



## Unravelling the genomic landscape of leukemia using NGS techniques: the challenge remains

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### Introduction

Leukemia is characterized by abnormal proliferation of hematopoietic cells, of which the number and complexity of genetic aberrations tend to increase during disease evolution. More is known about the molecular basis of leukemia than any other form of cancer, primarily due to the availability of abundant malignant cells to study with. The diagnosis and classification of hematologic malignancy has been described in the World Health Organization (WHO) classification of hematopoietic neoplasm, originally published in 2001, representing a paradigm shift by incorporating genetic information into the diagnostic algorithms [1]. The value of genetics is reinforced in the revised 2008 WHO acute myeloid leukemia classification scheme, which emphasized the importance of genetic test results to define clinically relevant disease entities in conjunction with morphology, immunophenotype, and other clinicopathologic features, mostly supported by relevant genetic technologies including karyotype, fluore-

scence in situ hybridization (FISH), polymerase chain reaction (PCR), sequencing, and microarrays [2]. The revised 4th edition of the WHO classification was released in 2016, due to continuing major advances that revealed new mechanisms of tumorigenesis and new potential therapeutic targets, which have been made through the application of high-throughput genetic technologies, such as gene expression profiling and next-generation sequencing (NGS) [3].

### Cytogenetics in the diagnosis of the hematologic malignancies

Conventional cytogenetics was the standard diagnostic tool to study chromosomal abnormalities in hematologic malignancies for a long time, which has allowed identification of most of the recurrent numerical and structural chromosomal abnormalities. This method provides a low-resolution whole-genome scan, particularly useful to detect balanced translocations associated with fusion genes and related inversions and aneuploidies, which are common in AML, ALL and CML. Since this methodology relies on the presence of dividing cells that can be blocked at the metaphase stage of mitosis, however, has main drawbacks in leukemic cells with a low mitotic index, low sensitivity from 5% to 10% (depending on the number of cells analyzed), and a limited resolution (typically 3–5 Mb) that minor clones with small deletions or cryptic translocations can be missed [4].

FISH analysis has been applied to hematologic oncology to overcome some of these difficulties which can be performed on interphase cells and therefore does not rely on the presence of dividing cells. By targeting specific genomic sequences of interest, it detects cryptic deletions and balanced translocations more sensitive than conventional cytogenetics (0.5–1%) and has higher resolution (100 kb, depending on the probe). However, the caution is needed to avoid false-positive and -negative results due to co-

localization or drop-out of signals, which laboratories should establish cut-off values for each probe set to define unambiguous results [5].

### Microarray-based techniques

Microarray-based techniques for whole-genome based scanning by molecular karyotyping, such as array comparative genomic hybridization (aCGH) and single nucleotide polymorphism (SNP) array were introduced, which are the method of choice for searching for chromosomal copy number changes, either losses or gains of whole or segmental chromosomes. The copy number changes with a resolution of 50 kb can be detected by aCGH by comparing the genetic material from a test individual to that of a reference normal individuals and through SNP array, minimal segment detectable can be decreased to 1 kb by estimation of gene copy number on hybridized signals representing genotype of polymorphisms directly in test DNA. This method is capable of identifying novel genomic imbalance throughout the entire genome even in the small population of malignant cells. The loss of heterozygosity (LOH) and copy number neutral LOH (CN-LOH) events detected by SNP array have enabled the identification of new-cancer related genes therefore expected to increase diagnostic yield when combined with metaphase cytogenetics [4].

### NGS approaches to improve genetic diagnostics

The use of the techniques described above allows 'gross' chromosomal abnormalities such as translocations, duplications and deletions to be identified, which served as the most important predictor for risk stratification. However, this is only the tip of the iceberg since genes can be altered in a number of ways (single nucleotide variants (SNVs), small indels, methylation and so on) that could be critical to the leukemogenesis or progression of hematologic malignancies. NGS provides more 'detailed' genetic abnormalities which is promising for the study of leukemia with the ability to fully sequence thousands of genes in a single test and simultaneously detect base substitutions, small indels, copy number variations, translocations depending on sequencing modalities such as whole-genome, exome, transcriptome, and targeted gene sequencing. Studies have been conducted successfully in cytogenetically normal AML and have demonstrated the power of whole genome sequencing (WGS) to discover novel cancer-associated mutations [6].

NGS holds great promise for the study of leukemia and playing as a state of the art, and has already moved into clinical diagnostics and suggested that this technology has the potential to eventually replace all other genetic analysis at diagnosis. There are three NGS approaches to improve genetic diagnostics: 1) WGS, 2) whole-exome sequencing (WES), and 3) targeted enrichment of a set of genes (gene

panel). WES is designed to selectively sequence the coding regions of the genome, which constitute about 1% of human genome. This method can be performed with low cost compared with WGS, but it cannot identify mutations outside the coding regions, nor chromosomal abnormalities, such as translocations and inversions, with breakpoint located in the introns. WGS can overcome these limitations in that it covers the entire genome allowing not only gene mutations but also structural abnormalities, such as deletions, amplifications and translocations. Unfortunately, its major drawbacks are the high cost, the large amount of data generated and the complexity of the bioinformatics analysis and clinical interpretation [7].

Targeted gene sequencing provides a comprehensive, unbiased mutational profiling of many genes of interest and may be a more ideal tool for understanding the overall impact of numerous mutations within an individual leukemia. It is able to identify the full spectrum of significant gene mutations in terms of prognosis, from SNVs to translocations, depending on the customization with less laborious and less expensive than WGS or WES.

A choice between these options requires that relevant outcome parameters first be chosen based on the followings: 1) sensitivity (false-negative rate) and specificity (false-positive rate), since false-negative rate is the major critical parameter in NGS, a complementary approach to identify mutations undetected by NGS, underscoring the power and significance of combining coverage-based analysis with additional target screening of low-depth regions is necessary, 2) diagnostic yield or variant yield, the percentage of variants detected in a given analysis which reflects a proxy for the likelihood of establishing a genetic diagnosis, and 3) cost-effectiveness, the choice of a particular approach must be justified by showing significant additional benefit in healthcare [8]. Besides the NGS approaches discussed above, there are alternative methods that provide information about alterations in gene expression and epigenetic modifications such as transcriptome sequencing or methylome sequencing. Since leukemogenesis is a multistep process involving post-transcriptional changes and epigenetic modifications, the comprehensive analysis of genetic alterations is desired to include these alternative approaches, which are powerful tool for gene expression analysis.

### NGS-based genetic diagnosis in hematologic malignancies

At present, only a small number of genetic abnormalities are used to predict prognosis and orientate therapy. As a consequence of NGS testing in the hematologic malignancy, several novel somatic mutations, including *SF3B1*, *IDH1*, *IDH2*, *DNMT3A*, *MYD88* and *MLL2*, have been discovered, and the characterization of these genetic alterations may substantially affect the clinical management and the therapeutic decisions. Furthermore, the genetic characterization of intrinsic drug resistance of the tumor cells may guide patients with alternative drug combinations

targeting novel genes or pathways discovered in chemo-resistant cases. Therefore, the discovery of novel genetic alterations not only increases our understanding of the leukemogenesis but also opens new therapeutic options.

With the dawn of increasing demand in NGS based genetic diagnosis in hematologic malignancies, there are several challenges that must be addressed. First, the management of the data obtained from sequencing must be addressed, since the complexity of the bioinformatics infrastructure and expertise needed to analyze the huge amount of data generated. Second, the disorders with inherited predisposition to AML or other hematologic malignancy needs to be considered in genetic analysis by discriminating somatic and germline variants. Furthermore, better understanding of genetic events by distinguishing the driver over the passenger mutations to reduce the complexity of the generated data. Moreover, re-evaluation of patient's genome should be considered since knowledge about genomics and disease is rapidly expanding [9].

### Future perspectives

Over 50 years since the first specific chromosomal abnormality identified in chronic myeloid leukemia, the so-called Philadelphia chromosome in 1960, the technology has much evolved, allowing the analysis of cancer genomes at an increasingly greater detail. Although the advancement of NGS unraveled the genomic landscape of hematologic malignancies and opened an era of genome-guided target therapies, the challenge remains how to set up regulatory standards for assuring analytical validity of the test. Transferring the NGS methods from the research laboratory to the clinical setting can be very challenging, and the goal in the post-genomic era of us is the successful transition from genetic discovery to therapeutic intervention [10].

### Authors' Disclosures of Potential Conflicts of Interest

No potential conflicts of interest relevant to this article were reported.

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