○	○
Chr13 FLT3 duplication	○	○
16 CBFB::MYH11 inversion/translocation	○	○
16 CBFA2T3::GLIS2 translocation	○	○
17p whole arm deletion	○	○
Chr17 TP53 deletion	○	○
Chr17 monosomy	○	○
Chr19 trisomy	○	○
Chr22 trisomy	○	○

Figure 6. Exmple Guideline selection panel.

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Generating a Report of Results

Results can be communicated easily by leveraging customizable MS Word templates. Templated reports can be configured based on site-specific preferences and information to quickly tag the export of relevant sample and variant information for comprehensive summary of results with supportive details. For hematological malignancy analyses, special sections can be included which provide a summary of the detected Guideline targets and tiered variants across the genome as illustrated in ***Figure 7***. Analyses in VIA are further supported by expansive audit trail of analyst actions and interpretations to preserve historical context with the case review and have confidence.

Figure 7. Example report sections for Guideline and Whole Genome Results.

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Acute Myeloid Leukemia Case Study

Figure 8. Case example of OGM data analyzed in VIA 7.0 for hematological malignancy. ***A:*** Circos plot representation of all structural and copy number variants passing through the filter pipelines. ***B:*** Chromosome browser display of a translocation on chromosome 6 disrupting region gene DEK with tracks displayed for the professional society guidelines and OGM coverage. ***C:*** Variant Detail pane displaying the impact to genes NUP214 and DEK by the Tier 1A translocation between chromosomes 6 and 9. ***D.***: Sample report of results for selected variants supported by interpretations and tables of results.

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Conclusion

Traditional cytogenetic methods for analyzing chromosomal aberrations in hematological malignancies have their limitations, often failing to detect critical variants and thus hindering our ability to fully understand and treat these deadly diseases. The emergence of Optical Genome Mapping (OGM) offers a promising alternative.

OGM has the potential to change the field by providing a comprehensive view of all structural variants, including those missed by conventional methods. Bionano's end-to-end OGM workflow, including sample prep kits, the Stratys system and VIA software, is at the forefront of this revolution, arming researchers with the necessary tools to unravel the secrets of these complex cancers. The insights gleamed from this deeper understanding of chromosomal aberrations can serve as a guide for the develoment of novel therapeutics.

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Technical Assistance

For technical assistance, contact Bionano Technical Support. You can retrieve documentation on Bionano products, SDS's, certificates of analysis, frequently asked questions, and other related documents from the Support website or by request through e-mail and telephone.

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