Direct RNA sequencing in Philadelphia-positive leukemia by nanopore sequencing

Philadelphia-positive (Ph+) leukemia clinical settings cannot do without molecular evaluation of the *BCR-ABL1* kinase domain (KD) to select the best therapeutic approach. Among TKIs, Ponatinib is the only currently approved drug able to suppress all *BCR-ABL1* single mutants in Ph+ leukemia, including the *BCR-ABL1* T315I mutant. However, the emergence of compound mutations in a *BCR-ABL1* allele may confer ponatinib resistance [1]. In this context, *BCR-ABL1* mutational analysis of these patients has become crucial.

Recently, we have demonstrated that nanopore sequencing (NS): a third-generation long-read sequencing approach, is suitable for this purpose, showing greater sensitivity than Sanger sequencing (SS) and the ability to distinguish between polyclonal and compound mutations [2]. Furthermore, NS strategy is easier, faster, and cheaper than other next-generation sequencing (NGS) techniques, making *BCR-ABL1* mutational analysis possible in all laboratories.

NS is also the only sequencing chemistry able to perform direct RNA sequencing, without the need to synthesize the complementary DNA (cDNA) like in other NGS approaches. Moreover, these latter methods risk losing RNA molecule information because of the short length of the reads generated and because RNA modifications are not carried forward in cDNA [3].

In light of these considerations, we intend to perform RNA direct sequencing of Ph+ leukemia cases to assess the mutational profile of the *BCR-ABL1* KD.

This new approach could allow a more accurate mutational detection, free from bias due to cDNA synthesis or PCR enrichment. Furthermore, NS will distinguish between polyclonal and compound mutations (as already demonstrated [2]), allowing a better identification of cases likely to fail Ponatinib treatment. These advantages should improve the management of Ph+ patients, reducing costs due to inappropriate therapeutic protocols.

Furthermore, in the era of the “-omic” sciences, the opportunity to directly sequence the RNA molecule could clarify unknown aspects of the transcriptomic profile of these diseases, such as detecting alternative isoforms of the genes implicated in the *BCR-ABL1* pathway or epitranscriptome modifications. These data could improve our biological knowledge of a class of diseases that has been widely studied but still needs further exploration.

References

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