Editorial Commentary

Challenges of predicting immune checkpoint therapy responders in lung cancer

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Non-small cell lung cancer (NSCLC) is the leading cause of cancer related death worldwide (1) and usually diagnosed in an already metastasized state, hence leaving systemic therapy as the preferred treatment with only a minority of patients eligible for targeted therapies. Recent developments from the so-called immune checkpoint blockade (ICB) or immunotherapy, mainly targeting programmed death-1 (PD-1) receptor or the ligand (PD-L1) (2-5), offer improved outcome of patients following relapse after platinum-based chemotherapy or even as first-line therapy (6,7). However, patient selection is a critical step and the development of predictive biomarkers is of utmost importance.

Current efforts to tackle patient stratification based on biomarkers addressed tumoral factors such as PD-L1 expression on the tumor, tumor-infiltrating lymphocytes (TIL), tumor mutational burden (TMB) and neo-antigen load as well as somatic mutations and factors related to genome integrity, microsatellite instability (MSI) and mismatch-repair deficiency (dMMR). In addition, peripheral biomarkers have been studied which assess T cell receptor clonality, circulating immune cells, serum protein signatures and soluble PD-L1 (8). Further emphasis has been also attributed to immune gene signatures and multiplexed immunohistochemistry (9) and pre-existing immune activity regarded as a prerequisite (10). However, there is currently no single biomarker that is indicative for predicting response and the most common one, PD-L1 expression on tumor cells and/or immune cells is exhibits a complicated cut-off value scheme, depending on the used antibody clone and PD-L1 negative patients can still benefit from either anti-PD-1 or anti-PD-L1 therapy. The present study of Duruisseaux *et al.* (11) aimed to tackle these obstacles and employ epigenetic techniques to investigate DNA methylation signatures for predicting anti-PD-1 therapy in stage IV NSCLC patients. They assembled one discovery cohort and two validation cohorts from a total of 162 patients in a multicenter study setting. Patients were eligible if a histologically proven stage IV NSCLC occurred and had undergone sampling before any antineoplastic treatment as well as being exposed to anti-PD-1 therapy. In case of relapse after surgery or chemo-radiotherapy, adjuvant chemotherapy or combined with radiotherapy was allowed. As study outcomes, progression-free survival (PFS), overall survival (OS) and disease-specific survival (DFS) were chosen.

Patient samples from the discovery cohort were categorized as responders with durable clinical benefit, as defined by absence of progression or death within the first 6 months of therapy (N=10), or as non-responders (progression or death within the first 6 months; N=24). A supervised classification model was then utilized to predict responders and non-responders which were denominated further as EPIMMUNE positive or EPIMMUNE negative according to the methylation status of significantly associated CpG sites (301 CpGs). This epigenetic signature was associated with PFS and OS and the EPIMMUNE negative signature was regarded as independent of poor

health and regarded as a biomarker for disease-specific death (HR 0.072, 95% CI: 0.015–0.334, P=0.0012; log-rank P=0.0010). The EPIMMUNE signature was found to be an independent predictor for PFS and OS in multivariate Cox regression analysis and not associated with any clinicopathological variable tested. Interestingly, neither PD-L1 expression on tumor cells nor CD8 cells in stroma or tumor were significantly associated with PFS or OS as well as TMB high patient groups. None of these factors added additional clinical value to the EPIMMUNE methylation signature.

The investigators further characterized the EPIMMUNE methylation signature associated with PD-1 response based on available DNA methylation patterns of different NSCLC cell lines: notably, inhibition of β-Catenin signaling, deficient DNA repair and activation of interferon (IFN) γ response were attributed to the EPIMMUNE positive signature. Available methylation data signatures from public databases were used to deduct immune cell signatures from both EPIMMUNE panels and revealed that non-responders/EPIMMUNE-negative patients are enriched for myeloid, mainly tumor-associated macrophages and tumor-associated neutrophils, while EPIMMUNE-positive patients, who responded to PD-1 therapy, were enriched for cells of the lymphoid lineage. More detailed bioinformatics analyses showed particular enrichment of $CD4^+$ α/β T cells with ability to produce IFN γ, $CD8^+$ α/β central memory T cells and natural killer cells, hence indicating a highly reactive immune response. EPIMMUNE negative patients further exhibited enrichment of cancer-associated fibroblasts (CAFs) as well as senescent endothelial cells and progenitor endothelial cells, consistent with a hypoxic microenvironment.

The findings from this discovery cohort were then sought to validate in an independent cohort, where the EPIMMUNE-positive signature was again associated with PFS and remained significant using Cox multivariate analyses. The authors then set out to cross-validate their findings with data obtained from TCGA datasets, where only 48% of the EPIMMUNE signature CpGs were present but still able to predict clinical response, PFS and OS in their own discovery cohort but not in their validation cohort. Noteworthy here is the finding that using the EPIMMUNE CpG panel computed from TCGA datasets, the authors were unable to predict OS in patients who did not receive immunotherapy (data obtained from TCGA).

To further simplify the EPIMMUNE panel, one single DNA methylation marker was chosen based on ANOVA results and CPG methylation difference between responders and non-responders. Here the T-cell-related forkhead box P1 (FOXP1) transcription factor methylation signal was used analyze association with outcome data and was found to be positively associated with PFS but not OS in the discovery cohort and was successfully replicated in the validation cohort based on pyrosequencing. The study added a new layer of promising biomarkers, which in the single version of FOXP1 CpG loci assessed by pyrosequencing offer more practical value for patient selection than array-based approaches. The authors did not omit to point out, that their approach and here, especially the EPIMMUNE negative signature, could be also addressed by classical immunohistochemistry staining methods. This is indeed a detail that easily occurs to the careful reader where the EPIMMUNE-positive signature is in line with the concept of inflamed tumors and the EPIMMUNE-negative with the so-called immunologically cold tumors (12), albeit assessed by a more complex and specimen-demanding technique than any histology-based cell type quantification. A plethora of immune-checkpoint therapies, especially in NSCLC treatment, are applied in patients with already metastasized disease when surgery is not feasible, small biopsies are the only sample material available in addition to serum or blood. It is therefore conceivable and to mention that these developments are likely or hopefully be adapted to liquid biopsies or even other non-invasive forms of specimen acquisition such as exhaled-breath condensate to allow repeated sampling for time-course analyses and closely monitored risk-assessment.

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Footnote

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aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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