Molecular testing for patients with non-small cell lung cancer at the Hôpitalde la Citadelle in Liège: striving for a fast test result with a fully-automated real-time PCR platform

Over the last decades, a greater understanding of the disease biology of non-small cell lung cancer (NSCLC) and the identification of recurrent oncogenic driver alterations has dramatically improved the therapeutic landscape. Indeed, we have moved away from a one-size-fits-all approach to a more personalized treatment strategy in which patients with a specific oncogenic driver mutations are treated with a suitable targeted therapy. However, to make this personalized treatment a reality, it needs to be accompanied by a fast and comprehensive molecular evaluation, allowing the accurate and timely detection of targetable oncogenic alterations. For this article, we asked *Dr. Henry Paridaens, medical biologist at the Hôpital de la Citadelle in Liège* to share how they currently approach the molecular testing of NSCLC patients.

Could you briefly talk us through your current workflow for molecular testing in patients with NSCLC?

Dr. Paridaens: At the Hôpital de la Citadelle we routinely test all NSCLC patients with a non-squamous histology for oncogenic driver alterations, irrespective of the disease stage. In addition, to this, also selected patients with a squamous histology can be tested. The latter is mainly restricted to very young patients and to never-smokers. From a technical point of view, we have opted to do an initial screening for oncogenic driver mutations using a real-time PCR system (Idylla™). With this fully-automated system we are able to rapidly assess the presence of mutations in *EGFR*, *BRAF* and *KRAS*. In addition to this, we also use the Idylla™ GeneFusion panel, which allows for the detection of gene fusions involving *ALK, ROS1* and *RET* and *MET* exon 14 skipping mutations.

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When these qPCR assays do not indicate the presence of an oncogenic driver mutation, we initiate next generation sequencing (NGS), first on DNA and if this test also turns out negative, we perform an RNA-based NGS. The latter is also the case when the qPCR test for fusion genes is negative. Whenever the qPCR test reveals an oncogenic driver mutation, we usually forego on further NGS analyses. The main reason for this is that most oncogenic driver mutations are mutually exclusive.

What is the rationale behind this strategy?

Dr. Paridaens: The main reason to opt for this strategy is that the qPCR results are available very fast. In our center patients are discussed during a weekly multidisciplinary tumor board and therefore our clinicians prefer to have the result of the molecular testing within a week. With our qPCR platform this is feasible as we need 4-5 working days at most to generate a result. Of course, if we need to do further NGS analyses, our turnaround time gets longer, especially if we need to do both the DNA and RNA NGS. In patients where we do qPCR, DNA and RNA NGS the turnaround time can increase to 3 weeks, which is becoming problematic for the clinic. Nevertheless, our clinicians are aware of these NGS turnaround time and already receive the results of the qPCR test beforehand.

What are the major challenges with the strategy you are using?

Dr. Paridaens: As indicated before, the strategy can generate a fast result in cases where the qPCR picks up an

DR. PARIDAENS

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oncogenic driver. However, in cases where we need to do an NGS we see a marked increase in the turnaround time. While the overall performance of the Idylla™ platform is good, it is not 100%. This especially the case for gene fusions. In fact, while the GeneFusion cartridge is able to respectively detect 93% and 97% of all known *ALK* and *ROS1* gene fusions, the detection rate for *RET*-fusions is lower at only 85%. As we have a RNA NGS as back-up, the fusions that are missed on qPCR will be picked up during the back-up testing. This generates a delay in *RET*-fusion detection for a limited group of patients (15% of the 1-2% of RET-fusion positive patients).

How did the molecular testing for NSCLC patients evolve over the last years and which changes do you expect for the near future?

Dr. Paridaens: My career at the Hôpital de la Citadelle only started in 2019, but nevertheless I have witnessed some important changes in the way molecular testing is being performed in NSCLC patients. First of all, the number of genes that we need to test has gradually increased over time and it is to be expected that this trend will continue in the years to come. For me, the introduction of the Idylla™ GeneFusion cartridge was a big step forward as is the routine use of NGS in clinical practice. In addition to this, we have invested substantially in optimizing molecular testing on liquid biopsies in lung cancer and we now routinely perform molecular tests on circulating tumor DNA. However, I think that the potential of liquid biopsies in lung cancer is not yet fully exploited. For example, over the years we have become very experienced in monitoring treatment responses based on liquid biopsies in patients with colorectal cancer and I hope that we can do the same in patients with lung cancer. Finally, I believe that we need to have more attention for copy number variations and genetic instability in lung cancer. In other cancer types, these things have already spurred research enthusiasm, but this is far less the case in lung cancer.