



Platinum Priority – Review – Urothelial Cancer – Editor's Choice  
Editorial by Haris Zahoor and Petros Grivas on pp. 445–447 of this issue

## The Cancer Immunogram as a Framework for Personalized Immunotherapy in Urothelial Cancer

Nick van Dijk<sup>a</sup>, Samuel A. Funt<sup>b</sup>, Christian U. Blank<sup>a</sup>, Thomas Powles<sup>c</sup>, Jonathan E. Rosenberg<sup>b</sup>, Michiel S. van der Heijden<sup>a,\*</sup>

<sup>a</sup>Department of Medical Oncology, Netherlands Cancer Institute, Amsterdam, The Netherlands; <sup>b</sup>Genitourinary Oncology Service, Department of Medicine, Memorial Sloan Kettering Cancer Center, New York, NY, USA; <sup>c</sup>Department of Medical Oncology, Barts Cancer Institute, London, UK

### Article info

#### Article history:

Accepted September 12, 2018

#### Associate Editor:

James Catto

#### Keywords:

Urothelial cell cancer  
Immunotherapy  
Immune checkpoint inhibitors  
Cytotoxic T lymphocyte-associated protein 4  
Programmed cell death 1  
Programmed cell death receptor ligand 1  
Biomarkers

### Abstract

**Context:** The abysmal outlook of urothelial cancer (UC) has changed with the introduction of immunotherapy. Still, many patients do not respond and distinctive biomarkers are currently lacking. The rise of this novel armamentarium of immunotherapy treatments, in combination with the complex biology of an immunological tumor response, warrants the development of a comprehensive framework that can provide an overview of important immunological processes at play in individual patients.

**Objective:** To develop a comprehensive framework based on tumor- and host-specific parameters to understand immunotherapy response in UC. This framework can inform rational, biology-driven clinical trials and ultimately guide us toward individualized patient treatment.

**Evidence acquisition:** A literature review was conducted on UC immunotherapy, clinical trial data, and biomarkers of response to checkpoint inhibition.

**Evidence synthesis:** Here, we propose a UC immunogram, based on currently available clinical and translational data. The UC immunogram describes several tumor- and host-specific parameters that are required for successful immunotherapy treatment. These seven parameters are tumor foreignness, immune cell infiltration, absence of inhibitory checkpoints, general performance and immune status, absence of soluble inhibitors, absence of inhibitory tumor metabolism, and tumor sensitivity to immune effectors.

**Conclusions:** Longitudinal integration of individual patient parameters may ultimately lead to personalized and dynamic immunotherapy, to adjust to the Darwinian forces that drive tumor evolution. Incorporating multiparameter biomarkers into quantitative predictive models will be a key challenge to integrate the immunogram into daily clinical practice.

**Patient summary:** Here, we propose the urothelial cancer immunogram, a novel way of describing important immunological characteristics of urothelial cancer patients and their tumors. Seven characteristics determine the chance of having an immunological tumor response. Using this immunogram, we aim to better understand why some patients respond to immunotherapy and some do not, to ultimately improve anticancer therapy.

© 2018 European Association of Urology. Published by Elsevier B.V. All rights reserved.

\* Corresponding author. Department of Medical Oncology, Netherlands Cancer Institute, Plesmanlaan 121, 1066CX, Amsterdam, The Netherlands. Tel. +31 20 512 2569; Fax +31 20-512 2572. E-mail address: [ms.vd.heijden@nki.nl](mailto:ms.vd.heijden@nki.nl) (M.S. van der Heijden).



## 1. Introduction

The introduction of checkpoint blockade (CPB) has changed the treatment landscape of metastatic urothelial cancer (mUC) [1,2]. Still, many patients do not experience clinical benefit to anti-PD-(L)1 alone. Although potentially important associations between biomarkers and clinical responses to CPB have been observed, these biomarkers are not yet ready for clinical practice until prospectively validated in clinical studies. Heterogeneity in prior therapies and use of archival tissue for biomarker development further cloud interpretation. In 2016, Blank and colleagues [3] proposed the cancer immunogram, a theoretical framework that integrates candidate biomarkers to ultimately inform individualized treatment with multiparameter biomarkers. The immunogram was constructed on the assumption that T-cell activity is the ultimate effector mechanism that is affected by seven unrelated immunogenic parameters: tumor foreignness, general immune status, immune cell infiltration capacity, absence of checkpoints, absence of soluble inhibitors, absence of inhibitory tumor metabolism, and tumor sensitivity to immune effector mechanisms. Recently, this concept has been extended to non-small cell lung cancer (NSCLC) [4]. Here, we propose a cancer immunogram specifically for urothelial cancer (UC) patients. The main goals are to: (1) better understand the complexity of the anticancer immune response in UC and thus facilitate translational research, and (2) help prioritize biomarkers that should be prospectively tested in clinical studies, eventually leading to a multifactorial model that can better predict clinical CPB response in UC.

## 2. Evidence acquisition

A PubMed/Medline search was conducted with terms including urothelial cancer, bladder cancer, immunotherapy, biomarkers, checkpoint inhibition, checkpoint inhibitors, checkpoint blockade, anti-CTLA-4, anti-PD-1, and anti-PD-L1. Additional literature was found using a snowballing approach. Relevant data from recent conferences were included.

## 3. Evidence synthesis

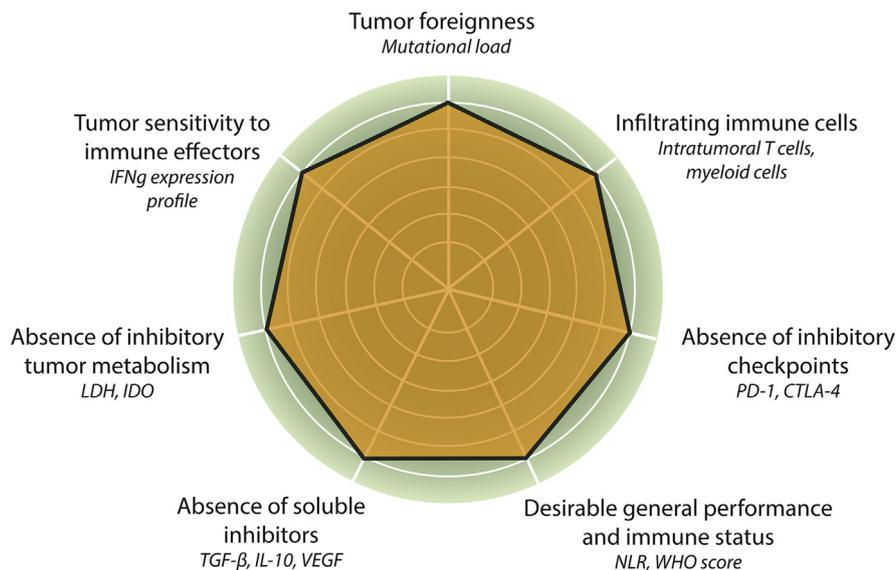
### 3.1. UC immunogram

The UC immunogram (Fig. 1) constitutes a theoretical framework with multiparameter candidate biomarkers, structured around seven axes, aiming to capture the most important factors determining an anticancer immune response. Available data for each axis, based on results obtained in UC where available, are reviewed below.

### 3.2. Tumor foreignness

#### 3.2.1. Tumor mutations

The adaptive immune system can recognize tumor features as “foreign” and elicit an immune response. Cancer antigens include immune-privileged peptides or genetically altered peptides (neoantigens) [5]. It has been postulated that high tumor mutational burden (TMB) and neoantigen load are associated with a higher likelihood of an immunotherapy response. Consistently, pembrolizumab showed remarkable



**Fig. 1 – Urothelial cancer immunogram.** The proposed cancer immunogram for UC patients reflects seven key immunological axes and their underlying biomarkers (*italic*) that facilitate successful immunotherapy treatment. The immunogram is constructed on the assumption that T-cell activity is the ultimate effector mechanism that is affected by these seven unrelated axes. The outer region of the plot depicts the most favorable immune status for immunotherapy treatment. In the hypothetical patient example above, the line connects the seven parameters in a highly favorable situation for immunological antitumor response. Several examples of cancer immunograms of UC patients who were treated with anti-PD-L1 in the IMvigor210 study can be found in Figure 2 and the Supplementary material (Applications of the urothelial cancer immunogram). IDO = indolamine 2,3-dioxygenase 1; IFN $\gamma$  = interferon gamma; IL = interleukin; LDH = lactate dehydrogenase; NLR = neutrophil-to-lymphocyte ratio; TGF- $\beta$  = transforming growth factor beta; UC = urothelial cancer; VEGF = vascular endothelial growth factor; WHO = World Health Organization.

activity in cancers with mismatch repair (MMR) deficiency [6], which leads to very high mutation rates. Preliminary analysis on MMR status in UC tumor samples revealed that MMR deficiency was particularly observed in upper tract urothelial cancer [7]. Interestingly, five of these MMR-deficient patients were treated with CPB and all showed robust responses, including three complete responses [7]. After melanoma and lung cancer, UC has the highest frequency of somatic mutations [8]. In clinical trials with atezolizumab [9] and nivolumab [10,11], tumor response was associated with TMB. Furthermore, *APOBEC3A/3B* expression [12] and mutations in genes involved in DNA damage response [13] were associated with TMB and response to CPB.

### 3.2.2. Molecular subtypes

Transcriptome profiling in The Cancer Genome Atlas (TCGA) project revealed that UC can be clustered into molecular subtypes [8,14]. These molecular subtypes were associated with a response to atezolizumab in the IMvigor210 trial [9]. Gene expression signatures were used to discriminate basal from luminal subtypes defined by TCGA in 195 UC patients. The objective response rate (ORR) was highest in luminal cluster II subtype (34%), compared with 10% for cluster I, 16% for cluster III, and 20% for cluster IV [9]. By contrast, in the Checkmate 275 trial with nivolumab, the highest response rate (30%) was observed in basal cluster III, whereas luminal cluster II showed a 25% response rate [11]. Since it is unclear why some molecular subtypes respond to treatment and some do not, larger data sets from phase III trials are needed to better understand molecular signatures as predictors of immunotherapy response.

### 3.2.3. Viral integrations

Genomic data from UC were used by the TCGA consortium to investigate viral integration in UC. These data showed that 6% of the investigated bladder tumors contained viral DNA and transcripts, including HPV and BK virus DNA [15]. Viral integrations may contribute to increased tumor foreignness by expressing viral oncogenes that may induce an immune response [16]. The role of viral integrations in UC immunotherapy treatment is currently unclear.

## 3.3. Immune cell infiltration

### 3.3.1. Intratumoral T cells

Tumor-infiltrating CD8<sup>+</sup> T cells play a key role in antitumor immunity, and their presence in the tumor-immune microenvironment (TME) has been associated with longer survival in several malignancies [17], including UC [18]. Data from the IMvigor210 study showed that CD8<sup>+</sup> density in the tumor area was associated with response to atezolizumab in mUC [9]. Intratumoral T-cell profiles can be characterized by three histologically distinct phenotypes: (1) the immune inflamed phenotype, marked by robust immune infiltrate and PD-L1 expression, (2) the immune-excluded phenotype, where T cells are particularly present in the stroma, and (3) the immune desert phenotype, characterized by the absence of infiltrating lymphocytes [19]. In the UC

IMvigor210 cohort, 47% of tumors were classified as immune excluded, 27% were classified as immune desert, and 26% exhibited the inflamed phenotype [12]. The latter demonstrated the highest response to atezolizumab and correlated with PD-L1 signal and CD8 T effector signature in gene expression analysis. Interferon gamma (IFN $\gamma$ )-stimulated genes and the chemokines CXCL9 and CXCL10 were significantly associated with PD-L1 positivity and response to atezolizumab as well [9,12]. Expression of immune genes, such as IFN $\gamma$ , CXCL9, and CXCL10, was also enriched in mUC tumors responding to nivolumab in the Checkmate 275 study [11]. Transforming growth factor beta (TGF- $\beta$ ) signaling was negatively associated with response in immune-excluded tumors (described below).

Several signaling pathways activated in UC have been associated with a lack of T-cell inflammation, including peroxisome proliferator-activated receptor gamma (PPAR- $\gamma$ ) and fibroblast growth factor receptor (FGFR) [20]. These signaling pathways promote tumor progression and anti-inflammatory features, and are particularly active in luminal I tumors [21–23]. Inhibition of the PPAR- $\gamma$  pathway enhanced inflammatory chemokines and cytokines in mouse models [22]. Recent data on erdafitinib (pan-FGFR inhibitor) in mUC patients with prespecified FGFR alterations demonstrated robust responses (ORR 70%) in patients with prior CPB [24]. Whether FGFR inhibitors can resensitize luminal I tumors to immunotherapy remains to be explored.

Using another approach, the CD-122 (interleukin [IL]-2 receptor) biased agonist NKTR-214 plus nivolumab showed preferential activation and expansion of effector T cells and NK cells over T-regs, with remarkable response rates in cancer patients [25]. Interestingly, robust responses were also observed in PD-L1-negative tumors. These preliminary data on PPAR- $\gamma$  modulators, FGFR inhibitors, and NKTR-214 show potential strategies to “ignite” immune-cold UC and restore immunosurveillance, as has been shown with BRAF inhibition in melanoma [26].

### 3.3.2. Inhibitory immune cells

Besides the presence of antitumor immune cells, other subpopulations of immune cells may facilitate cancer progression through activity toward an immunosuppressive environment. For example, T-regs inhibit CD8<sup>+</sup> T-cell function via release of immunosuppressive cytokines including IL-10 [27]. In a small UC cohort, the ratio of CD8<sup>+</sup> to T-reg tumor-infiltrating lymphocyte (TIL) densities was strongly associated with the response to neoadjuvant chemotherapy (NAC) [28]. Still, the exact role of T-regs in UC remains unclear. Macrophages are highly plastic cells, and when accumulated in tumors, they are termed tumor-associated macrophages (TAMs). Macrophages can become polarized and impair CD8<sup>+</sup> T-cell function after manipulation by tumor-derived signals including angiopoietin-2, M-CSF, CCL2, and vascular endothelial growth factor (VEGF) [29–31]. Post-translational modification of cytokines and chemokines induced by TAMs hinders T-cell infiltration into the tumor, resulting in the trapping of CD8<sup>+</sup> T cells in the stroma, thus supporting immune-excluded and immune

desert tumors [32]. Emerging data suggest that high intratumoral TAM density is associated with tumor stage and poor response to NAC in UC [33]. In addition, TAMs were found to express PD-L1 upon tumor cytokine release in bladder cancer [34]. Still, the precise role of suppressive immune cells in the TME is not well established in UC. This is needed, as depletion of inhibitory cells could potentially enhance T-cell-mediated responses and optimization of immunogram parameters, suggesting improved conditions for CPB [35].

### 3.4. Absence of inhibitory checkpoints

#### 3.4.1. PD-L1

In phase II trials with atezolizumab (Imvigor210) and nivolumab (Checkmate 275), numerically higher ORR and longer overall survival (OS) were observed in PD-L1-positive UC patients [9,11], whereas conflicting results exist for pembrolizumab [1,36]. Recent data demonstrated that PD-L1 relies on CMTM6/4 (molecule found to stabilize surface PD-L1 expression) to efficiently execute its immunosuppressive role. CMTM6 blockage reactivated effector T cells and may represent a novel strategy to target the PD-1/PD-L1 axis [37]. Beyond limited knowledge on PD-L1 regulation, variability in PD-L1 assays, accompanied by spatiotemporal dynamics in expression, explains the weakness of PD-L1 as a single-analyte biomarker and the need for a comprehensive multiparameter approach.

#### 3.4.2. Other immune checkpoints

Besides PD-1/PD-L1, many other immune checkpoints are studied in mUC. As shown for melanoma [38], combining anti-PD-(L)1 with anti-CTLA-4 treatment may induce higher response rates compared with anti-PD-1/PD-L1 alone [39]. Other interesting targets studied in clinical trials (Supplementary Table 1) include T-cell immunoglobulin and mucin-domain containing-3 (TIM-3), T-cell immunoreceptor with Ig and ITIM domains, lymphocyte-activation gene 3 (LAG-3), and NKG2A. These checkpoints can be expressed at baseline or may be induced by treatment targeting PD-1/PD-L1, indicating acquired resistance [40,41]. Recent data on anti-LAG-3 demonstrated that anti-PD-1 refractory melanoma patients had a 16% response rate to anti-PD-1/LAG-3 combination therapy [42,43]. Even higher response rates were observed in patients with LAG-3 positivity on intratumoral immune cells, suggesting that expression of LAG-3 might be a resistance mechanism to anti-PD-1 therapy. In patients with a previous response to anti-PD-1 monotherapy, LAG-3 upregulation may be a mechanism of acquired resistance, as described in preclinical models [44,45]. In an apparent contradiction to a model of acquired resistance by upregulation of inhibitory checkpoints, gene expression analysis in on-treatment biopsies showed increased expression of immune checkpoints (ie, *TIGIT*, *LAG-3*, and *TIM-3*) upon treatment with anti-PD-1/PD-L1, particularly in responders [46,47]. NKG2A is an inhibitory receptor expressed by both T and NK cells binding HLA-E, often exploited by tumors to evade immunosurveillance [48]. The introduction of anti-NKG2A

in early-phase clinical trials introduced strategies to simultaneously activate both effector cells and broaden antitumor responses [49,50]. Studies testing combination immunotherapy and strategies that target multiple effector cells will hopefully increase immunotherapy response.

### 3.5. General performance and immune status

To date, most of the data around prediction of response to immunotherapy have focused on intratumoral characteristics. Despite getting less attention, accessibility of a patient's blood makes blood-based biomarkers an attractive approach that might contribute to patient selection for immunotherapy treatment. A retrospective study on 720 metastatic melanoma patients treated with ipilimumab demonstrated that increased absolute neutrophil levels significantly decreased OS and progression-free survival (PFS) [51]. High CD4+/CD8+ lymphocyte counts were associated with improved survival upon anti-CTLA-4 treatment in melanoma, whereas decreased lymphocyte counts correlated with poor outcome [52]. Several other immune status-related biomarkers have been associated with ipilimumab response in melanoma patients, including a high absolute eosinophil count [53], enhanced peripheral T-cell levels, and high baseline peripheral FoxP3+ T-reg counts [53,54].

In UC, data on general immune status are sparse. Neutrophil-to-lymphocyte ratio (NLR) appears to be a prognostic marker in UC [55]. Recent preliminary analyses suggest that low NLR and high albumin are associated with tumor shrinkage and higher OS following treatment with durvalumab in UC patients [56]. Other adverse prognostic clinical parameters, such as low baseline performance status or presence of bone or liver metastases, are indicators of poor prognosis and a lack of response to CPB in UC [9]. Interestingly, Sharma et al (Sharma et al, AACR 2018) recently showed that low baseline levels of circulating myeloid-derived suppressor cells (MDSCs) were associated with longer OS in the Checkmate 275 trial testing nivolumab for mUC [57]. In addition, MDSCs in peripheral blood were negatively associated with pathological stage at cystectomy, most notably in patients treated with NAC [58]. Future research will unravel whether composite biomarkers derived from pretreatment and on-treatment blood have biomarker potential in UC, enforcing the immunogram framework and increasing our understanding of antitumor responses to ultimately predict clinical response.

### 3.6. Absence of soluble inhibitors

Soluble immunosuppressive factors (ie, cytokines and growth factors) can create a hostile and immunosuppressive TME. Immunosuppressive cytokines such as IL-10 and TGF- $\beta$  are often released by tumor cells, T-regs, MDSCs, or fibroblasts, and are crucial regulators of T-cell exhaustion in resistant tumors [29,59]. Elevated IL-10 can induce immunosuppression by promoting T-reg polarization [60] and enhancing PD-L1 expression on dendritic cells and TAMs,

resulting in PD-L1-mediated T-cell exhaustion [61]. In UC, higher levels of serum IL-10 were found in high-grade tumors compared with lower-grade tumors, whereas higher IL-10 urine levels were associated with poor recurrence-free survival.

TGF- $\beta$  plays an important role in angiogenesis and immunosuppression [62,63]. Recent data demonstrated that TGF- $\beta$  can directly impair CD8+ T-cell function by down-regulation of functional effector proteins (ie, granzymes and perforins) [62], and high TGF- $\beta$  levels were shown to be an indicator of poor prognosis in resectable muscle-invasive bladder cancer (MIBC) [64]. In an in-depth analysis of the IMvigor210 study, unresponsiveness to atezolizumab was associated with TGF- $\beta$  signaling in fibroblasts, particularly in patients with immune-excluded tumors [12]. In a mouse model exhibiting the immune-excluded phenotype, treatment with anti-TGF- $\beta$  plus anti-PD-L1 lowered TGF- $\beta$  signaling in stromal cells, enhanced intratumoral T-cell trafficking and induced T-cell-mediated tumor rejection [12].

T-cell function can also be impaired by adenosine. Adenosine binds the A2A receptor on T cells and inhibits T-cell proliferation and cytolytic function [65], while it is also known to promote metastasis via A2B signaling on tumor cells [66]. Moreover, CD73 converts AMP to adenosine and is known to be an indicator of poor prognosis in UC [67]. Interestingly, PD-L1-/CD73+ tumors showed lower TILs compared with PD-L1+/CD73- tumors, suggesting that CD73 might play a role in excluding T cells and promoting immune desert tumors [67].

Another mechanism utilized by tumor cells to impair T-cell function is the secretion of VEGF [68]. VEGF promotes tumor angiogenesis, directly impairs T-cell function, and contributes to tumor progression in UC [68,69]. Ramucirumab, an antibody targeting VEGF receptor-2, showed improved PFS when added to docetaxel in second-line UC [70]. In kidney and lung cancer, atezolizumab plus bevacizumab (anti-VEGF) showed clinical benefit in metastatic patients [71], and this strategy is currently being investigated in patients with advanced UC. Recent work demonstrated that inflammatory tumors are marked by high expression of cyclooxygenases (COX), prostaglandin E2 (PGE2), and IL-6, which are known for their immunosuppressive potency [72,73]. Particularly, IL-6 stimulates hepatocytes to synthesize CRP and, thus, marks CRP as a surrogate for immunosuppressive tumors [73]. A retrospective analysis of 88 patients with MIBC treated with chemoradiotherapy showed that elevated CRP prior to treatment predicts a poor prognosis [74]. No studies have linked CRP levels to clinical outcome upon CPB in UC.

### 3.7. Absence of inhibitory tumor metabolism

Recently, Renner et al. [75] published a review on metabolic hallmarks of cancer that described the metabolic interplay between tumor cells and immune cells as a dynamic system that can be re-educated by cancer therapies. High energy demand and antitumor immunity drive tumor cells, MDSCs, or granulocytes to highly express lactate dehydrogenase (LDH), indolamine 2,3-dioxygenase 1 (IDO1), COX, glucose

transporters, glutaminase, arginase, and oxidative phosphorylation [75,76]. As a result, essential fuel for efficient T-cell functioning, such as glucose and amino acids, becomes depleted in the TME and consequently impairs antitumor T-cell function [77]. Moreover, lactate and other metabolic products such as kynurenines and PGE2 further impair antitumor T-cell function [75]. IDO1 is an enzyme that converts tryptophan into kynurenine and is often upregulated by tumors to exhaust antitumor T cells [78]. In bladder cancer tissue, IDO1 was expressed in 57% of cases, while in healthy bladder tissue only 18% expressed IDO1. Higher IDO1 expression was associated with poor histological grade (tumor invasiveness) and poor clinical outcome in bladder cancer [79]. In a murine bladder cancer model, IDO1 was targeted with siRNA, resulting in enhanced antitumor immunity [80]. Epacadostat [81] and BMS-986205 [82], both selective blockers of IDO1, were recently tested in combination with anti-PD-1 in single-arm studies and showed efficacy in mUC [82]. However, recent randomized data in melanoma failed to show benefit of epacadostat, casting doubt on the validity of this strategy in unselected patients [83]. Reasons for failure may include a lack of appropriate biomarkers for patient selection. Despite the negative epacadostat trial in melanoma, randomized trials testing combinations of anti-IDO1 with CPB are ongoing in UC, based on efficacy signals in single-arm trials and preclinical rationale.

Other amino acids essential for T-cell and tumor cell metabolism and function are arginine and glutamine. Preclinical data demonstrated that arginine depletion inhibits T-cell and NK-cell activation and function, and promotes *in vivo* generation of MDSCs [84], whereas glutamine deprivation particularly promotes T-reg polarization [85]. CB-1158 targets arginase to prevent deprivation of arginine and is being currently tested with or without pembrolizumab in mUC. A drug that targets glutaminase (CB-839) is being currently tested in a phase I/II clinical trial evaluating CB-839 in combination with nivolumab in patients with melanoma, renal cell carcinoma, and NSCLC. High LDH levels are correlated with poor prognosis and lower ORR to CPB in melanoma [86]. In UC, patients with high serum lactate were found to have poor prognosis [87]. Additionally, LDH is incorporated in the six-factor prognostic model developed by Pond et al. [88]. This model was designed to predict OS in platinum-refractory mUC patients treated with atezolizumab, but needs further refinement and validation in larger datasets, including datasets with other agents targeting PD-1/PD-L1. Thus, the exact association between LDH levels and response to CPB warrants further investigation.

In conclusion, interfering with metabolic pathways might provide ways to eliminate tumor cells directly, or indirectly by reprogramming metabolic pathways to enhance CD8+ T-cell function.

### 3.8. Tumor sensitivity to immune effectors

#### 3.8.1. Antigen presentation and recognition

CD8+ T-cell activation depends on several simultaneous signal interactions, including T-cell receptor (TCR)

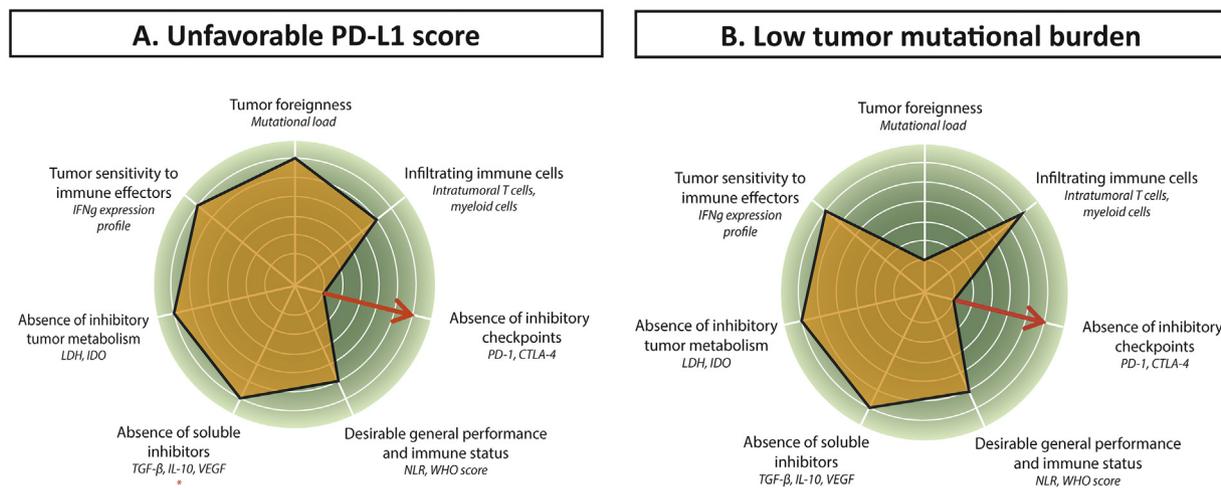
binding to the MHC-antigen complex on tumor cells and costimulatory signaling [89]. Tumors can evade CD8+ T-cell immune surveillance through genetic and epigenetic alterations in the antigen-presenting machinery. Early studies with epigenetic modifiers resulted in re-expression of tumor-associated antigens and MHC-antigen complexes, whereas potential synergy was observed when epigenetic modifiers were combined with CPB [90,91]. Likewise, point mutations and deletions in beta-2-microglobulin (B2 M), a crucial building block required for MHC class I assembly, were found in almost 30% of melanoma tumors upon CPB resistance [92]. Analysis of a progressive tumor lesion obtained from a patient with colorectal cancer treated with TIL therapy (anti-KRAS G12D presented by HLA-C\*08:02) showed a loss of HLA-C\*08:02 in the relapsing lesion [93]. In UC, early data suggest that HLA loss by mutations in  $\beta$ 2-microglobulin genes was not the underlying cause of low HLA class I presence. Instead, coordinated transcriptional downregulation of the HLA components B2 M and APM was found to be a key element of irreversible HLA loss [94]. While evidence is currently lacking, it is likely that immunotherapy-induced alterations in the antigen-presenting machinery also occur in UC.

### 3.8.2. TCR repertoire

The TCR repertoire is also involved in antigen recognition. In a retrospective analysis in melanoma and prostate cancer, patients responding to ipilimumab showed TCR clonotype stability in PBMCs 4 wk after treatment start [95]. In mUC, durable responses with atezolizumab treatment were associated with lower baseline TCR clonality in peripheral blood [96], suggesting that a higher variety of TCR receptors might increase the probability that a tumor-specific T-cell population is present. Recent provocative data showed that neoadjuvant treatment with ipilimumab plus nivolumab induced higher numbers of expanded and newly detectable TCR clones in the peripheral blood compared with the adjuvant setting in stage 3 melanoma [97].

### 3.8.3. IFN $\gamma$ signaling

CD8+ T-cell effector function can be impaired despite successful binding of tumor cells. Loss of IFN $\gamma$  signaling has been associated with resistance to anti-CTLA-4 immunotherapy [98]. In melanoma, mutational analysis showed that primary resistance to ipilimumab was associated with mutations in IFN $\gamma$  receptors 1 and 2 (*IFNGR1* and *IFNGR2*), interferon regulatory factor 1, and *JAK1* and *JAK2*, allowing cancer cells to escape from IFN $\gamma$ -mediated killing



**Fig. 2 – Example immunograms of urothelial cancer patients treated with second-line anti-PD-(L)1 checkpoint inhibition. In the UC immunograms, outer region of the plot depicts the most favorable status for a T-cell-mediated anticancer immune response, which is affected by seven unrelated axes. Immunogram scores are based on available data from individual patients on that specific axis. Orange arrow: shift of the immunogram upon anti-PD-(L)1 treatment. Immunogram axes with no available data are marked by an orange star (\*) and have been qualified as favorable (hypothetically) in the immunogram. (A) A patient with a high mutational burden, favorable TCGA class II, and substantial CD8+ T-cell infiltration. The patient had favorable CD8+ T-cell effector and IFN $\gamma$  immune activation signatures, while the PD-L1 score (IC2) was unfavorable and may have impaired natural antitumor response. The patient had a WHO performance score of 1, had no visceral metastases, and had favorable NLR ratio and LDH. All parameters, except high PD-L1 expression, were favorable for an immune response. Treatment with anti-PD-L1 corrects the only unfavorable parameter that may have prevented T cells from executing an antitumor response, and led to a complete response, which is still ongoing (OS currently 1230 d). (B) A patient with unfavorable tumor foreignness (low TMB, TCGA IV) with dramatic intratumoral CD8+ T-cell infiltration and favorable CD8+ effector and IFN $\gamma$  immune activation signatures. The tumor environment showed high PD-L1 IC expression (PD-L1 IC2), which may have prevented T cells from eliminating tumor cells. This patient had WHO 1 with no visceral metastases and favorable NLR ratio and LDH. While this patient had dramatic intratumoral CD8+ T-cell infiltration with a favorable immune gene signature, treatment with anti-PD-L1 did not result in a tumor response and OS (117 d) was limited. In this case, involvement of other inhibitory checkpoint pathways, regulatory T cells, or presence of soluble inhibitors (ie, TGF- $\beta$ ) may explain anti-PD-L1 resistance. Furthermore, despite having sufficient CD8+ T-cell infiltration, a limited tumor-specific T-cell repertoire may explain nonresponse despite having sufficient CD8+ T-cell infiltration. Treatment with anti-PD-(L)1/CTLA-4 might hypothetically have resulted in a broader and more effective immune response. More examples can be found in the Supplementary material (Applications of the urothelial cancer immunogram).**

IC = immune cell; IDO = indolamine 2,3-dioxygenase 1; IFN $\gamma$  = interferon gamma; IL = interleukin; LDH = lactate dehydrogenase; NLR = neutrophil-to-lymphocyte ratio; OS = overall survival; TCGA = The Cancer Genome Atlas; TGF- $\beta$  = transforming growth factor beta; TMB = tumor mutational burden; UC = urothelial cancer; VEGF = vascular endothelial growth factor; WHO = World Health Organization.

[98]. Additionally, TGF- $\beta$ -mediated downregulation of granzymes and perforins has been shown to impair CD8+ T-cell-mediated antitumor killing [62].

#### 4. Conclusions

In recent years, several biomarkers for immunotherapy response have been proposed. Still, these biomarkers are not ready for incorporation into clinical practice due to insufficient discriminatory power. Tissue acquisition for biomarker analyses has been heterogeneous (eg, transurethral resection vs cystectomy vs biopsy of a metastatic site), with variability in preceding treatments. A more homogeneous collection of tissue in prospective trials and incorporation of this bias into the interpretation of biomarkers are warranted. Additionally, some biomarkers may be more dynamic than others and should be monitored closely [99]. The UC immunogram provides a constantly evolving theoretical framework that incorporates multidimensional candidate biomarkers that should be measured and validated in clinical studies, ultimately informing clinical decision making. An individual patient can be assessed on each of the seven axes, to estimate the likelihood of a response to occur and to assess which factors may still prevent a response. We have provided such an assessment for several patients treated with CPB (Fig. 2 and Supplementary material, Applications of the urothelial cancer immunogram). Individualized data on immunogram parameters may be obtained by tumor genomics, immune gene signatures, immunohistochemistry, and blood-based assays, and could be monitored during the course of disease, to adjust treatment accordingly. A key challenge for the near future will be to explore whether data on the UC immunogram parameters can be incorporated into quantitative predictive models that can be used in clinical practice.

**Author contributions:** Michiel S. van der Heijden had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

**Study concept and design:** van Dijk, Blank, van der Heijden.

**Acquisition of data:** van Dijk, van der Heijden, Funt.

**Analysis and interpretation of data:** None.

**Drafting of the manuscript:** van Dijk, van der Heijden, Funt.

**Critical revision of the manuscript for important intellectual content:** Blank, Rosenberg, Funt, Powles, van der Heijden.

**Statistical analysis:** None.

**Obtaining funding:** None.

**Administrative, technical, or material support:** Rosenberg, Funt.

**Supervision:** van der Heijden.

**Other:** None.

**Financial disclosures:** Michiel S. van der Heijden certifies that all conflicts of interest, including specific financial interests and relationships and affiliations relevant to the subject matter or materials discussed in the manuscript (eg, employment/affiliation, grants or funding, consultancies, honoraria, stock ownership or options, expert testimony, royalties, or patents filed, received, or pending), are the following: N. van Dijk: none. S.A. Funt: research support—Genentech/Roche, AstraZeneca; equity ownership—Urogen Pharma and Allogene Therapeutics. C.U. Blank: consultancy fees (to institute)—BMS, Merck/MSD, Novartis,

Roche, Lilly, GSK, Pfizer, and GenMab; research funding—Novartis and BMS. T. Powles: consultancy fees—BMS, Pfizer, Merck/MSD, AstraZeneca, Lilly, Roche/Genentech, and Exelixis; research funding—AstraZeneca and Roche/Genentech. J.E. Rosenberg: consultancy fees—BMS, Merck/MSD, AstraZeneca/Medimmune, Lilly, Roche/Genentech, Agensys, Inovio Pharmaceuticals, and EMD Serono; research funding—AstraZeneca/Medimmune, Incyte, Roche/Genentech, Mirati Therapeutics, Oncogenex, Novartis, and Viralytics; stock/ownership—Merck and Illumina. M.S. van der Heijden: consultancy fees (to institute)—AstraZeneca/Medimmune, Roche/Genentech, BMS, and Merck/MSD; research funding—BMS and Astellas.

**Funding/Support and role of the sponsor:** None.

**Acknowledgments:** We would like to thank Sanjeev Mariathasan and Edward E. Kadel (both from Genentech, San Francisco, USA) for providing data on individual patients. We thank Ton Schumacher for providing valuable input.

#### Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at <https://doi.org/10.1016/j.eururo.2018.09.022>.

#### References

- [1] Bellmunt J, de Wit R, Vaughn DJ, et al. Pembrolizumab as second-line therapy for advanced urothelial carcinoma. *N Engl J Med* 2017;376:1015–26.
- [2] Powles T, Duran I, van der Heijden MS, et al. Atezolizumab versus chemotherapy in patients with platinum-treated locally advanced or metastatic urothelial carcinoma (IMvigor211): a multicentre, open-label, phase 3 randomised controlled trial. *Lancet* 2018;391:748–57.
- [3] Blank CU, Haanen JB, Ribas A, Schumacher TN. Cancer immunology. The “cancer immunogram”. *Science* 2016;352:658–60.
- [4] Karasaki T, Nagayama K, Kuwano H, et al. An immunogram for the cancer-immunity cycle: towards personalized immunotherapy of lung cancer. *J Thorac Oncol* 2017;12:791–803.
- [5] Gilboa E. The makings of a tumor rejection antigen. *Immunity* 1999;11:263–70.
- [6] Le DT, Uram JN, Wang H, et al. PD-1 blockade in tumors with mismatch-repair deficiency. *N Engl J Med* 2015;372:2509–20.
- [7] Iyer G, Audenet F, Middha S, et al. Mismatch repair (MMR) detection in urothelial carcinoma (UC) and correlation with immune checkpoint blockade (ICB) response. *J Clin Oncol* 2017;35:4511.
- [8] Lawrence MS, Stojanov P, Polak P, et al. Mutational heterogeneity in cancer and the search for new cancer-associated genes. *Nature* 2013;499:214–8.
- [9] Rosenberg JE, Hoffman-Censits J, Powles T, et al. Atezolizumab in patients with locally advanced and metastatic urothelial carcinoma who have progressed following treatment with platinum-based chemotherapy: a single-arm, multicentre, phase 2 trial. *Lancet* 2016;387:1909–20.
- [10] Galsky MD, Saci A, Szabo PM, et al. 848PD Impact of tumor mutation burden on nivolumab efficacy in second-line urothelial carcinoma patients: exploratory analysis of the phase ii checkmate 275 study. *Ann Oncol* 2017;28:v295–329.
- [11] Sharma P, Retz M, Siefker-Radtke A, et al. Nivolumab in metastatic urothelial carcinoma after platinum therapy (CheckMate 275): a multicentre, single-arm, phase 2 trial. *Lancet Oncol* 2017;18:312–22.

- [12] Mariathasan S, Turley SJ, Nickles D, et al. TGFbeta attenuates tumour response to PD-L1 blockade by contributing to exclusion of T cells. *Nature* 2018;554:544–8.
- [13] Teo MY, Seier K, Ostrovskaya I, et al. Alterations in DNA damage response and repair genes as potential marker of clinical benefit from PD-1/PD-L1 blockade in advanced urothelial cancers. *J Clin Oncol* 2018;36:1685–94.
- [14] Sjobahl G, Eriksson P, Liedberg F, Hoglund M. Molecular classification of urothelial carcinoma: global mRNA classification versus tumour-cell phenotype classification. *J Pathol* 2017;242:113–25.
- [15] Cancer Genome Atlas Research, Network. Comprehensive molecular characterization of urothelial bladder carcinoma. *Nature* 2014;507:315–22.
- [16] Moore PS, Chang Y. Why do viruses cause cancer? Highlights of the first century of human tumour virology. *Nat Rev Cancer* 2010;10:878–89.
- [17] Al-Shibli KI, Donnem T, Al-Saad S, Persson M, Bremnes RM, Busund LT. Prognostic effect of epithelial and stromal lymphocyte infiltration in non-small cell lung cancer. *Clin Cancer Res* 2008;14:5220–7.
- [18] Sharma P, Shen Y, Wen S, et al. CD8 tumor-infiltrating lymphocytes are predictive of survival in muscle-invasive urothelial carcinoma. *Proc Natl Acad Sci U S A* 2007;104:3967–72.
- [19] Chen DS, Mellman I. Elements of cancer immunity and the cancer-immune set point. *Nature* 2017;541:321–30.
- [20] Sweis RF, Spranger S, Bao R, et al. Molecular drivers of the non-T-cell-inflamed tumor microenvironment in urothelial bladder cancer. *Cancer Immunol Res* 2016;4:563–8.
- [21] Robertson AG, Kim J, Al-Ahmadie H, et al. Comprehensive molecular characterization of muscle-invasive bladder cancer. *Cell* 2017;171, 540–56 e25.
- [22] Korpala M, Puyang X, Jeremy Wu Z, et al. Evasion of immunosurveillance by genomic alterations of PPARgamma/RXRalpha in bladder cancer. *Nat Commun* 2017;8:103.
- [23] Allen LE, Maher PA. Expression of basic fibroblast growth factor and its receptor in an invasive bladder carcinoma cell line. *J Cell Physiol* 1993;155:368–75.
- [24] Siefker-Radtke AO, Necchi A, Park SH, et al. First results from the primary analysis population of the phase 2 study of erdafitinib (ERDA; JNJ-42756493) in patients (pts) with metastatic or unresectable urothelial carcinoma (mUC) and FGFR alterations (FGFRalt). *J Clin Oncol* 2018;36:4503.
- [25] Diab A, Hurwitz ME, Cho DC, Papadimitrakopoulou V, Curti BD, Tykodi SS, et al. NKTR-214 (CD122-biased agonist) plus nivolumab in patients with advanced solid tumors: Preliminary phase 1/2 results of PIVOT. *J Clin Oncol* 2018;36:3006.
- [26] Frederick DT, Piris A, Cogdill AP, et al. BRAF inhibition is associated with enhanced melanoma antigen expression and a more favorable tumor microenvironment in patients with metastatic melanoma. *Clin Cancer Res* 2013;19:1225–31.
- [27] Sundstedt A, O'Neill EJ, Nicolson KS, Wraith DC. Role for IL-10 in suppression mediated by peptide-induced regulatory T cells in vivo. *J Immunol* 2003;170:1240–8.
- [28] Baras AS, Drake C, Liu JJ, et al. The ratio of CD8 to Treg tumor-infiltrating lymphocytes is associated with response to cisplatin-based neoadjuvant chemotherapy in patients with muscle invasive urothelial carcinoma of the bladder. *Oncoimmunology* 2016;5: e1134412.
- [29] Mantovani A, Biswas SK, Galdiero MR, Sica A, Locati M. Macrophage plasticity and polarization in tissue repair and remodelling. *J Pathol* 2013;229:176–85.
- [30] Mantovani A, Ming WJ, Balotta C, Abdeljalil B, Bottazzi B. Origin and regulation of tumor-associated macrophages: the role of tumor-derived chemotactic factor. *Biochim Biophys Acta* 1986;865:59–67.
- [31] Murdoch C, Tazzyman S, Webster S, Lewis CE. Expression of Tie-2 by human monocytes and their responses to angiotensin-2. *J Immunol* 2007;178:7405–11.
- [32] Molon B, Ugel S, Del Pozzo F, et al. Chemokine nitration prevents intratumoral infiltration of antigen-specific T cells. *J Exp Med* 2011;208:1949–62.
- [33] Takeuchi H, Tanaka M, Tanaka A, Tsunemi A, Yamamoto H. Predominance of M2-polarized macrophages in bladder cancer affects angiogenesis, tumor grade and invasiveness. *Oncol Lett* 2016;11:3403–8.
- [34] Wang X, Ni S, Chen Q, et al. Bladder cancer cells induce immunosuppression of T cells by supporting PD-L1 expression in tumour macrophages partially through interleukin 10. *Cell Biol Int* 2017;41:177–86.
- [35] Tavazoie MF, Pollack I, Tanquero R, et al. LXR/ApoE activation restricts innate immune suppression in cancer. *Cell* 2018;172, 825–40.e18.
- [36] Balar AV, Castellano D, O'Donnell PH, et al. First-line pembrolizumab in cisplatin-ineligible patients with locally advanced and unresectable or metastatic urothelial cancer (KEYNOTE-052): a multicentre, single-arm, phase 2 study. *Lancet Oncol* 2017;18:1483–92.
- [37] Mezzadra R, Sun C, Jae LT, et al. Identification of CMTM6 and CMTM4 as PD-L1 protein regulators. *Nature* 2017;549:106–10.
- [38] Wolchok JD, Chiarion-Sileni V, Gonzalez R, et al. Overall survival with combined nivolumab and ipilimumab in advanced melanoma. *N Engl J Med* 2017;377:1345–56.
- [39] Sharma P, Callahan MK, Calvo E, et al. Efficacy and safety of nivolumab plus ipilimumab in previously treated metastatic urothelial carcinoma: First results from the phase I/II CheckMate 032 study. Presented at: 2016 SITC Annual Meeting; November 9–13, 2016; National Harbor, MD.
- [40] Pardoll DM. The blockade of immune checkpoints in cancer immunotherapy. *Nat Rev Cancer* 2012;12:252–64.
- [41] Topalian SL, Drake CG, Pardoll DM. Immune checkpoint blockade: a common denominator approach to cancer therapy. *Cancer Cell* 2015;27:450–61.
- [42] Ascierto PA, Melero I, Bhatia S, et al. Initial efficacy of anti-lymphocyte activation gene-3 (anti-LAG-3; BMS-986016) in combination with nivolumab (nivo) in pts with melanoma (MEL) previously treated with anti-PD-1/PD-L1 therapy. *J Clin Oncol* 2017;35:9520.
- [43] Ascierto PA, McArthur GA. Checkpoint inhibitors in melanoma and early phase development in solid tumors: what's the future? *J Transl Med* 2017;15:173.
- [44] Blackburn SD, Shin H, Haining WN, et al. Coregulation of CD8+ T cell exhaustion by multiple inhibitory receptors during chronic viral infection. *Nat Immunol* 2009;10:29–37.
- [45] Grosso JF, Kelleher CC, Harris TJ, et al. LAG-3 regulates CD8+ T cell accumulation and effector function in murine self- and tumor-tolerance systems. *J Clin Invest* 2007;117:3383–92.
- [46] Riaz N, Havel JJ, Makarov V, et al. Tumor and microenvironment evolution during immunotherapy with nivolumab. *Cell* 2017;171, 934–49.e15.
- [47] Herbst RS, Soria JC, Kowanetz M, et al. Predictive correlates of response to the anti-PD-L1 antibody MPDL3280A in cancer patients. *Nature* 2014;515:563–7.
- [48] Levy EM, Bianchini M, Von Euv EM, et al. Human leukocyte antigen-E protein is overexpressed in primary human colorectal cancer. *Int J Oncol* 2008;32:633–41.
- [49] Cohen R, Fayette J, Posner M, et al. Abstract CT158: phase II study of monalizumab, a first-in-class NKG2A monoclonal antibody, in combination with cetuximab in previously treated recurrent or metastatic squamous cell carcinoma of the head and neck (R/M SCCHN):

- preliminary assessment of safety and efficacy. *Cancer Res* 2018;78:CT158.
- [50] Segal NH, Naidoo J, Curigliano G, et al. First-in-human dose escalation of monalizumab plus durvalumab, with expansion in patients with metastatic microsatellite-stable colorectal cancer. *J Clin Oncol* 2018;36:3540.
- [51] Ferrucci PF, Gandini S, Battaglia A, et al. Baseline neutrophil-to-lymphocyte ratio is associated with outcome of ipilimumab-treated metastatic melanoma patients. *Br J Cancer* 2015;112:1904–10.
- [52] Martens A, Wistuba-Hamprecht K, Yuan J, et al. Increases in absolute lymphocytes and circulating CD4+ and CD8+ T cells are associated with positive clinical outcome of melanoma patients treated with ipilimumab. *Clin Cancer Res* 2016;22:4848–58.
- [53] Martens A, Wistuba-Hamprecht K, Geukes Foppen M, et al. Baseline peripheral blood biomarkers associated with clinical outcome of advanced melanoma patients treated with ipilimumab. *Clin Cancer Res* 2016;22:2908–18.
- [54] Simeone E, Gentilcore G, Giannarelli D, et al. Immunological and biological changes during ipilimumab treatment and their potential correlation with clinical response and survival in patients with advanced melanoma. *Cancer Immunol Immunother* 2014;63:675–83.
- [55] Marchioni M, Primiceri G, Ingrosso M, et al. The clinical use of the neutrophil to lymphocyte ratio (NLR) in urothelial cancer: a systematic review. *Clin Genitourin Cancer* 2016;14:473–84.
- [56] Powles T, Jin C, Zheng Y, et al. Tumor shrinkage and increased overall survival are associated with improved albumin, neutrophil lymphocyte ratio (NLR) and decreased durvalumab clearance in NSCLC and UC patients receiving durvalumab. *J Clin Oncol* 2017;35:3035.
- [57] Sharma P, Baron A, Necchi A, Plimack ER, Pal SK, Bedke J, et al. Abstract CT178: Nivolumab monotherapy in patients with advanced platinum-resistant urothelial carcinoma: efficacy and safety update and association between biomarkers and overall survival in CheckMate 275. *Cancer Res* 2018;78, CT178-CT.
- [58] Ornstein MC, Diaz-Montero CM, Rayman P, et al. Myeloid-derived suppressors cells (MDSC) correlate with clinicopathologic factors and pathologic complete response (pCR) in patients with urothelial carcinoma (UC) undergoing cystectomy. *Urol Oncol* 2018;36:405–12.
- [59] Lin RL, Zhao LJ. Mechanistic basis and clinical relevance of the role of transforming growth factor-beta in cancer. *Cancer Biol Med* 2015;12:385–93.
- [60] Roncarolo MG, Bacchetta R, Bordignon C, Narula S, Levings MK. Type 1T regulatory cells. *Immunol Rev* 2001;182:68–79.
- [61] Noguchi T, Ward JP, Gubin MM, et al. Temporally distinct PD-L1 expression by tumor and host cells contributes to immune escape. *Cancer Immunol Res* 2017;5:106–17.
- [62] Lebrun JJ. The dual role of TGFbeta in human cancer: from tumor suppression to cancer metastasis. *ISRN Mol Biol* 2012;2012:381428.
- [63] Jiang Y, Li Y, Zhu B. T-cell exhaustion in the tumor microenvironment. *Cell Death Dis* 2015;6:e1792.
- [64] Shariat SF, Kim J.-H., Andrews B, et al. Preoperative plasma levels of transforming growth factor (1 strongly predict clinical outcome in patients with bladder carcinoma. *Cancer* 2001;92:2985–92.
- [65] Zhang H, Conrad DM, Butler JJ, Zhao C, Blay J, Hoskin DW. Adenosine acts through A2 receptors to inhibit IL-2-induced tyrosine phosphorylation of STAT5 in T lymphocytes: role of cyclic adenosine 3',5'-monophosphate and phosphatases. *J Immunol* 2004;173:932–44.
- [66] Mittal D, Sinha D, Barkauskas D, et al. Adenosine 2B receptor expression on cancer cells promotes metastasis. *Cancer Res* 2016;76:4372–82.
- [67] Martin P, Spitzmueller A, Wu S, et al. Mutually exclusive expression of CD73 and PDL1 in tumors from patients (pt) with NSCLC, gastroesophageal (GE) and urothelial bladder carcinoma (UBC). *J Clin Oncol* 2017;35:3079.
- [68] Gavalas NG, Tsiatas M, Tsiatilonis O, et al. VEGF directly suppresses activation of T cells from ascites secondary to ovarian cancer via VEGF receptor type 2. *Br J Cancer* 2012;107:1869–75.
- [69] Dickinson AJ, Fox SB, Persad RA, Hollyer J, Sibley GN, Harris AL. Quantification of angiogenesis as an independent predictor of prognosis in invasive bladder carcinomas. *Br J Urol* 1994;74:762–6.
- [70] Petrylak DP, de Wit R, Chi KN, et al. Ramucirumab plus docetaxel versus placebo plus docetaxel in patients with locally advanced or metastatic urothelial carcinoma after platinum-based therapy (RANGE): a randomised, double-blind, phase 3 trial. *Lancet* 2017;390:2266–77.
- [71] Atkins MB, McDermott DF, Powles T, et al. IMmotion150: a phase II trial in untreated metastatic renal cell carcinoma (mRCC) patients (pts) of atezolizumab (atezo) and bevacizumab (bev) vs and following atezo or sunitinib (sun). *J Clin Oncol* 2017;35:4505.
- [72] Zelenay S, van der Veen AG, Bottcher JP, et al. Cyclooxygenase-dependent tumor growth through evasion of immunity. *Cell* 2015;162:1257–70.
- [73] Wang D, DuBois RN. Immunosuppression associated with chronic inflammation in the tumor microenvironment. *Carcinogenesis* 2015;36:1085–93.
- [74] Yoshida S, Saito K, Koga F, et al. C-reactive protein level predicts prognosis in patients with muscle-invasive bladder cancer treated with chemoradiotherapy. *BJU Int* 2008;101:978–81.
- [75] Renner K, Singer K, Koehl GE, et al. Metabolic hallmarks of tumor and immune cells in the tumor microenvironment. *Front Immunol* 2017;8:248.
- [76] Papadopoulos KP, Tsai FY-C, Bauer TM, et al. CX-1158-101: a first-in-human phase 1 study of CB-1158, a small molecule inhibitor of arginase, as monotherapy and in combination with an anti-PD-1 checkpoint inhibitor in patients (pts) with solid tumors. *J Clin Oncol* 2017;35:3005.
- [77] Cham CM, Driessens G, O'Keefe JP, Gajewski TF. Glucose deprivation inhibits multiple key gene expression events and effector functions in CD8+ T cells. *Eur J Immunol* 2008;38:2438–50.
- [78] Moon YW, Hajjar J, Hwu P, Naing A. Targeting the indoleamine 2,3-dioxygenase pathway in cancer. *J Immunother Cancer* 2015;3:51.
- [79] Yang C, Zhou Y, Zhang L, Jin C, Li M, Ye L. Expression and function analysis of indoleamine 2 and 3-dioxygenase in bladder urothelial carcinoma. *Int J Clin Exp Pathol* 2015;8:1768–75.
- [80] Yen MC, Lin CC, Chen YL, et al. A novel cancer therapy by skin delivery of indoleamine 2,3-dioxygenase siRNA. *Clin Cancer Res* 2009;15:641–9.
- [81] Smith DC, Gajewski T, Hamid O, et al. Epcadostat plus pembrolizumab in patients with advanced urothelial carcinoma: preliminary phase I/II results of ECHO-202/KEYNOTE-037. *J Clin Oncol* 2017;35:4503.
- [82] Tabernero J, Luke JJ, Joshua AM, et al. BMS-986205, an indoleamine 2,3-dioxygenase 1 inhibitor (IDO1i), in combination with nivolumab (NIVO): updated safety across all tumor cohorts and efficacy in pts with advanced bladder cancer (advBC). *J Clin Oncol* 2018;36:4512.
- [83] Long GV, Dummer R, Hamid O, Gajewski T, Caglevic C, Dalle S, et al. Epcadostat (E) plus pembrolizumab (P) versus pembrolizumab alone in patients (pts) with unresectable or metastatic melanoma: results of the phase 3 ECHO-301/KEYNOTE-252 study. *J Clin Oncol* 2018;36:108.
- [84] Choi BS, Martinez-Falero IC, Corset C, et al. Differential impact of L-arginine deprivation on the activation and effector functions of T cells and macrophages. *J Leukoc Biol* 2009;85:268–77.

- [85] Droge W, Eck HP, Betzler M, Schlag P, Drings P, Ebert W. Plasma glutamate concentration and lymphocyte activity. *J Cancer Res Clin Oncol* 1988;114:124–8.
- [86] Weide B, Martens A, Hassel JC, et al. Baseline biomarkers for outcome of melanoma patients treated with pembrolizumab. *Clin Cancer Res* 2016;22:5487–96.
- [87] Jiang F, Ma S, Xue Y, Hou J, Zhang Y. LDH-A promotes malignant progression via activation of epithelial-to-mesenchymal transition and conferring stemness in muscle-invasive bladder cancer. *Biochem Biophys Res Commun* 2016;469:985–92.
- [88] Pond GR, Niegisch G, Rosenberg JE, et al. New 6-factor prognostic model for patients (pts) with advanced urothelial carcinoma (UC) receiving post-platinum atezolizumab. *J Clin Oncol* 2018;36:413.
- [89] Milstein O, Hagin D, Lask A, et al. CTLs respond with activation and granule secretion when serving as targets for T-cell recognition. *Blood* 2011;117:1042–52.
- [90] Heninger E, Krueger TE, Lang JM. Augmenting antitumor immune responses with epigenetic modifying agents. *Front Immunol* 2015;6:29.
- [91] Wrangle J, Wang W, Koch A, et al. Alterations of immune response of non-small cell lung cancer with azacytidine. *Oncotarget* 2013;4:2067–79.
- [92] Sade-Feldman M, Jiao YJ, Chen JH, et al. Resistance to checkpoint blockade therapy through inactivation of antigen presentation. *Nat Commun* 2017;8:1136.
- [93] Tran E, Robbins PF, Lu YC, et al. T-cell transfer therapy targeting mutant KRAS in cancer. *N Engl J Med* 2016;375:2255–62.
- [94] Romero JM, Jimenez P, Cabrera T, et al. Coordinated downregulation of the antigen presentation machinery and HLA class I/beta2-microglobulin complex is responsible for HLA-ABC loss in bladder cancer. *Int J Cancer* 2005;113:605–10.
- [95] Cha E, Klinger M, Hou Y, et al. Improved survival with T cell clonotype stability after anti-CTLA-4 treatment in cancer patients. *Sci Transl Med* 2014;6:238ra70.
- [96] Snyder A, Nathanson T, Funt SA, et al. Contribution of systemic and somatic factors to clinical response and resistance to PD-L1 blockade in urothelial cancer: an exploratory multi-omic analysis. *PLoS Med* 2017;14:e1002309.
- [97] Rozeman EA, Akkooi ACJV, Menzies AM, Smith M, Scolyer RA, Pronk LM, et al. Multicenter phase 2 study to identify the optimal neo-adjuvant combination scheme of ipilimumab (IPI) and nivolumab (NIVO) (OpACIN-neo). *J Clin Oncol* 2018;36, TPS9606-TPS.
- [98] Gao J, Shi LZ, Zhao H, et al. Loss of IFN-gamma pathway genes in tumor cells as a mechanism of resistance to anti-CTLA-4 therapy. *Cell* 2016;167, 397–404 e9.
- [99] Lesterhuis WJ, Bosco A, Millward MJ, Small M, Nowak AK, Lake RA. Dynamic versus static biomarkers in cancer immune checkpoint blockade: unravelling complexity. *Nat Rev Drug Discov* 2017;16:264.