EDITORIAL

Routine characterization of biomarkers in non-small cell lung carcinoma: how much is enough?

In recent years there was an extraordinary development in targeted therapies for cancer. This development was quickly followed by the realization that successful application of these therapies is highly dependent on the identification and routine use of predictive biomarkers of therapy response. In this context, the fine characterization of tumours, both at the molecular and histopathological levels, might provide fundamental insight. In the study by Sousa et al.,[1] published in the current issue of the Portuguese Journal of Pulmonology, the authors show adenocarcinomas of the lung are highly heterogeneous from the histopathological standpoint and show different patterns of expression of immunohistochemical and genetic biomarkers. In their study the authors used a set of 10 immunohistochemical biomarkers and 3 genetic biomarkers. The authors further reinforce the need to carefully identify lung adenocarcinoma histological patterns with putative implications in diagnosis and pathogenesis understanding. Unfortunately, the study does not include data on the clinical follow-up of the patients precluding the analysis of the predictive value of the data presented. Nevertheless, the publication of this study may constitute a good opportunity to discuss the clinical impact of routine characterization of biomarkers in non-small cell lung carcinoma.

The diagnosis of lung cancer involves a variety of approaches ranging from pathology to medical imaging and molecular biology. As our understanding of the disease grows, and new knowledge is translated into clinically relevant procedures, there is an increasing pressure on diagnostic turnaround times and relevant sample material for the increased number of companion tests prior to decision-to-treat. Advanced lung cancer diagnostics are a showcase for this challenge; biological material for diagnostics procedures is often limited (cytology and biopsy material), and timely responses are needed for optimal therapeutic decisions. In this cancer model, tumour samples are necessary for routine pathology evaluation combined with advanced molecular biology analysis.

The diagnosis and histological classification of lung cancer into its major subtypes - small cell lung carcinoma, adenocarcinoma and squamous carcinoma - relies on morphological characterization and on the use of differentiation biomarkers. Although several markers are available for routine diagnosis, the immunohistochemical expression of TTF1 and p63 are currently markers of choice to put forth differential diagnosis between squamous carcinoma and adenocarcinoma of the lung. Expression of TTF1 favours the diagnosis of adenocarcinoma, whereas expression of p63 favours diagnosis of squamous carcinoma.

The addition of tyrosine kinase inhibitors into the therapeutic arsenal of non-small cell lung carcinoma, brought into routine clinical practice the analysis of mutations in the *EGFR* and *KRAS* genes, and the detection of chromosomal translocations involving the *ALK* and *ROS1* genes. Specific activating mutations in exons 18, 19, 20 and 21 of the *EGFR* gene allow selection of 10-20% of patients for treatment with the EGFR inhibitors gefitinib™ or erlotinib™. In contrast, patients with other *EGFR* mutations, namely insertions and deletions in exon 20, and mutations in the *KRAS* gene, are not likely to respond to the same EGFR inhibitors, and as such should receive alternative treatment. In addition, 3-5% of patients not eligible for EGFR-inhibitor treatment may still benefit from treatment with ALK-inhibitor crizotinib™ as long as they can be confirmed to carry chromosomal translocations involving the *ALK* or *ROS1* genes.

Currently, lack of integration between molecular assays, requires all the above described mutation and chromosomal translocations to be analysed sequentially in multiple assays. Each step demands its own biological aliquot for analysis and has its own turnaround time, challenging both sample requirement and laboratory diagnostics turnaround time. Typically, the biological material will be used first for histopathology analysis, which may include the study of protein markers such as TTF1 and p63 using immunohistochemistry (IHC). This step will typically take 5-10 working days. If diagnosis of non-small cell lung carcinoma is

confirmed (80-85% of the cases), first-line therapy with EGFR inhibitors should be considered, especially for adenocarcinomas. The second step will therefore include mutation screening for the *EGFR* gene alone or in conjunction with the *KRAS* gene. This step will typically take 5-10 working days. If not eligible for anti-EGFR therapy there will be further testing for *ALK* and *ROS1* translocations using fluorescence in-situ hybridization (FISH) alone, or a combination of IHC and FISH. This third step takes additional 5-10 working days. Altogether, this three-step testing strategy may take something between 15 and 30 working days, delaying timely oncology treatment initiation and incurring patient insecurity. Additional delays may occur if samples need to be transferred between laboratories.

While we all eagerly await technological developments that allow for single-assay methods/devices coping with the current challenges of minimizing both the need for analytical biological material and turnaround time, there is a pressing need to prioritize the type and number of companion tests in lung cancer diagnosis. In that respect, current recommendations are very straightforward: as long as lung cancer diagnosis has been confirmed, all available biological material shall be prioritized for the analysis of molecular biomarkers with predictive value to therapy response which, at the time of this writing, include and are limited to *EGFR* and *KRAS* mutations, and *ALK* and *ROS1* translocations. International organizations, such as the CAP/IASLC/AMP, clearly state physicians should order predictive testing at the time of diagnosis for patients with advanced stage lung adenocarcinoma, regardless of their clinical history. That is how much is enough.

Reference

1. Sousa V, Rodrigues C, Silva M, Alarcão AM, Carvalho L. Lung adenocarcinoma: Sustained subtyping with immunohistochemistry and EGFR, HER2 and KRAS mutational status. Rev Port Pneumol. 2015, http://dx.doi.org/10.1016/j.rppnen.2014.09.009.

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