Review Article

Current markers and their value in the era of immuno-oncology

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Abstract: Immunotherapy in urothelial cancer is a quickly evolving field as new agents are being investigated in multiple clinical trials and various clinical settings. The purpose of this review is to provide an insight into the mechanism of these treatments, potential targets to evaluate treatment response and to give an update on the current status of clinical trials. Urothelial cancer is a polyclonal disease with a substantial tumor heterogeneity and a high mutational load which may be beneficial as this may trigger a stronger T-cell mediated immune response. PD-1 expression has been shown to correlate with stage, grade, progression and poorer survival but it appears challenging to be utilized as a predictor for treatment response in urothelial cancer. Another important concept is immune cell (IC) infiltration, which is a reflection of the activated immune response within the target tissue. Marker genes may represent signaling pathways involved in T-cell recognition and lysis of T-cells. The complexity of the tumor and host interaction requires multiple concepts to be integrated into a future model to assess treatment response. We have evaluated multiple biomarker approaches currently investigated in clinical trials in urothelial cancer.

Keywords: Immunotherapy; bladder cancer; biomarker

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Immunotherapy

The concept of any type of Immunotherapy in oncology is the stimulation of the patient's own immune system to attack cancer cells.

William Bradley Coley—an American surgeon born in 1862—is considered to be the father of Immunotherapy in cancer medicine. He introduced the idea of stimulating the immune system by administering bacterial fluids containing Streptococcus pyogenes and Serratia marcescens. While the therapeutical effect of his treatments was heavily debated, his work can be considered part of the foundation of modern era Immunotherapy (1).

The most important detriment in these historical attempts in enhancing the immune response, however was the at best rudimentary knowledge of the immune system.

The focus of contemporary immunotherapy is the T cell activity which may be modulated by the tumor's immunogenic properties ("foreignness"), general immune status, IC infiltration, absence of checkpoints, absence of soluble inhibitors, absence of inhibitory tumor metabolism and tumor's sensitivity to immune effectors. All these parameters have been conceptualized in the "Cancer Immunogram" by Blank *et al.* in 2016 (2). It is a schematic approach with the goal of visualizing treatment interactions between the cancer and the immune system. All these parameters need to be addressed when markers in Immunotherapy are being discussed.

Tumor foreignness in urothelial cancer

The molecular characterization of urothelial cancer reported

by the "The Cancer Genome Atlas Project" has identified multiple genomic alterations, molecular subtypes based on expression characteristics and a mutational landscape reflecting the heterogeneous biology of muscle invasive tumors.

Urothelial cancer is a polyclonal disease, which undergoes significant changes. Faltas *et al.* performed whole exome sequencing and clonality analysis of 72 tumors including 16 tumors before and after chemotherapy (3). They found that chemotherapy treated tumors exhibit an intra-patient heterogeneity and that most mutations are not shared. This heterogeneity was spatial (different sites within the tumor) and temporal (changes of the course of the disease). The authors described for their analysis that branching off and metastatic spread appeared to be very early events in the natural history of the disease.

Intra-tumoral heterogeneity appears to be associated with the neo-antigen load and this again correlates and therefore with response to immunotherapy. Tumors with a high mutational load can benefit from immunotherapy as they may induce a greater T-cell mediated immune response (4). When arranging tumors in a hierarchical list from highest to lowest mutational load urothelial cancer is fourth on the list suggesting that it is an excellent candidate for Immunotherapy (5).

Expression profile of checkpoints

The expression of PDL-1 on the surface of urothelial cancer cells ranges from 21–28% and can be detected like most surface proteins by immunohistochemistry (IHC) on formalin-fixed and paraffin-embedded tissue sections.

It has been associated with more advanced stage, higher grade, progression and poorer survival (6-9). More relevant than its prognostic value however is its potential to predict response to treatment.

In a meta-analysis, the overall response rate to PDL-1 positive tumors was significantly higher than in PDL-1 negative tumors but this difference was not significant for Genitourinary Cancers. In addition, PDL-1 negative tumors did respond to immunotherapy as well which suggests that PDL-1 Expression is not a universally valid prediction tool (10). The authors also described the challenges in comparing PDL-1 expression studies: different thresholds for positivity of the stain in IHC, various methodologies using different antibodies, different staging techniques, visual interpretation (cell surface versus cytoplasmic expression and the definition for PDL-1 in a patient (single biopsy versus multiple biopsies from the same patient; primary versus metastatic lesion).

This is where intra-tumoral heterogeneity becomes a problem. A biopsy may not be representative of the tumor as a whole. In a typical histological exam, only 0.001% of a 5 cm sphere shaped tumor is examined under the microscope (11). The difference in PDL-1 expression by using different antibodies at different tumor localizations has been demonstrated by Ilie *et al.* in lung cancer patients and (11) conveys that IHC techniques may not provide a comprehensive evaluation of a tumor's immune status.

In the metastatic setting, it has also been reported for lung cancer that there is a significant difference in the microenvironment of tumor cells at the primary site or within the metastasis (lung or bone) based on the PDL-1 expression and the presence of tumor infiltrating lymphocytes (TILs). Of 73 cases analyzed in this report, there was corresponding PDL-1 expression in the primary and brain met in 63 patients. In ten patients there was a disagreement in expression (12).

While there were fewer discordant pairs in PDL-1 expression when samples of the primary and the metastasis were collected within less than 6 months this difference was not significant.

The immune cell infiltration

A pre-requisite for an anti-tumor response is the infiltration of T-cells into the tumor. Blank *et al.* have listed multiple reasons for a poor infiltration of a tumor such as deficient T-cell priming, mechanical barriers such as tumor related fibrosis or impermeable vasculature or the absence of T-cell inducing cytokines (2).

Simply spoken for an efficient tumor response the tumor needs to be "different" but you also require activated T-cells around to recognize the tumor as "foreign" and initiate an immune response.

A study, looking at the density of tumor-infiltrating lymphocytes in patients with brain metastases from various primaries. They analyzed tissues by IHC looking at CD3, CD8, CD45R0, FOXP3, PD1 and PDL-1 and calculated an Immunoscore (13). The Immunoscore is a numeration of CD8 and CD45R0 cells at the center of the tumor and the invasive margin and has been proposed as an adjunct to the AJCC/UICC TNM classification (14).

In the former study, looking at patients with brain metastases no correlation of PDL-1 status and Immunoscore was found. In a correlative analysis with survival, it was found that patients with a high Immunoscore had improved overall survival with or without adjuvant whole brain radiation therapy.

Marker genes in immunotherapy

Mutational changes within a tumor can result in very different effects in terms of response to Immunotherapy. While some mutations may result in neoantigens, which induce a potent T cell response others may lead to a resistance to an immunotherapy (15). A study recently published by Patel *et al.* used genetic manipulations to understand the interactions between T cells as effectors and melanoma cells as targets. They reported new genes and microRNAs that may exhibit an effect in tumor lysis by T cells (15). While not specifically looking at urothelial cancer cells, the authors describe the role of the *APLNR* gene regulating T cell response and most likely playing a role in other cancers as well. The gene is ultimately involved in the activation of the JAK-STAT signaling cascade inducing antigen expression and consecutively improving recognition and lysis by T cells. A novel aspect of this study is the utilization of the CRISPR technology, which allows for large-scale screening of the genome to identify biologically meaningful gene expression that correlates with resistance to immunotherapy. In the future, this sort of analysis may help to focus on genes relevant to T-cell activation in a tumor i.e., the patient specific situation.

Predictive/prognostic biomarkers across the published studies of advanced urothelial carcinoma (UC): PD-L1 expression and beyond

After decades of therapeutic stagnation in the field of UC, the advent of immune checkpoint inhibitors (ICI) has revolutionized the available therapeutic options for advanced disease. PD-1/PD-L1 inhibitors have already received United States Food and Drug Administration (USFDA) approval, either in the postchemotherapy indication or for the chemonaïve, cisplatin-ineligible setting, and some of them have been granted approval by the European Medicines Agency (16). In addition, a myriad of translational data are emerging from these studies. These data yield promise to expand our understanding of the biology underlying response to ICI and point to a future multicomponent biomarker.

Biomarker findings from the clinical trials conducted in the first-line setting

In the first-line metastatic setting, results have been disclosed for atezolizumab and pembrolizumab in patients who are ineligible to receive cisplatin-based chemotherapy.

IMvigor210 study enrolled a cohort of 119 patients (cohort 1) who received atezolizumab at the dose of 1,200 mg intravenously (IV), every 21 days, until unacceptable toxicity occurrence or evidence of disease progression (PD) (17).

The objective response rate (ORR) in the intention-to-treat (ITT) population was 23% (95% CI: 16–31%), including 9% complete responses (CR). In this study, PD-L1 expression was evaluated in ICs using the Ventana antibody clone SP142. Cut-off definitions were the following: PD-L1 IC2+/3+ if ≥5% positive IC, or 1+ if 1–5% positive IC.

Disappointingly, in cohort 1 there was no enrichment in response according to the expression of PD-L1, as the ORR was 28% (95% CI: 14–47%) in IC2/3+ and 21% in IC-negative patients. Similar trends were seen regarding OS, as median OS was 12.3 months [95% CI: 6.0-not estimable (NE)] for IC2/3+ patients and 19.1 months (95% CI: 9.8–NE) for IC0/1+ patients.

Keynote-052 study evaluated pembrolizumab in 370 patients in the same setting (18). In this study, like in all pembrolizumab studies in UC, PD-L1 expression was assessed using Dako antibody clone 22C3, and the combined positivity score (CPS) was developed. This score evaluated the number of PD-L1 staining cells (tumor cells and IC) out of the total number of viable tumor cells. Using receiver operating characteristic (ROC) curve analysis along with the ORR and biomarker prevalence profile, the CPS cut-off of 10% was determined to be the optimal enrichment cut-off for predicting response. At the time of the last update, the ORR in all comers was 29% (95% CI: 25–34%), in CPS >10% population was 37% in the training set, and 51% in the validation set, showing some enrichment (19).

Data on overall survival was not yet gave. Interestingly, appreciable numbers of additional responses were captured using an eighteen T-cell inflamed gene expression panel as compared to the PD-L1 immunohistochemical biomarker alone.

In summary, there is currently no evidence supporting the PD-L1 use as a biomarker for selecting patients for ICI therapy in chemotherapy-naive patients, and definitive conclusions will be likely drawn with the results of the ongoing, randomized, phase 3 studies that are currently open in the first-line setting (NCT02853305, NCT02807636, NCT02516241, NCT03036098).

Biomarkers of response and outcome for salvage immunotherapy in metastatic UC

Multiple studies with single-agent or combination

immunotherapy have disclosed or are currently recruiting patients who have failed platinum-based chemotherapy. Atezolizumab was the first approved immunotherapeutic drug in UC.

Approval was granted and is based on the results of the cohort 2 of IMvigor210 study that included 310 patients (20). In this study, in contrast with the results obtained in the cohort 1 of the same trial, patients with higher expression of PD-L1 (i.e., IC 2/3+) had better ORR (26%) than the ones with no or weak expression (IC 0/1+). Similar trend was observed for OS, as median OS in IC2/3+ population was 11.4 months (95% CI: 9.0–NE) versus 6.5 months (95% CI: 4.4–8.3) for IC0 patients.

Most noteworthy, several additional data were obtained that may be used for patient enrichment pending validation. These results included the association between the T-effector (T_{eff}) gene signature (i.e., expression of CD8, granzymes, perforin, cytokines and other factors) and PD-L1 IC status, as well as between the expression of multiple immune inhibitory regulators (e.g., *LAG3*, *HAVCR2*, *CTLA4*, *IDO1*, *FOXP3*, *CD244*) and PD-L1 status. Furthermore, two key factors were associated with atezolizumab response: T_{eff} gene signature and Cancer Genome Atlas (TCGA) luminal II subtype (P=0.0072). Luminal-I tumors displayed low T_{eff} expression, and may be regarded to as being characterized by an "immune desert" in their microenvironment, according to Rosenberg *et al.* (21).

Interestingly, this subtype is also enriched of alterations of the fibroblast growth-factor receptors (*FGFR*) genes, and therefore combination of ICI and pan-*FGFR* inhibitors might be particularly beneficial for their patients (22). Finally, tumor mutation burden (TMB), evaluated by means of quartile split and using the Foundation One test, was significantly associated with response and survival to atezolizumab in these patients (21). Confirmatory results may come from the translational body of evidences from concluded phase 3 IMvigor211 trial, which compared atezolizumab with standard of care in the same clinical setting (23). Based on the results available to date, PD-L1 expression was associated with improved OS in both arms: in the atezolizumab arm, median OS was 8.6 months in ITT population *vs.* 11.1 months in IC2/3+ patients.

Keynote-045 study was a phase 3 study, which compared pembrolizumab with standard chemotherapy (24). In this study, 542 patients were randomized to receive pembrolizumab 200 mg IV every 3 weeks for maximum 2 years *vs.* 3-weekly docetaxel, paclitaxel, or vinflunine. In this study, in contrast with IMvigor211 findings, PD-L1 expression was a negative prognostic factor in both pembrolizumab and chemotherapy arm: the median OS for pembrolizumab was 10.3 months in ITT population versus 8.0 months in CPS ≥10%.

For both durvalumab and avelumab, despite signals of improved responses were reported in PD-L1 positive patients (using Ventana SP263 antibody and Dako 73-10 antibody, respectively, and both evaluating tumor cells and ICs), conditional approval was granted for all comers regardless of PD-L1 expression (25-27).

Finally, translational findings from the nivolumab phase 2 CheckMate275 study have been reported (28). In this study, the ORR was 19.6% in all comers, and a trend toward enriched responses in PD-L1-positive patients was found (5% cutoff on tumor cells only; Dako antibody, clone 28-8). Of note, TMB showed a statistically significant positive association with ORR and PFS, and a strong association with OS, even when adjusted for baseline factors and tumor PD-L1 expression (29).

Conclusions

The efficacy of Immunotherapy can't be measured by a single biomarker. The complex biological interaction of these treatments will require a conceptual approach that integrates all possible variables. In conclusion, it is clear from the presented results that the search for the optimal biomarker approach will necessarily require harmonisation and novel research. Academic investigators and pharmaceutical companies will have to make an effort with the aim of promoting collaborative post-hoc evaluations of the datasets of the large concluded phase 2 and 3 studies.

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Footnote

Conflicts of Interest: The authors have no conflicts of interest to declare.

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