

## Immunotherapy of Bladder Cancer

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### Abstract

Bladder cancer used to be the only cancer treated by immunotherapy in form of intravesical BCG. Since approval of BCG for Non muscle invasive bladder cancer (NMIBC), there has been significant advancement in our knowledge about immune alteration in cancer and availability of immunotherapeutic agents. Tumor induced cell mediated immunosuppression is identified as a key factor for development and progression of cancer. Immune suppression in bladder cancer is predominantly through Macrophages. Myeloid derived suppressor cell, NK cells, Treg and expression of immune checkpoint receptor inhibitors also contribute to immune suppression. BCG induces innate immune response and its efficacy is limited to NMIBC. Novel immunotherapeutic agents evaluated in bladder cancer are administered locally or systemically to induce innate or adaptive immune response. Systemic administration of antibodies against PD-1/PD-L1 axis are now approved for treatment of locally advanced/metastatic bladder cancer as a first line as well as second line therapy. Pembrolizumab is also approved for BCG unresponsive NMIBC. Since response to immunotherapy are neither uniform nor universal, attempts are made to identify prognostic and predictive biomarkers. Identified biomarkers lack desired specificity and sensitivity. Several immune approaches using innate as well as adaptive mechanism are under evaluation to improve outcome of intravesical BCG or immune check point receptor inhibitors.

**Keywords:** *NMIBC; MIBC; Metastatic bladder cancer; BCG; Pembrolizumab; Nivolumab; Atezolizumab; Durvalumab; Avelumab; CADI-05; Novel therapy; Adaptive immune response; Innate immune response; Tumor infiltrating immune cells (TIL), Macrophage; Treg; Immune check point receptors; Desmocollin 3*

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### Introduction

Bladder cancer is 10<sup>th</sup> common cancer with incidence of approximately 550,000 (3% of all cancers) with age standardized rate of 9.6 per 100,000 for males and 2.4 per 100,000 for females globally [1]. It is characterized by the highest rate of somatic mutations with mutation frequency of transcription factor is equal to RNA binding protein [2,3]. Immunotherapy in the form of intravesical BCG to treat non muscle invasive bladder cancer (NMIBC) has a unique distinction of being the first approved immunotherapy. Intravesical BCG predominantly induces innate immune response. Innate immune response is a localized quick immune response mediated by macrophages/monocytes, NK cells, Neutrophils etc. Another type of immune response is known as adaptive immune response mediated by T cells (CD4, CD8) and dendritic cells.

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BCG immunotherapy though successful, is associated with recurrence of disease, probably because of inability to induce tumor specific immune response [4]. This has led to search for better immunotherapies to improve outcome. The immunotherapeutic agents evaluated in management of bladder cancer are known to induce innate immune response and/or adaptive immune response. For generating innate immune response, it is given at the site of action (Intravesically).

Cancer development and progression is associated with changes in immune profile of tumor microenvironment. There is increase in immunosuppression with progression of tumor. Cell mediated immune response is a key player in immunoediting associated with tumor progression as well as elimination. The cancer induced immunosuppression is also described as Th2 type of immune response.

Checkpoint inhibitors acting on PD-1/PD-L1 axis, induces adaptive immune response by eliminating tumor induced immunosuppression. They are known to induce proliferation of antigen experienced tumor infiltrating immune cells. Presence of antigen experienced immune cells is dependent on neoantigens which in turn depends on burden of mutation. Their introduction as immunotherapeutic agents have improved outcome of many cancer and are currently approved for the management of metastatic bladder cancer also.

In this article brief overview of immunosuppressive cells in bladder cancer, approved immunotherapy, biomarkers for immunotherapy and novel immunotherapeutic agents for bladder cancer is provided.

## **Immunosuppressive Immune Cells Relevant for Bladder Cancer**

Immunosuppressive cells are also grouped into innate and adaptive.

### **Immunosuppressive cells responsible for innate immunity**

#### **Macrophage**

Of all immune cells, macrophage is the predominant tumor-infiltrating immune cell in bladder cancer [5]. Macrophage, found in established tumors [tumor associated macrophage (TAM)], are immunosuppressive in nature (M2 macrophage). Tumor vascularity and angiogenesis seen in bladder cancer is ascribed to TAM and their no. is associated with higher stage and grade of disease [5,6]. In NMIBC as well muscle invasive bladder cancer (MIBC), higher M2 type (CD68+ve and CD163+ve) are associated with poor response to therapy (intravesical BCG for NMIBC and chemotherapy for MIBC; resistance to BCG, shorter recurrence free survival, progression free survival) [5,7-12].

#### **Myeloid derived suppressor cells (MDSCs)**

MDSCs are recruited to the tumor from circulation usually in response to tumor microenvironment (TME) mainly tumor cells. Their number in peripheral blood and tumor correlate with stage and grade. Higher number is associated with poor prognosis [13]. Intratumoral MDSCs are inversely proportional to intratumoral CD8+ T cells [14]. T cell to MDSC ratio of <1 correlating with a poorer recurrence free survival post treatment [15]. They express immunosuppressive factors like Arginase 1, inducible nitric oxide synthases (iNOS) and PD-L1 [16].

### **Neutrophils/Neutrophil-lymphocyte ratio**

Neutrophils seen in bladder cancer are immunosuppressive in nature unless altered by therapy [16]. They are essential for efficacy of intravesical BCG therapy [17,18]. They are associated with grade of tumor. Intratumoral high neutrophils are predictive of poor prognosis [19]. Circulating neutrophil-lymphocyte ratio (NLR) is identified as a prognostic parameter for response to therapy. High NLR appeared to correlate with worse overall, recurrence-free and cancer specific survival [20-22].

### **Immunosuppressive cells responsible for adaptive immunity**

#### **Treg cells (FoxP3 Regulatory Cells)**

High Treg counts are associated with higher stage (T1 or more) and high tumor grade [23]. Their number is associated with response to therapy with higher number predicting failure to respond or a shorter recurrence free survival post BCG treatment for NMIBC [9, 10, 23-25]. Their relative proportion amongst T cell population is more important [10].

#### **Immune inhibitory receptor (checkpoint) expression**

Expression of inhibitory receptors on immune cells or their ligands on tumor cells help tumor cells to escape immune surveillance. Widely studied inhibitory receptors (Immune Checkpoints) are PD-1/PD-L1, IDO1 and CTLA-4. PD-1/PD-L1 expression increases with increased stage and grade of disease. In one study PD-L1 expression was 7% - pTa, 16% - pT1, 23% - pT2, 30% - pT3/4, CIS - 45% and BCG unresponsive tumors - 90% [26]. Higher PD-1 expression on CD8+ T-cell is also associated with poor response or failure to respond to therapy (BCG in NMIBC and/chemotherapy for MIBC) [26-28]. The IDO expression is associated with a poorly differentiated tumors. IDO1 expressing tumors are more likely to be large and more aggressive [26]. All three immune inhibitory receptors are interrelated in bladder cancer [26].

### **Approved Immunotherapies for Non Muscle Invasive Bladder cancer**

Intravesical BCG has been a gold standard for management of NMIBC. The regimen for its use has been standardized over time. So far, efforts to have a therapeutic agent as good as or better than BCG are not successful.

#### **Intravesical BCG**

Intravesical BCG following transurethral resection (TUR) is a standard of care to prevent recurrence of tumor. It achieves complete response rate of around 70% after induction therapy. Maintenance therapy is useful to consolidate the outcome of induction therapy and preventing progression. Prevention of disease progression is maximum in CIS [29]. It provides better outcome compared to intravesical chemotherapy. The risk of recurrence following intravesical BCG is around 30% lower compared to intravesical chemotherapy [29]. Intravesical BCG works by inducing innate immune response. Response to BCG is seen in absence of expression of none or one of the four immunosuppressive markers (PD-L1, PD-1, FOXP3, and CD204) in the urine at 6 to 8 weeks [30]. Resistance is associated with the expression of two or more of PD-L1, PD-1, FOXP3 and CD204 [30]. Efficacy is dependent on tumor infiltration of neutrophils, NK cells and change in macrophage (CD68)/T cell (CD3) ratio [31-34].

There are efforts to predict outcome of intravesical BCG [35,36]. Tumor grade and stage, tumor recurrence pattern and presence/absence of lymphovascular invasion are easy to evaluate and are predictive of progression of disease. They are not good parameters to predict response to therapy. Age, sex and genetic polymorphism predicts poor response to intravesical

BCG. Response to therapy is poor in females, elderly and those harbouring polymorphisms in genes encoding IL-6, IL-17, IL-2, TNF-  $\alpha$ , MCP-1, TRAIL receptors. Other genes identified include hGPX1, NRMAP1.

### **Systemic pembrolizumab**

For the treatment of patients with BCG unresponsive, high-risk, NMIBC with carcinoma in situ (CIS) with or without papillary tumors who are ineligible for or have elected not to undergo cystectomy.

### **Approved Immunotherapies for locally advanced or Metastatic Bladder cancer**

#### **Pembrolizumab [37]**

For the treatment of patients:

- a) Who are not eligible for any platinum-containing chemotherapy, regardless of PD-L1 status?
- b) Who have disease progression during or following platinum-containing chemotherapy or within 12 months of neoadjuvant or adjuvant treatment with platinum-containing chemotherapy?

#### **Nivolumab [38]**

For the treatment of patients with locally advanced or metastatic urothelial carcinoma who have disease progression during or following platinum-containing chemotherapy or have disease progression within 12 months of neoadjuvant or adjuvant treatment with platinum-containing chemotherapy.

#### **Atezolizumab [39]**

For the treatment of adult patients with locally advanced or metastatic urothelial carcinoma who:

- a) Are not eligible for any platinum-containing chemotherapy regardless of PD-L1 status,
- b) Or have disease progression during or following any platinum-containing chemotherapy, or within 12 months of neoadjuvant or adjuvant chemotherapy.

#### **Durvalumab [40]**

For the treatment of:

- a) Locally advanced or metastatic urothelial carcinoma who have disease progression during or following platinum-containing.
- b) Have disease progression within 12 months of neoadjuvant adjuvant treatment with platinum-containing chemotherapy.

#### **Avelumab [41]**

Patients with locally advanced or metastatic urothelial carcinoma (UC) who:

- a) Have disease progression during or following platinum-containing chemotherapy
- b) Have disease progression within 12 months of neoadjuvant or adjuvant treatment with platinum-containing chemotherapy.

Drug		Relevant study	No. patients	ORR (%)	CR (%)	Median OS (MO)	Grade 3-4 TRAEs (%)
<i>Second-line treatment (in platinum refractory cases)</i>							
Anti PD-L1	Atezolizumab	IMvigor210, Cohort2 [2]	310	14.5	5.0	7.9	16.0
	Durvalumab	NCT01693562	191	17.8	3.7	18.2	6.8
	Avelumab	NCT01772004	249	17.0	6.0	6.5	8.0
Anti-PD-1	Pembrolizumab	KEYNOTE-045	521	21.1	6.0	10.3	15.0
	Nivolumab	Checkmate-275	265	19.6	2.3	8.7	18.0
<i>First-line treatment (in cisplatin-ineligible patients)</i>							
Anti PD-L1	Atezolizumab	IMvigor210, Cohort 1	119	23.0	9.2	15.9	16.0
Anti-PD-1	Pembrolizumab	KEYNOTE-052	370	24	5	-	15.0
<i>BCG unresponsive[37]</i>						<b>Median duration of response</b>	
Anti-PD-1	Pembrolizumab [39]	KEYNOTE-057	096	-	41	16.2	12.6 [43]

**Table 1:** US - FDA approved immune checkpoint inhibitors in metastatic urothelial carcinoma [42].

### Biomarkers for Immunotherapy of Bladder Cancer

Immune therapy works by changing immune tumor microenvironment (iTME). The changes are dependent on background iTME. The efforts are made to use this information related to iTME to provide prognostic and predictive biomarkers. Currently, there are no biomarkers with desired specificity and sensitivity to select therapy for treatment of NMIBC as well as MIBC. In general, lower amount of immunosuppressive cells indicates good prognosis. Predicting response is more challenging but higher GATA3 and lower Tbet predicts response to BCG and vice versa [44].

#### Prognostic biomarkers

##### Cytotoxic T cells; CD8+ T cells

Exhausted CD8 cells expressing immunosuppressive markers like PD-1 are associated with shorter RFS in MIBC. However, their presence indicate potential for treatment with immunotherapy to improve outcome [24].

##### CD4+ T cells

There are no good studies with functional phenotypic CD4 cells. Their presence in tumor in tumor is generally indicative of immunosuppression (immunosuppressive subtype) and is suggestive of poor prognosis in NMIBC [24].

##### PD-L1

Higher PD-L1 expression is associated with aggressive disease and decreased overall survival and negative PD-L1 status is associated with improved PFS and OS when treated with chemoradiotherapy [45].

### **Neutrophil, Neutrophil - lymphocyte ratio**

Higher intratumoral neutrophil and neutrophil - lymphocyte ration is associated with poor prognosis (RFS and OS) [19-22].

### **Natural resistance - associated protein1 (NRAMP1)**

NRAMP1 polymorphism is associated with increased recurrence rate following BCG therapy with decreased recurrence free survival and cancer specific survival [29].

### **Myeloid derived suppressor cells**

MDSC expression predicts poor prognosis (Overall Survival) [46].

### **Desmocollin3 (DSC3)**

DSC3 is described to be over expressed in a basal squamous subtype of MIBC [47]. It has an inverse correlation with GATA3. In other cancers, its role as a prognostic parameter is described [48,49] but this is not known in bladder cancer.

### **Predictive biomarker**

#### **Macrophage**

Higher no. of CD183 expressing macrophages predict resistance to BCG therapy [10]. They are negative predictor of recurrence-free survival, progression-free survival and disease-specific survival to BCG [7,8].

#### **GATA3**

Expression of GATA3 within tumor is indicative of immunosuppression. Its expression is higher in NMIBC and low grade tumors, its overexpression predicts response to BCG in NMIBC but poor response to therapy in MIBC. [44,50,51]. Its low expression predicts resistance/poor response to BCG [10].

#### **Tbet**

Expression of Tbet within tumor is indicative of Th2 response (immune - stimulation). Its expression is higher in MIBC compared to NMIBC and in high grade tumors compared to low grade tumors. Lower Tbet is predictive of response to BCG. Compared to evaluation of GATA3 and Tbet in isolation, their ratio (GATA3/Tbet) is better predictor of response to BCG with higher ratio predicting response to therapy [50,51].

#### **Treg cells (FoxP3 Regulatory Cells)**

Treg cells: Higher Treg cells are predictive of poor response (Shorter recurrence free survival)/resistance to BCG [10,25].

#### **Myeloid derived suppressor cells (MDSC)**

Higher intratumoral MDSC predicts poor response to therapy in NMIBC and MIBC [14,15]. Higher MDSC with lower T lymphocytes (higher ratio of MDSC and T cells) improves prediction.

#### **PD-1**

PD-1 is expressed by immune cells and not by tumor cells. PD-1 expressing immune cells are immunosuppressive in nature. The ratio of PD-1 and CD8+ cells provide an idea about iTME. Higher ratio is associated with reduced RFS in MIBC [52].

### **PD-L1**

Antibodies working on PD-1/PD-L1 axis PD-L1 were designed to neutralize immune suppression induced by presence of intratumoral PD-L1. FDA approved test for detection of PD-L1 expression is approved for use with Pembrolizumab and Atezolizumab for identifying patient population which is likely to respond to them [37,39]. However, PD-L1 expression is indicative and does not have desired specificity and sensitivity for use as a predictive biomarker for the treatment of metastatic bladder cancer [53].

### **DSC3**

In a preliminary study of BCG recurrent/refractory NMIBC, DSC3 expression was associated with response to CADI-05 [54].

### **Novel immunotherapy approaches for replacing intravesical BCG**

Intravesical BCG is associated significant side effects, which are severe in nature in few patients. Efforts are made to replace intravesical BCG by other immunotherapy.

### **Intravesical Therapy**

#### **Based on organism**

Organism based intravesical immunotherapy being evaluated include typhoid vaccine(TY21A), genetically modified BCG (VPM1002BC), COXSACKIE virus (CAVTAK), Recombinant oncolytic *E.coli* targeting integrin (VAX014), Mycobacterial Cell wall product (MCNA).

#### **Others**

#### **Durvalumab**

Anti PD-L1 antibody is being administered intravesically to minimize side effects of Durvalumab while retaining efficacy.

#### **Allogenic cancer vaccine**

HS 410: This is to induce tumor specific immune response.

#### **Imiquimod**

Topical TLR7 agonist.

### **Systemic therapy**

#### **CADI-05**

It is a TLR-2 agonist which is found useful in management of treatment naïve NMIBC [55]. It is administered intradermally and thus does not need frequent visits to hospital for intravesical administration of therapeutic agent. It provides results identical to intravesical BCG without side effects seen with intravesical BCG.

#### **Combination therapy**

Anti-PD1/PD-L1 therapeutics are also evaluated with intravesical BCG to improve outcome of BCG therapy.

## **Immunotherapy strategies previously tested in BCG unresponsive NMIBC [26]**

### **BCG plus IFN- $\alpha$**

While there was an evidence for synergy in early trials, it failed to provide benefit in those who have received prior BCG.

### **Quadruple immunotherapy**

Intravesical interleukin-2 and subcutaneous GMCSF when added to BCG/ IFN- $\alpha$  therapy, it improved outcome of BCG/ IFN- $\alpha$  combination.

### **Bropirimine**

It is a small molecule Th1 response enhancer. It did not provide significant benefit and so development was discontinued.

### **Keyhole limpet hemocyanin**

It was found immunogenic but failed to provide significant benefit.

### **MCNA**

Though found effective, it does not provide significant benefit in BCG unresponsive NMIBC.

## **Novel immunotherapy approaches in BCG unresponsive NMIBC [26]**

These include systemic agents, intravesical therapies and combinations.

### **BCG unresponsive bladder cancer**

#### **Systemic agents**

##### **Checkpoint inhibitors**

##### **Immune Checkpoint Inhibitors of the PD-1/PD-L1 Axis**

While Pembrolizumab is approved for CIS and is undergoing evaluation for papillary NMIBC. Nivolumab, Atezolimumab and Durvalumab are also under evaluation.

##### **Small molecule inhibitors of indoleamine (2,3)-dioxygenase 1 (linrodostatmesylate, epacadostat)**

They are under development as a single agent or in combination with anti PD-1. Early results are encouraging but their failure in melanoma has led to decreased enthusiasm for development of these products.

##### **Interleukin-2 super agonist (ALT-801)**

ALT-801 is a recombinant humanized fusion protein of T-cell receptor-IL-2 [56].

#### **Active immunotherapy:**

##### **Interleukin-2 super agonist (ALT-801)**

ALT-801 is a recombinant humanized fusion protein of T-cell receptor-IL-2 [56].

##### **CADI-05**

CADI-05 is a potent TLR 2 agonist which induces desmocollin-3 targeting Th1 type of immune response following intradermal administration [29]. Unlike other immunomodulators. It also reduces immunosuppressive tumor microenvironment by

decreasing intratumoral T reg, PD-1 while increasing activated intratumoral CD8+ T cells, NK cells and M1 macrophages [54,57]. It provides durable response in BCG recurrent/unresponsive DSC3 expressing NMIBC [53].

### **Keyhole Limpet Hemocyanin**

It is undergoing evaluation in comparison with Doxorubicin.

### **Intravesical therapies**

#### **Interleukin-15 superagonist (ALT-803)**

ALT-803 is a fusion complex comprising recombinant IL-15 fused to soluble receptor IL-15RaSushi-Fc. IL-15 is known to induce proliferation and activation of NK cells and CD8 cells. It is being evaluated in combination with intravesical BCG.

### **Viral gene therapies**

#### **Adstiladrin**

It is a non-replicating recombinant adenovirus gene transfer vector rAd-IFN/Syn-3 providing co-administration of the human IFN- $\alpha$ -2b gene with the excipient Syn-3. Following good outcome, it is undergoing phase III trial.

#### **CG0070**

CG0070 is an adenovirus which infects and proliferates in bladder cancer. It is designed to produce GM-CSF. In early clinical trial it provided best results in patients with borderline or high RB1 phosphorylation.

#### **VPM1002BC**

This is a genetically modified BCG wherein listeriolysin gene from *Listeria monocytogenes* is added and urease C gene is deleted to improve efficacy of BCG and reduce side effects [56].

#### **EN3348**

Also known as MCNA, Uricidin is Mycobacterial Cell wall-DNA Complex [MCC].

#### **EphB4-HAS**

This is a recombinant fusion protein wherein human receptor tyrosine kinase ephrin type-B receptor 4 (sEphB4) is fused, at its C-terminus, to full-length human serum albumin (HSA).

### **Combination therapies**

#### **With Intravesical BCG**

#### **Vaccines (PANVAC)**

PANVAC is a pox viral vaccine comprising transgenes for mucin 1, carcinoembryonic antigen and co-stimulatory molecules. It is being evaluated with intravesical BCG as it provides better immune response compared to BCG alone.

### **Immune Checkpoint Inhibitors of the PD-1/PD-L1 Axis**

Pembrolizumab, Nivolumab and Durvalumab are being evaluated with intravesical BCG.

**With intravesical novel agent - oportuzumab monatox**

Intravesical oportuzumab monatox is being tested in combination with systemic durvalumab [56].

**With external beam radiation**

Durvalumab is being evaluated with external beam radiotherapy.

**With IDO inhibitor**

Nivolumab is being evaluated in combination with IDO inhibitor BMS-986205 (CheckMate 9UT) [58].

**With peptide vaccine**

Durvalumab in combination with a five peptide cancer vaccine(S-488210/S-488211) is undergoing evaluation (DURANCE) [59].

**Novel immunotherapy approaches in locally advanced and or metastatic bladder cancer**

**Immune checkpoint inhibitors of the PD-1/PD-L1 axis**

**With Intravesical BCG**

Pembrolizumab, Nivolumab, Atezolizumab, Durvalumab, Avelumab are being evaluated with intravesical BCG in management of muscle invasive bladder cancer.

**With external beam radiation**

Pembrolizumab, Nivolumab, Atezolizumab, Durvalumab, Avelumab are being evaluated with radiotherapy and/or chemoradiotherapy for locally advanced MIBC.

**With IDO inhibitor**

Pembrolizumab is being evaluated with epacadostat.

**With HDAC inhibitor**

Pembrolizumab with etinostat.

**With Anti-GITR antibody**

**Oncolytic virus**

Recombinant oncolytic E.coli targeting integrin (VAX014) is being evaluated with pembrolizumab.

Pembrolizumab, Atezolizumab, Nivolumab alone and with Iplimumab are being evaluated with chemotherapy for first line treatment of metastatic bladder cancer.

Nivolumab is also being evaluated along with NKTR-214 (CD122 agonist).

Durvalumab is being evaluated with oleclumab (mAB against CD73).

## **Active immunotherapy**

### **CADI-05**

CADI-05 is found to be useful in management of locally advanced (MIBC) bladder cancer when used along with external beam radiotherapy. It achieves complete response which is durable at least for two years [60,61].

### **CDX1307**

CDX1307 vaccine generates response against  $\beta$ -HCG secreting tumors. It is being evaluated with chemotherapy.

### **Reolysin**

It contains reovirus to be administered intra - lesionally. It is being evaluated with chemotherapy.

## **Conclusion**

Immunotherapy was first approved to prevent recurrence of non-muscle invasive bladder cancer in form of intravesical BCG. Since then, significant advancement has been made in understanding of immune changes in cancer and now, systemic immunotherapy in the form of checkpoint inhibitors are approved to treat wider spectrum of bladder cancer and it is possible to treat BCG unresponsive as well as locally advanced and metastatic bladder cancer using immunotherapy.

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