Targeted RNA sequencing for the detection of actionable oncogenic driver mutations in NSCLC: experience from the University Hospitals Leuven

Over the last decade we have witnessed a dramatic evolution in the treatment paradigm for patients with advanced non-small cell lung cancer (NSCLC). In this, targeted therapy has become the standard of care for subgroups of patients harboring specific oncogenic driver alterations. To facilitate an accurate and timely detection of these targetable molecular alterations, hospitals have adopted different strategies to optimize the molecular testing for patients with NSCLC. In this article, *Prof. Christophe Dooms (thoracic oncologist)*, *Prof. Birgit Weynand (pathologist)* and *Prof. Isabelle Vanden Bempt (clinical biologist)* discuss how they addressed the challenges posed by the evolving molecular testing needs in patients with NSCLC.

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

Prof. Dooms: In our center, all patients with a NSCLC diagnosis are tested for PD-L1 by immunohistochemistry. In addition to this, next generation sequencing (NGS) is performed in all patients with a non-squamous tumor histology. In fact, while the clinical relevance of the level of PD-L1 expression and the presence of targetable oncogenic driver mutations used to be limited to patients with advanced disease, these factors now also hold relevance for patients with an earlier disease stage. Only for patients with very small, stage I tumors we currently forgo these tests.

Prof. Weynand: To speed up the molecular testing we have adopted a reflex testing strategy. In this, all samples with a non-squamous histology are immediately processed for NGS analysis. In addition to the PD-L1 testing mentioned by ***Prof. Dooms***, we are also doing an immunohistochemical (IHC) analysis for ALK-fusions in all samples with a non-squamous histology. For samples with a spinocellular histology, the reflex tests consist of PD-L1 and pan-TRK IHC.

Prof. Vanden Bempt: According to the Belgian reimbursement criteria for NGS, it is recommended to first do a DNA-based NGS or alternative analysis for the detection of mutations in driver oncogenes and only perform an RNA-based NGS analysis for gene fusion detection if the first analysis did not reveal an oncogenic driver mutation. As you can imagine, this sequential NGS strategy comes with a prolonged turnaround time for a large proportion of patients. To overcome this, you can opt to do DNA and RNA NGS analysis in parallel. However, this is associated with a higher cost and a larger tissue need, which can be problematic in NSCLC patients. As an alternative, the UZ Leuven has invested in the development of an RNA-based NGS test that allows the simultaneous detection of all relevant oncogenic drivers in NSCLC, including mutations as well as gene fusions. This approach was extensively validated[1] and accredited after which the RIZIV/INAMI accepted this strategy as an alternative for the sequential DNA-RNA NGS workflow.

Prof. Dooms: *"I believe that this operational optimization played a major role in achieving the low failure rate with our RNA NGS analysis (~5%)."*

Prof. Weynand: Apart from a reduced turnaround time, this RNA-based NGS strategy also has the advantage that it requires a small amount of tissue. This is of particular relevance given the 'issue of tissue' in lung cancer. In fact, in contrast to many other tumor types, resection samples are rare in lung cancer where we usually have to perform the pathological and molecular work-up on small biopsies, or cytological samples.

Prof. Dooms: Apart from optimizing the RNA NGS analysis, we have also invested in improving the pre-analytical steps in the process. To this end, we have developed a number of standard operating procedures for tumor sampling. In doing so, we are able to systematically deliver a suitable sample for subsequent IHC and molecular analysis. I believe that this operational optimization played a major role in achieving the low failure rate with our RNA NGS analysis (~5%).

PROF. DOOMS

PROF. WEYNAND

PROF. VANDEN BEMPT

What is the turnaround time you are able to achieve with this RNA-based strategy?

Prof. Vanden Bempt: With our current workflow we have a turnaround time of about 8 business days. In the clinic, this comes down to approximately 2 weeks between the day the sample was taken and the molecular test result. In theory we could do the RNA testing faster, but that would not be cost-effective. As a compromise, we have opted to bundle samples into two RNA NGS runs per week.

Prof. Dooms: With the current turnaround time, the time for molecular testing is no longer a bottleneck in our diagnostic work-up of NSCLC patients. When a patient with a suspicion for lung cancer comes in, we try to schedule the biopsy or cytology within 1-2 days and the necessary medical imaging for staging are planned in parallel. Taking into account this planning, the second consult with the patient is usually scheduled about 2-4 weeks after the first consult. At that time, we generally have all the molecular and imaging data at our disposal allowing us to make an informed treatment decision. In this respect, I do need to underscore that we are privileged to have the molecular testing facility in house. I can imagine that this workflow is somewhat more complex if you need to send the sample to an external site for molecular analysis.

How do you think the molecular testing of NSCLC patients will evolve in the years to come?

Prof. Dooms: The future of molecular testing in NSCLC will in part be determined by the research into predictive biomarkers for immunotherapy and targeted therapy. As such, I believe that the future will be much more nuanced than what we have now. In addition to this, I think that the treatment personalization in the advanced setting will increasingly trickle down to earlier disease stages. In fact, with the use of adjuvant osimertinib in early-stage EGFR-positive NSCLC and the indication for neoadjuvant immunochemotherapy in PD-L1 positive stage II/III NSCLC we are already seeing examples of this evolution.

Prof. Vanden Bempt: Currently, our RNA NGS analysis is based on a targeted panel of about 25 genes. In the future we would like to broaden this significantly. In this respect, we are currently working on a research project to assess whether we can obtain additional clinically relevant information when sequencing the entire transcriptome (whole genome transcriptome sequencing). Apart from the different targetable alterations, whole transcriptome sequencing will allow us to get a complete picture of the genetic make-up of NSCLC samples, including the tumor mutational burden, the presence of important co-mutations, and perhaps even expression profiles that predict a response to immune checkpoint inhibition. Compared to parallel DNA and RNA NGS with large gene panels, which remains a targeted approach, whole transcriptome sequencing offers un-biased comprehensive detection of gene mutations and fusions as well as expression profiles, at limited cost and with minimal input of archival tumor material.

Prof. Dooms: On a final note, I would also like to mention our virtual molecular multidisciplinary oncology consult (MOC). During this weekly virtual meeting we discuss the targeted molecular findings in NSCLC patients within our multidisciplinary team. Once we implement whole transcriptome sequencing into daily clinical practice, the interpretation of the molecular findings will become increasingly more complex. In this respect, these virtual molecular MOCs will certainly become more important in the years to come.

Reference: 1. Claerhout S, et al Lung Cancer 2022;166:242-9.