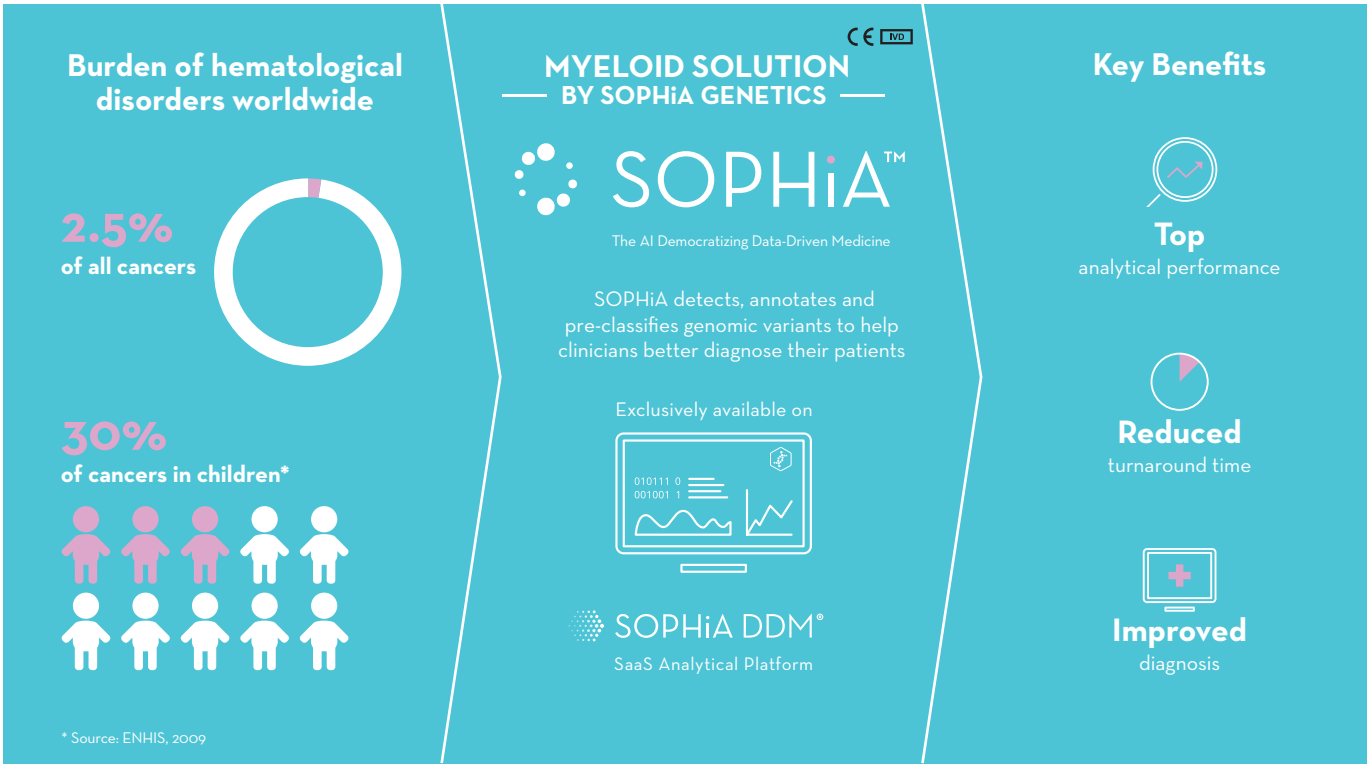
Oncology

SOPHiA GENETICS®

Myeloid Solution™ by SOPHiA GENETICS CE IVD

The state of the art molecular diagnostic application for hematological disorders



Introduction

To date, more than 100 types of cancers have been described.[1] Hematological cancers, such as leukemia, originate from the bone marrow. According to the most recent Global Cancer data, there were 352'000 new cases (201'000 males, 151'000 females) of leukemia diagnosed in 2012.[2] This incidence represents 2.5% of all cancers (excluding non-melanoma skin cancer), making leukemia the 11th most common cancer in 2012. The 265'500 associated deaths (151'300 males and 114'200 females) represent 3.2% of all cancers.[2] Hematological disorders are characterized by several gene mutations, which are currently used as markers to define different subtypes and better diagnose them. The application of Next-Generation DNA Sequencing (NGS) to hematological malignancies over the past several years has provided novel insights into disease characterization and diagnosis, favouring personalized treatment options.[3] The main categories of leukemia and hematological disorders listed below are covered by the Myeloid Solution by SOPHiA GENETICS (see figure on page 2):

- **Acute Myeloid Leukemia** (AML), **the most common form of acute leukemia**[4]
- **Myelodysplastic Syndrome** (MDS)[5, 6]
- **Myeloproliferative Neoplasms** (MPN)[7]
- **Juvenile Myelomonocytic Leukemia** (JMML)[8]
- **Acute Lymphoid Leukemia** (ALL)[9]

The clinical grade analytical performance of the Myeloid Solution by SOPHiA GENETICS are the subject of this white paper.

Hematological disorders covered by the Myeloid Solution by SOPHiA GENETICS, including the genes associated with them

DISEASE	GENES
AML	*ASXL1, BRAF, CEBPA, DNMT3A, EZH2, FLT3, IDH1, IDH2, KIT, KRAS, NPM1, NRAS, RUNX1, SRSF2, TET2, TP53, U2AF1, WT1*
MDS	*ASXL1, BRAF, CBL, CEBPA, CSF3R, DNMT3A, EZH2, FLT3, HRAS, IDH1, IDH2, KRAS, MPL, NPM1, NRAS, RUNX1, SF3B1, SRSF2, TET2, TP53, U2AF1, WT1, ZRSR2*
MPN	*CALR, JAK2, MPL, SETBP1*
JMML	*CBL, KRAS, NRAS, PTPN11, RUNX1, SETBP1, ZRSR2*
ALL	*ABL1, BRAF, FLT3, HRAS, JAK2, KRAS, NRAS, PTPN11*

Leukemia and other hematological pathologies in numbers

The incidence rates of leukemia continue to rise each year.[10, 11, 12] In Europe, approximately 82'300 new cases of leukemia were diagnosed in 2012.[2] In the US, leukemia has been estimated to account for 62'130 of 1'688'780 new cancer cases in 2017[13], representing 3.68% of the total cases.

In Western countries, AML is the most common type of leukemia among adults and represents about 33% of all leukemia cases, followed by CLL, which accounts for ~25% of new cases[10] and ALL for 9%.[14] Worldwide, leukemia most often occur in adults older than 55 years but are also the most common cancers in children younger than 15 years.[1] 76% of these leukemia in children are ALL and most of the remaining cases are classified as AML.[4, 12]

Between 2009 and 2016, leukemia death rates significantly decreased in all categories of the world population.[15, 16] This decrease is a direct result of improved diagnoses and treatments in recent years. As the number of new cases is steadily increasing[16], understanding the causes of leukemia and improving diagnoses remain major public health concerns.

Diagnosis of leukemia and other hematological pathologies

Clinicians have a range of options for patient diagnosis and often choose a combination of approaches according to different factors: the type of cancer suspected, signs and symptoms, age and medical condition, and previous medical tests. As the number of leukemia cells increases in the blood and bone marrow, there is less room for healthy white blood cells, red blood cells, and platelets. This can lead to infections, anemia, and easy bleeding, signs indicative of such disease. Characterizing the specific type of leukemia is important to establish each patient's prognosis and select a personalized treatment.[8, 10, 17]

Traditional diagnostic tests can be invasive and/or time consuming. The first of many such tests is often a complete blood count that measures the amount of red and white blood cells and platelets. Additionally, clinicians can perform microscopic analysis of cell morphology or bone marrow aspiration and biopsy. Cytogenetic analysis (karyotyping) is a molecular test used to examine the chromosome structure in blood cells and bone marrow to determine if they show specific abnormalities. Karyotyping of all chromosomes usually takes weeks of expert analysis. Alternatively, Fluorescent In Situ Hybridization (FISH) can reveal chromosomal aberrations within a few days, sometimes even at the level of specific genes. Another time-consuming approach is immunophenotyping (flow cytometry) that enables characterization of antigens on the surface of cells.[18]

Sequence-based diagnosis of leukemia and other hematological pathologies

Hematological cancers frequently have mutations at specific genomic loci. These mutations can be identified by sequencing the regions of interest. For instance, the most commonly mutated genes in MDS are *ASXL1, DNMT3A, EZH2, RUNX1, SF3B1, SRSF2, TET2, TP53,* and *U2AF1*.[6] For JMML, approximately 90% of patients carry either somatic or germline mutations of *CBL, KRAS, NF1, NRAS,* or *PTPN11*.[6, 8, 19] AML displays mutations in critical genes for normal cell development, cellular survival, proliferation, and maturation, such as *CEBPA, DNMT3A, FLT3, IDH1, IDH2, KIT, KRAS, NPM1* and *NRAS*.[18]

The first DNA sequencing method was developed in 1977 by Frederick Sanger and his coworkers.[20] Since that time, Sanger sequencing has been widely used to detect specific mutations. However, even though it is highly accurate this technique only produces a few thousand bp significantly limiting the detections of mutations per run. Additionally, potentially pathogenic variants within low frequency clonal populations can be difficult to identify due to the higher threshold of detection.[21]

In contrast to Sanger sequencing, Next-Generation DNA Sequencing (NGS) is a cost effective high-resolution method to simultaneously detect mutations in all genes involved in leukemia. With NGS, billions of DNA bases can be sequenced in a day. Such high-throughput molecular characterization and classification of leukemia offers a powerful cancer survey for clinicians, directly leading to improved and personalized diagnostics for patients. When analyzed with advanced pattern recognition and machine learning based algorithms, the patient genome data produced by NGS enables the identification and characterization of the pathogenicity levels of all types of mutations (Single Nucleotide Variants (SNVs), small Insertions or deletions (Indels), Internal Tandem Duplications (ITDs) and Copy Number Variants (CNVs)) with clinical grade sensitivity and specificity. These diagnostic tests are accurate and comprehensive, providing a better understanding of the disease and its progression, enabling healthcare providers to define the most relevant treatment plan.

The detection of large or small variants within the huge amount of NGS sequence data is not trivial, especially with somatic samples. Unlike germline analysis, where expected variant fractions are typically 50% or 100%, variants associated with leukemia are more challenging to detect with confidence, due to very low frequency variants and mixed clonal cell populations.[21] As NGS data contains more sequencing noise than Sanger sequencing, the identification of real variants versus artefacts is not easy. Additionally, current NGS technology generates short (150 - 300 bp) reads. While this is not an issue to capture SNVs or small Indels, it can be challenging to detect long insertions (e.g. ITDs in *FLT3*), large deletions, CNVs or repeats in GC-rich regions (e.g. *CEBPA*). By understanding the complexity of NGS, SOPHiA GENETICS through SOPHiA AI overcomes all these technical challenges.

Myeloid Solution by SOPHiA GENETICS

CE IVD

The Myeloid Solution by SOPHiA GENETICS is a molecular diagnostic application that harnesses NGS technology, combining the analytical power of SOPHiA with a capture-based target enrichment kit and a full access to SOPHiA DDM®

platform, using blood as sample source.
This solution has recently obtained the CE-IVD certification for kit as well as SNV and Indel detection algorithms.

A Comprehensive Solution

The knowledge acquired by SOPHiA through the analysis of large amounts of data has allowed the development of a highly optimized kit (Knowledge-Driven Kit Design) to improve the quality of raw data, which will be better analyzed by SOPHiA.

The Myeloid Solution is designed to detect difficult variants, such as large deletions, insertions and ITDs. It provides the means to detect all main myeloid disorders (previously cited: AML, MDS, MPN and ALL) with a panel targeting 30 genes (see figure on page 2). The gene content of the Myeloid Solution has been carefully evaluated together with hematology experts from several leading institutions in Europe. Laboratories in France, Belgium, Germany and Spain have contributed to the most accurate definition of diagnostically relevant target regions, thus allowing the design of the best curated myeloid panel on the market.

The NGS sequence files obtained from sequencing are uploaded for processing to SOPHiA DDM platform empowered by SOPHiA. Within 4h, SOPHiA generates comprehensive results and customizable variants reports can be generated.

Top analytical performance

Healthcare institutions using NGS to detect mutations in myeloid disorders commonly face difficulties in the analysis of key biomarkers, such as *CALR, CEBPA*, and *FLT3*. For example, *CEBPA* has a high GC-content that hinders sequencing, and consequently, downstream variant calling. This results in low coverage of the region of interest. However, the Myeloid Solution by SOPHiA GENETICS guarantees uniform coverage of *CEBPA* necessary for clinical diagnostics. In addition, PEPPER®, one of the core technologies of SOPHiA, allows the detection of the *CALR* 52 bp deletions and the *FLT3* ITD up to 177 bp. Then, MUSKAT®, another proprietary algorithm powered by SOPHiA, enables CNV detection.

SOPHiA GENETICS simultaneously designs the capture assay and optimizes the data analysis for clinical-grade analytical performance. Thanks to this rigorous approach, SOPHiA GENETICS provides the best solution for NGS analysis of myeloid disorders and it overcomes all the frequently encountered limitations of other commercial offerings available for the diagnosis of these diseases.

Reference partner

Aarhus University Hospital

Aarhus University Hospital is one of the largest hospitals in Denmark, harbouring a very advanced center for molecular diagnostics with strong experience in NGS. MOMA, their core facility for NGS, performs the genomic tests, while the Hemo Diagnostics Laboratory is in charge of the interpretation of the data. Aarhus University Hospital was looking for a NGS-based solution to improve their diagnostics. They chose SOPHiA to perform their genomic tests, for the diagnosis of leukemia and other hematological disorders, because of its superior analytical performance and robustness. Indeed, SOPHiA detects all types of variants (SNVs, Indels and CNVs) in a very short turnaround time, such as *CALR* deletion, *FLT3* tandem repeats and an exceptional coverage of *CEBPA*.
Today, Aarhus University Hospital offers a rapid and precise

diagnosis to all their patients. So far, they have provided reliable diagnoses to over 120 patients and are expecting to run 150 to 200 tests within a year.

"SOPHiA GENETICS has supported our NGS lab with a clear and intuitive workflow, allowing us to obtain excellent results in routine use."

Dr. Kasper Thorsen, Groupleader, Aarhus University Hospital, Department of Molecular Medicine (MOMA)

"The Myeloid Solution by SOPHiA GENETICS guarantees uniform coverage of essential regions, which are otherwise methodologically hard to access. Results obtained by SOPHiA are intuitively presented on SOPHiA DDM platform that provides an overview of both sequencing quality and variant calls."

Anni Aggerholm MSc, PhD, Aarhus University Hospital, HemoDiagnostic Lab (HDL)

Conclusion

Genomic testing of leukemia, through the Myeloid Solution by SOPHiA GENETICS, allows clinicians to diagnose and treat their patients faster and accurately. The strength of this CE-IVD labeled solution lies in the optimal combination of technologies, a Knowledge-Driven Kit Design and SOPHiA AI exclusively available on SOPHiA DDM analytical platform.

Therefore, the Myeloid Solution by SOPHiA GENETICS leads to a performance that reaches the standards required for clinical diagnostic testing (see table on the right).

Moreover, using the Myeloid Solution, clinicians will become member of the world's largest clinical genomics community, allowing them to anonymously and safely share knowledge for better variant interpretation.

PERFORMANCE MEASUREMENT	OBSERVED	LOWER 95% CI
Sensitivity	99,85%	96,78%
Specificity	99,99%	99,98%
Accuracy	99,99%	99,98%
Precision	99,27%	96,78%
Repeatability	98,69%	
Reproducibility	99,30%	

A total of 242 samples were processed on MiSeq® to obtain the above-mentioned metrics

About Us

Global leader in Data-Driven Medicine, SOPHiA GENETICS is a health tech company which has developed SOPHiA AI, the most advanced technology for clinical genomics, helping healthcare professionals better diagnose and treat patients.

The global network of hundreds of institutions worldwide that use the SOPHiA DDM analytical platform powered by SOPHiA form the world's largest clinical genomics community.

By enabling the rapid adoption of genomic testing, turning data into actionable clinical insights, and sharing knowledge through its community, SOPHiA GENETICS is democratizing Data-Driven Medicine to save lives.

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