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Emerging role of immunotherapy in urothelial carcinoma - immunobiology/biomarkers

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Abstract

Urothelial bladder cancer is one of the first cancers recognized to be immunogenic since 40 years ago when the use of bacillus Calmette–Guerin (BCG) was shown to prevent recurrence. Since that time, our knowledge of immune biology of cancer has expanded tremendously, and bladder cancer patients finally have new active immunotherapeutic drugs with on the horizon. Anti-programmed cell death-1 (PD-1)/(programmed cell death ligand-1 (PD-L1) therapy has shown impressively durable responses in urothelial bladder cancer (UBC), but the reported response rates warrant improvement. To outline potential strategies to overcome tumor immune resistance, herein, we summarize current models of tumor immunology with a specific focus on bladder cancer. Recognition of tumor-specific antigens through cross-presentation, T cell priming and activation, and trafficking of immune cells to the tumor microenvironment are some of the critical steps we now understand to be necessary for an effective anti-tumor immune response. Many of the involved steps are important targets for therapeutic interventions. As new immunotherapies are developed, predictive biomarkers will also be important to select patients most likely to respond and to better understand tumor biology. Several potential biomarkers are reviewed including PD-L1 expression, identification of T cell-inflamed/non-T cell-inflamed tumors based on immune gene expression, intrinsic molecular subtyping based on luminal/basal or the cancer genome atlas (TCGA) groups, T cell receptor (TCR) sequencing, and somatic mutational density. Within even the past few years our current knowledge of immune biology has exploded, and we are highly optimistic about the future of UBC therapy that will be available to patients.

Introduction

Decades of efforts aimed at elucidating the biology of cancer immunity have now finally begun to bear fruit for patients in the clinic. Immunotherapy for bladder cancer was reported as early as 1976, when Alvaro Morales reported the successful intravesicular treatment of nine patients with tuberculosis vaccine bacillus Calmette–Guerin (BCG) [1]. Expanded studies confirmed the efficacy of BCG leading to United States Food and Drug

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Administration (FDA)-approval in 1990 as the second cancer immunotherapy, behind only interferon-alpha. Since that time only a single additional drug has secured regulatory approval for the treatment of bladder cancer, valrubicin, in 1998 [2]. Given the paucity of new treatments over the past 20 years (Figure 1) for a disease responsible for over 165,000 deaths per year worldwide [3], identification of novel therapeutic targets has been a key priority in the field.

Our understanding of bladder cancer immunobiology has grown tremendously since the approval of BCG. Several potential mechanisms underlying the anticancer effects of BCG have been identified including activation of the innate immune response through toll-like receptors (TLRs), recruitment of immune cells through cytokine production, and direct cytotoxicity [4]. With the recent successes of immune checkpoint inhibitors in metastatic UBC [5–8], researchers have again brought bladder cancer to the forefront of immunotherapy. There now remains a continued urgency to build upon the early success with immune checkpoint blockade and identify biomarkers to guide patient selection and identify rational combination approaches. This article will examine our current knowledge of the immunobiology of UBC and discuss potential future approaches to improve therapeutic responses to immunotherapy.

Components of effective anti-tumor immunity

When intact and unperturbed, the human immune system can recognize and eradicate abnormal malignant cells. This process is clearly disrupted in patients who develop cancers or have progression during therapy. To develop novel strategies for immunotherapy in UBC, the events required for an effective anti-tumor immune response must be appreciated. First, the process of oncogenesis generally leads to genetic instability and the occurrence of non-synonymous somatic mutations. Such mutations encode for tumor-specific neoantigens that can be recognized by the immune system, allowing the immune response to target malignant cells while sparing normal host cells [9]. Tumor cells are usually poor antigen-presenting cells, so developing cancer immunity depends on cross-presentation of neoantigens by activated dendritic cells, which is dependent on Type I interferon signaling and generally occurs in tumor-draining lymph nodes [10, 11]. This fundamental step is necessary for the development of spontaneous T cell priming against cells recognized as non-self and the generation of adaptive immunity. This selectivity underpins a major advantage of immunotherapies over cytotoxic antineoplastic therapies.

Comprehensive genomic profiling of UBC has confirmed that it is associated with a relatively higher mutational burden compared with other cancer types [9, 12–14]. Also notable is the high inter-patient variability of UBC, with some tumors developing over one thousand non-synonymous mutations and others developing several orders of magnitude fewer mutations. An important challenge in current UBC immunotherapy development will be to facilitate responses in patients with tumors that fail to elicit anti-tumor immune priming. It may be that a subset of specific neoantigens is necessary to drive T cell priming in UBC. Additionally, recruitment of important immune cells, such as dendritic cells or lymphocytes, to the tumor microenvironment is often circumvented through tumor-specific mechanisms.

Priming and activation of tumor antigen-specific T cells is dependent on both the engagement of the T cell receptor (TCR) with the MHC:neoantigen complex and the presence of a co-stimulatory signal resulting from binding of CD28 with B7-1 (CD80) or B7-2 (CD86) on activated dendritic cells [15, 16]. In the absence of this second signal, T cells not only fail to become activated and proliferate, but also become prone to a hyporesponsive state called anergy or apoptosis [17]. While still accurate, this general historical model is somewhat over-simplified. Indeed, additional factors including the strength of the TCR signal and the presence or absence of other signals also influences the fate of the T cell.

Once a tumor-antigen specific T cell is activated, expression of other surface molecules is induced. These molecules may be co-stimulatory, such as members of the tumor necrosis factor (TNF) receptor superfamily 4-1BB (CD137), OX-40 (CD134), GITR, and ICOS (CD 278) [18–20]. Paradoxically, the expression of costimulatory molecules such as 4-1BB has also been noted on dysfunctional T cells [21]. T cell activation also results in the expression of inhibitory molecules, which act as a rheostat on the immune response. These inhibitory molecules have now been well described and include CTLA-4 (CD152), LAG-3 (CD223), Tim-3, VISTA, BTLA-4, and PD-1 (CD279) [19, 22]. These are typically induced early in T cell activation, but persistence of negative signals results in T cell dysfunction and tolerance. In bladder cancer, the presence of inhibitory and co-stimulatory markers has been observed by immunohistochemistry, flow cytometry, and gene expression [5, 23–28]

A principle component of the entire anti-tumor immune response is the recruitment and activation of immune cells to the tumor. To this effect, a supportive tumor microenvironment is critical to initiating the cascade of adaptive immunity. Bladder tumors, like other cancers, show variability in infiltrating immune cells and increased tumor-infiltrating lymphocytes has been linked with better prognosis (Figure 2) [28, 29]. The lack of recruitment of immune cells may be secondary to altered cytokine/chemokine production, tumor cell-intrinsic oncogenic pathways, or variability in patient immune function secondary to genetics or environmental factors. When infiltrating immune cells are present, an impeded immune response may be secondary to expression of the aforementioned immune checkpoints, but also due to the presence of other immune regulatory cells such as Tregs (CD4+CD25+FoxP3+), monocytes, myeloid-derived suppressor cells, or fibroblasts.

Therapeutic targeting of dysfunctional anti-tumor immunity

Strategies to reestablish the elements of cancer immunity that are circumvented by tumors have now resulted in an eruption of novel cancer immunotherapeutics. In tumors believed to lack a tumor-antigen specific T cell response, the use of therapeutic vaccines to stimulate an adaptive immune response are being investigated [30]. Vaccines use one or more tumor-associated or tumor-specific antigens. The latter is preferred since tumor-associated antigens may be variably expressed in normal tissues, which likely results in deletion of high-avidity TCRs through immunologic development.

In addition to selection of the most active and specific antigens, the use of adjuvants is now understood as critical to generating an effective immune response. The ideal adjuvant

stimulates CD8⁺ T cells and activates T_H1 helper cells, which produce interferon-gamma. Adjuvants with immunogenic properties include microbial products such as BCG, liposomal microspheres, immune modulators such as GM-CSF, and viral vectors. Given that many historical vaccine trials were plagued by the development of tolerance and poor response rates, there is now hope that recent advances in immune checkpoint blockade can be adopted in combination to enhance response to therapeutic vaccines.

Vesigenurtacel-L (HS-410) is now in clinical development for non-muscle invasive bladder cancer. This drug is thought to produce potent CD8⁺ T cell responses against multiple bladder cancer antigens and is currently being investigated in combination with BCG. The vaccine employs the chaperone protein gp96 (HSP90B1) as an adjuvant to stimulate dendritic cell activation. Preliminary data indicate both an increase in CD8⁺ tumor-infiltrating lymphocytes and an increase in T cell clonality by TCR sequencing in clinically responding patients [31].

For muscle invasive disease, treatment with a personalized vaccine was recently showed to improve overall survival versus best supportive care [32]. In this study, patients were randomized to best supportive care with or without a personalized vaccine that included 2–4 of 31 candidate peptides selected based on HLA types and peptide-reactive IgG titers. The personalized vaccine was given with an adjuvant (Montanide ISA 51 VG). In addition to increasing overall survival, significant immune responses were noted after 7 weeks as measured by IgG titers and interferon- γ ELISPOT. A positive immune response also correlated with longer progression free survival. However, progression-free survival for the total assessed group was not significantly prolonged with vaccine treatment. Given that significant number of patients in this trial did not demonstrate an immune response, investigation into causes for lack of response could inform future vaccine studies in UBC.

Adoptive immunotherapy is another approach used to induce tumor antigen-specific T cell responses [33]. In this approach, lymphocytes are harvested and expanded, then re-infused into the patient. In UBC, a pilot study of infusion of T cells that were expanded ex-vivo from tumor draining lymph nodes reported 2 out of 6 patients with responses [34]. This process may be enhanced through genetic engineering of T cells to express chimeric antigen receptors (CARs). Remarkable responses using this approach to target CD19 in hematologic malignancies such as ALL have been reported and this technology has expanded to other targets [35]. However, the development of CARs for solid tumors such as UBC has been limited by selection of ideal antigens with expression limited to tumor cells. Antigens such as L1-CAM (CD 171) and prostate stem cell antigen (PSCA) are expressed on bladder cancer cells and might be amenable to targeting by CAR T cell therapy [36, 37]

Research teams are also investigating the use oncolytic viruses to induce direct cytotoxicity in cancer cells and stimulate an anti-tumor immune response [38]. In non-muscle invasive bladder cancer the use of an oncolytic adenovirus, CG0070, is underway [39]. CG0070 is a replication-competent oncolytic adenovirus that selectively replicates in RB-deficient tumors, a tumor suppressor commonly lost in bladder cancer [40]. This therapy uses granulocyte macrophage-colony stimulating factor (GM-CSF) under control of the human E2F-1 promoter, which is upregulated in RB-deficient cells [41]. CG0070 is hypothesized to

stimulate an adaptive immune response through the release of tumor-specific antigens and stimulation of dendritic cells by GM-CSF. A phase II trial of CG0070 is currently underway.

Even when T cells recognize tumor-specific antigens, lack of co-stimulation or the presence of inhibitory pathways prevents effector T cells from clonal expansion or carrying out effector function. Reversing this T cell dysfunction through inhibition of immune checkpoints has resulted in a sea change in cancer immunotherapy. Targeting CTLA-4 with ipilimumab was the first clinically successful immune checkpoint inhibitor therapy, which gained FDA approval in melanoma [42, 43]. The duration of disease control and plateau in survival curve after three years [44] has even led researchers to entertain the concept of cure in a fraction of treated melanoma patients [45].

A window of opportunity study explored two doses of ipilimumab in 12 patients with localized invasive UBC prior to cystectomy to determine the pharmacodynamic effects of treatment [27]. Ipilimumab treatment was shown to increase tumor infiltration of immune cells positive for CD3, CD4, and CD8. The specific population of tumor-infiltrating CD8⁺ICOS^{hi} and CD4⁺ICOS^{hi} cells increased, and was shown to produce interferon gamma and recognize the tumor-associated NY-ESO-1 antigen [46]. ICOS is a member of the extended B7/CD28 superfamily that is induced upon T cell activation. Preclinical studies have led to the development of an agonistic ICOS antibody (JTX-2011) that is currently being investigated as a novel immunotherapeutic [47, 48]. Ipilimumab has also been explored in combination with chemotherapy for metastatic UBC. In that setting it was shown to increase circulating CD4 and CD8 T cells and alter the peripheral blood cytokine/chemokine milieu in a subset of patients [49].

Soon after the development of anti-CTLA-4 antibodies, several antibodies targeting PD-1/PD-L1 were developed and have resulted in regulatory approvals in melanoma, non-small cell lung cancer, and renal cancer [50–52]. This class of immunotherapies has generated the most excitement in UBC, given the reports of early-phase clinical trials of atezolizumab and pembrolizumab demonstrating impressive responses in a subset of heavily pre-treated patients including several complete responses [5, 53, 54]. These antibodies are now being explored in clinical trials involving virtually every clinical disease state of UBC.

Rescuing T cell function is now being studied with several other inhibitory checkpoints including LAG3 (CD223) [55], Tim-3 [23, 56], BTLA-4 [57], and VISTA [58]. Similarly, agonistic antibodies targeting co-stimulatory pathways is a complimentary approach to enhance T cell activation and effector function. Targets being investigated include 4-1BB (CD137), OX-40 (CD134), GITR, and ICOS (CD 278) [59]. Reports in bladder cancer tumor models indicated that agonism of GITR leads to reduction in Treg populations within the tumor [60]. Finally, deranged metabolic pathways also impact T cell function. For instance, the expression of indoleamine-2,3-dioxygenase (IDO) leads to catabolism of tryptophan by conversion into kynurenine. Depletion of tryptophan and accumulation of kynurenine in tumors results in effector T cell dysfunction and accumulation of Tregs. [61, 62]. Inhibition of this enzyme in combination with anti-PD-1/PD-L1 therapy has show response rates of over 50% in a Phase I/II study with advanced solid tumor patients, including 2 with UBC [63].

In addition to enhancing T cell function, regulation of the tumor microenvironment will be critical to enhance immunotherapeutic efficacy. Inhibitory and costimulatory pathway upregulation is linked with activation of T cells and dependent on their presence in the tumor microenvironment [64]. Consistent with this experimental observation, clinical response to immunotherapy in several cancer types is correlated with the presence of a pre-existing immune cell infiltrate or “T cell-inflamed” phenotype (Figure 2) [65–67]. Causes for the development of a non-T cell-inflamed phenotype remain incompletely understood, although recent evidence implicating tumor cell oncogenic pathways, such as activation of Wnt/beta-catenin or PTEN loss, has been reported [68, 69]. In UBC, certain targetable pathways such as FGFR3, appear to be activated exclusively in non-T cell inflamed tumors and might represent novel targets [28]. The induction of T cell infiltration and an adaptive immune response can be achieved experimentally by activation of the STING pathway [70–72]. The effect is driven by Type I interferon production in response to sensing of cytosolic DNA. Finally, environmental effects may even play a role in mediating a T cell-inflamed phenotype. The presence of specific commensal microbiota in the gut was recently shown to impact response to immune checkpoint blockade in an experimental model [73]. Going forward, the recruitment of immune cells into tumors lacking a pre-existing immune response will be a major challenge for advancing current immunotherapeutic strategies [74].

Development of predictive biomarkers

As new immunotherapeutic drugs are being developed at a rapid pace, there is an ongoing need to concurrently identify predictive biomarkers to personalize our therapeutic algorithms. Much excitement has been generated in UBC regarding anti-PD-1/PD-L1 therapy, but the majority of patients’ cancers still fail to respond. Ideally, clinicians could identify patients highly unlikely to respond and direct them to clinical trials investigating novel approaches. Considerable work has been aimed at this goal in both UBC and other tumor types.

PD-L1 expression by immunohistochemistry has been studied extensively as a biomarker for immune response (Table 1). As previously mentioned, expression of inhibitory molecules such as PD-1 occurs after T cell activation, and its persistence leads to T cell dysfunction. The PD-1 ligand, PD-L1, can be expressed by both tumor cells and/or tumor-infiltrating immune cells. In UBC, it has been observed in some studies that expression by tumor cells is infrequent (<10%), but expression of PD-L1 on tumor-infiltrating immune cells occurs in up to one-third of samples [5, 25]. The latter has demonstrated prognostic value as it is associated with improved overall survival in metastatic disease [25]. Clinical studies for each individual anti-PD1/PD-L1 antibody in UBC have reported different variations of PD-L1 expression as associated with clinical response. For atezolizumab, tumor infiltrating immune cell expression of PD-L1 was associated with objective response rate [54]. In contrast, expression of PD-L1 on both tumor cells and tumor-infiltrating immune cells has been linked with objective response rate for durvalumab and pembrolizumab [53, 75]. Significant challenges remain for using PD-L1 as a predictive biomarker. As with any immunohistochemical assay, widespread adoption would necessitate extensive validation and the development of uniform standards of interpretation. Ideally, a biomarker would be predictive of response to all drugs in the anti-PD-1/PD-L1 class, as opposed to a single

agent. Most importantly, up to 10% of patients with PD-L1 negative tumors have been shown to respond anti-PD-L1 therapy. This suggests that the negative predictive value is insufficient to exclude patients from potentially life-prolonging therapy. However, data being generated from ongoing trials may refine our use of this biomarker to improve its predictive value.

Aside from PD-L1, other immune checkpoints are induced upon activation of T cells present in the tumor microenvironment. CD8A expression in UBC strongly correlates with PD-L1, LAG-3, Tim-3, IDO, and FoxP3 [28]. Perhaps the assessment of tumor-infiltrating immune cells alone by either immunohistochemistry or immune gene expression may be a more robust biomarker of response to immune checkpoint blockade. In melanoma, NanoString technology was used to develop an immune gene expression score that associated with clinical response [76]. Similarly, response to anti-PD-L1/PD-L1 therapy in head and neck cancer could also be predicted by immune gene expression [77]. In UBC, expression of interferon γ -inducible, T-helper-1-type chemokines, CXCL9 and CXCL10, were linked with response to atezolizumab [54].

The mutational landscape is also important for response to immunotherapies, as the adaptive cancer immunity relies on recognition of neoantigens encoded by nonsynonymous mutations. Recent studies in melanoma and lung cancer have correlated the neoantigen density of a tumor with the efficacy of immune checkpoint blockade [78, 79]. Furthermore, patients with colon cancers carrying high somatic mutation rates due to mismatch-repair deficiency demonstrated a 40% response rate to anti-PD-1 therapy with pembrolizumab as compared with a 0% response rate in patients with mismatch repair-proficient tumors [80]. Recently published data on atezolizumab in urothelial bladder cancer showed that responders had a higher median mutational load than non-responders (12.4 versus 6.4 mutations/Mb) [54]. However, the implication that mutational density alone can predict response remains unconfirmed. There is wide variation with the upper range in non-responders being over 50 mutations/Mb and many responders having fewer than 10 mutations/Mb. Even cancers with relatively fewer neoantigens, such as renal cancer, respond to immunotherapy [52]. Additionally, some data indicate that immune infiltration in UBC does not correlate with mutational density alone [28]. Likely there is more complexity to the relationship between mutations and immunotherapy response. Novel strategies at defining immunogenic epitopes are underway including predictive MHC binding algorithms and tandem mass spectrometry and exome sequencing [81, 82].

In contrast to tumor sequencing, next-generation sequencing of T cell receptor (TCR) transcripts has recently emerged as a method to characterize the tumor infiltrating T cell repertoire of an individual patient [83–85]. T cells contains a pair of receptor chains, most commonly alpha and beta. Three complementarity-determining regions (CDRs) on each chain bind amino acid residues of peptides presented by MHC molecules. The CDR3, or third region, of a TCR beta chain is generated by rearrangement of the variable (V), diversity (D), and joining (J) gene segments to generate a diversity of T cell clones. Thus, this region is what enables recognition of myriad peptide sequences, including neoantigens, and defines the clonal specificity of a T cell. TCR alpha chains undergo similar rearrangement, but the resulting CDR3s only contain V and J segments. Using TCR sequencing of the CDR3

regions, the clonality of a population of tumor infiltrating lymphocytes can be assessed by decomposition of the V-(D)-J transcripts. In UBC, one study indicated that a low TCR diversity, which is suggestive of expansion of tumor-antigen specific T cell clones within the tumor, correlated with longer recurrence free survival after cystectomy [86]. Interestingly, in recurring patients the diversity index was highly correlated with the ratio of CD8/FOXP3 expression. In other words, recurring tumors either demonstrated high diversity, which indicates a lack of clonal expansion of antitumor CD8⁺ T cells, or low diversity due to clonal expansion of immunosuppressive Treg cells. As in this example, TCR sequencing is a useful strategy to understand intrapatient variability in prognosis, but might also be applied in the future to understand specific responses to immunotherapies.

Several genomic analyses of UBC have identified intrinsic molecular clusters, such as the luminal and basal subtypes or TCGA clusters I to IV [14, 87, 88]. These subtypes likely define biologically distinct cancers that might have differential sensitivity to immunotherapies. In the recent phase II study of atezolizumab in UBC, differential responses were noted in TCGA subtypes, with cluster II having a significantly higher objective response rate (34%) versus cluster I, III, and IV (10%, 16%, and 20%, respectively) [54]. Interestingly, PD-L1 expression on tumor-infiltrating immune cells was predictive of response, but not PD-L1 expression on tumor cells. Basal subtype tumors had significantly higher PD-L1 expression on tumor infiltrating immune cells versus luminal subtype (60% versus 23%). CD8 T-effector gene expression was increased in all TCGA clusters except luminal I, which had the lowest response rate. Using logistic regression modeling, TCGA subtype, PD-L1 expression, and mutational burden each provided non-redundant, complimentary predictive information in the atezolizumab study. This was the first study to assess biomarkers of response to anti-PD-L1 therapy in UBC and provided thorough insight into biological mechanisms at play.

Conclusion and future directions in bladder cancer immunotherapy

Our growing understanding of immune biology coupled with recent successes in immune checkpoint blockade in UBC now has the potential to truly impact clinical therapeutics for UBC. However, it is humbling to note patients with advanced disease benefiting from immune checkpoint blockade remain in the minority, only modestly above the historical 10% response rate with chemotherapies. It is therefore incumbent upon us to continue to improve our immunotherapeutic strategies rationally from our expanding understanding of tumor immunology. Conceptual strategies gaining interest include combining immunotherapy with chemotherapy, radiotherapy, or molecularly-targeted therapy [89]. However, clinically evaluating all, or even most, of the possible combinations employing immunotherapies in UBC would be impossible. Priority should be given to approaches with the most rational and abundant preclinical and/or clinical supporting data. It is important to borrow knowledge from advances in tumor immunology from other cancers such as melanoma, however UBC has unique biologic features warranting investigation in a disease-specific manner. With any approach tested in the clinic, the development and validation of biomarkers will also be critical.

It is highly likely that separate strategies will need to be developed for individuals with tumors with a pre-existing, but suppressed, immune response, or T cell-inflamed UBCs, versus non T cell-inflamed UBCs. Published data indicate the former group is more likely to respond to anti-PD-L1 targeted therapy, but even in this enriched population less than half of patients respond. It is likely that dominant negative regulatory pathways drive resistance mechanisms in this subgroup. Thus, the addition to anti-PD-L1 of additional antagonist antibodies targeting immune checkpoints or agonistic antibodies for co-stimulatory targets therapy may overcome this resistance.

Non-T cell-inflamed UBC tumors may not benefit from such an approach as they lack pre-existing tumor-antigen specific immune priming. This group may prove more challenging to treat with immunotherapies. A logical approach in this subgroup would to enhance recruitment of immune cells to the tumor microenvironment and priming of dendritic cells. Treatment with vaccines to generate tumor antigen-specific T cells, or the use of chemotherapy or radiotherapy to expose neoantigens for dendritic cell priming, followed by the addition of immune checkpoint blockade should be pursued for non-T cell-inflamed UBC. Cisplatin based chemotherapy remains the standard of care for metastatic UBC and localized disease in the peri-operative setting. Most patients respond to chemotherapy, but often have a short duration before progression. The addition of immunotherapy could potentially extend the tail on the survival curve after chemotherapy provides an acute response. Finally, as oncogenic pathways that cause immune exclusion are validated, the use of targeted therapy to inhibit those pathways should be tested for the induction of an immune infiltrate.

Anti-PD-L1 therapy has now become approved in melanoma, non-small cell lung cancer, and renal cancer. The excitement around its activity in UBC is giving hope to patients and providers desperate for improvement in our current treatment paradigm. Teamwork between basic, translational, and clinical scientists across different institutions has an ability to change the field more rapidly now than ever before.

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Figure 1. Cumulative number of United States Food and Drug Administration (FDA) drug approvals in genitourinary cancers beginning in 1995 through 2015. Only a single drug, valrubicin, was approved for UBC during this time period.

Figure 2.

Schema depicting the development of T cell-inflamed versus non-T cell-inflamed tumor microenvironments in UBC. T cell-inflamed tumors are associated with upregulation of immune inhibitory pathways that suppress the immune response and result in resistance to anti-PD-1/PD-L1 therapy. In contrast, non-T cell inflamed tumors lack infiltrating T cells, thus generally do not respond to immune checkpoint blockade. This group may require immune priming strategies (vaccines, chemotherapy, radiation therapy, etc.) or targeting of oncogenic pathways that are resulting in T cell exclusion.

Table 1

Characteristics of predictive biomarkers for anti-PD-1/PD-L1 immunotherapy in UBC

Biomarker	Clinical Data	Advantages	Disadvantages
PD-L1 on tumor cells (TC)	<ul style="list-style-type: none"> Atezolizumab phase II study: Only 20% of samples showed any visible TC staining. No correlation with response Pembrolizumab phase 1b study: ORR 9% (TC-) vs. 33% (TC+) 	<ul style="list-style-type: none"> Extensive experience with immunohistochemistry assay in multiple cancers including UBC Rapid turnaround time 	<ul style="list-style-type: none"> Mixed results for prediction of response with pembrolizumab and atezolizumab Insufficient negative predictive value for clinical use, responses seen in each group
PD-L1 on infiltrating immune cells (IC)	<ul style="list-style-type: none"> Atezolizumab phase II study: ORR 8% (IC0) vs. 10% (IC1) vs. 26% (IC2/3) Pembrolizumab phase 1b study: ORR 0% (TC/IC-) vs. 29% (TC/IC+) 	<ul style="list-style-type: none"> Correlated with response to therapy with two different drugs Extensive experience with immunohistochemistry assay in multiple cancers including UBC Rapid turnaround time 	<ul style="list-style-type: none"> Mixed results for prediction of response with pembrolizumab and atezolizumab Insufficient negative predictive value for clinical use. While no responses to pembrolizumab were seen in TC and IC negative patients, data limited to 4 (TC/IC-) patients
T cell immune gene signature	<ul style="list-style-type: none"> Atezolizumab phase II study: Higher expression of each 8 T cell signature genes correlated with improved clinical outcome, with CXCL9 and CXCL10 reaching statistical significance (p=0.0057 and p=0.0079, respectively) Pembrolizumab phase 1b study: 13-gene T cell signature associated with PFS (p= 0.024) 	<ul style="list-style-type: none"> Correlated with response to therapy with two drugs Likely most immunologically relevant classification 	<ul style="list-style-type: none"> No standardized assay (several different T cell immune signatures currently being explored) Limited predictive clinical data published to date
Intrinsic molecular subtypes	<ul style="list-style-type: none"> Atezolizumab phase II study: ORR highest in TCGA luminal subtype II at 34% vs. 10% (subtype I), 16% (subtype III), and 20% 	<ul style="list-style-type: none"> Well characterized subtypes by TCGA analysis and prior studies Likely representative of biologically distinct UBC cancers 	<ul style="list-style-type: none"> No standardized assay (varying number of clusters in various analyses) Longer turnaround time, would require

Biomarker	Clinical Data	Advantages	Disadvantages
Neoantigen density	(subtype IV) (p=0.0017)		<ul style="list-style-type: none"> gene expression profiling and bioinformatic analyses Insufficient negative predictive value for clinical use, responses seen in each group
	<ul style="list-style-type: none"> Atezolizumab phase II study: High mutational density in responders (12.4 per megabase) non-responders (6.4 per megabase) (p<0.0001) 	<ul style="list-style-type: none"> Sequencing data increasingly obtained for other purposes (i.e. to provide potential predictive markers for molecular targeted therapy) 	<ul style="list-style-type: none"> No standardized assay (assessment of Longer turnaround time as requires genomic sequencing

ORR=objective response rate defined according to RECIST v1.1