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Clinical Cancer Research

Predictive Biomarkers for PD-1 Axis Therapies: The Hidden Treasure or a Call for Research

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Blockade of the PD-1 axis has emerged as an effective anticancer immunotherapy against various tumor types. Diverse studies have identified biomarkers associated with response to these therapies. However, the clinical use of such tests is limited by their variable performance and restricted understanding of their biologic significance. *Clin Cancer Res; 22(9); 1–3. ©2016 AACR.*

See related article by Ock et al., p. 2261

In this issue of *Clinical Cancer Research*, Ock and colleagues analyzed data from The Cancer Genome Atlas (TCGA) to classify tumors into four groups based on their levels of PD-L1 and CD8A mRNA transcripts (e.g., TMIT-I to IV; ref.1). Notably, they identified differential frequency of these subgroups across tumor types, with predominance of cases with high PD-L1 and CD8A (e.g., TMIT-I) in lymphoid malignancies, lung, and renal carcinomas and overall low frequency in brain, prostate, liver, and endocrine tumors. In addition, they found a positive association between TMIT-I tumors and the total mutational load, *in silico*–predicted mutant neoantigens, DNA repair defects [e.g., microsatellite instability (MSI) and POLE mutations], PD-L1 gene amplification, and infection by Epstein–Barr virus and human papillomavirus (but not hepatitis B virus). It is tempting to speculate that relatively simple marker signatures, such as the one proposed by Ock and colleagues including binarized scores of only 2 targets, will capture enough information to effectively predict response to PD-1 therapies. However, validation of such signatures in independent populations and using outcome information is necessary to prove this hypothesis.

Blockade of the PD-1 axis induces prominent and lasting clinical responses in a proportion of patients with diverse tumor types, including melanoma, non–small cell lung cancer (NSCLC), renal cell carcinoma, bladder urothelial malignancies, and Hodgkin lymphoma (2–8). These agents are well tolerated and display considerably more antitumor activity than standard chemotherapy agents. To date, two PD-1–targeting antibodies, nivolumab (Bristol-Myers Squibb) and pembrolizumab (Merck) have received regulatory approval by the FDA for clinical use in advanced melanoma and NSCLC. In addition, nivolumab was recently approved for use as second-line treatment in metastatic renal cell carcinoma. FDA clearance for use in other tumor types and clinical settings is anticipated as further data emerge. In addition, antibodies blocking the PD-1 ligand PD-L1 have shown comparable antitumor properties and are expected to enter the market. For their potential of benefit in so many different tumor types and long duration of responses, PD-1 axis therapies are candidates to become one of the most impactful anticancer agents ever used in oncology. However, there are prominent costs associated with these therapies, and they are not without toxicities, raising concerns about the impact of their expanded (and massive) use.

One of the major challenges in the field of immuno-oncology is the identification of predictive biomarkers. Using correlative studies of clinical trial samples, diverse biomarkers have shown association with benefit from PD-1 axis agents (Fig. 1). Elevated expression of PD-L1 protein by immunohistochemistry (IHC) in tumor and/or stromal cells has been associated with increased response in melanoma, bladder cancer, and NSCLC (2–6). However, the predictive value of the IHC test is limited, and the strength of the association is variable between studies and tumor types. For instance, PD-L1 IHC expression (even using the same PD-1 compound and IHC assay) is not clearly associated with benefit in squamous head and neck (HNSCC) and renal cell carcinomas (7). The biologic determinants for this discrepancy are unclear and question the direct biologic link between the presence of the biomarker and treatment. In addition, different companies have developed distinct IHC assays utilizing PD-L1 as a biomarker for their individual compounds. The analytic properties and concordance of different PD-L1 antibodies seem to be limited (9). To date, only pembrolizumab has been approved by the FDA in association with a companion IHC test, the Dako 22C3 pharmDx, for use as second-line therapy in NSCLC (but not in melanoma). Interestingly, the approval of nivolumab for non-squamous NSCLC was associated with the PD-L1 IHC 28-8 pharmDx test, but it was not defined as a companion biomarker. Despite being provided by the same company and targeting an epitope in the same domain of the same protein, the threshold for positivity of both approved IHC assays in tumor cells is different. The assay developed with the anti–PD-L1 agent atezolizumab (Ventana SP142) is associated with response to therapy when the signal is present in tumor and/or stromal cells (4). The apparent discordances in the IHC tests and their dissimilar association with outcome challenge the current understanding of the mechanism of action of these compounds and highlight the need for more research in the field. Two multi-institutional efforts led by the National Comprehensive Cancer Network (NCCN/BMS PD-L1 partnership) and the International Association for the Study of

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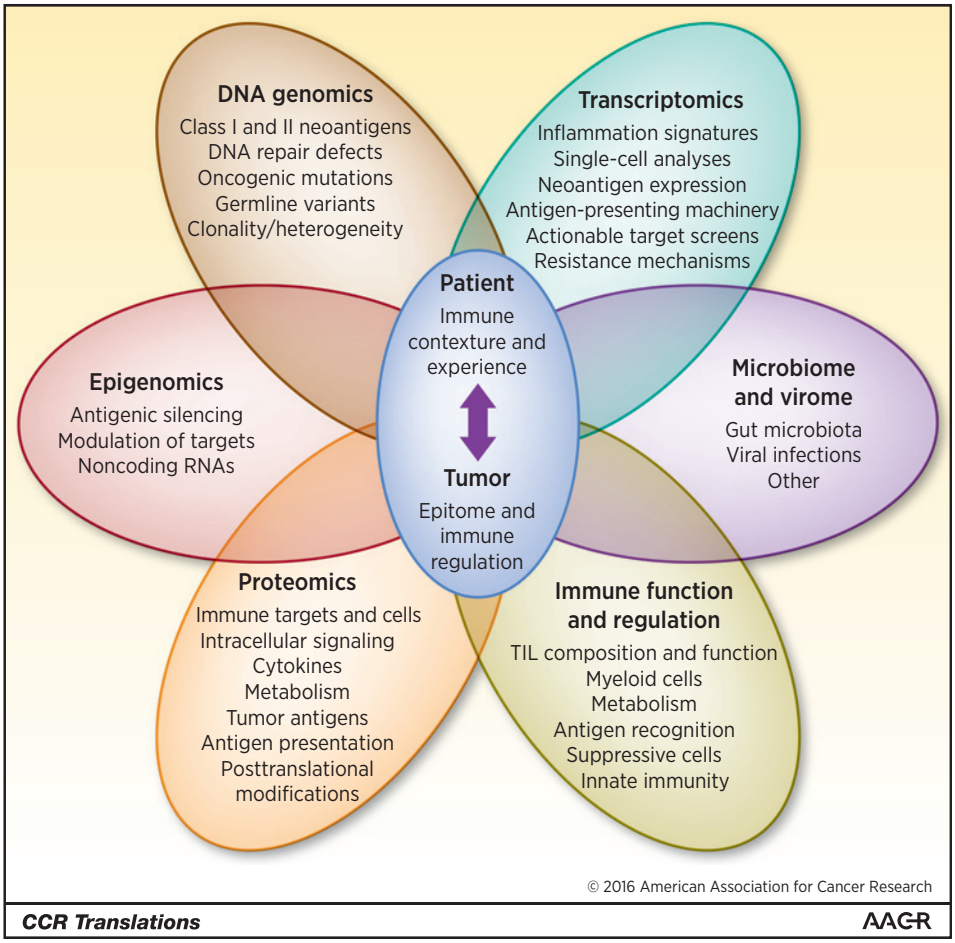

Figure 1.
Translational view of the complex patient–tumor immune interactions. Central to the antitumor immune response are the patient contexture and tumor antigenic potential. Diverse patient factors can affect the antitumor immune response, including particular genotypes, antigenic exposure, comorbidity, treatments, aging, and environmental exposures. Tumor features involved include the genomic landscape of mutations/neoantigens, genomic instability, heterogeneity, immunosuppressive signals, oncogenic viruses, and antigen silencing, among others. Diverse strategies can be used in translational research using patient samples or model systems to interrogate specific components of these interactions.

Lung Cancer (IASLC, the Blueprint study) are ongoing to assess the comparability and performance of different PD-L1 IHC assays in lung cancer.

Although widely discussed in the literature, the preexistence of $CD8^+$ tumor-infiltrating lymphocytes (TILs) has only been directly associated with anti–PD-1 benefit in melanoma and MSI-high colorectal carcinomas (2, 10). This supports the notion that a preexisting antitumor immune response is required for immune reinvigoration using these agents. However, the association between increased TILs and benefit from PD-1 axis agents has not been found in other solid tumors (11) and NSCLC (4). Specific inflammation and IFNγ-related mRNA-based signatures have also been suggested to correlate with response to PD-1 blockade in melanoma, bladder, gastric, and HNSCC (various abstracts in 2015 American Society of Clinical Oncology annual meeting). Finally, expanded T-cell receptor (β-chain) clonality was associated with response to PD-1 blockade in melanoma (2). The predictive value and independence of these markers requires further validation.

A high nonsynonymous mutational burden or *in silico*–predicted class I neoantigen load detected by whole-exome sequencing analysis was associated with clinical benefit to pembrolizumab in NSCLC (12). In this study, the sensitivity of PD-L1 IHC (any positive) was higher than the median mutational load (91% vs. 83%, respectively), but the specificity was prominently lower (67% vs. 28%). Refinement of the mutational signatures incorporating the allelic frequency of the mutant epitopes, clonality of the variants, and the relative expression of the immunogenic transcripts are ongoing and could increase the predictive value.

Over the last years, we have seen a number of studies addressing immuno-oncology questions using DNA and RNA sequencing data from public databases. In particular, data from TCGA have provided novel insights about the association between genomic and immune characteristics across diverse tumor types (13, 14). It is relevant to consider that the TCGA project was designed to study the tumor DNA/RNA and not the surrounding stroma containing most immune cells and related signals and that most of the TCGA samples were selected against the presence of stroma requiring more than 80% tumor content to be included. In addition, samples were only from resection specimens of primary, early stage tumors (stages I–III) without extensive prior therapy that are likely to be immunologically different from the advanced malignancies currently being treated with PD-1–blocking agents. The lack of treatment annotation and immune characteristics of the tumors and patients also limits some of the immuno-oncology perspectives of this extraordinary collection. Future cancer initiatives including key immunologic parameters (Fig. 1), and the development of training programs specific to the uniqueness of the field of immuno-oncology should be considered to support adequate progress in this emerging field.

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In conclusion, diverse studies have identified tumor markers associated with response to PD-1 axis therapies. However, the clinical use of these tests is limited by their suboptimal performance and restricted understanding of their biologic significance. Although valuable connections between tumor genomics and immune characteristics of human cancer have been obtained from analyses of public cancer databases, clear limitations indicate the need for new initiatives specifically focused in immuno-oncology. Considerable advancement in the fundamentals of cancer immunobiology, translational studies from clinical samples, animal models, and computational/bioinformatic tools will be required to secure progress in the field and have immunology lead the war on cancer.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Authors' Contributions

Conception and design: K.A. Schalper, R.S. Herbst
Development of methodology: K.A. Schalper, R.S. Herbst
Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): K.A. Schalper, R.S. Herbst
Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): K.A. Schalper, R.S. Herbst
Writing, review, and/or revision of the manuscript: K.A. Schalper, E. Kaftan, R.S. Herbst
Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases): E. Kaftan

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