Review Article

A narrative review of predictive immuno-histo/cyto chemistry in lung cancer: focus on gene fusions and PD-L1 expression

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Background and Objective: Personalized medicine has significant modified the way advanced stage non-small cell lung cancer (NSCLC) patients have been managed. In fact, the development of different target drugs has significantly improved the clinical outcomes of these patients. In this scenario, gene rearrangements play a crucial role. Gene fusions, including those involving anaplastic lymphoma receptor tyrosine kinase (*ALK*), ROS proto-oncogene 1, receptor tyrosine kinase (*ROS1*), rearranged during transfection (*RET*) and neurotrophic receptor tyrosine kinase (*NTRK*) genes, occur very rarely in advanced stage NSCLC patients. Remarkably, these genomic alterations represent an important target for treatment decision algorithm in these patients. In addition, programmed death-ligand 1 (PD-L1) protein expression evaluation is crucial for immune-checkpoint inhibitors (ICIs) administration.

Methods: We reviewed the recent literature on PubMed focusing the attention on the role of IHC/ICC in advanced stage NSCLC patients harboring gene fusions and for the evaluation of PD-L1 expression.

Key Content and Findings: Thus, immunohistochemistry/immunocytochemistry (IHC/ICC), beyond the traditional role in the assessment of the immunophenotype of neoplastic cells to refine uncertain morphological diagnosis, plays a pivotal role in advanced stage NSCLC patients in order to administrate the best treatment option and avoid to leave any patient behind. IHC/ICC is a cost-effective, easy to use and rapid technique that can be adopted even on very scant neoplastic samples. Conclusions: Here, we focalize the attention on the adoption of IHC/ICC approach for gene fusions detection and PD-L1 protein expression evaluation in advanced stage NSCLC patients.

Keywords: Immunohistochemistry (IHC); immunocytochemistry (ICC); non-small cell lung cancer (NSCLC); molecular pathology; molecular oncology

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Introduction

Lung cancer is still the leading cause of cancer related deaths worldwide (1). Nevertheless, significant steps forwards have been made during the last decades in the field of cancer treatment for these patients. In particular, the identification of several genomic alterations, as point mutations, insertions/deletions (indels), abnormal splicing events and gene fusions, that are or may be potential target for personalized treatments has significant modified the management of advanced stage non-small cell lung cancer (NSCLC) patients (2-4). To this end, international societies have established a panel of so-called "must test genes" [including epidermal growth factor receptor (*EGFR*) and V-raf murine sarcoma viral oncogene homolog B (*BRAF*)

gene mutations and anaplastic lymphoma receptor tyrosine kinase (*ALK*) and ROS proto-oncogene 1, receptor tyrosine kinase (*ROS1*) gene rearrangements] that represents the minimum standard of care panel of genes that must be necessarily tested in advanced stage NSCLC patients (5-8). This rapid evolving field has significant modified the role of pathologists in the diagnostic algorithm of NSCLC patients. In fact, it is crucial the correct management of cancer tissue specimens for both morph-molecular analysis, in order to avoid to leave any patient behind (9). It should be borne in mind that several molecular platforms are being currently available for predictive purposes in molecular predictive pathology laboratories. Among these, immunohistochemistry/immunocytochemistry (IHC/ICC), beyond the classical role in the assessment of the immunophenotype of neoplastic cells, by evaluating the protein expressed by cells, have acquired a crucial predictive role. As a general role, IHC/ICC has been adopted to make classification of advanced stage NSCLC patients more accurate, in a time-efficient, easy and cost-limited way (10,11). In addition, IHC/ICC play a pivotal role in the identification of aberrant proteins production as a consequence of gene fusion events (12-15). These latter can determine tumor development and progression either with a constitutive kinase activation or an altered transcription process of the involved genes (16). In addition, IHC/ICC is currently the only approved approach for the evaluation of the expression of programmed death-ligand 1 (PD-L1) for immune-checkpoint inhibitors (ICIs) administration (17-20). IHC/ICC can be adopted even on very scant neoplastic samples (12). In addition, this morphological approach has the ability to identify fusion events even in cases with an unknown gene partner. However, IHC/ICC suffers from inter-observer variability and may require orthogonal validation of positive results (13).

Here we reviewed the role of IHC/ICC in advanced stage NSCLC patients harboring gene fusions and for the evaluation of PD-L1 expression. We present the following article in accordance with the Narrative Review reporting checklist (available at https://jxym.amegroups.com/article/view/10.21037/jxym-22-7/rc).

Methods

We reviewed the recent literature on PubMed focusing the attention on the role of IHC/ICC in advanced stage NSCLC patients harboring gene fusions and for the evaluation of PD-L1 expression.

ALK gene

As early as 2007, Soda *et al.* described the echinoderm microtubule-associated protein-like 4 (*EML4*)/*ALK* gene fusion in NSCLC patients (21). Despite a number of different partners have been identified, in all cases the chimeric protein shows the presence of the carboxy-terminal portion with kinase activity of ALK protein (14,21-24). Overall, *ALK* gene fusions are reported in about 3-5% of advanced stage NSCLC patients, in particular young, light or never-smokers, featuring an adenocarcinoma with extracellular mucin, cribriform pattern and signet ring cell histology (21,25). Advanced stage NSCLC patients harboring *ALK* rearrangement benefit from ALK tyrosine kinase inhibitors (TKIs) administration (26). Currently, different antibodies are being commercially available for IHC/ICC evaluation of ALK fusion protein (27). Among these, 5A4 clone (Abcam, Cambridge, UK) and D5F3 (Ventana, Tucson, AZ, USA) are the most commonly adopted in molecular predictive pathology laboratories (14). Clone 5A4 staining is evaluated by a 4 scores system (0, 1+, 2+, and 3+) (28). Overall, despite it has been demonstrated a full concordance with fluorescent in situ hybridization (FISH) for scores of 0 and 3+, less reproducibility has been reported for scores 1+ and 2+; thus a FISH confirmatory analysis is required in these cases (29,30). Conversely, D5F3 clone has demonstrated a higher sensitivity and specificity with respect to 5A4 clone (31). D5F3 clone with the Ventana automated immunoassay (Ventana ALK D5F3 CDx Assay, Ventana Medical Systems, Tucson, AZ) has obtained the Food and Drug Administration (FDA) approval as a companion diagnostic assay for ALK TKIs administration (12). Overall, through a tyramide-based amplification step in addition to the polymeric phase (OptiView; Ventana Medical Systems) it is possible the selectively intensification of the specific immunosignal while contemporary reducing the background noise (31,32). This process determines the generation of a positive, strong, granular cytoplasmic staining, or negative result (31). For all these reasons, D5F3 clone was adopted to select advanced stage NSCLC patients in the phase 3 trial comparing alectinib to crizotinib (33). Basing on these results, recent recommendations indicate that a strong positive staining with D5F3 clone is enough to start a treatment with ALK TKIs (34). However, it should be borne in mind that, due to the rarity of *ALK* rearrangements in advanced staged NSCLC patients, it must be mandatory to adopt ALK positive and negative controls samples (27). Beyond

histological specimens, it has been widely demonstrated the suitability of different cytological preparation for ALK ICC analysis (35-41).

ROS1 gene

ROS1 gene fusions were reported in advanced stage NSCLC patients for the first time in 2007 by Rikova *et al.* (42) Similar to *ALK* gene fusions, *ROS1* rearranged cases occur very rarely (about 1–2%), in particular in young, never-smoker women, with adenocarcinoma histology characterized by extracellular mucin, cribriform pattern, and signet ring cells (22,43). In addition, *ROS1* rearranged tumors show a hepatoid cytology, with abundant eosinophilic cytoplasm, round and relatively monomorphic nuclei, and prominent nucleoli (44). As well as for *ALK* fusions, *ROS1* fused advanced stage NSCLC patients showed a significant improvement in clinical outcome after treatment with TKIs (45). IHC/ICC approach may represent a valid option to detect *ROS1* fusion events (12-14). A limited number of ROS1 specific antibodies have been developed and adopted into the clinical diagnostic routine practice (46-48). Among these, the most common employed in molecular predictive pathology laboratories is represented by D4D6 clone (Cell Signaling Technology, Leiden, The Netherlands), due to a high sensitivity and specificity (49-51). However, D4D6 clone has not obtained FDA approval as a companion diagnostic due to challenging in interpretation of results. In fact, the major issue of IHC/ICC approach is related to the possibility of "false positive" results. This phenomenon is due to the reactivity of non-neoplastic cells, such as hyperplastic type 2 pneumocytes, alveolar epithelial and basal cells, bronchial epithelial and metaplastic bronchiolar cells, and peribronchial glands (49). As a general role, only tumors with 2+ or 3+ staining signal in more than half neoplastic cells should be scored as ROS1 positive (52,53). Another ROS1 specific clone, namely SP384 (Ventana Medical Systems, Oro Valley, AZ, USA), showed a very high sensitivity and specificity (54). However, it is strongly recommended to confirm all ROS1 IHC/ICC positive cases with FISH, polymerase chain reaction (PCR) based approaches or next generation sequencing (NGS) approaches (12,53).

RET gene

The first report of kinesin family member 5B (*KIF5B*)/rearranged during transfection (*RET*) gene fusion in advanced stage NSCLC dates back to 2012 (55). Similar to *ROS1* gene fusions, *RET* gene rearrangements account very rarely (1–2%) in advanced stage NSCLC patients (55-58). Overall, *RET* gene fusions account more frequently in young, female, non-smoker adenocarcinoma patients (56,58-63). In addition, it seems to be associated with poorly differentiation and solid pattern of growth (64). Despite the rarity, the increasing attention on *RET* fusions is associated with the recent approval of selpercatinib and pralsetinib for the treatment of advanced stage NSCLC patients (65-71). Regarding RET IHC, a global poor concordance with results obtained by PCR-based and FISH approaches is reported (72,73). However, among the different clones, the most commonly adopted is EPR2871 antibody. Yang *et al.* demonstrated that the sensitivity of IHC is related to the fusion partner, and in particular the highest sensitivity has been registered for KIF5B (100%), followed by CCDC6 (88.9%) and NCOA4 (50%). Interestingly a specificity of 82% was reached (74).

Neurotrophic receptor tyrosine kinase (NTRK) gene

NTRK1 and *NTRK2* gene fusions were identified for the first time in advanced stage NSCLC patients by Vaishnavi *et al.* in 2013 and by Stransky *et al.* in 2014, respectively (75,76). Overall, gene fusions involving *NTRK1-2-3* are very rarely (<1%) reported in advanced stage NSCLC patients (75,77,78). As for other gene fusions, these genomic alterations seem to occur more frequently in younger, non-smoker adenocarcinoma patients (79). The necessity to detect these very uncommon gene fusion events is related to the approval of larotrectinib and entrectinib in patients harboring *NTRK* gene fusions, regardless the age and the histotype (80-82). Different immunostaining approaches have been reported in literature (83,84). Among these, the pan-Trk antibody EPR17341 (Abcam, MA, USA) showed the highest performance. This clone is able to detect a homologous region of Trk-A, Trk-B, and Trk-C proteins near the C-terminus (84-86). In particular Hechtman *et al.* reported a sensitivity of 95.2% and a specificity of 100.0% among different previously genotyped cancer types. Noteworthy, However, the sensitivity differs in Trk-A (96%), Trk-B (100%) and Trk-C (79%) fused proteins. Overall, regarding NSCLC, the EPR17341 clone showed a sensitivity of 87.5% and a specificity of 100% (87). It should be borne in mind that staining intensity may be variable. Thus, it has been proposed that neoplasms with at least 1% of positive neoplastic cells should be considered

as positive (88). An important point is represented by the localization (nuclear, peri-nuclear, cytoplasmic, cell membrane) of the staining pattern, that is correlated with the specific fusion partner (89). A crucial issue in NTRK immunostaining is associated with the constitutive expression of Trk proteins in some normal human adult tissues, including testis, colonic ganglia, and nervous tissue (88). However, in lung cancer patients, the European Society for Medical Oncology (ESMO) guideline suggested to adopt IHC/ICC as a screening tool when NGS is not available. Nevertheless, it is recommended to further confirm positive results before treatment administration (83).

PD-L1

ICIs have dramatically changed the management of advanced stage NSCLC patients (90-92). Notably, IHC/ICC approach is the only approved for the evaluation of the expression level of PD-L1 in order to administrate ICIs (93). In particular, both histological (IHC) and cytological (ICC) samples have been demonstrated to be suitable for tumor proportion score (TPS) evaluation of PD-L1 expression (94,95). As a general rule, Reck *et al.* reported, in advanced stage NSCLC patients who displayed a PD-L1 expression on at least 50% of tumor cells, a significantly higher progression-free survival and overall survival with limited adverse events of pembrolizumab with respect to platinum-based chemotherapy (90). However, despite these encouraging results, discordant data have been reported in literature. In fact, it has been highlighted that patients with a negative PD-L1 IHC/ICC may respond to ICIs whereas other with a high PD-L1 expression can show no responsiveness to immunotherapy (96,97). This may be due to the heterogeneous expression of PD-L1 in different histological sub-types (98,99). Currently, different clones are commercially available for PD-L1 testing (100). Among these, 22C3 clone (pharmDx, Agilent Technologies, Inc., Santa Clara, CA, USA) is the only companion diagnostic test for the administration of pembrolizumab (101). Conversely, 28-8, SP142 and SP263 clones for nivolumab, atezolizumab and durvalumab, respectively are only being adopted as complementary diagnostics (101,102). However, several efforts have been spent to evaluate the reproducibility and interchangeability of the different clones. In particular, the Blueprint phase 1 and 2 projects clearly demonstrated the interchangeability of clones 22C3, 28-8, and SP263 assays and the lower sensitivity of the SP142 assay for the evaluation of PD-L1 expression on both histological and cytological samples (94,95). Beyond histological specimens, in fact, even cytological samples demonstrated to be a suitable starting specimen to assess PD-L1 expression status before ICIs administration (19,103,104).

Conclusions

In conclusion, although NGS should be preferred over the other molecular diagnostic approaches in advanced stage NSLC patients, enabling to simultaneously analyze different DNA- and RNA-based biomarkers (13), IHC/ICC still represents a valid diagnostic tool for gene fusions detection. In addition, IHC/ICC approach is currently the only available diagnostic tool for the assessment of PD-L1 expression in order to administrate ICIs.

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