SCIENCE CHINA
Life Sciences

•REVIEW•

April 2021 Vol.64 No.4: 512–533
https://doi.org/10.1007/s11427-020-1768-y

Current status and future perspectives of immunotherapy in bladder cancer treatment

Zhangsong Wu[1,2†], Jinjian Liu[1,2†], Ruixiang Dai[1,2] & Song Wu[1,2,3*]

[1]*Department of Urological Surgery, The Third Affiliated Hospital of Shenzhen University, Shenzhen University, Shenzhen 518000, China;*
[2]*Shenzhen Following Precision Medical Institute, The Third Affiliated Hospital of Shenzhen University, Shenzhen University, Shenzhen 518000, China;*
[3]*Department of Urological Surgery, The First Affiliated Hospital of Guangzhou Medical University, Guangzhou Medical University, Guangzhou 510120, China*

Received April 21, 2020; accepted June 23, 2020; published online August 26, 2020

The treatment strategy of bladder cancer has evolved not only through the traditional modalities of surgery and chemotherapy but also by immunotherapy over the past several decades. Immunotherapies such as intravesical Bacillus Calmette-Guérin (BCG) vaccines and immune checkpoint blockades (ICBs) are sometimes used for treating patients with bladder cancer, especially those who develop resistance to conventional first-line treatments such as surgery and chemotherapy. Unfortunately, it is a limited number of individuals that see clinical benefits from this approach, and complicating matters more is that many of these patients suffer severe immune-related adverse events (irAEs). If current momentum continues to result in improved response rates and managed irAEs, immunotherapy could be poised to revolutionize the landscape of urothelial carcinoma therapeutics.

bladder cancer, urothelial carcinoma, immunotherapy, predicative biomarkers, adverse events

Citation: Wu, Z., Liu, J., Dai, R., and Wu, S. (2021). Current status and future perspectives of immunotherapy in bladder cancer treatment. Sci China Life Sci 64, 512–533. https://doi.org/10.1007/s11427-020-1768-y

Introduction

Urothelial carcinoma of the bladder (UCB) is the most prevalent malignancy worldwide with ~430,000 new diagnoses each year (Siegel et al., 2019). Smoking, gender, and age are established risk factors for bladder cancer (Antoni et al., 2017). Based on the clinical TNM classification of malignant tumors, bladder cancer can be categorized into three distinctly different entities including non-muscle-invasive bladder cancer (NMIBC) with high recurrence rate, muscle-invasive bladder cancer (MIBC) with a significant risk of metastasis, and metastatic disease (mUCB) with high mortality (Antoni et al., 2017). Approximately 75% of newly diagnosed patients present with NMIBC, with the rest diagnosed with MIBC or mUCB. In general, the 5-year survival rate of NMIBC is up to nearly 90%, but this declines precipitously to no more than 50% in MIBC and less than 15% in mUCB, respectively (Berdik, 2017; Cumberbatch et al., 2018). The treatment paradigm for urothelial carcinoma is thus unique among these three disease states: NMIBC may be managed by transurethral resection of the bladder tumour (TURBT) with or without adjuvant intravesical agents such as Bacillus Calmette-Guérin (BCG) or chemotherapeutic drugs dependent on the unique clinical and pathological factors (Babjuk et al., 2017). The preferred option for MIBC is radical resection with neoadjuvant cisplatin-based chemotherapy (Milowsky et al., 2016). For patients who have progressed to mUCB, intravenous chemotherapy administration is considered the best available treatment option. Although surgery, radiotherapy, chemotherapy, and targeted

†Contributed equally to this work
*Corresponding author (email: doctor_wusong@126.com)

therapies for bladder cancer have been studied over the past three decades, a substantial portion of patients who suffer from bladder cancer still fail to be cured (Chism, 2017). Clinical management for urothelial carcinoma is challenging due to its heterogeneity with diverse histopathology, molecular subtypes, and variable responses to the various therapies (Apolo and Burger, 2015; Ghasemzadeh et al., 2016). Clinicians have been stuck with the same limited range of treatment options to offer patients until the emergence of two significant advancements: (i) a remarkable advancement in UCB biology due to multicenter gene sequencing and expression efforts, and (ii) the introduction of immunotherapy (e.g., immune checkpoint blockades (ICBs) and BCG) (Felsenstein and Theodorescu, 2018). However, the current primary issue with immunotherapy is that only a fraction of patients benefit from it. Thus, it is critical to understand the determinants driving response and causing resistance. In this review, our overarching aim is to provide comprehensive insights into cancer immunotherapy, especially as it relates to bladder cancer.

Categories and mechanism of immunotherapy

Crosstalk between a tumor and the immune system was exploited and recognized as a promising target for cancer treatment as early as 1891 (Coley, 1991). For instance, BCG, the first Food and Drug Administration (FDA)-approved immunotherapy for bladder cancer, has been used to reduce the risk of NMIBC recurrence as the result of stimulating the innate and adaptive immune response (Redelman-Sidi et al., 2014). Based on novel insights into immunotherapy, enormous clinical investigations have thus led to the accelerated development of the following five methods: ICBs, adoptive immunotherapy, cancer vaccines, co-stimulatory receptor agonists, and cytokines (Table 1). Other emerging immunotherapies such as bispecific antibodies and oncolytic viruses are not discussed here; if these are of interest, here are some relevant review articles we recommend (Krishnamurthy and Jimeno, 2018; Lawler et al., 2016; Riley et al., 2019; Smith and Zaharoff, 2016; Twumasi-Boateng et al., 2018).

Immune checkpoint blockades

To date, ICBs are the most prominently investigated among these immunotherapies, and CTLA4 inhibition and PD-1/PD-L1 blockade are the two most promising treatments among current ICB strategies (Figure 1) (Ribas and Wolchok, 2018); other checkpoint inhibitors have been reviewed in detail elsewhere (Granier et al., 2017; Pardoll, 2012; Webb et al., 2018). Immune checkpoints are immune cell surface receptors that regulate immunity. Physiologically, immune checkpoints play vital roles in maintaining immune balance and preventing autoimmunization (Pardoll, 2012). More precisely, PD-1 (CD279), as a transmembrane protein, can not only be expressed on activated T cells but also be slightly expressed on B cells, double-negative ($CD4^{-}CD8^{-}$) T cells in the thymus, monocytes, activated natural killer T cells, and immature Langerhans cells. The ligand of PD-1 is PD-L1 (B7-H1/CD274), which is constitutively expressed at low levels on a wide variety of non-hematopoietic cells and antigen-presenting cells (APCs). Under normal conditions, cells use the PD-L1/PD-1 interaction to suppress the proliferation of $CD8^{+}$ T cells and inhibit T cell receptor (TCR)-mediated cytotoxic function to avoid autoimmunity and resolve inflammation (Song et al., 2019). To escape recognition and elimination from T lymphocytes, cancerous cells can also themselves upregulate PD-L1 expression or stimulate PD-L1 expression in tumor microenvironment (TME) cells, including macrophages, stromal cells, and dendritic cells (DCs) such that PD-L1 targets its receptor PD-1 on antitumor immune response of cytotoxic T cells to render those cells inactive (Alsaab et al., 2017). Consequently, blocking this crosstalk with monoclonal antibodies (mAbs) that bind to either PD-1 or PD-L1 enhances T cell-mediated cancer cell death (Taha et al., 2019). For details, please see these review articles (Boussiotis, 2016; Song et al., 2019). CTLA4, as a co-inhibitory molecule, is the first FDA-approved ICB for cancer therapeutics. The crosstalk between CTLA4 and its ligands (e.g., CD80 and CD86) delivers inhibitory signals for T cell-mediated tumor cell death and promotes tumor survival via the CTLA4 cytoplasmic tail (Webb et al., 2018). Thus, blocking the interaction between CTLA4 and its ligands can facilitate T cells to recognize and obliterate cancer cells. However, it is necessary to mention that the immunotherapeutic effect of anti-CTLA-4 mAbs may extend beyond simple receptor stimulation; indeed, recent preliminary studies have proposed that the activity of anti-CTLA-4 mAbs mainly relies on several additional capacities, including the depletion of regulatory T (Treg) cells in TME by antibody-dependent cell-mediated cytotoxicity or the blockade of trans-endocytosis of B7 on DCs (Arce Vargas et al., 2018; Du et al., 2018).

The clinical impact of checkpoint blockade strategies, providing a survival advantage compared with traditional chemotherapies, has grown considerably, and has been tested in various tumors including melanoma, renal cell carcinoma, non-small cell lung cancer, and urothelial carcinoma over the past several decades. For instance, a Phase III clinical trial that used nivolumab to treat patients with metastatic melanoma demonstrated improved responses, prolonged progression-free survival (PFS) and overall survival (OS) as compared to those with chemotherapy treatment (Robert et al., 2014). Similarly, another Phase III clinical trial illustrated that pembrolizumab was associated with a lower rate of

Table 1 Summary of current immunotherapy being investigated for bladder cancer[a)]

Therapy	Type	Delivery Methods	Disease Targeted	Mechanism	Ref or NCT number
Immune checkpoint inhibitors					
Ipilimumab	CTLA4 mAb	IV IV	Met LMIBC	Normalization	CheckMate032 CheckMate091 NCT03520491 NCT02553642 NCT03844256
Tremelimumab	CTLA4 mAb	IV	Met LMIBC	Normalization	NCT03601455 NCT03234153 NCT03472274 NCT03601455 NCT03682068
Pembrolizumab	PD-1 mAb	IV, Ives	Met, NMIBC LMIBC	Normalization	NCT03504163 NCT02324582
Nivolumab	PD-1 mAb	IV, Ives	Met, NMIBC MIBC	Normalization	NCT03519256
Atezolizumab	PD-L1 mAb	IV, Ives	Met, NMIBC MIBC	Normalization	NCT02792192 NCT03799835
Avelumab	PD-L1 mAb	IV, Ives	Met, NMIBC MIBC	Normalization	NCT03892642
Durvalumab	PD-L1 mAb	IV, Ives	Met, NMIBC MIBC	Normalization	NCT03759496 NCT03528694
KHK2455	IDO mAb	IV	Met	Normalization	NCT03915405
Epacadostat	IDO mAb	IV	Met	Normalization	NCT03832673
Cytokines for lymphocyte promotion					
Intron A	Recombinant IFNα 2b	Ives SC	NMIBC	Immune-enhancement	NCT00004122 NCT00082719
SCH 721015	Recombinant IFNα	Ives	NMIBC	Immune-enhancement	NCT01162785 NCT01687244
Roferon A	Recombinant IFNα	Ives	NMIBC	Immune-enhancement	NCT00082719
ALT-801	Recombinant IL-2	IV, Ives	Met, NMIBC	Immune-enhancement	NCT01326871 NCT01625260
ALT-803	Recombinant IL-15	Ives	NMIBC	Immune-enhancement	NCT03022825 NCT02138734
Imiquimod	Stimulates TNF, IL-12 and IFNγ production	IV	CIS	Immune-enhancement	NCT01731652 NCT03872947
Adoptive T cell therapies					
NY-ESO-1 tumour antigen	TAA-engineered T lymphocytes	IV	BC	Immune-enhancement	NCT02869217 NCT02457650
MAGE-A4	TAA-engineered T lymphocytes	IV	BC	Immune-enhancement	NCT03132922
MAGE-A10	TAA-engineered T lymphocytes	IV	BC	Immune-enhancement	NCT02989064
4SCAR-PSMA 4SCAR-FRa	TAA-engineered T lymphocytes	IV	MIBC	Immune-enhancement	NCT03185468
HER2-AdVST	TAA-engineered T lymphocytes	IV	BC	Immune-enhancement	NCT03740256
MASCT-I	block PD1 receptor	IV	Met	Immune-enhancement	NCT03034304
Cancer vaccines					
Bacillus Calmette-Guérin	Strain of mycobacterium tuberculosis variant bovis	Ives	NMIBC CIS	Immune-enhancement	(Morales et al., 1976)
pPJV7611	Plasmid DNA cancer vaccine	IV	BC	Immune-enhancement	NCT00199849
NY-ESO-1	Peptide vaccine	IV	BC	Immune-enhancement	NCT00070070
AGS-003-BLD	Tumor cell-derived vaccine therapy	IV	MIBC	Immune-enhancement	NCT02944357

(*To be continued on the next page*)

(*Continued*)

Therapy	Type	Delivery Methods	Disease Targeted	Mechanism	Ref or NCT number
CDX-1307	Against hCG-β	IV	MIBC Met	Immune-enhancement	NCT01094496 NCT00709462 NCT00648102
HS-410	NA	SC	NMIBC	Immune-enhancement	NCT02010203
PGV 001	Personalized cancer vaccine	IV	Met	Immune-enhancement	NCT03359239
NYESO-1	Dendritic cell based vaccines	IV	Met	Immune-enhancement	(Smith and Zaharoff, 2016)
Modified vaccinia virus ankara vaccine expressing p53	Vaccine	IV	Met	Immune-enhancement	NCT02432963
Co-stimulatory receptor agonists					
CpG	TLR-9 agonist	IT	NMIBC	Immune-enhancement	(Smith and Zaharoff, 2016)
HP-NAP	TLR-2 agonist	IVes, IT	NMIBC	Immune-enhancement	(Smith and Zaharoff, 2016)
TMX-101	TLR-7 agonist	IVes	NMIBC CIS	Immune-enhancement	NCT01731652
RGX-104	LXR agonist	IV	Met	Immune-enhancement	NCT02922764
DPX-Survivac	Survivin agonist	IV	Met	Immune-enhancement	NCT03836352
Oncolytic viruses					
AdCD40L	Adenovirus vector serotype 5	IT	BC	Immune-enhancement	NCT00891748
CG0070	Oncolytic adenovirus serotype 5	IT	NMIBC	Immune-enhancement	NCT02365818 NCT02143804 NCT00109655 NCT01438112
Instiladrin	Adenovirus vector harbouring the human IFNα 2b	IT	MIBC	Immune-enhancement	NCT02773849

a) IT: intratumoral; IV: intravenously or systemically; Ives: intravesical; BC: bladder cancer; Met: metastatic; MIBC: muscle-invasive bladder cancer; NMIBC: non-muscle-invasive bladder cancer; SC: subcutaneous; CIS: carcinoma in situ; hCG-β: human chorionic gonadotropin-beta; CpG: cytidine-phosphate-guanosine; LXR: liver X receptor; NA: not applicable.

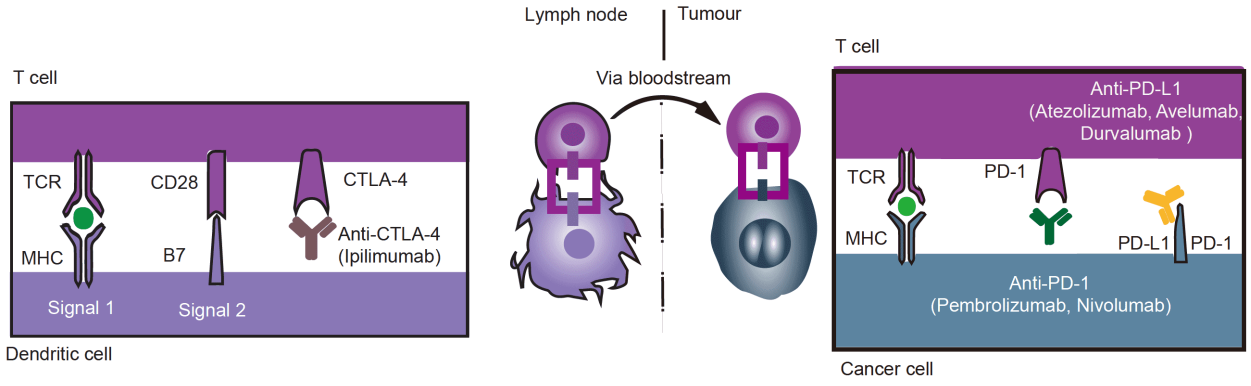

Figure 1 (Color online) CTLA-4 and PD-1 and PD-L1 blockade to induce antitumor responses. (Left) CTLA-4 is presented by an antigen-presenting cell and is a negative regulator of co-stimulation that is required for initial activation of an antitumor T cell in a lymph node upon recognition of its specific tumor antigen. The activation of CTLA-4 can be inhibited by anti-CTLA-4 antibodies. (Right) Once T cells are activated, they circulate throughout the body to find their cognate antigen presented by cancer cells. Upon recognition, triggering of the TCR leads to the expression of negative regulatory receptor PD-1, which turns off the antitumor T cell response. This negative interaction can be blocked by anti-PD-1 or anti-PD-L1 antibodies. Copyright 2018, University of California, Antoni Ribas (Ribas and Wolchok, 2018).

treatment-related adverse events and with significantly longer OS than chemotherapy as a second-line therapy for platinum-refractory advanced urothelial carcinoma (Bellmunt et al., 2017). To date, five FDA-approval PD-L1/PD-1 blockades (pembrolizumab, avelumab, atezolizumab, durvalumab, and nivolumab), one FDA-approved CTLA4 inhibitor (ipilimumab), and one novel IDO-1 inhibitor (epacadostat) are currently in clinical development and have been investigated in ongoing clinical trials for bladder cancer.

Adoptive immunotherapy

Adoptive immunotherapy, also called engineered immune

cells, involves genetically engineering immune cells to recognize tumor-associated antigens (TAAs). In general, adoptive immunotherapy utilizes two approaches involving TCR T cells and chimeric antigen receptor T (CAR T) cells and is perceived as a novel promising approach for patients. For instance, TCR T cells are engineered to express maximal-affinity TCRs that respond to TAAs presented by major histocompatibility complexes (MHCs) on the tumor cell surface (Cohen and Reiter, 2013). The TAAs targeted by TCR T cells include melanoma-associated antigens (MAGEAs), cancer-testis antigens (CTA), and New York esophageal squamous cell carcinoma 1 (NY-ESO-1) antigens (Linnemann et al., 2013). For instance, the NY-ESO-1 targeted T cell therapy has been implemented for melanoma treatment (Hunder et al., 2008), and the therapeutic efficacy can be improved via combination with several cytokines including IL-7, IL-15, and IL-21 or eliminating Treg cells (Klebanoff et al., 2005; Wrzesinski et al., 2010). Ongoing trials are also investigating the safety of the TCR T cell strategy in urothelial carcinoma, such as targeting NY-ESO-1 antigens and MAGEAs. However, the limitations of TCR T cell therapy are of concern, for instance, it is known that tumors have a high likelihood of downregulating MHC molecules and could thus evade TCR-mediated antitumor immunity.

Unlike MHC-dependent TCR T cells, CAR T cells can directly bind to cancerous cell surfaces to induce tumor cell death, such that CAR T therapies circumvent the problem of MHC downregulation (Lim and June, 2017). This provides a new direction for immunotherapy in the treatment of bladder cancer. Unfortunately, although the CAR T cell strategy has garnered a high reputation from its success in several types of cancer including leukemia and lymphoma, clinical results in bladder cancer are not yet available. Additional major considerations for the pervasive application of CAR T cell therapies is that they are technically complex, time intensive, and expensive (Levine et al., 2017).

Cytokine therapeutics

Cytokines are general glycoproteins or polypeptides with molecular weights usually below 30 kD, which could evoke coordinated and robust immunity in response to a foreign antigen by promoting the effects of immunocyte-immunocyte interactions. To date, the four main varieties of cytokines that have been investigated for tumor immunotherapy include interleukins (ILs), interferons, granulocyte-macrophage colony-stimulating factor (GM-CSF), and chemokines. Large studies have demonstrated that ILs have broad proinflammatory effects on antitumor activity in animal tumor models. It is important to note that many ILs, including IL-2, IL-10, IL-12, IL-15, and IL-21, have already been widely explored in urothelial carcinoma through several clinical trials (Smith and Zaharoff, 2016). Despite promising outcomes for those IL molecules in preclinical studies, results from clinical studies are not satisfactory. Interferons released by host cells in response to the presence of several viruses trigger the immune system to eradicate pathogens. In general, human interferons can be classified into three major categories: interferon type I including IFN-α, IFN-β, IFN-ε, IFN-κ, and IFN-ω, interferon type II including IFN-γ in humans, and interferon type III for IFN-λ (Riley et al., 2019). More specifically, IFN-α and IFN-λ, as either a monotherapy or combination therapy, have entered clinical trials for patients with bladder cancer (Smith and Zaharoff, 2016). GM-CSF, which is secreted by a range of cells including macrophages, endothelial cells, T cells, and tumor cells, can stimulate the production of monocytes and granulocytes from hematopoietic progenitors. The antitumor mechanisms by which GM-CSF strengthens immune responses involve two main strategies: (i) facilitating T lymphocyte proliferation and homeostasis and (ii) improving the efficacy of processing and presentation of TAA by supporting DC differentiation (Yan et al., 2017). However, GM-CSF can also act as an immune suppressor as it can expand immature myeloid subsets to cause negative signals for antitumor response, such as myeloid-derived suppressor cells. Similarly, there are several clinical trials studying the use of GM-CSF for patients suffering from bladder cancer, but the outcomes have been insufficient. Chemokines are small, secreted proteins best known for their roles in lymphoid tissue development and mediating immune cell trafficking (Griffith et al., 2014). The potential role of chemokine molecules in serving as a monotherapy or in combination with canonical or immunomediated therapies for cancer patients has also recently gained traction due to its positive effects in modulating cancer stem-like cell properties, tumor cell proliferation, and invasion and metastasis of neoplasms (Mollica Poeta et al., 2019).

Cytokines play pivotal roles in eliminating pathogens and preventing the development of neoplasia. Unfortunately, the therapeutic efficacies resulting from clinical trials failed to meet the promising results derived from preclinical models. However, it is worth noting the limitations of these approaches, including short therapeutic windows, the short half-life of cytokines, and that large quantities are associated with severe toxicities. Thus, in future implementations, two vital aspects should be considered: (i) the delivery method of cytokines to the TEM to avert systemic proinflammatory effects and (ii) combination strategies of immunotherapy with other approaches.

Co-stimulatory receptor agonists

Immunization activities are generally modulated by co-sti-

mulatory and co-inhibitory receptors (e.g., immune checkpoints). In general, co-stimulatory receptors are triggered to stimulate an immune response to extrinsic antigens (Mayes et al., 2018). Co-stimulatory receptors are equally important in mediating anticancer immune responses and driving productive anticancer immunity as compared to co-inhibitory receptors (Peggs et al., 2009); even in terms of cancer immune surveillance, co-stimulatory receptors are more nuanced with respect to duration and timing than co-inhibitory entities to some extent. Generally, synthetic agonistic antibodies can specifically target co-stimulatory receptors leading to activation of downstream intracellular pathways that give rise to the proliferation and survival of T lymphocytes (Peggs et al., 2009). Thus, more attention is paid to the role of co-stimulatory receptor agonists in cancer therapeutics. For instance, Toll-like receptors (TLRs), which are expressed on innate immune cells such as DCs, play a pivotal role in both innate and adaptive immune responses. The level of TLRs is negatively correlated with the invasiveness of bladder cancer. Thus, several TLRs agonists (TLRs-2, TLRs-7, and TLRs-9) serving as therapeutic agents have entered clinical trials. Other co-stimulatory receptors expressed on the surface of APCs, including CD28 and the tumor necrosis factor receptor super family, also show potency in promoting cell growth and anticancer activity (Riley et al., 2019).

Cancer vaccines

Therapeutic cancer vaccines destroy tumors by stimulating the acquired cellular immune response. The most successful vaccine applied for bladder cancer is BCG. In general, BCG is a unique strain of mycobacterium bovis that was developed for treating tuberculosis in 1927 (Calmette et al., 1927). Concurrent with its advancement was an increasing appreciation of the relationship between malignancies and the immune system, Morales et al. first reported exciting outcomes of intravesical BCG for the treatment of NMIBC in 1976 (Morales et al., 1976). To date, intravesical BCG has become the standard therapy for high- or intermediate-risk NMIBC (e.g., stage T1 neoplasms, carcinoma in situ, high-grade cancer, and multiple and recurring stage Ta tumors>3 cm) (Babjuk et al., 2017). It is currently accepted that BCG-induced antitumor activity is a multistep process: BCG first attaches to urothelial cells followed by internalization via micropinocytosis; the epithelium then upregulates MHC-II molecules and releases several cytokines to recruit immunocytes to the TME; such immunocytes generate so-called predominantly Th1 cytokine milieu, which ultimately leads to cancer eradication via induced cytotoxicity through NK cells, $CD8^+$ lymphocytes, and granulocytes. However, 30%–50% of individuals are still refractory to BCG, with recurrence odds ranging from 32.6% to 42.1% and progression rates from 9.5% to 13.4% (Zuiverloon et al., 2012). The alternative treatment for BCG-resistance is radical cystectomy or trimodal therapy, but the survival rate is poor (Babjuk et al., 2017). According to a recent report, the current most reliable predictive markers for intravesical BCG response are still clinical stage and clinical tumor type. Other studies have suggested additional potential predictors including panels of urinary cytokines, fluorescent in situ hybridization (FISH) patterns, and nomograms (e.g., CUETO and EORTC tables) (Kamat et al., 2018). Despite it being more than four decades after the initial application of BCG in bladder cancer, it is still not yet completely understood how BCG stimulates an antitumor immune response or how to choose suitable bladder cancer patients for personalized intravesical BCG. Thus, large amounts of data must be amalgamated from multiple platforms to approach the cusp of understanding the molecular mechanisms that drive BCG-induced tumors and to identify molecular subtypes that may predict response to therapy, as a suggestion for MIBC (Kim et al., 2019) and as a prediction of its response (Gontero et al., 2015).

Aside from BCG, other types of cancer vaccines, such as DCs, cancerous cell lysates, neoantigens, and nucleic acids (Guo et al., 2013), have recently been investigated. DC vaccines in particular, collected from patient-derived DCs following a modification to express TAAs *in vitro*, are the most studied class of cell-based cancer vaccines owing to their ability to directly activate T cells to eliminate cancer cells (Garg et al., 2017).

Cancerous cell lysate vaccines refer to the direct use of the tumor lysates to produce vaccines. Despite their simple production, they tend to show poor immunogenicity. Thus, this is critical in the evaluation and inclusion of effective adjuvants in tumor lysates.

Nucleic acid regimens, including mRNA- and DNA-based vaccines, have been recognized as effective alternatives to conventional methods and mainly depend on delivery tools that transfer exogenous nucleotides into target cells (Riley et al., 2019). In these tools, mRNA or DNA is assimilated by APCs and then translated to catalyze antigen expression, consequently activating the recognition and elimination of tumor cells by T cells presented with such targeted antigens (Riley et al., 2019).

Neoantigens derived from somatic mutation of tumor cells could also serve as vaccines to boost the adaptive immune system (Lauss et al., 2017). One prominent advantage of using neoantigens is that they can specifically target neoplastic cells, thus virtually eliminating off-target effects (Li et al., 2017). For more details regarding these four categories of vaccines, this published review article is recommended (Riley et al., 2019).

Biomarkers of response and prognostic factors

To date, BCG is one of the most effective intravesical therapies for NMIBC (Kamat et al., 2018), and ICBs represent a prominent improvement over previous first- or second-line therapies for bladder cancer. Despite these encouraging results, unfortunately only a small proportion of subjects treated with ICBs or BCG respond to these agents, requiring the development of reliable predictors to provide personalized treatment for those patients who stand to benefit from these immunotherapies. Thus, we focus our attention herein on the molecular markers that would allow prediction of ICB and BCG response given that those are the two most promising types of immunotherapy for bladder cancer.

Immune checkpoint inhibitors

In 2016, the concept of a "cancer immunogram (including general immune status, tumor foreignness, tumor sensitivity to immune effector mechanisms, absence of inhibitory tumor metabolism, immune cell infiltration capacity, absence of checkpoints, and absence of soluble inhibitors)" was brought forward (Blank et al., 2016). This immunogram was soon thereafter applied to lung cancer (Karasaki et al., 2017). Recently, a study has suggested that this concept could also be extended to bladder cancer for the prediction of ICB response (van Dijk et al., 2019). Additionally, other factors including age, gender, general clinical condition, and commensal microorganisms could potentially play vital roles in understanding the influential factors in ICB response and antitumor immunity (Havel et al., 2019). Based on recent work, we suggest herein that the efficacy of ICBs could be influenced by at least four main parameters: clinical characteristics-based biomarkers (including gender, age, and general performance status), blood-based biomarkers (referring to general immune status, absence of soluble inhibitors, and liquid biopsy), tumor tissue-based biomarkers (including tumor foreignness, immune cell infiltration capacity, absence of checkpoints, and absence of inhibitory tumor metabolism), and commensal microorganisms (Buder-Bakhaya and Hassel, 2018; Nakamura, 2019).

Clinical characteristics-based biomarkers. As discussed above, gender and age are established risk factors for bladder cancer. Several studies have demonstrated that both gender and age serve as reasonable predictors for tumor response to ICBs. For instance, one study has revealed that both OS and PFS of male patients treated with ICBs were significantly longer than those of female patients (Wu et al., 2018). As for age, several investigations have identified that the tumor response to ICBs in elderly subjects was significantly higher than for younger subjects in both preclinical and clinical studies (Kugel 3rd et al., 2018; Nosrati et al., 2017). However, the mechanisms behind these effects remain to be elucidated. Further studies are therefore warranted to validate the predictive value of gender and age for ICB antitumor immunity. The general condition of patients might also play an influential role in ICB efficacy. Specifically, the Eastern Cooperative Oncology Group (ECOG) Performance Status is a widely used method to assess the functional status of a patient with scores ranging from 0 to 5. A low ECOG score, representing good clinical condition, is correlated with a high overall response rate and prolonged OS time for patients receiving ICB treatment according to results from some studies (Dobbin et al., 2016; Nakamura et al., 2016).

Blood-based biomarkers. As is known, blood-derived predictors can be tested in a straightforward and minimally invasive manner. Another preferential characteristic is that blood-based biomarkers could enable doctors to indirectly discover a variety of information about a tumor (e.g., general immune status, absence of soluble inhibitors, and liquid biopsy). Thus, there is great interest in the development of serum- or whole blood-derived biomarkers that could characterize immune status to predict response to ICBs. Analysis of general immune status seems mundane but will likely be of relevance in many clinical settings. For instance, immune status-related biomarkers have been associated with ipilimumab response in patients with melanoma, such as enhanced peripheral T cell levels, high absolute eosinophil counts, high peripheral $FoxP3^+$ Treg numbers, and high $CD4^+/CD8^+$ lymphocyte counts (Martens et al., 2016; Simeone et al., 2014; van Dijk et al., 2019). Likewise, low baseline levels of circulating myeloid-derived suppressor cells, low peripheral blood neutrophil to lymphocyte ratio, and high albumin may appear to be associated with longer OS time following treatment with ICBs in UCB patients (van Dijk et al., 2019). Additionally, the diversity of TCR repertoires is correlated with antigen recognition and presentation. In mUCB, one study indicated that lower baseline TCR clonality in peripheral blood increases the probability of durable responses with atezolizumab, suggesting that a higher diversity of TCR receptors in peripheral blood is involved in the increased population of tumor-specific T cells (Snyder et al., 2017).

Moreover, the absence of soluble inhibitors is involved with the outcome of PD-1 blockade because soluble immunosuppressive factors can induce an immunosuppressive and hostile TME (Mantovani et al., 2013). For example, upregulation of IL-10 in the tumor immune microenvironment (TIME) can trigger immunosuppression by inducing Treg polarization (Lin and Zhao, 2015). TGF-β contributes to the induction of angiogenesis and immunosuppression leading to activated tumor escape (Jiang et al., 2015). Additionally, T cell function can be impaired by adenosine (Martin et al., 2017) and vascular endothelial growth factor (VEGF) (Atkins et al., 2017). In patients with locally me-

tastatic bladder cancer treated with PD1/PD-L1 blockade, IFNγ signatures have been investigated and shown to be positively involved with prognosis (Bais et al., 2017). In addition, loss of IFNγ signaling has been correlated with resistance to anti-CTLA-4 therapy (Gao et al., 2016).

Liquid biopsy. The analysis of tumors using biomarkers circulating in fluids such as the blood can potentially play an active role in informing treatment decisions in the emerging field of immune-oncology. The two well-developed biomarkers detected by liquid biopsy are circulating cell-free tumor DNA (ctDNA) and circulating tumor cells (CTCs). A decrease in CTCs and ctDNA is linked to the high treatment response and prolonged PFS and OS for a variety of malignancies treated through the use of ICBs (Buder-Bakhaya and Hassel, 2018; Nakamura, 2019). Furthermore, through CTCs and ctDNA analysis, interesting messages involving underlying tumors are revealed. For instance, a study has shown the potential to guide ICB therapies through the characterization of CTCs if they express specific markers such as PD-L1 in urothelial carcinoma (Anantharaman et al., 2016). In addition, the mutation burden of ctDNA, in accordance with the association of a tumor mutation burden (TMB), has also been assessed, and the results suggest that it is closely correlated with ICB response (Khagi et al., 2017).

Tumor tissue-based biomarkers. In general, there are several vital rate-determining steps that the immune system conducts during the activity of eradicating tumor cells: it initially determines if the immune system could detect tumor cells as "non-self or foreign" (e.g., tumor foreignness); second, the capacity of immune cell infiltration enables immune cells to have a chance to kill tumor cells (e.g., immune cell infiltration capacity). Finally, tumor sensitivity to immune effectors ensures that $CD8^+$ T cells are activated to destroy the tumor cells (e.g., absence of checkpoints and inhibitory tumor metabolism).

To understand the differential ICB treatment response in individuals, it is indispensable to first investigate how the immune system detects and recognizes cancers as non-self or foreign. As is known, the activation of a T cell response by APCs requires the presence of an altered repertoire of MHC-associated peptides. In general, such a repertoire of tumor peptides can be categorized into two broad classes: non-mutated self-antigens and tumor-specific neoantigens (Coulie et al., 2014). More precisely, non-mutated self-antigens are mainly derived from aberrant expression in neoplastic cells as the result of transcriptional or epigenetic reprogramming, which is normally restricted to male gametes and trophoblasts, known as MAGEAs, CTA, and NY-ESO-1 (Gjerstorff et al., 2015). Notably, a somewhat surprising association between dissatisfactory ICB response and upregulation of MAGEA and CTA molecules has been reported (Shukla et al., 2018). Tumor-specific neoantigen peptides are the primary targets of many species, including humans, to activate antitumor immunity, which normally originates from viral or mutated gene product somatic mutations in tumor genomes (Gubin et al., 2014). Some studies have indicated that a higher neoantigen burden and TMB are close correlated with increased efficacy of immunotherapies in diverse cancer types, including bladder cancer (Legrand et al., 2018), small cell lung cancer (Hellmann et al., 2019), independent cohorts of non-small-cell lung cancer (NSCLC) (Forde et al., 2018), melanoma (Goodman et al., 2017), and human papilloma virus (HPV) and negative head and neck squamous cell carcinoma (HNSCC) (Hanna et al., 2018). A common type of mutations in most of these analyses regarding the relationship between ICB response and TMB is in the form of non-synonymous single nucleotide variants (nsSNVs). Both somatic copy number alterations (SCNAs) (Taylor et al., 2018) and indel mutations (Saeterdal et al., 2001) could also further explain the differential response in individuals. However, even when high TMB exists within treated individuals, some patients do not respond to ICBs, suggesting that TMB alone cannot completely discriminate all responders from non-responders and vice versa (Lesterhuis et al., 2017). These identifications clarify that, although the connection between ICB response and TMB is remarkably robust, other factors are involved; for instance, oncogenic viruses including BK virus, HPV, and merkel cell polyomavirus could generate several proteins that may act as immunogenic neoantigens to increase tumor foreignness with capable immunogenic peptides. Indeed, bioinformatic analysis of The Cancer Genome Atlas datasets has identified increased infections with oncogenic viruses in gastric cancer, urothelial carcinoma, and HNSCC and has verified a strong association between endogenous antitumor immunity and virus-driven neoplasms (Rooney et al., 2015). However, the role of viral integration in urothelial carcinoma immunotherapy treatment is currently unclear; thus, validation in multicenter prospective studies is needed prior to clinical application. Moreover, molecular subtypes may be another reliable predictor for determining the therapeutic efficacy of ICBs in bladder cancer (Petrylak et al., 2017; Robert et al., 2014). However, their predictive role is not clear yet due to the lack of uniform approach in molecular categorization and an unclarified mechanism of how molecular subtypes influence response rates. Multicenter data are thus required to better determine molecular signatures for predicting immunotherapy response in the future. Other surrogate factors for assessing ICB response include mutations in genes involved in DNA repair mechanisms (e.g., mismatch repair defects and microsatellite instability) or other specific mutated genes (e.g., β2M, JAK2, POLE, STK11, SERPINB3, SERPINB4, and APOBEC3A/3B) (Aggen and Drake, 2017; Mehnert et al., 2016; Rizvi et al., 2015).

Another potential prognostic indicator of ICB response is

tumor-infiltrating lymphocytes density (Fridman et al., 2012). For instance, tumor-infiltrating $CD8^+$ T cells show predictive characteristics in antitumor immune response, and their emergence in the TME has been correlated with longer survival across diverse cancer types (Al-Shibli et al., 2008), including UCB (Sharma et al., 2007). For instance, the IMvigor210 study showed that $CD8^+$ T lymphocyte density in a local tumor was positively correlated with atezolizumab response in advanced/metastatic urothelial cancer (Rosenberg et al., 2016). Beyond the advent of antitumor immunocytes, other subpopulations of immune cells like Treg and tumor-associated macrophages may facilitate cancer progression by activating several tumor-associated signals, including angiopoietin-2, MCSF, CCL2, and VEGF, to induce an immunosuppressive environment (van Dijk et al., 2019). However, the precise role of suppressive immunocytes in the tumor environment has not been well established in urothelial carcinoma.

As mentioned above, a vital step for antitumor response is the activation of $CD8^+$ T lymphocytes, which is initiated following the recognizing of antigens presented by APCs. Thus, the destruction of TAA recognition and presentation signaling pathways in neoplastic cells, including significant mutations in the HLA-I coding gene, which could downregulate HLA-I expression and heterozygosity or induce the disruption of HLA-I function, could induce tumor immune escape. For example, deletions and point mutations in β_2-microglobulin (β_2M), a crucial molecule for MHC I molecular assembly, account for up to nearly 30% of ICB resistance in melanoma (Sade-Feldman et al., 2017). In urothelial cancer, coordinated transcriptional dual downregulation of HLA components (such as antigen-presenting machinery and β_2M) (Romero et al., 2005) is essential in irreversible HLA loss, but the evidence correlating mutations in the HLA-I coding gene and ICB response is still lacking.

The efficacy of ICBs understandably relies on the expression level of immune checkpoints as well. It is estimated that the expression of PD-L1 is between 20% and 30% in UCB tissue samples. Importantly, PD-L1 expression, as measured by immunohistochemical analysis in urothelial tumors of the bladder, is involved in increased all-cause mortality and a higher pathologic stage at resection, suggesting that increased levels may be associated with more aggressive disease. These data show that PD-L1 expression is prognostic in terms of clinical outcome, whereas even in a population enriched for upregulation of PD-L1 expression there are still numerous patients who do not respond to ICBs (Ribas and Tumeh, 2014). Nevertheless, under certain circumstances, patients without detectable levels of PD-L1 still positively respond to PDL1-targeted treatments (Drake et al., 2016). Thus, this evidence indicates the weakness of solely opting to use the expression of PD-L1 as a biomarker for predicting response and requires a comprehensive multiparameter approach. Apart from PD-1/PD-L1, various other immune checkpoints are currently undergoing investigation in clinical trials of mUCB, including CTLA-4, T cell immunoreceptor with Ig and ITIM domains (TIGIT) (Riaz et al., 2017), T cell immunoglobulin and mucin-domain containing-3 (TIM-3) (Topalian et al., 2015), lymphocyte activation gene 3 (LAG-3) (Riaz et al., 2017), Siglec-15 (Wang et al., 2019), and NKG2A (Segal et al., 2018). These effectors may be induced by treatment targeting PD-1/PD-L1 or may be expressed at baseline (Topalian et al., 2015). Research assessing the combination of multiple ICBs will hopefully result in enhanced ICB efficacy.

Finally, tumor metabolism is also closely correlated with antitumor immunity. As is known, the glycometabolism of tumor cells differs from that of normal cells. Specifically, the conversion of pyruvate to lactate occurs even under conditions of sufficient oxygen, leading to a high level of lactate dehydrogenase (LDH) in TME (Blank et al., 2016). Some studies have suggested that lactic acid and low local pH can impair crucial T cell functions. Moreover, the high energy demand of cancer cells also compromises the function of T cells in TME because the fuel in the TME is insufficient for supplying T cell antitumor activities. Thus, intratumoral pH level and glucose depletion deserve attention as potential biomarkers for antitumor immunity. Furthermore, other metabolism-related enzymes (e.g., IDO1, cyclooxygenases, arginase, glutaminase, oxidative phosphorylation, and glucose transporters) potentially show predictive value (Renner et al., 2017); thus, the exact association between their molecular levels and response to ICBs warrants further investigation.

Commensal microorganisms. One point worth emphasizing is that, in human evolution, pathogens are considered as one of the strongest selective forces, and the long persistent crosstalk between microorganisms and host have likely evolved a variety of immunologically related genetic variations found in humans (Fumagalli et al., 2011). Physiologically and pathologically, it is anticipated that microbiota play a vital role in influencing human immunity (Garrett, 2015; Zitvogel et al., 2016). Indeed, the efficacy of ICBs appears to be in close accordance with the patient's gut microbiome in both mouse models (Vétizou et al., 2015) and humans (Zitvogel et al., 2018). One investigation in particular has indicated that the ratio of immunotherapy response-associated bacteria to immunotherapy resistance-associated bacteria could contribute to definitively stratifying responders from non-responders (Matson et al., 2018). Intriguingly, patients treated with antibiotics during the course of ICB therapy had minimized antitumor activity (Routy et al., 2018). These results are tantalizing and implicate the potential role of gut microbiota in influentially affecting ICB response and antitumor immunity.

BCG

Since its first application in 1976 (Morales et al., 1976), the clinical effects of intravesical BCG therapy for urothelial carcinoma have been confirmed. In fact, intravesical BCG is the standard care for high-risk and now even for intermediate-risk NMIBC (Babjuk et al., 2011). Still, nearly 30%–50% of patients are refractive to BCG treatment; even worse, 15% progress to MIBC (Zuiverloon et al., 2012). Clinically applicable and reliable tools to predetermine NMIBC recurrence and progression are desperately required to decrease the mortality, morbidity, and expenditure budget for bladder cancer (Kamat et al., 2018). Multiple factors are involved with the high-risk of BCG unresponsiveness. First, high intra- and inter-observer variability among pathologists, leading to incorrect histologic staging of tumors, could help to explain BCG failure; second, full compliance with the current protocol is affected by BCG-associated side effects (van Rhijn et al., 2010). In 2012, Zuiverloon et al. published a review that hallmarks predicting response to BCG high-risk urothelial carcinomas patients. In this review, the markers could be mainly divided into four groups: clinicopathologic, intracellular cell cycle, inflammatory, and gene polymorphisms. The authors indicated that the measurement of urinary IL-2 levels seems to be the most potent marker of all of the clinically predictive parameters (Zuiverloon et al., 2012). In addition, recent data show that the best predictors of BCG response are clinicopathologic features including tumor grade and stage, and FISH patterns of cytologic anomalies as well as panels of urinary cytokines appear to be promising biomarkers (Kamat et al., 2018).

Toxicity profiles

Immunotherapy stimulates and arouses the body's innate and adaptive immune response to defend against neoplasm; unfortunately, it can have inflammatory side effects that are often termed irAEs (Table 2) (Baxi et al., 2018; Delanoy et al., 2019; Haanen et al., 2018; Johnson et al., 2018; Postow et al., 2018). ICBs, for example, have recently shown remarkable benefits toward multiple cancer treatments. Meanwhile, they may also initiate autoimmune activity against potentially any organ because immune checkpoints could arrest autoimmunity by inducing T cell exhaustion at sites of inflammation (PD-1/PD-L1) or by prohibiting DC-mediated T cell activation (CTLA-4) under normal physiologic conditions.

ICB drugs are overall less toxic than standard chemotherapy for mUCB, but side effects still exist (Bellmunt et al., 2017). For instance, systemic T cell activation induced by an anti-CTLA-4 antibody strongly correlates with an immunotherapy-related adverse effect that mitigates its therapeutic functionality. Although irAEs have been less frequently and less severe as reported with PD1/PD-L1 blockade than with anti-CTLA4 inhibition (Doyle et al., 2019; Hodi et al., 2010), irAEs induced by anti-PD-1 or anti-PD-L1 therapy have actually been reported in clinical trials. Generally, the incidence rate of irAEs varies from 12% to 17% (Doyle et al., 2019). The most commonly reported organ-specific irAEs are the endocrine glands, gastrointestinal, skin, tract, and liver. Less often, the cardiovascular and central nervous system, musculoskeletal, hematologic systems, and pulmonary system are involved (Table 2) (Haanen et al., 2018; Postow et al., 2018). Other adverse events, such as musculoskeletal problems derived from systemic inflammation, have also been described to negatively influence the quality of life (Cappelli et al., 2017). Moreover, a recent case report showed that checkpoint inhibitors seem to fuel tumor "hyperprogression" in some patients, especially in patients with several gene mutations such as EGFR or extra copies of MDM2 or MDM4 (Kaiser, 2019).

Causes of organ-specific irAEs are unclear, but they are thought to represent bystander effects from activated T cells and are consistent with the mechanism of action of ICBs. Specifically, one set of studies suggests that the same antigens present on both inflamed organs and tumor cells perhaps accounts for irAEs, which is closely dependent on antitumor response. For instance, in a report of two cases of myocarditis, the investigator identified similar T cell clones in both infiltrating T cells of normal tissue (myocardium) and tumor tissue, and thus speculated that common antigens are shared between the myocardium and tumor (Johnson et al., 2016). Similarly, cross-reactivity between T cells directed against normal tissue and T cells directed against a related antigen in tumors was reported in a study involved with a depigmentation disorder called vitiligo (Byrne and Fisher, 2017). Other studies suggest there may be mechanisms of autoimmune toxicity that are independent of antitumor response. For example, several reports have found that normal pituitary glands express CTLA-4 and suggest that such pre-existing pituitary-associated CTLA-4 antigen expression may be one cause of hypophysitis during ipilimumab treatment, which can be explained by the activation of local complement-mediated inflammation without representing a shared effect from antitumor activity (Caturegli et al., 2016; Iwama et al., 2014). Beyond the pre-existing antigen, pre-existing antibodies could also contribute to the organ dysfunctions; more precisely, patients who have pre-existing antithyroid antibodies could develop a deteriorating situation in thyroid disorders after receiving anti-PD-1 therapy, which can be explained due to the increase in pre-existing antithyroid antibodies correlated with humoral immunity (Osorio et al., 2017). As such, elucidating the mechanisms of irAEs presents a formidable opportunity to understand the mole-

Table 2 Immune-related adverse events and their management for urothelial carcinoma[a)]

Toxicities grade	irAEs	Possible symptoms	Frequency of organ-specific irAEs (%)				Grading	Management
			Anti-PD-1		Anti-PD-L1			
			all	3/4	all	3/4		
Skin	Rash	Maculopapular rash. Pruritus.	7	1	1–7	<1–2	Grade 1–2: Covers ≤30% of body surface area±Pruritus. Grade 3–4: ≤30% of body surface area±Pruritus Limits self-care ADLs life-threatening consequences.	Grade 1–2: Continue ICB Start topical steroid cream, anti-itch cream, oral antihistamine; cold compresses, oatmeal baths; If rash persists for >1 week or interferes with daily living, start moderate potency steroid cream. Grade 3: If serious or with desquamation, hold ICB Start MPS 1.0–2.0 mg $(kg{\cdot}day)^{-1}$. If imAE resolves to Grade 1 or less, taper steroid dose over 4–6 weeks and consider resuming ICB.
	Pruritus		9–20	NR	1	<1		
Gastroint-estinal	Diarrhoea	Abdominal pain. Cramping Change in bowel pattern. Increase in ostomy output. Mucous or blood in stool.	2–5	1–2	<1–2	0–1	Grade 1: <4 stools over baseline. Asymptomatic. Grade 2: 4–6 stools over baseline. IV fluids <24 h indicated colitis with abdominal pain, blood in stool, no ADL interference. Grade 3: ≥7 stools/day over baseline. IV fluids >24 h. Interference with ADLs severe abdominal pain, peritoneal signs; medical intervention indicated. Grade 4: life-threatening perforation.	Grade 2: Hold ICB until Grade 1; If recurrent or if lasting >5 days, consider starting steroid dose (prednisone 1.0–2.0 mg $(kg{\cdot}day)^{-1}$ or equivalent). Grade 3: Hold ICB. Start MPS 1.0–2.0 mg $(kg{\cdot}day)^{-1}$. If imAE resolves to Grade 1 or less, taper steroid dose over 4–6 weeks and consider resuming ICB. Grade 4: Permanently discontinue ICB; Start steroid followed by tapering as for Grade 3. Refractory: Consider additional immunosuppressant (e.g. infliximab).
	Colitis	Abdominal pain Cramping. Change in bowel pattern. Increase in ostomy output. Mucous or blood in stool.	2–5	1–2	1	0–1		
Endocrine (thyroid)	Hypothyroi-dism	Weight loss/gain. Feeling hot/cold. Changes in mood behavior. Fatigue. Increased sweating. Faster/slower heart rate. Diarrhoea/constipation. Hair loss. Heat/cold intolerance.	6–11	NR	3–6	0–1	Grade 1: Asymptomatic. Grade 2: Symptomatic. Requiring hormone replacement or medical intervention. Grade 3–4: Severe symptoms, life-threatening. Requiring hospitalization or urgent medical intervention Limiting self-care ADL.	Grade 1: Continue ICB. Grade 2–4: Hold ICB; Manage symptoms; Hyperthyroidism; Medical management for severe symptoms; Hypothyroidism. Initiate hormone replacement if TSH >10. Adjust replacement hormone dosing to maintain T4 in mid-range. Consider resuming ICB when symptoms resolve to ≤Grade 1.
	Hyperthyro-idism		1	NR	2–4	NR		
	Thyroiditis		1<	NR	NR	NR		
Endocrine (HPA axis)	Hypophysis	Hypophysis: visual changes, headaches, fatigue, weakness confusion, hallucinations, memory loss, labile mood, insomnia, anorexia. Adrenalitis: fatigue, malaise, hypotension, vague gastrointestinal symptoms, weight loss, hypoglycaemia.	1	1<	1<	NR	Grade 1: Asymptomatic. Grade 2: Symptomatic. Grade 3–4: Severe symptoms. Requiring hospitalization or urgent medical intervention. Limiting self-care ADL. Life-threatening.	Grade 1: Continue ICB. Grade 2–4: Hold ICB; Hypophysis; Stress dose IV MPS with mineral corticoid if also adrenal crisis; Hormone repletion; Adrenalitis; Hormone repletion (may require lifetime hormone replacement). Requirement for stress dosing of steroid. If imAE resolves to Grade 1 or less, taper steroid dose over 4–6 weeks and consider resuming ICB.
	Adrenal insufficien-cy		<1–2	1<	<1–2	1<		
	Diabetes mellitus		1<	NR	1<	NR		

(*To be continued on the next page*)

(*Continued*)

Toxicities	irAEs	Possible symptoms	Frequency of organ-specific irAEs (%)				Grading	Management
			Anti-PD-1		Anti-PD-L1			
grade			all	3/4	all	3/4		
Hepatic	AST increased	Nausea. Decreased appetite. Fever. Vague abdominal discomfort. RUQ pain. Dehydration. Jaundice. Bleeding bruising. Dark urine.	NR	NR	<1–2	<1	Grade 1: AST or ALT>ULN to 3×ULN and/or total bilirubin>ULN to 1.5×ULN. Grade 2: AST or ALT>3× to<5×ULN and/or total bilirubin >1.5–3×ULN. Grade 3–4: AST or ALT>5×ULN and/or total bilirubin>3×ULN.	Grade 1: Continue ICB. Stop hepatotoxic medications. Grade 2: Hold ICB. Monitor laboratory results (e.g. 3× per week). Consider MPS 0.5–1.0 mg $(kg\cdot day)^{-1}$. If imAE resolves to Grade 1, consider resuming treatment after steroid tapered over 4–6 weeks. Grade 3–4: Permanently discontinue ICB. MPS 1.0–2.0 mg $(kg\cdot day)^{-1}$ with taper as listed for Grade 2. Refractory/recurrent: Consider additional immunosuppressant (eg, mycophenolate mofetil).
	ALT increased		NR	NR	1	NR		
	Hepatitis		1	<1	1–2	1		
Lung	Pneumonitis	Coughing. Wheezing. Tachypnoea/tachycardia. Shortness of breath at exertion. Hypoxia. Increased oxygen requirements. Radiographic changes.	2–4	1–2	1	0–1	Grade 1: Asymptomatic. Clinical or diagnostic observations. Grade 2: Mild-to-moderate symptoms, limiting instrumental ADLs. Medical intervention indicated. Grade 3–4: Severe symptoms limiting self-care ADLs. New or worsening hypoxia. Life-threatening urgent intervention indicated.	Grade 1: Consider holding ICB. Oxygen support; albuterol nebulizer, PRN; steroid inhaler, PRN. Monitor every 2–3 days. Grade 2: Hold ICB. MPS 1.0–2.0 mg $(kg\cdot day)^{-1}$. Daily monitoring. If imAE resolves to baseline, consider resuming treatment after steroid tapered over 4–6 weeks. Grade 3–4: Permanently discontinue ICB. MPS 1.0–2.0 mg $(kg\cdot day)^{-1}$ increasing to 2.0–4.0 mg $(kg\cdot day)^{-1}$ if needed; taper as listed for Grade 2. Refractory: Consider additional immunosuppressant (e.g. infliximab).
Renal	Renal diseases	Often asymptomatic. Increase in serum creatinine. Vague nausea. Emesis. Decreased urine output. Cloudy/dark urine. Blood in urine. Ankle swelling.	1	0–1	1	0–1	Grade 1: Creatinine level increased >0.3 mg dL^{-1}; creatinine 1.5–2.0>baseline. Grade 2: Creatinine 2.3× above baseline. Grade 3: Creatinine>3× baseline or >4.0 mg dL^{-1}. Grade 4: Life-threatening.	Grade 1: Continue ICB. Hold all nephrotoxic drugs. Hydration. Grade 2–3: Hold ICB. Monitor serum creatinine every 2–3 days. MPS 0.5–1.0 mg $(kg\cdot day)^{-1}$; if no improvement increase to 1.0–2.0 mg $(kg\cdot day)^{-1}$. If imAE resolves to Grade 1, consider resuming treatment after steroid tapered over 4–6 weeks. Grade 4: Permanently discontinue ICB. MPS 1.0–2.0 mg $(kg\cdot day)^{-1}$ with taper as listed for Grade 2–3.

a) ICB, immune checkpoint blockades; ADL, activities of daily living; ALT, alanine transaminase; AST, aspartate transaminase; imAE, immune-mediated adverse event; IV, intravenous; MPS, methylprednisolone; PRN, as needed; RUQ, right upper quadrant; TSH, thyroid-stimulating hormone; ULN, upper limit of normal.

cular underpinnings and identify predictive biomarkers of irAEs. Indeed, scientists have identified several biomarkers with merit for predicting the occurrence of irAEs during the ICB treatments. For instance, body composition parameters (Daly et al., 2017), gender (Valpione et al., 2018), T cell repertoire, gut microbiome (Chaput et al., 2017; Dubin et al., 2016), and cytokines (e.g., IL-6 and IL-17) (Callahan et al., 2011; Valpione et al., 2018) may be involved in the pathophysiology of ipilimumab-induced adverse events. Pre-existing autoantibodies (e.g., antithyroglobulin and type 1 diabetes antibodies), blood cell counts (e.g., total white blood cells and absolute lymphocyte and eosinophil numbers) (Fujisawa et al., 2017; Schindler et al., 2013) and cytokines (e.g., IL-6) (Tanaka et al., 2017) are responsible for the irAEs caused by anti-PD-1/PD-L1 drugs.

Recommendations have therefore been published to carefully monitor patients treated with ICBs (Table 2) (Haanen et al., 2018; Nagai and Muto, 2018): First is education of patients and health-care professionals; patients should be educated using patient-specific educational materials about the symptoms and signs of irAEs and also be informed that irAEs may occur at any time, which is vital for early recognition and successful management of irAEs; to detect patients who potentially suffer from irAEs early, oncologists should be aware of the symptom profile of irAEs while providing ICBs for patients, especially those with autoimmune diseases or chronic viral infections. Second is the necessity for checklists describing laboratory tests and symptoms of irAEs that could provide useful references for any new symptoms occurring during ICBs, such as a study defining an "Immunotherapy Baseline Checklist" and "Examination Checklists for irAEs" based on physical examination, laboratory tests, and imaging methods (Nagai and Muto, 2018). Moreover, once irAEs are identified, their prompt and judicious management is important. Currently, the majority of irAEs are effectively treated by delaying administration of the checkpoint inhibitor or by inducing temporary immunosuppression with agents including oral glucocorticoids or other additional immunosuppressive agents (e.g., infliximab, mycophenolate, immunoglobulins, and mTOR inhibitors) in more severe cases (Postow et al., 2018). Finally, additional multidisciplinary cooperation among oncologists, other internal medicine specialists, and emergency medicine physicians is needed, which could contribute to oncologists learning the appropriate management of specific immune toxicities and also for organ specialists to increase their knowledge regarding these new drug-mediated toxicities (Nagai and Muto, 2018). In addition, other immunotherapies including BCG, cytokines, and oncolytic viruses can also result in irAEs; these irAEs and their management approaches are published in multiple review articles (Cousin et al., 2018; Gan et al., 2013).

Combination strategies to enhance the therapeutic effects of immunotherapy

As already discussed, not all people are sensitive to ICB treatment. The reasons behind ICB failure are not clearly understood. Aberrations in priming signals, activation of negative signals by recruitment of Treg cells or immunosuppressive cytokines, and deficiencies in antigens or APCs and stromal interactions are possible measures adopted by tumors to resist antitumor immune responses (Massari et al., 2018). Fortunately, various studies have identified that the combination ICBs with other drugs could be a key option to overcome cancer immune evasion. Nowadays, there are a number of ongoing clinical trials combining ICBs with nearly all available cancer therapeutics, including radiotherapy, chemotherapy, targeted therapy, local therapy, and other immunotherapies, such as adoptive cell therapies, to assess the effects of combinatorial therapies (Table 3) (Tang et al., 2018).

Radiotherapy and chemotherapy could play vital roles in overcoming immune-tumor escape. Tumor irradiation fiercely triggers inflammation at the site of application, stimulates the expression of MHC I and adhesion molecules, and leads to the activation of $CD8^+$ T cells (Massari et al., 2018). A phase I–II study is underway exploring the safety and efficacy of fixed-dose stereotactic body radiotherapy with concurrent or sequential pembrolizumab in individuals with mUCB (Sundahl et al., 2017). Additionally, the combination between radiotherapy and durvalumab is also under investigation as an adjuvant treatment. It is likely that chemotherapy could also show synergic effects with ICBs (Gandhi et al., 2018). Possible mechanisms by which chemotherapy can overcome immune-tumor escape may include the following: (i) The lytic effect induced by chemotherapeutic drugs could lead to the presentation of antigens and neoantigens resulting in $CD8^+$ T cell activation; (ii) the depletion of immunosuppressive cells in TME could conserve immune power against tumors; (iii) it could contribute immune cells to better penetrate tumor stroma. Indeed, there is reliable evidence to suggest a higher response to immunotherapy in patients who have previously been treated by chemotherapy than for those who have not (Zitvogel et al., 2011). This combinatorial approach is currently being explored in several clinical trials on bladder cancer.

Local therapy means directly injecting a variety of chemical and biological agents into tumor sites. Such agents include RIG-I-like receptor agonists, Toll-like receptor agonists, STING pathway modulators, and oncolytic viruses (Sanmamed and Chen, 2018). It is believed that local therapy initiates innate immune response so as to trigger adaptive immunity, makes more tumor antigens available from the death of tumor cells, and causes a better T cell response by generating a more inflammatory environment. Thus, it seems

Table 3 Immunotherapy (monotherapy and combination strategies) for the treatment of urothelial[a)]

Types	Clinical setting	mechanism	Interventions and treatments	Clinical setting: phase (*n*)	Primary outcome	NCT identifier
For locally advanced or metastatic urothelial carcinoma						
Monotherapy	First-line immunotherapy for cisplatin-ineligible patients	ICBs	Nivolumab	II (*n*=120)	ORR	NCT02553642
			Pembrolizumab	II (*n*=100)	ORR PFS OS	NCT02335424
			Atezolizumab	III (*n*=1,200)	ORR PFS OS	NCT02807636
			Ipilimumab	II (*n*=3)	OS	NCT01524991
		Cytokine	ALT-801	Ib–II (*n*=90)	Safety	NCT01326871
	Second-line therapy for metastatic disease	ICBs	Atezolizumab	IV (*n*=NA)	NA	NCT02589717
			Atezolizumab	III (*n*=931)	OS	NCT02302807
			Atezolizumab	II (*n*=439)	ORR	NCT02108652
			Avelumab	III (*n*=668)	OS	NCT02603432
			Nivolumab	II (*n*=242)	ORR	NCT02387996
			Pembrolizumab	II (*n*=350)	ORR	NCT02335424
			Pembrolizumab	III (*n*=470)	OS PFS	NCT02256436
			Pembrolizumab	II (*n*=200)	PFS	NCT02500121
			Pembrolizumab	III (*n*=542)	ORR OS	NCT02256436
			Pembrolizumab	Ib (*n*=33)	ORR OS	NCT01848834
			Avelumab	I (*n*=1,670)	Safety	NCT01772004
		Co-stimulatory receptor agonists	MGD009	I (*n*=114)	Safety	NCT02628535
			MGA271	I (*n*=114)	Safety	NCT01391143
Combination strategies	First-line immunotherapy for cisplatin-ineligible patients	Dual ICBs	Nivolumab+ipilimumab	I–II (*n*=130)	ORR	NCT01928394
			Nivolumab+ipilimumab	III (*n*=897)	OS PFS	NCT03036098
			Durvalumab+tremelimumab	I (*n*=380)	Safety ORR	NCT02261220
			Durvalumab+tremelimumab	III (*n*=1,200)	OS	NCT02516241
		Oncolytic virus+ICBs	CVA21+Pembrolizumab	I (*n*=90)	Safety	NCT02043665
		Cytokine+ICBs	NKTR+nivolumab	NA	Safety and toxicity, ORR	NCT02983045
			NKTR-214+nivolumab	1–II (*n*=393)	Safety ORR	NCT03435640
			NKTR-214+(atezolizumab (OR pembrolizumab))	I (*n*=75)	Safety	NCT0313889
		Antiangiogenesis+ICBs	Ramicirumab+pembrolizumab	I (*n*=NA)	Safety	NCT02443324
			Bevacizumab+atezolizumab	II (*n*=2)	OS	NCT03133390
			Cabozantinib+nivolumab±ipilimumab	I (*n*=NA)	Safety	NCT02496208
			Axitinib+avelumab	II (*n*=NA)	ORR	NCT03472560
		FGFR+ICBs	AZD4547+durvalumab	I (*n*=NA)	Safety	NCT02546661
			Rogaratinib+atezolizumab	1–II (*n*=210)	Toxicity PFS	NCT03473756
		PARP inhibitor +ICBs	Olaparib+durvalumab	I (*n*=NA)	Safety	NCT02546661
			Olaparib+durvalumab	II (*n*=150)	PFS	NCT03459846
		Antibody-drug conjugate+ICBs	Enfortumabvedotin+(pembrolizumab OR atezolizumab)	I (*n*=159)	Safety Toxicity	NCT03288545
	Second-line therapy for metastatic disease	Dual ICBs	Nivolumab+ipilimumab	III (*n*=897)	OS PFS	NCT03036098
			Durvalumab+tremelimumab	III (*n*=1,200)	OS	NCT02516241
			Durvalumab+MEDI0680	I (*n*=90)	ORR	NCT02118337
			Tremelimumab, followed by Durvalumab vs. combo	II (*n*=76)	ORR	NCT02527434
			Lirilumab+nivolumab	I (*n*=NA)	Safety	NCT01714739
		Metabolic interventions +ICBs	Nivolumab+cabozantinib	I (*n*=75)	ORR	NCT02496208
			Pembrolizumab+INCB024360	1–II (*n*=NA)	ORR	NCT02178722
			aCP-196+pembrolizumab	II (*n*=74)	ORR	NCT02351739
			EphB4-HAS+pembrolizumab	II (*n*=64)	OS	NCT02717156
			Pembrolizumab+vorinostat	Ib–II (*n*=42)	Safety	NCT02619253
			CPI-444±atezolizumab	I (*n*=NA)	Safety	NCT02655822
		Cytokine	PLX3397+pembrolizumab	Ib–II (*n*=NA)	Safety	NCT02452424
			Ulocuplumab+nivolumab	Ib–II (*n*=NA)	Safety	NCT02472977
			Interferon gamma+nivolumab	I (*n*=15)	Safety	NCT02614456
		Co-stimulatory receptor agonists+ICBs	Urelumab+nivolumab	Ib–II (*n*=200)	Safety	NCT02253992
			Varlilumab+atezolizumab	Ib–II (*n*=55)	Safety ORR	NCT02543645
			Ipililumumab+MGA271	I (*n*=NA)	Safety	NCT02381314
		Antiangiogenesis+ICB	Lenvatinib+pembrolizumab	Ib–II (*n*=150)	Safety ORR	NCT02501096
		vaccines+ICBs	CDX-1401+Poly ICLC+ Pembrolizumab	Ib–II (*n*=26)	Safety	NCT02661100
			P53MVA+pembrolizumab	I (*n*=15)	Safety	NCT02432963
		PARP+FGFR+ICBs	Durvalumab±AZD4547, Olaparib, AZD1775	Ib (*n*=40)	Safety	NCT02546661
		Oncolytic virus+ICBs	Enadenotucirev+Pembrolizumab	I (*n*=NA)	Safety	NCT02636036

(*To be continued on the next page*)

(*Continued*)

Types	Clinical setting	mechanism	Interventions and treatments	Clinical setting: phase (*n*)	Primary outcome	NCT identifier
For muscle-invasive bladder cancer						
Monotherapy	Adjuvant after radical cystectomy or chemoradiotherapy	ICBs	Pembrolizumab	III (*n*=739)	OS DFS	NCT03244384
		ICBs	Atezolizumab	III (*n*=700)	OS	NCT02450331
		ICBs	Nivolumab	III (*n*=640)	DFS	NCT02632409
	Neoadjuvant before radical cystectomy or chemoradiotherapy	ICBs	Pembrolizumab	II (*n*=40)	PCR	NCT03212651
		ICBs	Pembrolizumab	II (*n*=90)	PCR	NCT02736266
		ICBs	Pembrolizumab	II (*n*=81)	Safety	NCT03319745
		ICBs	Atezolizumab	II (*n*=85)	PCR	NCT02662309
combination strategies	Adjuvant after radical cystectomy or chemoradiotherapy	ICBs+chemoradiation	Nivolumab following chemoradiation	II (*n*=28)	DFS	NCT03171025
	ladder preservation management in combination with chemoradiotherapy	ICBs+chemoradiation	Pembrolizumab cisplatin Radiation	II (*n*=64)	Safety	NCT02662062
		ICBs+radiation therapy	Pembrolizumab Radiation therapy	II (*n*=34)	Safety	NCT02560636
		ICBs+chemoradiation	Pembrolizumab gemcitabine Radiation therapy	II (*n*=54)	DFS	NCT02621151
		ICBs+radiation therapy	Durvalumab Radiation therapy	Ib–II (*n*=42)	Safety DCR PFS	NCT0289116
	Neoadjuvant before radical cystectomy or chemoradiotherapy	vaccines+ICBs	Atezolizumab+PGV001	I (*n*=15)	Safety	NCT03359239
		Dual ICBs	Durvalumab+tremelimumab	I (*n*=15)	Safety	NCT02812420
		Dual ICBs	Durvalumab+tremelimumab	II (*n*=68)	PCR	NCT03234153
		Co-stimulatory receptor agonists+ICBs	Nivolumab with or without urelumab	II (*n*=44)	Immune response	NCT02845323
		ICBs+chemotherapy	Pembrolizumab gemcitabine+cisplatin	II (*n*=30)	Pathological down-staging (<pT2)	NCT02690558
		ICBs+chemotherapy	Pembrolizumab gemcitabine+cisplatin	Ib–II (*n*=81)	OS RFS	NCT02365766
		ICBs+chemotherapy	Pembrolizumab gemcitabine+cisplatin	II (*n*=41)	Safety	NCT03294304
		ICBs+chemotherapy	Pembrolizumab gemcitabine+cisplatin	II (*n*=30)	Safety	NCT02989584
For non-muscle-invasive bladder cancer						
Monotherapy	Intermediate risk recurrent NMIBC	ICBs	Pembrolizumab	I–II (*n*=36)	Safety	NCT03167151
	BCG-unresponsive NMIBC	ICBs	Pembrolizumab	II (*n*=260)	CRR RFS	NCT02625961
		ICBs	Atezolizumab	II (*n*=148)	CRR	NCT02844816
		ICBs	Durvalumab	II (*n*=34)	CRR	NCT02901548
combination strategies	High risk BCG- and chemotherapy naïve NMIBC	Cytokine+BCG	BCG±ALT-803	I–II (*n*=81)	Safety	NCT02138734
	BCG naïve high-risk NMIBC and BCG-relapsing NMIBC	ICBs+BCG	Pembrolizumab+BCG	I (*n*=27)	Safety	NCT02808143
	BCG-relapsing NMIBC	BCG+ICBs radiation therapy	Durvalumab±BCG radiation therapy	I–II (*n*=186)	RFS	NCT03317158
	BCG-relapsing NMIBC	ICBs+Vicinium	Durvalumab+oportuzumab	I (*n*=40)	Safety Tolerability	NCT03258593
	BCG-unresponsive NMIBC	Cytokine+BCG	BCG±ALT-803	II (*n*=100)	CRR	NCT03022825
	BCG-unresponsive NMIBC	BCG+ICBs	Atezolizumab+BCG	I–II (*n*=70)	Safety CRR	NCT02792192
	BCG-unresponsive NMIBC	BCG+ICBs radiation therapy	Durvalumab±BCG radiation therapy	I (*n*=186)	NA	NCT03317158

a) ICBs: immune checkpoint blockades; CRR: complete response rate; OS: overall survival; RFS: relapse-free survival; PFS: progression-free survival; BCG: Bacillus Calmette-Guérin; DFS: disease-free survival; ORR: objective response rate; OS: overall survival; DCR: disease control rate; PFS progression free survival; PCR: pathological response; PARP: poly ADP-ribose polymerase; FGFR: fibroblast growth factor receptor; NA: not applicable.

reasonable to combine local therapies with anti-PD therapy to neutralize immunosuppressive mechanisms. Oncolytic viruses (e.g., Coxsackievirus A21 (CVA21) and enadenotucirev) are novel promising agents against cancer. Some recent reports have illustrated the synergistic effect of local virotherapy as a way to increase tumor T cell infiltration and thus enhance the curative effect of anti-PD therapy (Ribas et al., 2017). Regarding bladder cancer, the safety profiles of CVA21 (Annels et al., 2015) and enadenotucirev (Calvo et al., 2014) have already been demonstrated, and the safety of the combination between pembrolizumab and CVA21 or enadenotucirev is currently under evaluation in clinical trials (Table 3).

No targeted therapy or anti-angiogenic agent has shown remarkable clinical effects in patients with bladder cancer as there has been no durable therapeutic effects. Contrarily, combinations of targeted therapies and ICBs has been explored extensively in animal models and clinical investigations. Indeed, blocking several significant signaling pathways such as fibroblastic growth factor, VEGF, and mesenchymal epithelial transition could lead to the direct exposure of cancer cells to immunocytes, a reduction of Treg, and eventually to tumor cell lysis with presenting antigens and neoantigens to initiate an immune chain reaction

(Galluzzi et al., 2012; Terme et al., 2013).

Currently, multiple immunotherapies are under development that could be combined with anti-PD therapy; these potential combinations have been reviewed elsewhere (Melero et al., 2015; Smyth et al., 2016). In UCB, there are several clinical trials for the combination two different ICBs; for instance, a dual therapy obtains a better clinical outcome than those from single agents such as CTLA-4 or PD-L1/PD1 monotherapy (Postow et al., 2015; Valsecchi, 2015). Combination therapy with ICBs and vaccines or tumor-associated lymphocytes could help the recruitment of activated T cells and memory T cells, and this is currently being assessed in several clinical investigations in patients with recurrent or advanced malignancies.

As for intravesical BCG, several combination strategies are under development for improving efficacy, including cytokines, ICBs, and targeted therapy, which can be reviewed in some published review articles (Kamat et al., 2017; Pettenati and Ingersoll, 2018). A recent study identified that PD-L1-expressing regulatory T cells are enriched during BCG therapy and may limit their efficacy, thus supporting the significance of ICBs for the treatment of bladder cancer with BCG (Chevalier et al., 2018).

Discussion

As discussed above, many patients have experienced minimal or no clinical benefit from immunotherapeutic intervention, and even worse, the immunotherapies may induce irAEs and sometimes even lethal side effects to the patients. Just like other available cancer therapeutics, such as radiotherapy, chemotherapy, and targeted therapy, it is equally vital for cancer immunotherapy to propose reliable biomarker to predict antitumor abilities before offering the treatment for patients. Luckily, with advances in science and technology, various predictors for antitumor response have been identified. Still, there is not likely to be a single predictor for distinguishing immunotherapy-sensitive patients from those likely to develop resistance to immunotherapy. Thereby, the development of a predictive model that incorporates multidimensional candidate biomarkers, such as the clinical characteristics-based biomarkers (including gender, age, and general performance status), blood-based biomarkers (referring to general immune status, absence of soluble inhibitors, and liquid biopsy), tumor tissue-based biomarkers (including tumor foreignness, immune cell infiltration capacity, absence of checkpoints, and absence of inhibitory tumor metabolism), and commensal microorganisms, that affect tumor-host interactions is needed. Furthermore, such a quantitative model should be continuously updated along with the advancement of knowledge on the molecular determinants of response to immunotherapies. Notably, this predictive model will accurately predict patients' susceptibility to immunotherapies and guide personalized treatments.

Moreover, the problem of the low response rate for ICBs and BCG cannot be ignored. As discussed above, combination strategies that enhance the therapeutic effects of immunotherapy are worthy of future consideration for the management of bladder and other cancers. In addition, the development of a novel delivery system for immunotherapies is equally important due to their excellent ability to target therapeutics to cancer lesions. In general, it is believed that engineered drug delivery systems are able to improve drug accumulation at and retention within target cells and tissues, which could not only enhance therapeutic efficacy but also simultaneously reduce off-target effects. For instance, nanomedicines have been implemented to transfer therapeutic components into tumor sites with systemic administration. They could target tumors by enhanced permeation and retention (also called passive) effects characterized by tumor vessels having greater retention and permeability of molecules than normal vessels owing to their poor lymphatic clearance (Xu et al., 2015). Aside from approaches that rely on systemic administration, technologies for local delivery directly targeting immune cells and immune-related organs, such as injectable hydrogels, implantable biomaterials, and microneedles, are also being explored (Figure 2).

Finally, other challenges that must be addressed are how to avoid the occurrence of, identify early, and accurately manage irAEs. Clarification of the underlying mechanism of irAEs may help to avoid the advent of irAEs. For instance, after learning that irAEs are induced by off-target effects, we could develop a novel drug delivery system that specifically anchors the drug to the tumor cells to mitigate irAEs. Given that most irAEs are mild and reversible if they are detected early and properly managed, biomarkers for predicting the occurrence of irAEs are essential. However, the evidence of who may be at an elevated risk remains unclear. Currently, body composition parameters, gender, T cell repertoire, gut microbiome, pre-existing autoantibodies, blood cell counts, and cytokines may be involved in the pathophysiology of immunotherapy-induced adverse events. Notably, we cannot draw a conclusion about whether a patient's biologic profile predisposes them to the occurrence of irAEs solely on the basis of a single biomarker. Thus, a predictive pattern needs to be proposed for identifying patients at increased risk for developing irAEs, which contributes to determining the need for surveillance and prompt treatment. In terms of severity and incidence, the adverse events induced by immunotherapies are generally less than those of other therapeutic strategies. Even so, adverse events induced by immunotherapies could also cause irreversible damage and even death for subjects not promptly and properly managed.

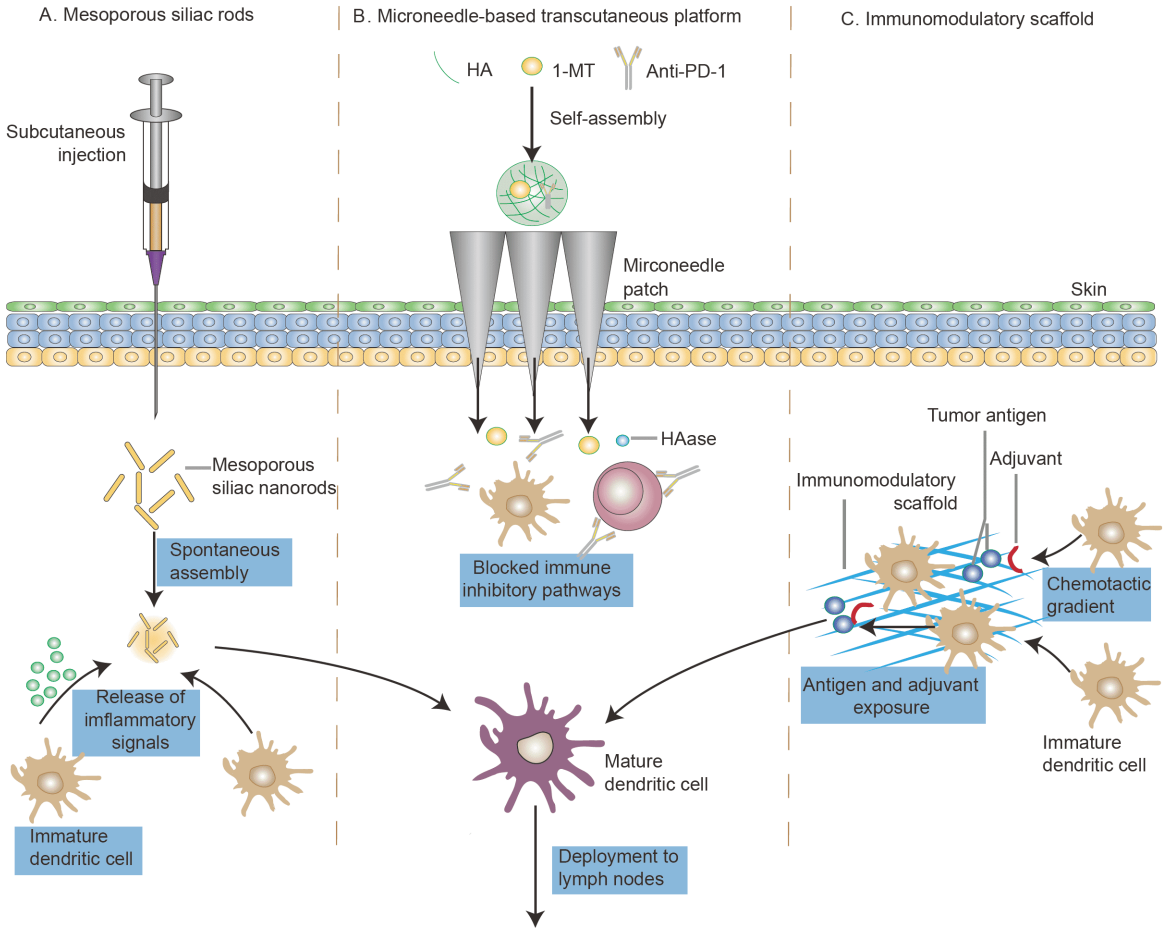

Figure 2 (Color online) Biomaterials for localized delivery of cancer immunotherapy. There are several biomaterials for reducing off-target effects via localized methods in response to tumor elimination. A, Mesoporous silica rods (MSRs) spontaneously assemble *in vivo* and recruit host cells for maturation. A phosphate-buffered saline (PBS) dispersion of MSRs is injected into the subcutaneous tissue of mice to form a pocket. After diffusion of PBS from the pocket, in situ spontaneous assembly of MSRs, analogous to the random assembly of thrown matchsticks, results in the formation of 3D interparticle spaces into which host cells can be recruited and educated by the therapeutics delivered with the MSRs. Educated cells can then emigrate from the structure to interact with other immune cells. B, In another approach, a microneedle-based transcutaneous platform loaded with self- assembled immunotherapeutic nanocarriers was used. Nanoparticle-mediated encapsulation and release of the indoleamine 2,3-dioxygenase (IDO) inhibitor 1-MT and an PD-1 antibody from self-assembled nanoparticles are mediated through a multistep process. First, the 1-MT is conjugated to hyaluronan (HA); then, this conjugate self-assembles around the anti-PD-1 antibody to form a nanoparticle for delivery. Once it has been delivered, the nanoparticle is dissociated by hyaluronidase (HAase), resulting in release of the drugs into the tumor microenvironment. These therapeutics can be delivered using microneedles as shown. C, A subcutaneously delivered porous biomaterial scaffold that releases a chemoattractant recruits naive dendritic cells into its void space. Scaffold-resident dendritic cells are exposed to tumor antigens and adjuvants, resulting in increased presentation of peptides on MHC-peptide complexes and phenotypic maturation. Mature dendritic cells traffic out of the scaffold to lymph nodes where they can stimulate antitumor immunity. Copyright 2019, University of Pennsylvania, Rachel S. Riley (Riley et al., 2019).

Thus, guidelines for the management and surveillance of irAEs are critical and will require interdisciplinary co-operation.

Conclusions

Since the first introduction of intravesical BCG as a treatment for NMIBC in the 1970s, immunotherapy has not achieved remarkable success for bladder cancer treatment over the past several decades, until recently. The development of five FDA-approved antibodies targeting the PD1/PD-L1 axis fortunately marks the end of this stalemate in treatment and can redefine the standard of first-line care for mUCB. In this review, we first introduced the mechanisms of the five main types of immunotherapies in bladder cancer and then described biomarkers for predicting ICB and BCG response; we additionally raised awareness of irAEs and proposed recommendations for the management of immunotherapy toxicity. Finally, we suggested that a dual immunotherapy approach or combining immunotherapy with other therapeutics could serve as promising therapeutic approaches in the care of patients with bladder cancer. However, many questions involving immunotherapy remain unanswered. What is the BCG mechanism in the treatment of NMIBC and how can we increase the low response rate?

What is different in patients who do not respond to immunotherapy? How can we unify the predictive model for immunotherapy efficacy? How can we prevent treatment intolerance? Why do irAEs occur? How are irAEs generally treated? Additional clinical trials and response biomarker research will provide clarity to these questions hopefully providing better prognoses for patients suffering from bladder cancer.

Compliance and ethics *The author(s) declare that they have no conflict of interest.*

Acknowledgements *This work was supported by the National Key Research and Development Program of China (2017YTA0105900).*

References

Aggen, D.H., and Drake, C.G. (2017). Biomarkers for immunotherapy in bladder cancer: a moving target. J Immunother Cancer 5, 94.

Al-Shibli, K.I., Donnem, T., Al-Saad, S., Persson, M., Bremnes, R.M., and Busund, L.T. (2008). Prognostic effect of epithelial and stromal lymphocyte infiltration in non-small cell lung cancer. Clin Cancer Res 14, 5220–5227.

Alsaab, H.O., Sau, S., Alzhrani, R., Tatiparti, K., Bhise, K., Kashaw, S.K., and Iyer, A.K. (2017). PD-1 and PD-L1 checkpoint signaling inhibition for cancer immunotherapy: mechanism, combinations, and clinical outcome. Front Pharmacol 8, 561.

Anantharaman, A., Friedlander, T., Lu, D., Krupa, R., Premasekharan, G., Hough, J., Edwards, M., Paz, R., Lindquist, K., Graf, R., et al. (2016). Programmed death-ligand 1 (PD-L1) characterization of circulating tumor cells (CTCs) in muscle invasive and metastatic bladder cancer patients. BMC Cancer 16, 744.

Annels, N.E., Simpson, G., Arif, M., Denyer, M., Iqbal, A., Mansfield, D., Sandhu, S., Melcher, A., Harrington, K., Au, G., et al. (2015). Oncolytic immunotherapy for the treatment of non-muscle invasive bladder cancer using intravesical coxsackievirus A21. J Immunother Cancer 3, P331.

Antoni, S., Ferlay, J., Soerjomataram, I., Znaor, A., Jemal, A., and Bray, F. (2017). Bladder cancer incidence and mortality: a global overview and recent trends. Eur Urol 71, 96–108.

Apolo, A.B., and Burger, M. (2015). A precision, personalized approach to the management of bladder cancer. Curr Opin Urol 25, 416–417.

Arce Vargas, F., Furness, A.J.S., Litchfield, K., Joshi, K., Rosenthal, R., Ghorani, E., Solomon, I., Lesko, M.H., Ruef, N., Roddie, C., et al. (2018). Fc effector function contributes to the activity of human anti-CTLA-4 antibodies. Cancer Cell 33, 649–663.e4.

Atkins, M.B., McDermott, D.F., Powles, T., Motzer, R.J., Rini, B.I., Fong, L., Joseph, R.W., Pal, S.K., Sznol, M., Hainsworth, J.D., et al. (2017). 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 35, 4505.

Babjuk, M., Böhle, A., Burger, M., Capoun, O., Cohen, D., Compérat, E. M., Hernández, V., Kaasinen, E., Palou, J., Rouprêt, M., et al. (2017). EAU guidelines on non-muscle-invasive urothelial carcinoma of the bladder: update 2016. Eur Urol 71, 447–461.

Babjuk, M., Oosterlinck, W., Sylvester, R., Kaasinen, E., Böhle, A., Palou-Redorta, J., and Rouprêt, M. (2011). EAU guidelines on non-muscle-invasive urothelial carcinoma of the bladder, the 2011 update. Eur Urol 59, 997–1008.

Bais, C., Kuziora, M., Morehouse, C., Higgs, B.W., Raja, R., Lee, Y., de Los Reyes, M., Rebelatto, M., Steele, K., Jin, X., et al. (2017). Biologic and clinical relevance of an IFNG mRNA signature (IFNGS) and PD-L1 protein expression in tumor and immune cells in urothelial cancer (UC) patients (pts) treated with durvalumab (D). J Clin Oncol 35, 3037.

Baxi, S., Yang, A., Gennarelli, R.L., Khan, N., Wang, Z., Boyce, L., and Korenstein, D. (2018). Immune-related adverse events for anti-PD-1 and anti-PD-L1 drugs: systematic review and meta-analysis. BMJ 360, k793.

Bellmunt, J., de Wit, R., Vaughn, D.J., Fradet, Y., Lee, J.L., Fong, L., Vogelzang, N.J., Climent, M.A., Petrylak, D.P., Choueiri, T.K., et al. (2017). Pembrolizumab as second-line therapy for advanced urothelial carcinoma. N Engl J Med 376, 1015–1026.

Berdik, C. (2017). Unlocking bladder cancer. Nature 551, S34–S35.

Blank, C.U., Haanen, J.B., Ribas, A., and Schumacher, T.N. (2016). The "cancer immunogram". Science 352, 658–660.

Boussiotis, V.A. (2016). Molecular and biochemical aspects of the PD-1 checkpoint pathway. N Engl J Med 375, 1767–1778.

Buder-Bakhaya, K., and Hassel, J.C. (2018). Biomarkers for clinical benefit of immune checkpoint inhibitor treatment—A review from the melanoma perspective and beyond. Front Immunol 9, 1474.

Byrne, E.H., and Fisher, D.E. (2017). Immune and molecular correlates in melanoma treated with immune checkpoint blockade. Cancer 123, 2143–2153.

Callahan, M.K., Yang, A., Tandon, S., Xu, Y., Subudhi, S.K., Roman, R.A., Heine, A.I., Pogoriler, E., Kuk, D., Panageas, K., et al. (2011). Evaluation of serum IL-17 levels during ipilimumab therapy: Correlation with colitis. J Clin Oncol 29, 2505.

Calmette, A., Guérin, C., Boquet, A., and Nègre, L. (1927). La vaccination préventive contre la tuberculose par le "BCG". Paris: Masson et cie.

Calvo, E., Gil-Martin, M., Machiels, J.P.H., Rottey, S., Cubillo, A., Salazar, R., Mardjuadi, F.I., Geboes, K.P., Ellis, C., Beadle, J.W., et al. (2014). A first-in-class, first-in-human phase I study of enadenotucirev, an oncolytic Ad11/Ad3 chimeric group B adenovirus, administered intravenously in patients with metastatic epithelial tumors. J Clin Oncol 32, 3103.

Cappelli, L.C., Gutierrez, A.K., Baer, A.N., Albayda, J., Manno, R.L., Haque, U., Lipson, E.J., Bleich, K.B., Shah, A.A., Naidoo, J., et al. (2017). Inflammatory arthritis and sicca syndrome induced by nivolumab and ipilimumab. Ann Rheum Dis 76, 43–50.

Caturegli, P., Di Dalmazi, G., Lombardi, M., Grosso, F., Larman, H.B., Larman, T., Taverna, G., Cosottini, M., and Lupi, I. (2016). Hypophysitis secondary to cytotoxic T-lymphocyte-associated protein 4 blockade. Am J Pathol 186, 3225–3235.

Chaput, N., Lepage, P., Coutzac, C., Soularue, E., Le Roux, K., Monot, C., Boselli, L., Routier, E., Cassard, L., Collins, M., et al. (2017). Baseline gut microbiota predicts clinical response and colitis in metastatic melanoma patients treated with ipilimumab. Ann Oncol 28, 1368–1379.

Chevalier, M.F., Schneider, A.K., Cesson, V., Dartiguenave, F., Lucca, I., Jichlinski, P., Nardelli-Haefliger, D., and Derré, L. (2018). Conventional and PD-L1-expressing regulatory T cells are enriched during BCG therapy and may limit its efficacy. Eur Urol 74, 540–544.

Chism, D.D. (2017). Urothelial carcinoma of the bladder and the rise of immunotherapy. J Natl Compr Canc Netw 15, 1277–1284.

Cohen, M., and Reiter, Y. (2013). T-cell receptor-like antibodies: targeting the intracellular proteome therapeutic potential and clinical applications. Antibodies 2, 517–534.

Coley, W.B. (1991). The treatment of malignant tumors by repeated inoculations of erysipelas. With a report of ten original cases. 1893. Clin Orthop Relat Res 105, 3–11.

Coulie, P.G., Van den Eynde, B.J., van der Bruggen, P., and Boon, T. (2014). Tumour antigens recognized by T lymphocytes: at the core of cancer immunotherapy. Nat Rev Cancer 14, 135–146.

Cousin, S., Seneschal, J., and Italiano, A. (2018). Toxicity profiles of immunotherapy. Pharmacol Ther 181, 91–100.

Cumberbatch, M.G.K., Jubber, I., Black, P.C., Esperto, F., Figueroa, J.D., Kamat, A.M., Kiemeney, L., Lotan, Y., Pang, K., Silverman, D.T., et al. (2018). Epidemiology of bladder cancer: A systematic review and contemporary update of risk factors in 2018. Eur Urol 74, 784–795.

Daly, L.E., Power, D.G., O'Reilly, Á., Donnellan, P., Cushen, S.J., O'Sullivan, K., Twomey, M., Woodlock, D.P., Redmond, H.P., and

Ryan, A.M. (2017). The impact of body composition parameters on ipilimumab toxicity and survival in patients with metastatic melanoma. Br J Cancer 116, 310–317.
Delanoy, N., Michot, J.M., Comont, T., Kramkimel, N., Lazarovici, J., Dupont, R., Champiat, S., Chahine, C., Robert, C., Herbaux, C., et al. (2019). Haematological immune-related adverse events induced by anti-PD-1 or anti-PD-L1 immunotherapy: a descriptive observational study. Lancet Haematol 6, e48–e57.
Dobbin, K.K., Cesano, A., Alvarez, J., Hawtin, R., Janetzki, S., Kirsch, I., Masucci, G.V., Robbins, P.B., Selvan, S.R., Streicher, H.Z., et al. (2016). Validation of biomarkers to predict response to immunotherapy in cancer: Volume II—Clinical validation and regulatory considerations. J Immunother Cancer 4, 77.
Doyle, E., Crew, J., Mostafid, H., Tuthill, M., Cerundolo, V., Gerristen, W., and Protheroe, A. (2019). Urothelial cancer: a narrative review of the role of novel immunotherapeutic agents with particular reference to the management of non-muscle-invasive disease. BJU Int 123, 947–958.
Drake, C.G., Bivalacqua, T.J., and Hahn, N.M. (2016). Programmed cell death ligand-1 blockade in urothelial bladder cancer: To select or not to select. J Clin Oncol 34, 3115–3116.
Du, X., Tang, F., Liu, M., Su, J., Zhang, Y., Wu, W., Devenport, M., Lazarski, C.A., Zhang, P., Wang, X., et al. (2018). A reappraisal of CTLA-4 checkpoint blockade in cancer immunotherapy. Cell Res 28, 416–432.
Dubin, K., Callahan, M.K., Ren, B., Khanin, R., Viale, A., Ling, L., No, D., Gobourne, A., Littmann, E., Huttenhower, C., et al. (2016). Intestinal microbiome analyses identify melanoma patients at risk for checkpoint-blockade-induced colitis. Nat Commun 7, 10391.
Felsenstein, K.M., and Theodorescu, D. (2018). Precision medicine for urothelial bladder cancer: update on tumour genomics and immunotherapy. Nat Rev Urol 15, 92–111.
Forde, P.M., Chaft, J.E., and Pardoll, D.M. (2018). Neoadjuvant PD-1 blockade in resectable lung cancer. N Engl J Med 379, e14.
Fridman, W.H., Pagès, F., Sautès-Fridman, C., and Galon, J. (2012). The immune contexture in human tumours: impact on clinical outcome. Nat Rev Cancer 12, 298–306.
Fujisawa, Y., Yoshino, K., Otsuka, A., Funakoshi, T., Fujimura, T., Yamamoto, Y., Hata, H., Gosho, M., Tanaka, R., Yamaguchi, K., et al. (2017). Fluctuations in routine blood count might signal severe immune-related adverse events in melanoma patients treated with nivolumab. J Dermatol Sci 88, 225–231.
Fumagalli, M., Sironi, M., Pozzoli, U., Ferrer-Admetlla, A., Ferrer-Admettla, A., Pattini, L., and Nielsen, R. (2011). Signatures of environmental genetic adaptation pinpoint pathogens as the main selective pressure through human evolution. PLoS Genet 7, e1002355.
Galluzzi, L., Senovilla, L., Zitvogel, L., and Kroemer, G. (2012). The secret ally: immunostimulation by anticancer drugs. Nat Rev Drug Discov 11, 215–233.
Gan, C., Mostafid, H., Khan, M.S., and Lewis, D.J.M. (2013). BCG immunotherapy for bladder cancer—The effects of substrain differences. Nat Rev Urol 10, 580–588.
Gandhi, L., Rodríguez-Abreu, D., Gadgeel, S., Esteban, E., Felip, E., De Angelis, F., Domine, M., Clingan, P., Hochmair, M.J., Powell, S.F., et al. (2018). Pembrolizumab plus chemotherapy in metastatic non-small-cell lung cancer. N Engl J Med 378, 2078–2092.
Gao, J., Shi, L.Z., Zhao, H., Chen, J., Xiong, L., He, Q., Chen, T., Roszik, J., Bernatchez, C., Woodman, S.E., et al. (2016). Loss of IFN-γ pathway genes in tumor cells as a mechanism of resistance to anti-CTLA-4 therapy. Cell 167, 397–404.e9.
Garg, A.D., Coulie, P.G., Van den Eynde, B.J., and Agostinis, P. (2017). Integrating next-generation dendritic cell vaccines into the current cancer immunotherapy landscape. Trends Immunol 38, 577–593.
Garrett, W.S. (2015). Cancer and the microbiota. Science 348, 80–86.
Ghasemzadeh, A., Bivalacqua, T.J., Hahn, N.M., and Drake, C.G. (2016). New strategies in bladder cancer: A second coming for immunotherapy. Clin Cancer Res 22, 793–801.
Gjerstorff, M.F., Andersen, M.H., and Ditzel, H.J. (2015). Oncogenic cancer/testis antigens: prime candidates for immunotherapy. Oncotarget 6, 15772–15787.
Gontero, P., Sylvester, R., Pisano, F., Joniau, S., Vander Eeckt, K., Serretta, V., Larré, S., Di Stasi, S., Van Rhijn, B., Witjes, A.J., et al. (2015). Prognostic factors and risk groups in T1G3 non-muscle-invasive bladder cancer patients initially treated with bacillus Calmette-Guérin: Results of a retrospective multicenter study of 2451 patients. Eur Urol 67, 74–82.
Goodman, A.M., Kato, S., Bazhenova, L., Patel, S.P., Frampton, G.M., Miller, V., Stephens, P.J., Daniels, G.A., and Kurzrock, R. (2017). Tumor mutational burden as an independent predictor of response to immunotherapy in diverse cancers. Mol Cancer Ther 16, 2598–2608.
Granier, C., De Guillebon, E., Blanc, C., Roussel, H., Badoual, C., Colin, E., Saldmann, A., Gey, A., Oudard, S., and Tartour, E. (2017). Mechanisms of action and rationale for the use of checkpoint inhibitors in cancer. ESMO Open 2, e000213.
Griffith, J.W., Sokol, C.L., and Luster, A.D. (2014). Chemokines and chemokine receptors: positioning cells for host defense and immunity. Annu Rev Immunol 32, 659–702.
Gubin, M.M., Zhang, X., Schuster, H., Caron, E., Ward, J.P., Noguchi, T., Ivanova, Y., Hundal, J., Arthur, C.D., Krebber, W.J., et al. (2014). Checkpoint blockade cancer immunotherapy targets tumour-specific mutant antigens. Nature 515, 577–581.
Guo, C., Manjili, M.H., Subjeck, J.R., Sarkar, D., Fisher, P.B., and Wang, X.Y. (2013). Therapeutic cancer vaccines: past, present, and future. Adv Cancer Res 119, 421–475.
Haanen, J.B.A.G., Carbonnel, F., Robert, C., Kerr, K.M., Peters, S., Larkin, J., and Jordan, K. (2018). Management of toxicities from immunotherapy: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up. Ann Oncol 29, iv264–iv266.
Hanna, G.J., Lizotte, P., Cavanaugh, M., Kuo, F.C., Shivdasani, P., Frieden, A., Chau, N.G., Schoenfeld, J.D., Lorch, J.H., Uppaluri, R., et al. (2018). Frameshift events predict anti-PD-1/L1 response in head and neck cancer. JCI Insight 3.
Havel, J.J., Chowell, D., and Chan, T.A. (2019). The evolving landscape of biomarkers for checkpoint inhibitor immunotherapy. Nat Rev Cancer 19, 133–150.
Hellmann, M.D., Callahan, M.K., Awad, M.M., Calvo, E., Ascierto, P.A., Atmaca, A., Rizvi, N.A., Hirsch, F.R., Selvaggi, G., Szustakowski, J.D., et al. (2019). Tumor mutational burden and efficacy of nivolumab monotherapy and in combination with ipilimumab in small-cell lung cancer. Cancer Cell 35, 329.
Hodi, F.S., O'Day, S.J., McDermott, D.F., Weber, R.W., Sosman, J.A., Haanen, J.B., Gonzalez, R., Robert, C., Schadendorf, D., Hassel, J.C., et al. (2010). Improved survival with ipilimumab in patients with metastatic melanoma. N Engl J Med 363, 711–723.
Hunder, N.N., Wallen, H., Cao, J., Hendricks, D.W., Reilly, J.Z., Rodmyre, R., Jungbluth, A., Gnjatic, S., Thompson, J.A., and Yee, C. (2008). Treatment of metastatic melanoma with autologous $CD4^+$ T cells against NY-ESO-1. N Engl J Med 358, 2698–2703.
Iwama, S., De Remigis, A., Callahan, M.K., Slovin, S.F., Wolchok, J.D., and Caturegli, P. (2014). Pituitary expression of CTLA-4 mediates hypophysitis secondary to administration of CTLA-4 blocking antibody. Sci Transl Med 6, 230ra45.
Jiang, Y., Li, Y., and Zhu, B. (2015). T-cell exhaustion in the tumor microenvironment. Cell Death Dis 6, e1792.
Johnson, D.B., Balko, J.M., Compton, M.L., Chalkias, S., Gorham, J., Xu, Y., Hicks, M., Puzanov, I., Alexander, M.R., Bloomer, T.L., et al. (2016). Fulminant myocarditis with combination immune checkpoint blockade. N Engl J Med 375, 1749–1755.
Johnson, D.B., Chandra, S., and Sosman, J.A. (2018). Immune checkpoint inhibitor toxicity in 2018. JAMA 320, 1702–1703.
Kaiser, J. (2019). Cancer immunotherapy may have a dark side. Science 363, 1377.
Kamat, A.M., Colombel, M., Sundi, D., Lamm, D., Boehle, A., Brausi, M., Buckley, R., Persad, R., Palou, J., Soloway, M., et al. (2017). BCG-unresponsive non-muscle-invasive bladder cancer: recommendations

from the IBCG. Nat Rev Urol 14, 244–255.
Kamat, A.M., Li, R., O'Donnell, M.A., Black, P.C., Roupret, M., Catto, J. W., Comperat, E., Ingersoll, M.A., Witjes, W.P., McConkey, D.J., et al. (2018). Predicting response to intravesical Bacillus Calmette-Guérin immunotherapy: Are we there yet? A systematic review. Eur Urol 73, 738–748.
Karasaki, T., Nagayama, K., Kuwano, H., Nitadori, J.I., Sato, M., Anraku, M., Hosoi, A., Matsushita, H., Morishita, Y., Kashiwabara, K., et al. (2017). An immunogram for the cancer-immunity cycle: Towards personalized immunotherapy of lung cancer. J Thorac Oncol 12, 791–803.
Khagi, Y., Goodman, A.M., Daniels, G.A., Patel, S.P., Sacco, A.G., Randall, J.M., Bazhenova, L.A., and Kurzrock, R. (2017). Hypermutated circulating tumor DNA: Correlation with response to checkpoint inhibitor-based immunotherapy. Clin Cancer Res 23, 5729–5736.
Kim, J., Kwiatkowski, D., McConkey, D.J., Meeks, J.J., Freeman, S.S., Bellmunt, J., Getz, G., and Lerner, S.P. (2019). The cancer genome atlas expression subtypes stratify response to checkpoint inhibition in advanced urothelial cancer and identify a subset of patients with high survival probability. Eur Urol 75, 961–964.
Klebanoff, C.A., Khong, H.T., Antony, P.A., Palmer, D.C., and Restifo, N. P. (2005). Sinks, suppressors and antigen presenters: how lymphodepletion enhances T cell-mediated tumor immunotherapy. Trends Immunol 26, 111–117.
Krishnamurthy, A., and Jimeno, A. (2018). Bispecific antibodies for cancer therapy: A review. Pharmacol Ther 185, 122–134.
Kugel 3rd, C.H., Douglass, S.M., Webster, M.R., Kaur, A., Liu, Q., Yin, X., Weiss, S.A., Darvishian, F., Al-Rohil, R.N., Ndoye, A., et al. (2018). Age correlates with response to anti-PD1, reflecting age-related differences in intratumoral effector and regulatory T-cell populations. Clin Cancer Res 24, 5347–5356.
Lauss, M., Donia, M., Harbst, K., Andersen, R., Mitra, S., Rosengren, F., Salim, M., Vallon-Christersson, J., Törngren, T., Kvist, A., et al. (2017). Mutational and putative neoantigen load predict clinical benefit of adoptive T cell therapy in melanoma. Nat Commun 8, 1738.
Lawler, S.E., Speranza, M.C., Cho, C.F., and Chiocca, E.A. (2016). Oncolytic viruses in cancer treatment. JAMA Oncol 3, 841.
Legrand, F.A., Gandara, D.R., Mariathasan, S., Powles, T., He, X., Zhang, W., Jhunjhunwala, S., Nickles, D., Bourgon, R., Schleifman, E., et al. (2018). Association of high tissue TMB and atezolizumab efficacy across multiple tumor types. J Clin Oncol 36, 12000.
Lesterhuis, W.J., Bosco, A., Millward, M.J., Small, M., Nowak, A.K., and Lake, R.A. (2017). Dynamic versus static biomarkers in cancer immune checkpoint blockade: unravelling complexity. Nat Rev Drug Discov 16, 264–272.
Levine, B.L., Miskin, J., Wonnacott, K., and Keir, C. (2017). Global manufacturing of CAR T cell therapy. Mol Ther Methods Clin Dev 4, 92–101.
Li, L., Goedegebuure, S.P., and Gillanders, W.E. (2017). Preclinical and clinical development of neoantigen vaccines. Ann Oncol 28, xii11–xii17.
Lim, W.A., and June, C.H. (2017). The principles of engineering immune cells to treat cancer. Cell 168, 724–740.
Lin, R.L., and Zhao, L.J. (2015). Mechanistic basis and clinical relevance of the role of transforming growth factor-beta in cancer. Cancer Biol Med 12, 385–393.
Linnemann, C., Heemskerk, B., Kvistborg, P., Kluin, R.J.C., Bolotin, D.A., Chen, X., Bresser, K., Nieuwland, M., Schotte, R., Michels, S., et al. (2013). High-throughput identification of antigen-specific TCRs by TCR gene capture. Nat Med 19, 1534–1541.
Mantovani, A., Biswas, S.K., Galdiero, M.R., Sica, A., and Locati, M. (2013). Macrophage plasticity and polarization in tissue repair and remodelling. J Pathol 229, 176–185.
Martens, A., Wistuba-Hamprecht, K., Geukes Foppen, M., Yuan, J., Postow, M.A., Wong, P., Romano, E., Khammari, A., Dreno, B., Capone, M., et al. (2016). Baseline peripheral blood biomarkers associated with clinical outcome of advanced melanoma patients treated with ipilimumab. Clin Cancer Res 22, 2908–2918.
Martin, P., Spitzmueller, A., Wu, S., Widmaier, M., Korn, R., Althammer, S., Zha, J., Higgs, B.W., Cooper, Z., and Steele, K. (2017). Mutually exclusive expression of CD73 and PDL1 in tumors from patients (pt) with NSCLC, gastroesophageal (GE) and urothelial bladder carcinoma (UBC). J Clin Oncol 35, 3079.
Massari, F., Di Nunno, V., Cubelli, M., Santoni, M., Fiorentino, M., Montironi, R., Cheng, L., Lopez-Beltran, A., Battelli, N., and Ardizzoni, A. (2018). Immune checkpoint inhibitors for metastatic bladder cancer. Cancer Treat Rev 64, 11–20.
Matson, V., Fessler, J., Bao, R., Chongsuwat, T., Zha, Y., Alegre, M.L., Luke, J.J., and Gajewski, T.F. (2018). The commensal microbiome is associated with anti-PD-1 efficacy in metastatic melanoma patients. Science 359, 104–108.
Mayes, P.A., Hance, K.W., and Hoos, A. (2018). The promise and challenges of immune agonist antibody development in cancer. Nat Rev Drug Discov 17, 509–527.
Mehnert, J.M., Panda, A., Zhong, H., Hirshfield, K., Damare, S., Lane, K., Sokol, L., Stein, M.N., Rodriguez-Rodriquez, L., Kaufman, H.L., et al. (2016). Immune activation and response to pembrolizumab in POLE-mutant endometrial cancer. J Clin Invest 126, 2334–2340.
Melero, I., Berman, D.M., Aznar, M.A., Korman, A.J., Pérez Gracia, J.L., and Haanen, J. (2015). Evolving synergistic combinations of targeted immunotherapies to combat cancer. Nat Rev Cancer 15, 457–472.
Milowsky, M.I., Rumble, R.B., Booth, C.M., Gilligan, T., Eapen, L.J., Hauke, R.J., Boumansour, P., and Lee, C.T. (2016). Guideline on muscle-invasive and metastatic bladder cancer (European Association of Urology guideline): American Society of Clinical Oncology Clinical Practice Guideline Endorsement. J Clin Oncol 34, 1945–1952.
Mollica Poeta, V., Massara, M., Capucetti, A., and Bonecchi, R. (2019). Chemokines and chemokine receptors: new targets for cancer immunotherapy. Front Immunol 10, 379.
Morales, A., Eidinger, D., and Bruce, A.W. (1976). Intracavitary Bacillus Calmette-Guerin in the treatment of superficial bladder tumors. J Urol 116, 180–182.
Nagai, H., and Muto, M. (2018). Optimal management of immune-related adverse events resulting from treatment with immune checkpoint inhibitors: a review and update. Int J Clin Oncol 23, 410–420.
Nakamura, Y. (2019). Biomarkers for immune checkpoint inhibitor-mediated tumor response and adverse events. Front Med 6, 119.
Nakamura, Y., Kitano, S., Takahashi, A., Tsutsumida, A., Namikawa, K., Tanese, K., Abe, T., Funakoshi, T., Yamamoto, N., Amagai, M., et al. (2016). Nivolumab for advanced melanoma: pretreatment prognostic factors and early outcome markers during therapy. Oncotarget 7, 77404–77415.
Nosrati, A., Tsai, K.K., Goldinger, S.M., Tumeh, P., Grimes, B., Loo, K., Algazi, A.P., Nguyen-Kim, T.D.L., Levesque, M., Dummer, R., et al. (2017). Evaluation of clinicopathological factors in PD-1 response: Derivation and validation of a prediction scale for response to PD-1 monotherapy. Br J Cancer 116, 1141–1147.
Osorio, J.C., Ni, A., Chaft, J.E., Pollina, R., Kasler, M.K., Stephens, D., Rodriguez, C., Cambridge, L., Rizvi, H., Wolchok, J.D., et al. (2017). Antibody-mediated thyroid dysfunction during T-cell checkpoint blockade in patients with non-small-cell lung cancer. Ann Oncol 28, 583–589.
Pardoll, D.M. (2012). The blockade of immune checkpoints in cancer immunotherapy. Nat Rev Cancer 12, 252–264.
Peggs, K.S., Quezada, S.A., and Allison, J.P. (2009). Cancer immunotherapy: co-stimulatory agonists and co-inhibitory antagonists. Clin Exp Immunol 157, 9–19.
Petrylak, D.P., de Wit, R., Chi, K.N., Drakaki, A., Sternberg, C.N., Nishiyama, H., Castellano, D., Hussain, S., Fléchon, A., Bamias, A., et al. (2017). 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 390, 2266–2277.

Pettenati, C., and Ingersoll, M.A. (2018). Mechanisms of BCG immunotherapy and its outlook for bladder cancer. Nat Rev Urol 15, 615–625.
Postow, M.A., Sidlow, R., and Hellmann, M.D. (2018). Immune-related adverse events associated with immune checkpoint blockade. N Engl J Med 378, 158–168.
Postow, M.A., Chesney, J., Pavlick, A.C., Robert, C., Grossmann, K., McDermott, D., Linette, G.P., Meyer, N., Giguere, J.K., Agarwala, S.S., et al. (2015). Nivolumab and ipilimumab versus ipilimumab in untreated melanoma. N Engl J Med 372, 2006–2017.
Redelman-Sidi, G., Glickman, M.S., and Bochner, B.H. (2014). The mechanism of action of BCG therapy for bladder cancer—A current perspective. Nat Rev Urol 11, 153–162.
Renner, K., Singer, K., Koehl, G.E., Geissler, E.K., Peter, K., Siska, P.J., and Kreutz, M. (2017). Metabolic hallmarks of tumor and immune cells in the tumor microenvironment. Front Immunol 8.
Riaz, N., Havel, J.J., Makarov, V., Desrichard, A., Urba, W.J., Sims, J.S., Hodi, F.S., Martín-Algarra, S., Mandal, R., Sharfman, W.H., et al. (2017). Tumor and microenvironment evolution during immunotherapy with nivolumab. Cell 171, 934–949.e16.
Ribas, A., Dummer, R., Puzanov, I., VanderWalde, A., Andtbacka, R.H.I., Michielin, O., Olszanski, A.J., Malvehy, J., Cebon, J., Fernandez, E., et al. (2017). Oncolytic virotherapy promotes intratumoral T cell infiltration and improves anti-PD-1 immunotherapy. Cell 170, 1109–1119.e10.
Ribas, A., and Tumeh, P.C. (2014). The future of cancer therapy: selecting patients likely to respond to PD1/L1 blockade. Clin Cancer Res 20, 4982–4984.
Ribas, A., and Wolchok, J.D. (2018). Cancer immunotherapy using checkpoint blockade. Science 359, 1350–1355.
Riley, R.S., June, C.H., Langer, R., and Mitchell, M.J. (2019). Delivery technologies for cancer immunotherapy. Nat Rev Drug Discov 18, 175–196.
Rizvi, N.A., Hellmann, M.D., Snyder, A., Kvistborg, P., Makarov, V., Havel, J.J., Lee, W., Yuan, J., Wong, P., Ho, T.S., et al. (2015). Mutational landscape determines sensitivity to PD-1 blockade in non-small cell lung cancer. Science 348, 124–128.
Robert, C., Long, G.V., Brady, B., Dutriaux, C., Maio, M., Mortier, L., Hassel, J.C., Rutkowski, P., McNeil, C., Kalinka-Warzocha, E., et al. (2014). Nivolumab in previously untreated melanoma without *BRAF* mutation. N Engl J Med 372, 320–330.
Romero, J.M., Jiménez, P., Cabrera, T., Cózar, J.M., Pedrinaci, S., Tallada, M., Garrido, F., and Ruiz-Cabello, F. (2005). Coordinated downregulation of the antigen presentation machinery and HLA class I/β2-microglobulin complex is responsible for HLA-ABC loss in bladder cancer. Int J Cancer 113, 605–610.
Rooney, M.S., Shukla, S.A., Wu, C.J., Getz, G., and Hacohen, N. (2015). Molecular and genetic properties of tumors associated with local immune cytolytic activity. Cell 160, 48–61.
Rosenberg, J.E., Hoffman-Censits, J., Powles, T., van der Heijden, M.S., Balar, A.V., Necchi, A., Dawson, N., O'Donnell, P.H., Balmanoukian, A., Loriot, Y., et al. (2016). 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 387, 1909–1920.
Routy, B., Le Chatelier, E., Derosa, L., Duong, C.P.M., Alou, M.T., Daillère, R., Fluckiger, A., Messaoudene, M., Rauber, C., Roberti, M.P., et al. (2018). Gut microbiome influences efficacy of PD-1-based immunotherapy against epithelial tumors. Science 359, 91–97.
Sade-Feldman, M., Jiao, Y.J., Chen, J.H., Rooney, M.S., Barzily-Rokni, M., Eliane, J.P., Bjorgaard, S.L., Hammond, M.R., Vitzthum, H., Blackmon, S.M., et al. (2017). Resistance to checkpoint blockade therapy through inactivation of antigen presentation. Nat Commun 8, 1136.
Saeterdal, I., Bjørheim, J., Lislerud, K., Gjertsen, M.K., Bukholm, I.K., Olsen, O.C., Nesland, J.M., Eriksen, J.A., Møller, M., Lindblom, A., et al. (2001). Frameshift-mutation-derived peptides as tumor-specific antigens in inherited and spontaneous colorectal cancer. Proc Natl Acad Sci USA 98, 13255–13260.
Sanmamed, M.F., and Chen, L. (2018). A paradigm shift in cancer immunotherapy: from enhancement to normalization. Cell 175, 313–326.
Schindler, K., Harmankaya, K., Postow, M.A., Frantal, S., Bello, D., Ariyan, C.E., Michielin, O.A., Hoeller, C., Pehamberger, H., and Wolchok, J.D. (2013). Pretreatment levels of absolute and relative eosinophil count to improve overall survival (OS) in patients with metastatic melanoma under treatment with ipilimumab, an anti CTLA-4 antibody. J Clin Oncol 31, 9024.
Segal, N.H., Naidoo, J., Curigliano, G., Patel, S., Sahebjam, S., Papadopoulos, K.P., Gordon, M.S., Wang, D., Gómez Rueda, A., Song, X., et al. (2018). First-in-human dose escalation of monalizumab plus durvalumab, with expansion in patients with metastatic microsatellite-stable colorectal cancer. J Clin Oncol 36, 3540.
Sharma, P., Shen, Y., Wen, S., Yamada, S., Jungbluth, A.A., Gnjatic, S., Bajorin, D.F., Reuter, V.E., Herr, H., Old, L.J., et al. (2007). CD8 tumor-infiltrating lymphocytes are predictive of survival in muscle-invasive urothelial carcinoma. Proc Natl Acad Sci USA 104, 3967–3972.
Shukla, S.A., Bachireddy, P., Schilling, B., Galonska, C., Zhan, Q., Bango, C., Langer, R., Lee, P.C., Gusenleitner, D., Keskin, D.B., et al. (2018). Cancer-germline antigen expression discriminates clinical outcome to CTLA-4 blockade. Cell 173, 624–633.e8.
Siegel, R.L., Miller, K.D., and Jemal, A. (2019). Cancer statistics, 2019. CA A Cancer J Clin 69, 7–34.
Simeone, E., Gentilcore, G., Giannarelli, D., Grimaldi, A.M., Caracò, C., Curvietto, M., Esposito, A., Paone, M., Palla, M., Cavalcanti, E., et al. (2014). Immunological and biological changes during ipilimumab treatment and their potential correlation with clinical response and survival in patients with advanced melanoma. Cancer Immunol Immunother 63, 675–683.
Smith, S.G., and Zaharoff, D.A. (2016). Future directions in bladder cancer immunotherapy: Towards adaptive immunity. Immunotherapy 8, 351–365.
Smyth, M.J., Ngiow, S.F., Ribas, A., and Teng, M.W.L. (2016). Combination cancer immunotherapies tailored to the tumour microenvironment. Nat Rev Clin Oncol 13, 143–158.
Snyder, A., Nathanson, T., Funt, S.A., Ahuja, A., Buros Novik, J., Hellmann, M.D., Chang, E., Aksoy, B.A., Al-Ahmadie, H., Yusko, E., et al. (2017). 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 14, e1002309.
Song, D., Powles, T., Shi, L., Zhang, L., Ingersoll, M.A., and Lu, Y.J. (2019). Bladder cancer, a unique model to understand cancer immunity and develop immunotherapy approaches. J Pathol 249, 151–165.
Sundahl, N., De Wolf, K., Rottey, S., Decaestecker, K., De Maeseneer, D., Meireson, A., Goetghebeur, E., Fonteyne, V., Verbeke, S., De Visschere, P., et al. (2017). A phase I/II trial of fixed-dose stereotactic body radiotherapy with sequential or concurrent pembrolizumab in metastatic urothelial carcinoma: evaluation of safety and clinical and immunologic response. J Transl Med 15, 150.
Taha, T., Meiri, D., Talhamy, S., Wollner, M., Peer, A., and Bar-Sela, G. (2019). Cannabis impacts tumor response rate to nivolumab in patients with advanced malignancies. Oncol 24, 549–554.
Tanaka, R., Okiyama, N., Okune, M., Ishitsuka, Y., Watanabe, R., Furuta, J., Ohtsuka, M., Otsuka, A., Maruyama, H., Fujisawa, Y., et al. (2017). Serum level of interleukin-6 is increased in nivolumab-associated psoriasiform dermatitis and tumor necrosis factor-α is a biomarker of nivolumab recativity. J Dermatol Sci 86, 71–73.
Tang, J., Shalabi, A., and Hubbard-Lucey, V.M. (2018). Comprehensive analysis of the clinical immuno-oncology landscape. Ann Oncol 29, 84–91.
Taylor, A.M., Shih, J., Ha, G., Gao, G.F., Zhang, X., Berger, A.C., Schumacher, S.E., Wang, C., Hu, H., Liu, J., et al. (2018). Genomic and functional approaches to understanding cancer aneuploidy. Cancer Cell 33, 676–689.e3.
Terme, M., Pernot, S., Marcheteau, E., Sandoval, F., Benhamouda, N.,

Colussi, O., Dubreuil, O., Carpentier, A.F., Tartour, E., and Taieb, J. (2013). VEGFA-VEGFR pathway blockade inhibits tumor-induced regulatory T-cell proliferation in colorectal cancer. Cancer Res 73, 539–549.

Topalian, S.L., Drake, C.G., and Pardoll, D.M. (2015). Immune checkpoint blockade: a common denominator approach to cancer therapy. Cancer Cell 27, 450–461.

Twumasi-Boateng, K., Pettigrew, J.L., Kwok, Y.Y.E., Bell, J.C., and Nelson, B.H. (2018). Oncolytic viruses as engineering platforms for combination immunotherapy. Nat Rev Cancer 18, 419–432.

Valpione, S., Pasquali, S., Campana, L.G., Piccin, L., Mocellin, S., Pigozzo, J., and Chiarion-Sileni, V. (2018). Sex and interleukin-6 are prognostic factors for autoimmune toxicity following treatment with anti-CTLA4 blockade. J Transl Med 16, 94.

Valsecchi, M.E. (2015). Combined nivolumab and ipilimumab or monotherapy in untreated melanoma. N Engl J Med 373, 1270–1271.

van Dijk, N., Funt, S.A., Blank, C.U., Powles, T., Rosenberg, J.E., and van der Heijden, M.S. (2019). The cancer immunogram as a framework for personalized immunotherapy in urothelial cancer. Eur Urol 75, 435–444.

van Rhijn, B.W.G., van der Kwast, T.H., Kakiashvili, D.M., Fleshner, N.E., van der Aa, M.N.M., Alkhateeb, S., Bangma, C.H., Jewett, M.A.S., and Zlotta, A.R. (2010). Pathological stage review is indicated in primary pT1 bladder cancer. BJU Int 106, 206–211.

Vétizou, M., Pitt, J.M., Daillère, R., Lepage, P., Waldschmitt, N., Flament, C., Rusakiewicz, S., Routy, B., Roberti, M.P., Duong, C.P.M., et al. (2015). Anticancer immunotherapy by CTLA-4 blockade relies on the gut microbiota. Science 350, 1079–1084.

Wang, J., Sun, J., Liu, L.N., Flies, D.B., Nie, X., Toki, M., Zhang, J., Song, C., Zarr, M., Zhou, X., et al. (2019). Siglec-15 as an immune suppressor and potential target for normalization cancer immunotherapy. Nat Med 25, 656–666.

Webb, E.S., Liu, P., Baleeiro, R., Lemoine, N.R., Yuan, M., and Wang, Y.H. (2018). Immune checkpoint inhibitors in cancer therapy. J Biomed Res, 32: 5–14.

Wrzesinski, C., Paulos, C.M., Kaiser, A., Muranski, P., Palmer, D.C., Gattinoni, L., Yu, Z., Rosenberg, S.A., and Restifo, N.P. (2010). Increased intensity lymphodepletion enhances tumor treatment efficacy of adoptively transferred tumor-specific T cells. J Immunother 33, 1–7.

Wu, Y., Ju, Q., Jia, K., Yu, J., Shi, H., Wu, H., and Jiang, M. (2018). Correlation between sex and efficacy of immune checkpoint inhibitors (PD-1 and CTLA-4 inhibitors). Int J Cancer 143, 45–51.

Xu, X., Ho, W., Zhang, X., Bertrand, N., and Farokhzad, O. (2015). Cancer nanomedicine: from targeted delivery to combination therapy. Trends Mol Med 21, 223–232.

Yan, W.L., Shen, K.Y., Tien, C.Y., Chen, Y.A., and Liu, S.J. (2017). Recent progress in GM-CSF-based cancer immunotherapy. Immunotherapy 9, 347–360.

Zitvogel, L., Ayyoub, M., Routy, B., and Kroemer, G. (2016). Microbiome and anticancer immunosurveillance. Cell 165, 276–287.

Zitvogel, L., Kepp, O., and Kroemer, G. (2011). Immune parameters affecting the efficacy of chemotherapeutic regimens. Nat Rev Clin Oncol 8, 151–160.

Zitvogel, L., Ma, Y., Raoult, D., Kroemer, G., and Gajewski, T.F. (2018). The microbiome in cancer immunotherapy: Diagnostic tools and therapeutic strategies. Science 359, 1366–1370.

Zuiverloon, T.C.M., Nieuweboer, A.J.M., Vékony, H., Kirkels, W.J., Bangma, C.H., and Zwarthoff, E.C. (2012). Markers predicting response to Bacillus Calmette-Guérin immunotherapy in high-risk bladder cancer patients: a systematic review. Eur Urol 61, 128–145.