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Letter to Editor

Application of Deep Sequencing on Leukemia Clinical Practice: How to Use?

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The Human Genome Project (HGP) was an international collaborative research with the goal to sequence and map the whole human DNA. It was believed that determining the sequence of the base pairs (A; T; C; and G) on human DNA would allow better understanding of the genetic basis of different diseases, including cancer. The draft of the human genome published in 2001 [1,2] did not ensure us the complete knowledge of diseases; however, it had started an important step to scientific research. The HGP was accomplished with first-generation sequencing (or Sanger sequencing), and this was one of the reasons it took more than 10 years to be completed and cost about $3 billion. The delay and cost of the HGP stimulated the development of new laboratory strategies for genome studies beginning the high throughput DNA sequencing era. The next generation sequencing (NGS) comprises a set of different platforms able to generate large amount of data in short time with high throughput capacity. Currently, there are a number of commercially available NGS machines, each using a particular nucleotide detection system. There are many possibilities of applying NGS into scientific and clinical support, allowing from whole genome sequencing (WGS) or exome (WES) - subset of genome that is protein coding - to the mutational evaluation of target genes [3,4]. Here we will discuss the application of NGS and Sanger sequencing in hematological malignancies, focusing on Chronic Myeloid Leukemia (CML) and Myelodysplastic Syndrome (MDS).

CML is a clonal proliferative disorder of hemopoietic stem cells characterized by a chromosomal translocation which is detectable cytogenetically as the Philadelphia (Ph) chromosome [5]. The translocation forms a novel gene by fusing the *BCR* gene on chromosome 22 with the *ABL* proto-oncogene on chromosome 9 (t(9;22) / *BCR-ABL*). Ph chromosome discovery was the first consistent chromosome abnormality associated with a human cancer. So, either the presence of Ph chromosome identified by karyotype or *BCR-ABL* gene identified by PCR is sufficient to diagnostic this type of leukemia. CML has a unique place in oncology due to the fact that virtually all patients express the genetic rearrangement formed by reciprocal translocation. It makes *BCR- ABL* a perfect biomarker, where its presence could be translated to a clinical space. The understanding of CML molecular pathogenesis led to the development of a specific tyrosine-kinase inhibitor (TKI). TKI are small molecules that compete for ATP site on *BCR-ABL* protein in CML cells. When they are covalently linked in the protein they interrupt the intracellular signaling, leading to cell death by apoptosis [6].

In CML, the best-characterized mechanism of resistance is BCR-ABL kinase domain point mutations that impair or prevent TKI binding. There are more than hundreds of mutations described, but not all have clinical importance [7]. Of these, only seven mutated amino acid sites remained problematic for selecting effective "next-line" tyrosine kinase inhibitor for treating CML [8]. Thus, the mutation evaluation is important to guide the clinicians to choose the better second-line TKI available, individualizing the therapy and improving outcome. The international recommendation considered Sanger sequencing as standard methodology to evaluate these mutations, based on a wide availability and robustness [9,10]. However, it has been discussed if the low frequencies these mutation detected by NGS are clinically relevant changing the clinical decision algorithms [11,12]. The importance of this methodology to evaluate the presence of compound mutation is almost a consensus in the

area. Distinguish if ≥2 mutations are in the same BCR-ABL locus (compound mutation) or in separately clones (polyclonal or non-compound mutation) may influence on the selection of the TKI in order to avoid resistance. Unfortunately, Sanger sequencing cannot definitively distinguish frequencies and clonal relationships of those mutations. Several compound mutations have been shown to confer resistance to specifics TKI, and this is likely to apply to other third-line TKIs as well. It has been suggested that sequential therapy with different TKI may inadvertently foster the development or selection of BCR-ABL compound mutations. While each of multiple mutant clones is expected to retain its individual sensitivity to a given TKI, compound mutations can dramatically affect TKI sensitivity and catalytic fitness of the tyrosine kinase [13].

In contrast to the unique biomarker in CML, MDS is a clonal disease with a variety of genetic damage. It is also arising from a malignant transformation of hematopoietic stem cell [14] and is characterized by ineffective hematopoiesis; cytopenias in peripheral blood; hypo- or hyperplasia in bone marrow; and increased risk of transformation into Acute Myeloid Leukemia (AML) [15].

Due to its clinically heterogeinety, MDS is classified into several different subtypes by French-American-British (FAB) cooperative group and World Health Organization (WHO). About 50% of patients have one or more cytogenetic abnormalities, generally chromosomal gain/loss, in those patients lacking cytogenetic markers establishment of a MDS diagnosis might be difficult [16]. In addition, cytogenetic is an important prognostic tool allowing stratification of patients into different risk groups [17].

Since 2008, few research groups discovered a series of mutations in different targets in patients with MDS, allowing the identification of new clonality markers in addition to cytogenetic [18–21]. In most studies, WES was used as the high throughput screening method, confirming the high capacity of this approach to discovery novel genetic markers. Currently, approximately 50 novel genes had been established, including epigenetic regulators; spliceosome proteins; transcriptional factor; cohesion proteins; and signaling pathway proteins. Epigenetic regulators and spliceosome genes are the gene category most frequently mutated in MDS, even though their clinical importance is still uncertain [22]. It has been shown that the same patient may have different mutations, and not necessarily mutations occur in the same clone, supporting the oligoclonal progression of the disease. Walter *et al*. demonstrated that the AML secondary from MDS may be due to increasing of a sub cloning [23]. Even if it is early to bring all novel markers into clinical practice, mutations in *ETV6, EZH2, ASXL1, RUNX1* and specially in *TP53*, are associated with an adverse prognosis. Besides, the identification of only one clonal mutation can discriminate MDS from a non-clonal disease. [24]. The mutational analysis of several genes using Sanger sequencing is laborious and expensive, therefore NGS target sequencing will probably the method of choice to handle molecular characterization. Furthermore, the high sensibility and coverage of NGS make this technique an important tool for evaluating MDS genomic landscape. Whether patients with the same genomic profile will behave in a clinical homogeneous way is to be seen.

In conclusion, currently either NGS or Sanger sequencing are relevant to predict clinical behavior and therapeutic response, the best methodology will mostly depend on laboratory expertise. For CML, the recommended methodology to identify point mutations is still Sanger sequencing and a positive finding can suggest therapeutic changes. Whether mutations detected at low level at diagnosis by NGS are to be considered clinically is still under discussion. In contrast, when two or more mutations are found in *BCR-ABL*, NGS can be a perfect tool to distinguish whether those mutations are on the same clone or not. The establishment of a polyclonal pattern is helpful to improve clinical management and overcome a resistant phenotype. For MDS, a more complex genetic disease, the picture is different and NGS seems to be essential to identify clinical relevant variants. The high sensitivity and multiples targets detection are already important for MDS clinical management. NGS is transforming the conduction of diagnostic investigation, prognostic stratification and therapeutic individualization. However, at this time point NGS creates new challenges, including the unwanted and unsought results, which should be carefully interpreted and managed while managing patients.

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Conflict of Interest

The authors have no conflict of interest to declare.

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