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TOPIC PAPER

Molecular predictors of response to PD-1/PD-L1 inhibition in urothelial cancer

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Abstract

Introduction The survival of patients with metastatic urothelial cancer (mUC) is poor. During the last 40 years, chemotherapy was the predominant treatment modality for mUC. The discovery of the immune checkpoint inhibitors (ICI), especially the inhibitors of the programmed cell death 1 and its ligand (PD-1/PD-L1), has revolutionized cancer immunotherapy. The PD-1 and PD-L1 inhibitors provide a new and effective treatment option for patients with UC, particularly for patients with recurrence after platinum-based therapy and those who are ineligible for cisplatin.

Methods A literature search on PubMed, ClinicalTrials.gov and selected annual congress abstracts was conducted in May 2018, using a combination of keywords, medical subject headings (MeSH) terms and free text incorporating urothelial bladder cancer; immunotherapy; immune checkpoint inhibition, biomarkers, PD1/PD-L1.

Results Although some patients demonstrate complete and/or durable responses under ICI, the reliable prediction of response to ICI is not possible. In the clinical setting, physicians are not able to predict response to ICI in mUC and to adequately select patients who will benefit. Exploratory analysis of clinical trial data revealed that PD-L1 expression, tumor mutation burden, tumor-infiltrating lymphocytes and gene expression profiles might have some predictive and/or prognostic value in different patient populations.

Conclusion Validated robust biomarkers are still needed to overcome this hurdle to forecast response of ICI in UC patients.

Keywords Urothelial bladder cancer (UBC) · Immunotherapy · Checkpoint inhibition · Biomarkers · PD1/PD-L1

Background

For localized muscle-invasive bladder carcinoma, radical cystectomy is the current standard of care [1, 2]. Nevertheless, cancer-specific survival (CSS) after cystectomy for localized disease ranges from 72 to 25% within the first 5 years. Two years after surgery, about 50% of patients with advanced tumor stages (T2b–T4a) develop metastases [3, 4] and chemotherapy has been the predominant treatment modality for these patients in the past 40 years [5]. The widely used first-line regimens include a combination of cisplatin plus gemcitabine and options of methotrexate, vinblastine and doxorubicin (MVAC) for metastatic urothelial cancer (mUC). Median overall survival (OS) is in the range of 14–15 months and 5-year OS rate is 5% [6, 7]. In the second-line setting, paclitaxel, docetaxel, gemcitabine, and pemetrexed are widely used as a mono- or combinatory therapy, while vinflunine has been only approved in Europe [5, 7].

In addition, around half of the patients with mUC are ineligible for cisplatin-based chemotherapy due to impaired kidney function, poor ECOG performance status or other comorbidities like polyneuropathy or impaired hearing. So far, these patients received carboplatin-based combinations or best supportive care [8]. In this respective setting of mUC treatment, immune checkpoint inhibitors (ICI) have revolutionized anti-cancer therapy. The PD-1/PD-L1 checkpoint inhibitors have shown clinical activity in advanced or UC. Nevertheless, a certain proportion of patients have no benefit from ICI due to initial disease progression, lack of response or later progression. Therefore, biomarkers are needed to

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predict response to ICI in mUC. An overview of currently available ICI and their possible biomarkers is outlined.

Methods

A comprehensive literature search on PubMed, ClinicalTrials.gov and selected annual congress abstracts was conducted in May 2018, using a combination of keywords, medical subject headings (MeSH) terms and free text incorporating urothelial bladder cancer; immunotherapy; immune checkpoint inhibition, biomarkers, and PD1/PD-L1.

ICI in mUC and PD-L1 expression

The US Food and Drug Administration (FDA) approved the monoclonal anti-PD-L1 antibodies atezolizumab, durvalumab, avelumab and the monoclonal anti-PD-1 antibodies nivolumab and pembrolizumab for the treatment of cisplatin-refractory mUC patients. Furthermore, atezolizumab and pembrolizumab are FDA approved for the first-line treatment of cisplatin-ineligible patients with high PD-L1 expression [9–15]. The recent studies which led to FDA approval demonstrated objective response rates (ORR) between 13 and 24% irrespective of PD-L1 expression. The potential of PD-1/PD-L1 ICI in UC as a new treatment modality as well as their respective biomarkers has been observed in multiple clinical trials. Below, data from these phase II and III trials are described in detail with a focus on the PD-1/PD-L1 axis. For other targets of ICI like the cytotoxic T-lymphocyte-associated protein-4 (CTLA-4), no agent has been currently approved in mUC.

Atezolizumab—IMvigor210 and 211

The IMvigor210 trial investigates atezolizumab in the second-line setting in 310 patients with locally advanced or mUC [9]. The ORR and median overall survival (mOS) with regard to PD-L1 expression are given in Table 1 [9, 16]. Increased PD-L1 expression on tumor-infiltrating immune cell (IC) was associated with a higher ORR and a longer mOS [17, 18]. In contrast, there was no correlation between PD-L1 expression on tumor cells (TC) and patient´s outcome. This observation suggests that PD-L1 expression, especially on IC, might serve as a potential biomarker.

A second cohort of 119 cisplatin-ineligible treatment-naïve patients was enrolled in IMvigor210 [15]. The data are summarized in Table 1. There was no significant association between PD-L1 expression levels and clinical response [14]. In contrast to the cisplatin-ineligible patients, a high expression of PD-L1 (IC2/3) was associated with increased ORR in cisplatin-refractory patients [9]. One possible explanation for this observation might be that the previous chemotherapy induced changes in the molecular gene expression patterns [19]. These findings were not confirmed in the subsequent phase 3 study IMvigor211 [20]. IMvigor211 (NCT02302807), a randomized phase 3 study, compared atezolizumab with the investigator's choice of chemotherapy (paclitaxel, docetaxel or vinflunine) after platinum-based chemotherapy and did not reach its primary end point of OS in the predefined IC2/3 subgroup. ORR and mOS are given in Table 1. Median OS in PD-L1 high expressers (IC2/3; ≥ 5% staining) was not significantly different 11.1 vs. 10.6 months in patients treated with atezolizumab vs. chemotherapy [20].

Nivolumab—CheckMate275 and Checkmate-032

CheckMate275 is a phase II trial of 265 patients with unresectable or mUC and progression or recurrence after platinum-based chemotherapy treated with nivolumab [10]. Data for ORR and mOS are given in Table 1. In this trial, analysis of the PD-L1 expression is based on TC rather than tumor-infiltrating immune cells. There was no association between PD-L1 expression and response to nivolumab, but ORR and OS were elevated among patients with PD-L1 expression on TC ≥ 1% [10].

The study Checkmate-032 examined nivolumab as monotherapy or in combination with ipilimumab in patients with advanced or metastatic solid tumors including mUC. Here, only the data for nivolumab in mUC are so far available and summarized in Table 1 [21].

Pembrolizumab—KEYNOTE-045, 052 and 012

Pembrolizumab was investigated in the phase III KEYNOTE-045 trial that compared pembrolizumab versus investigator's choice of chemotherapy (paclitaxel, docetaxel, vinflunine) in 542 patients with advanced or mUC as second-line treatment. ORR and mOS are summarized in Table 1 [13, 22]. In this trial, pembrolizumab demonstrated a benefit in OS compared to chemotherapy irrespective of PD-L1 expression (10.3 vs. 7.4 months) [22, 23]. For the PD-L1 expression, the "Tumor PD-L1 combined positive score" (CPS) was used. The CPS measures the total amount of TC and IC which express PD-L1. ORR was 21.6% if CPS was ≥ 10. In addition, there was no difference in PFS between patients treated with pembrolizumab or chemotherapy, also if stratified to the respective PD-L1 expression status. Here, the CPS was of prognostic importance, but not predictive [13, 22].

In the phase II study KEYNOTE-052 pembrolizumab was administered in 370 cisplatin-ineligible mUC patients as a first-line therapy. ORR and mOS are given in Table 1 [15, 24]. Here, PD-L1 status was prognostic. Patients with

Table 1 Summary of results of the different ICI in UC with regard to PD-L1 expression

Drug	Clinical trial	Disease type		Efficacy	PD-L1 biomarker
Atezolizumab	IMvigor 210	Metastatic second line	Phase 2 trial in 310 patients with locally advanced or mUC with progression during or within 1 year after platinum-based chemotherapy	Among all PD-L1 subgroups ORR 18% mOS 8.8 months Patients with ≥5% PD-L1 expression on IC ORR 26% mOS 11.4 months Patients with <1% PD-L1 expression on IC ORR 8% mOS 6.5 months	Increased PD-L1 expression on IC was associated with higher ORR to atezolizumab No correlation between PD-L1 expression on TC and patient's outcome
Atezolizumab	IMvigor 211	Metastatic cis-ineligible	Phase 2 trial in 119 cisplatin-ineligible treatment-naïve patients with locally advanced or mUC	Among all subgroups ORR 23% mOS 15.9 months Patients with ≥5% PD-L1 expression on IC ORR 28% mOS 12.3 months Patients with <1% PD-L1 expression on IC ORR 21 mOS 19.1 months	Responses occurred across all PD-L1 subgroups No significant association between PD-L1 expression levels and clinical response A high expression of PD-L1 (IC2/3) was associated with increased ORR
Atezolizumab	ABACUS	Neoadjuvant setting	Single-arm phase 2 study investigating atezolizumab in the neoadjuvant setting in muscle-invasive UC	pCR rate: 29% Tumor downstaging in non-muscle-invasive UC: 39% of patients	Results of biomarker candidates including PD-L1 status before and after therapy have not been published yet
Nivolumab	CheckMate 275	Metastatic second line	Phase 2 trial with 265 patients with metastatic or unresectable UC and progression or recurrence after platinum-based chemotherapy	Among all subgroups ORR 19.6% mOS 8.74 months Patients with ≥1% PD-L1 expression on TC ORR 23.8% mOS 11.30 months Patients with <1% PD-L1 expression on TC 16.1 ORR mOS 5.95 months	No significant association between PD-L1 expression and clinical response, but ORR and OS were elevated among patients with PD-L1 expression on tumor cells ≥ 1%

Table 1 (continued)

Drug	Clinical trial	Disease type		Efficacy	PD-L1 biomarker
Nivolumab ± ipilimumab	CheckMate-032	Metastatic second line	Multi-arm, phase 1/2 trial investigating nivolumab ± ipilimumab in patients with advanced or metastatic solid tumors including mUC, 78 patients with mUC received nivolumab monotherapy	Only data for nivolumab monotherapy: Among all subgroups ORR 24.4% mOS 9.7 months Patients with ≥ 1% PD-L1 expression on TC ORR 24% mOS 16.2 months Patients with < 1% PD-L1 expression on TC ORR 26.2% mOS 9.9 months	PD-L1 expression on TC was not associated with ORR Patients with high PD-L1 expression had higher mOS
Pembrolizumab	KEYNOTE -012	Metastatic second line	Phase 1b study investigating pembrolizumab in 33 patients with advanced solid tumors including mUC, all PD-L1 positive	Among all subgroups (only PD-L1 positive): mOS 13 months Patients with ≥ 1% PD-L1 expression on TC ORR 14% Patients with ≥ 1% PD-L1 expression on TC + IC ORR 24% Patients with < 1% PD-L1 expression on TC ORR 26% Patients with < 1% PD-L1 expression on TC + IC ORR 0%	Increased PD-L1 expression on TC and IC was associated with higher ORR
Pembrolizumab	KEYNOTE-045	Metastatic second line	Phase 3 trial with 542 patients with advanced or metastatic UBC and progression after platinum-based chemotherapy	Among all subgroups ORR 21.1% mOS 10.3 months Patients with tumor PD-L1 CPS ≥ 10 ORR 21.6% mOS 8.0 months	The benefit of pembrolizumab was independent of PD-L1 expression
Pembrolizumab	KEYNOTE-052	Metastatic cis-ineligible	Phase 2 study with 370 cisplatin-ineligible patients with mUBA as first-line therapy	Among all subgroups ORR 24% Patients with with tumor PD-L1 CPS ≥ 10 ORR 39% Patients with tumor PD-L1 CPS < 1 ORR 11%	PD-L1 status was prognostic. Patients with a CPS ≥ 10 had a higher ORR

Table 1 (continued)

Drug	Clinical trial	Disease type		Efficacy	PD-L1 biomarker
Pembrolizumab	PURE-01	Neoadjuvant setting	Single-arm phase 2 trial evaluating pembrolizumab in the neoadjuvant setting in UC (T2-T3bN0 and residual disease after TUR-B)	pCR in 39.5% of patients pCR: CPS score 30 no pCR: CPS score 10 Downstaging to pT < 2 in 51.2% Mean CPS score For pT0 patients: 30 For non-pT0 patients 10	With PD-L1 CPS score ≥ 20 Pathologic rate of CR increased to 50%
Avelumab	JAVELIN	Metastatic 2nd line	Phase 1 study in patients with metastatic solid tumors, including 249 patients with mUC that had progressed after platinum-based chemotherapy	Among all subgroups ORR 17% mOS 6.5 months Patients with ≥ 5% PD-L1 expression on TC ORR 24% mOS 8.2 months Patients with < 5% PD-L1 expression on TC ORR 13% mOS 6.2 months	An antitumor activity could not be linked to PD-L1 expression
Durvalumab	NCT01693562	Metastatic 2nd line	Phase 1/2 trial in 191 patients with locally advanced or mUC	Among all subgroups ORR 17.8% mOS 18.2 months Patients with ≥ 5% PD-L1 expression on TC ORR 27.6% Patients with < 1% PD-L1 expression on TC ORR 5.1%	ORR observed regardless of PD-L1 expression

IC immune cells, *TC* tumor cells, *mOS* median overall survival, *ORR* objective response rate, *CPS* tumor PD-L1 combined positive score, *pCR* pathologic complete response, *TUR-B* transurethral resection of bladder

a CPS ≥ 10 had a higher ORR. But low or absent PD-L1 expression did not preclude response [10, 21].

A summary of the results from KEYNOTE 012 investigating pembrolizumab in patients with advanced solid tumors including mUC is additionally given in Table 1 [25].

Avelumab—JAVELIN and durvalumab (NCT01693562)

JAVELIN (NCT01772004) and NCT01693562 are phase I studies that investigated the clinical activity and safety of avelumab and durvalumab in patients with metastatic solid tumors, including mUC that had progressed after platinum-based chemotherapy [12]. Detailed information is summarized in Table 1 [26, 27].

CTLA-4-inhibition—ipilimumab

Other ICIs are directed against the immune checkpoint molecule CTLA-4. A well-known example is ipilimumab [28]. An ongoing phase II trial combines the chemotherapeutic agents gemcitabine and cisplatin with ipilimumab. By the addition of ipilimumab to chemotherapy, a significant expansion of peripheral blood CD4 positive T cells was observed and correlated with an improved survival. ORR was 69%, and 17% of patients achieved a complete response with a median OS of 13.9 months [29].

In summary, PD-L1 expression is often used as a biomarker in ICI studies to select patients. Overexpression of PD-L1 in the pretreatment tissue correlated with a relatively higher response rate and an improved clinical outcome, although a significant proportion of patients did not benefit. Contradictorily, some patients with PD-L1-negative tumors also responded to anti-PD-1/PD-L1 therapy [30]. The survival benefit in patients with advanced UC treated with nivolumab, durvalumab or pembrolizumab does not correspond to PD-L1 expression in tumors [11, 15, 21]. But, in some trials overall response is strongly associated with PD-L1 expression, for example for durvalumab [11]. On the other hand, no association is detected in IMvigor210 cohort 2 [9], Keynote-045 [13] and Checkmate-275 [10].

To explain these differences, several factors must be considered: This includes the heterogeneity of PD-L1 expression levels within tumor tissues, the type of tumor tissue (fresh or archival samples), different used antibodies for immunohistochemistry (IHC) and great differences in the evaluation of PD-L1 positivity. PD-L1 IHC scoring was conducted in the reported studies with four distinct assays (Ventana PD-L1 SP142 and SP263 in IMvigor 210; Dako PD-L1 IHC 22C3 pharmDx assay in KEYNOTE-012 and KEYNOTE-052; Dako PD-L1 IHC 28-8 pharmDx assay in Checkmate275 and Dako PD-L1 IHC 73-10 pharmDx assay in JAVELIN).

Another important aspect is the applied method to quantify PD-L1 expression in clinical trials. There is no standardized format to assess PD-L1 immunohistochemically. The only similarity between the PD-L1 assays from the different companies is that they are all IHC tissue based and detect membrane expression of PD-L1. The scoring components differ for each trial including selected staining of TC or IC and also thresholds to define PD-L1 positivity are different. Already described above is that the PD-L1 expression on TC was used for nivolumab, IC and TC for pembrolizumab, whereas the expression of PD-L1 on IC was used with atezolizumab. The technical comparability and the inter-reader agreement of the used clinical assays investigating PD-L1 expression on IC and TC were recently assessed. Formalin-fixed paraffin-embedded tumor tissue of the bladder was stained with the four different assays to analyze PD-L1 expression, including the antibodies SP142, SP263 (VENTANA), 22C3 and 28-8 (DAKO). Depending on the used assay, the percentage of IC staining varied between 6.54 to 8.18% and TC staining from 5.46 to 15.85%. The inter-reader variation was slight and there was no significant difference between the assays. Only SP142 had a measurable significantly lower staining of TC compared to the other assays. As a result of this investigation there was a high concordance across all four assays achieved by trained readers for scoring PD-L1 on immune and tumor cells [31].

To date, there has been no reliable method to predict response to immunotherapy resulting in a possible overtreatment in non-responders with unnecessary toxicity. Furthermore, the phenomenon of hyperprogression of tumors with accelerated tumor growth rate under treatment with checkpoint inhibitors has been reported followed by a worsened prognosis [32]. Potential other investigated biomarkers to predict response to immunotherapy for mUC include cytokines, chemokines and tumor antigen-specific antibodies in the blood, the tumor mutational burden (TMB), gene expression profiles (GEP), the "The Cancer Genome Atlas" (TCGA) clustering and tumor-infiltrating lymphocytes (TILs). These are described in detail in the following sections. An overview of potential biomarkers as predictors of clinical response in patients with mUC and ICI is summarized in Fig. 1.

Tumor mutational burden (TMB)

Recent studies indicated an association between increased tumor mutational burden (TMB) and inflamed tumors with a favorable response in patients treated with immunotherapy [33–35]. According to data from the TCGA UC has the third highest mutation rate after melanoma and lung carcinoma [36]. The high prevalence of tumor somatic mutations shall translate into a higher neoantigen burden, which plays a role

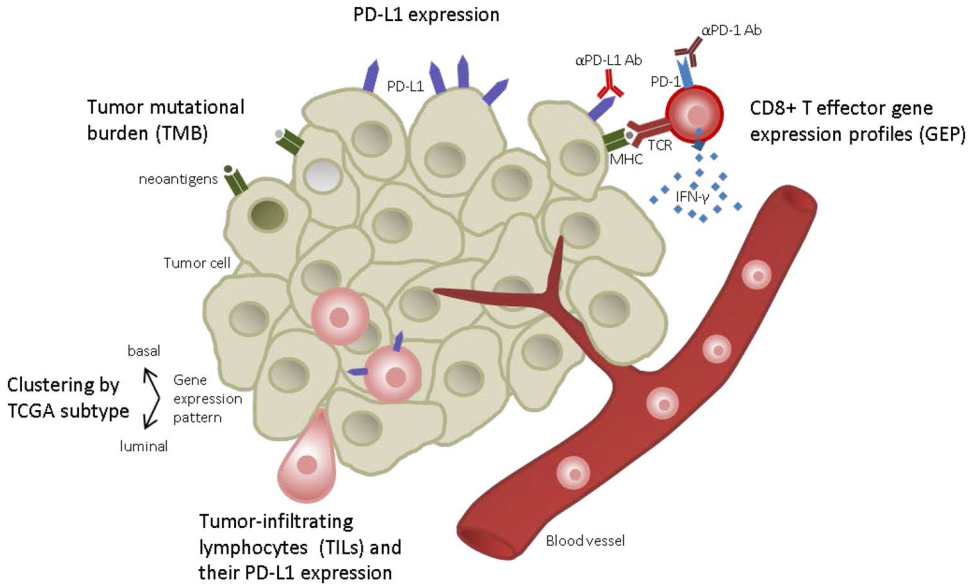

Fig. 1 Overview of potential biomarkers as predictors of response to ICI in patients with mUBC. The cellular biomarkers comprise of tumor-infiltrating lymphocytes (TILs) and their density and phenotype. The molecular biomarker consists of the gene expression profile (IFN-γ signature) of CD8 + TILs, the PD-L1 expression level of the tumor cells and immune cells, the tumor mutational burden and TCGA clustering

in tumor cell recognition by activated T cells [37]. Therefore, ICI enhance antitumor T-cell immunity [38]. Anti-PD-1/PD-L1 and anti-CTLA-4 monoclonal antibodies have shown significant effectiveness in the treatment of patients with advanced or mUC, including those with poor prognostic factors [9, 10, 14, 25]. Data from a subgroup analysis of IMvigor210 suggest that a higher TMB was associated with a significantly more favorable ORR to atezolizumab and longer OS. The median mutation load in atezolizumab-responders was increased with 12.4 compared to non-responders with 6.4 mutations per megabase [9, 13, 24]. In this subgroup analysis, smoking status, TCGA subtype and PD-L1 expression did not correlate with TMB [9]. These tumor samples were reanalyzed by whole exome sequencing and showed no confirmation for the potential correlation between mutational load, mutation-derived neoantigen load and clinical response to atezolizumab. This could be possible due to the small sample size [39]. In contrast, the Checkmate 275 study confirmed that patients with high TMB had a better ORR (31.9% vs. 17.4%) and median PFS (3.02 months vs. 1.87 months) [40].

In other tumor entities like melanoma and NSCLC high somatic mutational load correlates with clinical benefit in patients treated with PD-1 or CTLA-4 ICI [33, 41]. In further studies patients with high TMB often harbor specific DNA damage response defects, such as microsatellite instability (MSI-H) or mismatch repair deficiency (dMMR) [42]. It is known that patients with MSI-H or mismatch repair defects have a very high response rate to PD-1 blockade with an ORR of 39.6% to pembrolizumab and a response rate of 78% for 6 months or longer in other malignancies [43, 44]. Based on these findings, the FDA has approved pembrolizumab for tumors with MSI-H or dMMR [45]. High TMB was also detected in mUB patients who harbor additional mutations of the ERBB2 (HER2) and ERBB3 (HER3) region. Thereby, a combinatory approach with targeting treatments and ICI could be a feasible option [46]. Furthermore, important somatic gene mutations in DNA repair, like BRCA1 mutation, correlated with the responsiveness to anti-PD-1 therapy in glioblastoma [33, 47]. Data for mUC are missing in this setting.

As described the phase III IMvigor211 trial did not meet its primary end point of OS in PD-L1 pre-selected patients, but median OS was improved in the intent-to-treat (ITT) population. Therefore, the clinical outcomes in ITT and the pre-specified PD-L1 subgroup were compared with those of defined immune transcriptional gene expression (tGE) signatures and TMB. Results indicated that PD-L1 expression did not correlate with TMB. But a higher TMB was associated with favorable survival under atezolizumab [48]. These results indicate that TMB may play an independent role in predicting response to immunotherapy in mUC. Prospective validations in larger cohorts are still necessary. However, the negative predictive value of TMB remains unclear as some patients respond to immunotherapy even if they had a low TMB.

ICI and TMB as a predictive biomarker are also analyzed in the neoadjuvant setting. PURE-01 is a open-label, single-arm phase 2 trial that evaluated pembrolizumab prior to radical cystectomy in patients with T2 to T3bN0 stage and residual disease after transurethral resection of the bladder. The primary end point of pathological complete response (pCR) was observed in 39.5% and pathologic downstaging to pT < 2 in 51.2% of treated patients. Biomarker analyses included: IHC PD-L1 combined positive score (CPS, Dako 22C3) and TMB. In patients with pCR median CPS score was 30 vs. 10 in patients without pCR. TMB was higher in patients with pCR (13.16 vs. 11.41 mutations per megabase).

In the matched analysis of TUR-B and cystectomy samples median CPS score increased from baseline (10 vs. 30, not significant) and TMB decreased (10.1 vs. 4.4 mutations per megabase). The pCR rate increased to 50% in patients with PD-L1 CPS ≥ 20 and to 60% in patients with deleterious DNA damage response and/or *RB1* genomic alterations and to 90% among patients with all these features [49, 50].

ABACUS is a single-arm phase 2 study investigating atezolizumab in the neoadjuvant setting in muscle-invasive UC. The pCR rate was 29%. The results of biomarker candidates including T cell infiltration and PD-L1 status before and after therapy have not been fully published yet [51].

Tumor-infiltrating lymphocytes (TILs) and gene expression profiles (GEP)

T cell inflammation of tumors is characterized by a high number of CD8+ T cells, TH1-like chemokine and interferon (INF) expression [52]. Upon activation, these T cells can proliferate, differentiate and release pro-inflammatory cytokines such as IFN-γ and attack cells bearing specific antigens. IFN-γ leads to an intrinsic upregulation of PD-L1 and PD-L2 [53]. Depending on the number of tumor-infiltrating lymphocytes (TILs) and the expression of PD-L1 protein, the tumor microenvironment (TME) can be distinguished in either immunogenic, also called "hot" TME, or non-immunogenic, also known as "cold" TME [54]. Biomarker driven monitoring of the TME could be a valuable indicator to guide therapy and to decide for or against an ICI therapy. Gene expression profiles are useful as predictive biomarkers for chemotherapy and immunotherapy [55]. Perhaps most importantly, a specific quantification of isolated RNA from formalin-fixed paraffin-embedded whole tumor tissue could be a useful tool to characterize the TME. Here, the immune gene expression profiling has a major advantage as the RNA is quantified from several cell types within the tumor and so the GEP represents the whole TME. Taking the different components of chemokines, cytokines and cell surface proteins into account, the immune expression profiling can be used to determine the inflammatory status of a tumor tissue into "hot" or "cold".

For example, in metastatic melanoma the CD8+ T cell density in the tumor, the invasive margin and the clonality within the T cell repertoire were predictive for the response to ICI [56]. A special population of tumor-infiltrating CD8+ T cells expressing high levels of CTLA-4, are named "partially exhausted" cytotoxic T lymphocytes (peCTL) [57]. In metastatic melanoma, a strong correlation between peCTLs and the response to immunotherapy with PD-1 inhibitors is present [57, 58].

In the IMvigor210 trial, high levels of IFN-γ-induced chemokine expression determined by a CD8 + T cell effector gene expression profiling (GEP) and a higher density of infiltrating CD8+ T cells surrounding the tumor were associated with an increased ORR to atezolizumab [9]. In the same cohort a clear correlation between a higher percentage of TILs and higher peripheral T cell receptor clonal expansion in the blood with durable clinical response to atezolizumab was present [9]. In CheckMate275, a higher 25-gene IFN-γ signature assessed in 177 tumor samples correlated to a response to nivolumab. However, the negative predictive value remains unclear as some patients with a non-inflamed cytokine signature also responded [10]. These results suggest that defects in the signalling pathway of INF-γ can lead to resistance to ICI and PD-L1 expression seems to be regulated through the interferon-Jak/StaT IRF axis [59].

Based on results from a small cohorts of melanoma patients, a subset of 18 specific genes linked to INF-signaling (IDO1 and STAT1), antigen presentation (HLA-DRB1, HLA-DQA1, HLA-E), NK cell signaling pathway (NKG7, CMKLR1) and proteins from immunomodulation are actually under investigation in patients with different tumor types treated with anti-PD-1 therapy [60]. In a cohort of patients with head and neck or gastric cancer from the KEYNOTE-028 trial, this 18-gene panel correlated positive to pembrolizumab therapy across multiple solid tumors [61]. In the KEYNOTE-052 trial, the GEP score was significantly associated with a response to pembrolizumab ($p < 0.0001$) and predicted 70 out of 81 responders [24]. The studies CheckMate275 and Keynote-052 confirmed this observation with a strong relationship between a T cell-inflamed GEP score and response to nivolumab or pembrolizumab. In Keynote-052 patients with a negative PD-L1 status, but a high T cell-inflamed GEP score responded to ICI [10, 24]. Taken together, increased TILs within the TME of muscle-invasive bladder carcinoma specimens correlated with improved PFS and OS [62]. In the IMvigor211 trial, PD-L1 expression was positively correlated with the immune transcriptional gene expression (tGE) signatures, but not with TMB. High tGE signatures were associated with improved OS with chemotherapy and atezolizumab [48]. The utility of GEP still needs validation in large prospective studies.

A four-gene IFN-γ mRNA signature (IFN- γ, LAG3, CXCL9, and PD-L1 mRNAs) in tumor biopsies was a positive predictive biomarker for a longer OS and PFS in patients with non-small cell lung carcinoma (NSCLC) or UC for durvalumab treatment. In this study, PD-L1 positivity by IHC and IFN-γ signature gene expression at baseline independently correlated with a response to durvalumab therapy [63].

Clustering by TCGA subtype/intrinsic molecular subtypes

Other possible biomarkers include the molecular tumor subtypes based on The Cancer Genome Atlas (TCGA) [64, 65]. As part of these gene analyses UBC can be distinguished into basal and luminal tumors [64, 66]. In several trials, these molecular TCGA subtypes of mUC correlated with response to PD-1/PD-L1 ICI [64]. In the IMVigor210 trial, PD-L1 expression on IC was highly enriched in the basal subtype (60% vs. 23%) and PD-L1 expression on TC was almost exclusive in basal subtypes (39% vs. 4% in luminal). However, an enriched PD-L1 expression in the basal subtype did not correlate with ORR to atezolizumab. Nevertheless, the highest response rates were observed in patients with the molecular subtype luminal cluster II TCGA (ORR 34%) relative to the luminal cluster I (10%), the basal cluster I (16%), and the basal cluster II (20%) [9, 24]. By contrast, these findings are inconsistent with data from a similar cohort of the Checkmate-275 study. Here, patients with mUC and the TCGA subtype basal I cluster III had the highest response rate to nivolumab with 30% ORR, luminal cluster II tumors had an ORR of 25% [10]. Possible explanations for theses discrepancies from IMvigor210 and Checkmate275 might be related to tissue source. In both trials, biopsies from different specimens were used for the TCGA analysis, for example primary tumor, lymph nodes or metastatic lesions. In addition, there was a difference in clustering the molecular subtypes due to a lack of standardization of the TCGA classification. Therefore, final conclusions on the impact of TCGA subtyping as a predictive biomarker for response to checkpoint inhibition in mUC cannot be drawn. Other molecular classifications despite the TCGA subtyping are described, but they have no relevant role in the molecular profiling of ICI in mUC so far [67].

Conclusion

Immune checkpoint inhibition therapy targeting PD-1 and PD-L1 significantly improved OS and long-term disease control. The existing possibilities to select patients who will benefit from a PD-1 or PD-L1 ICI by biomarker-driven strategies are limited. Further prospective evaluation is needed to avoid overtreatment or undertreatment, toxicity and costs. Based on the available data, PD-L1 expression on IC and TC alone is insufficient as a biomarker in ICI of mUC. A harmonization may potentially solve these hurdles of different methods, antibodies and cutoffs in PD-L1 expression. Interestingly, patients with low or missing PD-1 or PD-L1 expression in tumor tissue can have durable responses. One possible strategy to improve the biomarker prediction in ICI is the combination of different variables like the TIL density, TMB, targeted gene expression profiling directed to T cell gene signatures and TCGA profiling. All these factors have to be validated prospectively in large cohorts. But, so far none of these potential biomarkers have yet been translated into clinical practice, although the currently available results clearly define patients who benefit most from ICI.

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Compliance with ethical standards

Conflict of interest Bedke: honoraria/research funding: AstraZeneca, Astellas, BMS, MSD, Pfizer, Roche; Stenzl: honoraria/research funding: BMS, MSD, Pfizer, Roche; Todenhöfer: BMS, MSD, Roche

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