Cytopathology in focus: Non-small cell lung carcinoma

Cytology samples and immunotherapy predictive testing

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January 2020—Requests for predictive biomarkers in oncology patients are becoming increasingly common in the cytology laboratory. At the time of rapid on-site evaluation, cytologists are now keenly aware of the need to collect adequate material not just for a diagnosis of malignancy but also for diagnostic and predictive molecular and immunohistochemical testing. This article provides an overview of current practices and some of the recent literature regarding predictive testing for immunotherapy in cytologic preparations in non-small cell lung carcinoma.

Biological rationale of immunotherapy. Tumor cells may evade the immune system through inhibition of the immune synapse between T cells and antigen presenting cells.[1] The inhibitory molecules programmed cell death-1 (PD-1) and cytotoxic T-lymphocyte-associated protein 4 (CTLA-4) are referred to as immune checkpoint molecules. Immunotherapy using PD-1, PD-L1/2 (programmed cell death ligand 1/2), and CTLA-4 targeted antibodies are immune checkpoint inhibitors. These therapies may restore the ability of the cytotoxic T cells to recognize and attack tumor cells.[1] In the appropriate context, high levels of PD-L1 expression on tumor cells suggest that the patient may be more likely to respond to inhibition of PD-1.[2]

Notably, immune checkpoint inhibitors have a unique set of side effects including immune-related adverse events as well as others seemingly unrelated to the immune system.[3] Rarely, these adverse events may prove fatal. Thus, efforts continue to better predict which patients will be most likely to benefit from immunotherapy. While the most evidence has accumulated for non-small cell lung carcinoma, other cancers with the potential to respond to immune checkpoint inhibitors include gastroesophageal adenocarcinoma, cervical carcinoma, urothelial carcinoma, esophageal squamous cell carcinoma, head and neck squamous cell carcinoma, and triple-negative breast carcinoma.[4]

PD-L1 immunohistochemistry. Although specific immune checkpoint inhibitors may be used clinically without testing of the tumor itself, many situations require quantification of the level of PD-L1 expression on tumor cells to allow the patient to receive specific therapies or to participate in clinical trials.[2] It is important to know which antibody clone or specific assay is requested, as some assays have been FDA approved/cleared as companion diagnostic tests for a specific drug,[4] while others may not be.

PD-L1 is quantified by the percentage of tumor cells with membranous staining. For PD-L1 clone 22C3 in advanced non-small cell lung carcinoma, greater than or equal to 50 percent staining may result in the use of pembrolizumab as first-line therapy, while a greater than or equal to one percent staining is considered positive and pembrolizumab may be considered as second-line therapy.[5,6] Interpretive criteria are likely to vary by antibody clone/testing platform,[7] and some investigational applications may explore PD-L1 expression in immune cells and/or stroma in addition to tumor cell expression.

Significant intratumoral, intersite, and temporal heterogeneity in PD-L1 expression has been reported. Cytologic preparations, owing to their small size, present a dilemma with respect to sampling. In cases where a small number of cells is present in the cell block (for example, fewer than 100 cells), both false-positives and false-negatives due to intratumoral heterogeneity are a concern. PD-L1 interpretive criteria were developed for histologic sections; in many cases the same criteria may be applied to adequately cellular cytologic preparations. Several studies have shown high concordance between cytologic samples and core biopsy or surgical resection specimens.[5,8-11] One study suggests that interobserver reproducibility may be lower in cytologic samples

specifically, as compared with histologic samples.[12] Reproducibility may improve as pathologists gain more experience in interpreting PD-L1 results in cytologic samples.

An important consideration in predictive immunohistochemical stains is the impact of fixation on quantitative results. Previous studies have shown the potential for both false-negative[13] and false-positive[14] results with alcohol fixation of cell blocks. To this end, validation may be necessary for predictive immunohistochemical stains if the fixation conditions of cytologic preparations (smears, liquid-based preparations, or cell blocks) differ from those used in the routine validation study.[15]

Other testing options under investigation. High rates of somatic mutations lead to the production of neoantigens that may be integral to the effectiveness of immune checkpoint inhibitors.[16] Thus, molecular testing for the tumor mutation burden, or a global measurement of somatic mutations, may aid in predicting response to immune checkpoint inhibitors. Efforts are underway to standardize tumor mutation burden estimation and reporting.[17] Although next-generation sequencing may be successfully performed in cytology samples with adequate tumor cellularity, tumor proportion, and assay validation,[18] few studies report tumor mutation burden in cytology samples at this time.

Likewise, mismatch repair defects may result in high rates of mutations and production of neoantigens. Thus, either mismatch repair immunohistochemistry or nucleic acid-based microsatellite instability testing may also help in predicting the potential response to immune checkpoint inhibitors. A CAP template exists for reporting DNA mismatch repair biomarkers for tumors other than endometrium and colon.[19] This template is not tailored to cytologic preparations. As with PD-L1 testing, validation may be needed when fixation conditions for cytologic preparations vary from the conditions used in the routine validation.[15]

In conclusion, cytology samples of non-small cell lung carcinoma represent an opportunity to provide predictive biomarkers with a minimally invasive approach. CAP guidelines on the collection and handling of thoracic small biopsy and cytology specimens are expected soon.[20] Guidelines regarding PD-L1 testing are also in progress.[21]

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