



# Novel Therapeutic Strategies for BCG-unresponsive Non-muscle Invasive Bladder Cancer

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## Abstract

Development of therapeutic strategies for non-muscle-invasive bladder cancer (NMIBC) that failed intravesical Bacillus Calmette - Guerin (BCG) therapy remains an urgent priority for clinicians. Currently, radical cystectomy is the recommended standard of care treatment options for these patients. Intravesical chemotherapy using gemcitabine and docetaxel are regarded as the most effective treatment options for unresponsive NMIBC, however, these options are ineffective in the control of bladder cancer. In this review, we present the definition of BCG unresponsive NMIBC and discuss about the recent management options that include immunotherapy, intravesical chemotherapy, gene therapy, and targeted individualized therapy. Notably, immunotherapy is the most recent strategy utilizing the PD-1/PD-L1 and other immune checkpoint inhibitors (ICIs). Pembrolizumab (KEYNOTE-057), Atezolizumab (SWOG S1605) and Nivolumab were developed and are efficacious in BCG –unresponsive NMIBC. In summary, ICIs are considered as the most promising agent for BCG unresponsive NMIBC in the future.

**Key words** BCG-unresponsive NMIBC, Intravesical chemotherapy, immunity inhibitors, PD-1 inhibitory

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## Introduction

Intravesical chemotherapy or immunotherapy combined with transurethral resection of bladder tumor (TURBT) is a standard of care for patients with non-muscle invasive bladder cancer (NMIBC). Studies have shown that one-third of the NMIBC patients will not respond to BCG and half of these patients having intravesical BCG treatment will undergo recurrence or progression during long-term follow-up [1, 2]. It is true that the prevalence of BCG-unresponsive NMIBC is on the rise and how to deal with the problem of BCG-unresponsive NMIBC remains a serious clinical concern [3-5]. Currently, a few clinical trials on the treatment for BCG unresponsive NMIBC are approved to advance, which involve the use of immune checkpoint inhibitors (ICIs), viral gene therapy, and fibroblast growth factor receptor inhibitor [6]. Traditional salvage treatments for BCG failure NMIBC include intravesical chemotherapy such as valrubicin, gemcitabine and device-assisted treatments such as chemohyperthermia (CHT), electromotive drug administration (EMDA), and photodynamic therapy (PDT) [7]. However, cystectomy is regarded as the standard treatment option for BCG-unresponsive NMIBC following adequate BCG treatment [8]. Here we conduct a review on these studies on novel therapeutic options of BCG-unresponsive NMIBC together with defining the characteristics of BCG-unresponsive tumors.

## How to define BCG-unresponsive NMIBC

Intravesical BCG therapy following TURBT is commonly used on patients with superficial bladder cancer. It is difficult task to distinguish the response of BCG therapy [9-11]. Adequate BCG therapy include at least five doses of an initial induction course plus at least two doses of maintenance therapy or a second induction course [12]. There are several ways to define BCG-treatment failure which are based on the responsiveness to BCG therapy and duration until tumor recurrence. These include i) BCG-refractory (presence of the disease at 6-month follow-up after BCG therapy, or any progression in stage, grade, or disease extent at 3-month follow-up), ii) BCG-resistant (disappearance of disease at 6-month follow-up despite presence of disease that was of a lesser degree, stage, or grade 3 months after induction BCG therapy), iii) BCG-relapsing (recurrence after disease-free status at 6-month follow-up), and iv) BCG-intolerant (recurrence after administering inadequate BCG therapy because of BCG toxicity) [12, 13].

Next, we review recent therapeutic options that immunotherapy, intravesical chemotherapy, gene therapy, and targeted individualized therapy for BCG-unresponsive NMIBC.

## Intravesical Chemotherapy

### Gemcitabine

Gemcitabine is effective in inhibiting DNA synthesis and is commonly administrated in systemic chemotherapy for NMIBC [14]. Its single postoperative dose setting and combined regimens as a current clinical management strategy has been used for NMIBC patients [13, 15]. As early as in 2006, Dalbagni et al. found that two courses of intravesical gemcitabine is effective in the control of tumor progression in patients with superficial bladder cancer refractory or intolerant to intravesical BCG therapy and unwilling to undergo cystectomy [16]. The study comprises of 30 patients with the encouraging results [16]. Another randomized controlled trial conducted with 40 patients in each group of gemcitabine versus second BCG induction for the treatment of recurrent NMIBC after initial BCG induction shows gemcitabine to have a significantly improved recurrence-free survival (RFS)

compared with a second induction course of BCG [17].

### Docetaxel

In 2006, the first clinical trial reported 18 cases with at least one prior induction course of BCG in patients that experienced recurrent Ta, T1, or carcinoma in situ, with the majority receiving two or more courses of BCG. 10 of 18 patients demonstrated a complete response 12 weeks after initiating therapy of six weekly intravesical instillations of docetaxel after TURBT [18]. Recently, Shantharam et al. performed a case series of study with 13 patients with high-risk NMIBC treated with  $\geq 1$  course of intravesical BCG who received salvage intravesical docetaxel and concluded that salvage intravesical docetaxel was well tolerated, and associated with an encouraging initial response rate of 69% and 24-month recurrence-free survival (RFS) of 25% [19]. In a large sample cohort with 54 patients suggests that thirty-two patients (59 %) had a complete initial response 12 week after initiating therapy [20]. One-year and 3-year RFS rate were 40% and 25%, respectively. The long-term 5 years disease-specific survival was 86%, and 5-year overall survival was 69% [20]. In addition, paclitaxel-hyaluronic acid was applied on bladder carcinoma in situ refractory to BCG and satisfactory response rates with minimal toxicity was observed in this clinical trial [21].

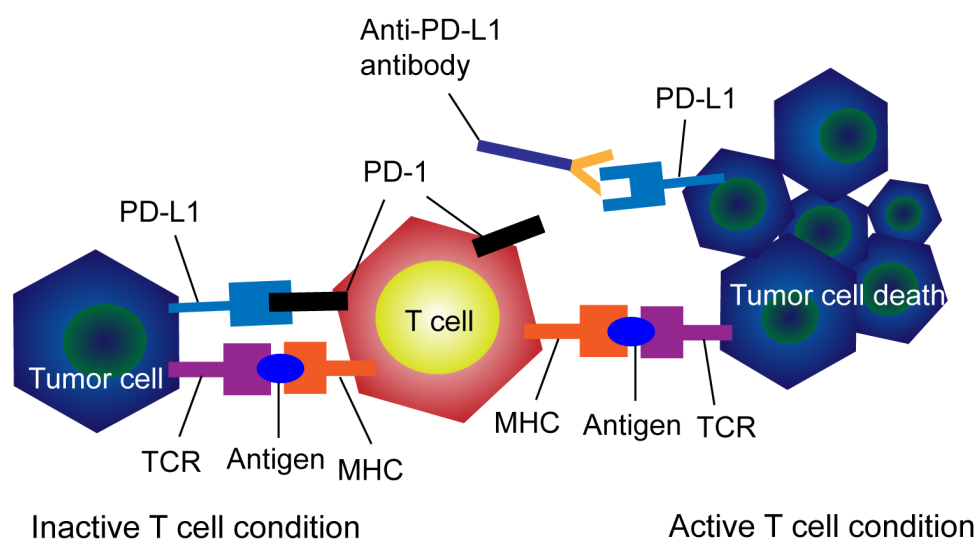
## Combination chemotherapy instillations

### BCG plus Interferon- $\alpha$ (IFN- $\alpha$ )

IFN- $\alpha$  is a pleiotropic immune modulator that has been demonstrated for its anti-proliferative activity in several preclinical studies. Combination therapy of intravesical IFN- $\alpha$  and BCG for NMIBC has been demonstrated to be more effective than either single agent in animal studies and suggested greater efficacy in clinical studies [22]. A Cochrane systematic review of assessment on the effects of intravesically administered BCG plus IFN- $\alpha$  compared with BCG alone for treating NMIBC found that there were very low-quality evidence suggesting no clear differences in the recurrence or progression with BCG plus IFN- $\alpha$  compared with BCG alone for NMIBC patients [23]. Steinberg et al. conducted a study comparing the effects of gemcitabine and docetaxel versus BCG plus interferon in patients with recurrent non-muscle invasive bladder cancer following a single induction course of BCG and their results suggested that patients with recurrent NMIBC after induction BCG had similar oncologic outcomes with induction gemcitabine/docetaxel as repeat induction with reduced dose BCG/IFN [24]. On these reports, it is anticipated that the effectiveness of BCG plus IFN- $\alpha$  on BCG-unresponsive NMIBC is need to be identified further.

### Device-assisted chemotherapy instillation

Device-assisted chemotherapy instillation as a therapy for BCG-unresponsive NMIBC was reported in some clinical studies. Such as radiofrequency-induced thermos-chemotherapeutic effect (RITE), conductive hyperthermic chemotherapy (HCT), and electromotive drug administration (EMDA) were demonstrated as a promising attractive alternative to BCG therapy [25]. The theoretical basis is that high temperatures may enhance drug function by encouraging tumor cells to absorb more chemotherapeutic agents, redistributing their intracellular concentrations, altering metabolic patterns, and/or inhibiting repair of DNA damage [26]. Hyperthermia combined with intravesical chemotherapy has been applied to increase the effects of chemotherapy because of increased temperature leading to an enhanced blood perfusion and cell permeability, in which allows



**Figure 1.** Schematic diagram on the mechanism of programmed death receptor and programmed death receptor ligand (PD-1/PD-L1) signals and Immune checkpoint inhibitors (ICIs). TCR: T cell receptor; MHC: major histocompatibility complex.

an increased MMC uptake [27]. A randomized controlled study by Tan et al. found that there was no significant difference in disease-free survival time (DFS) between RITE and institutional standard second-line therapy (control) in NMIBC patients with recurrence following BCG induction/maintenance [28]. Thermo-chemotherapy was reported there being an efficacy in patients refractory to intravesical therapies before considering early cystectomy [29]. Racioppi et al. conducted a clinical study on electroMotive drug administration (EMDA) of Mitomycin C (MMC) for high-risk “BCG failure” NMIBC with 3 years follow-up outcomes and the survival curves showed statistically significant differences ( $p$  value  $<0.05$ ) [30]. The authors assumed that the EMDA®-MMC could be considered safe and effective in high-risk NMIBC unresponsive to BCG, as a “bladder sparing” therapy in selected patients [30]. The study on the assessment of effectiveness of Hyperthermic IntraVesicalChemotherapy (HIVEC®) in BCG unresponsive NMIBC patients got a positive outcome that HIVEC® seems a valid treatment option for BCG unresponsive NMIBC patients [31]. A previous systematic review in 2011 on the combination treatment of intravesical chemotherapy and hyperthermia suggest that a 59% relative reduction in NMIBC recurrence when chemohyperthermia (C-HT), compared with MMC alone [32].

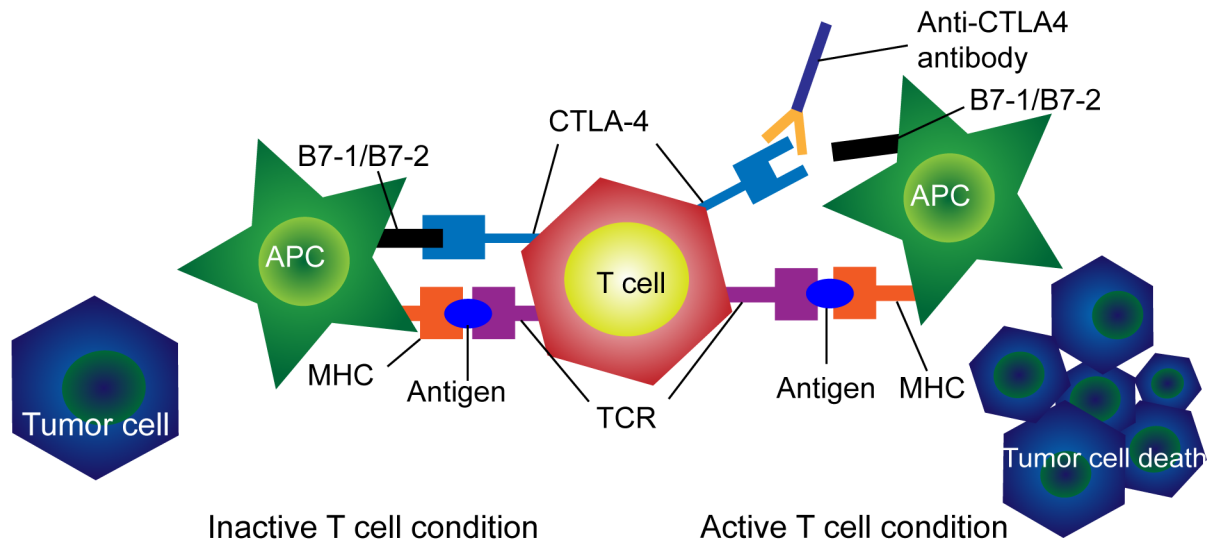
#### Photodynamic therapy

Photodynamic therapy (PDT) interacting between absorbed light and a retained photosensitizing agent to destroy tissue has been applied to treat NMIBC. PDT with Radachlorin was applied in a prospective, single-arm study with 34 patients and the study result show that recurrence free rates were 90.9% at 12-months, 64.4% at 24 months, and 60.1% at 30 months which suggest that PDT therapy is safe and effective treatment for NMIBC refractory or intolerant to BCG therapy in selected patients [33]. In 2010, a pilot study suggest that PDT using chlorin e6-polyvinylpyrrolidone (Ce6-PVP) was a feasible technique as a bladder sparing therapy for NMIBC refractory to intravesical BCG therapy [34].

#### Immunotherapy for BCG unresponsive NMIBC

Since intravesical BCG therapy was introduced for NMIBC in 1970s, there is no major breakthrough in drugs coming out until recently. Immune-checkpoint inhibitors (ICIs) targeting the programmed death 1/programmed death-ligand 1 (PD-1/PD-L1) and cytotoxic T-lymphocyte-associated protein 4 (CTLA-4) pathways has been proved significant anti-tumor activity (**Figure 1 and Figure 2**) [35-37]. Immune cells such as dendritic cells, tumor-associated macrophages, CD4 T-cells, CD8 T-cells, and natural killer cells play an important role in bladder cancer [37]. Anti-tumor immune response inhibition remains the major aim of immune suppression. Tumor growth and metastasis is supported by a microenvironment from tumor development progress which reduces potential adaptive immunity to a tumor antigen [38]. In this tumor microenvironment, dendritic cells population exert profound immune suppressive effects on T cells [39]. Dendritic cells are located in a hub position in the regulation of the balance between CD8 T-cell immunity versus tolerance to tumor antigens [39]. The tumor microenvironment with function of suppression and modulation of tumor-infiltrated DCs (TIDCs) can inhibit their function in initiating potent anti-tumor immunity as a result of tumor progression [40].

Tumor-associated macrophages (TAMs) classified as M1 and M2 on the different polarization play a dual role in bladder cancer [41]. For M1 macrophages and M2 macrophages, the former can be induced by tumor necrosis factor (TNF)- $\alpha$ , interferon- (IF- $\gamma$ ), interleukin (IL)-1 $\beta$ , IL-6, IL-23, lipopolysaccharide (LPS) and suggest an inhibitory action in the initiation and/or progression of bladder cancer [42]. IL-4, IL-10, IL-13, or transforming growth factor- $\beta$  (TGF- $\beta$ ) can activate the latter M2 macrophages which is associated with the promotion of cancer cell proliferation, migration, invasion, metastasis, and suppression of anti-tumor immune responses. Myeloid-derived suppressor cells (MDSCs) can prevent bladder cancer from attacking by CD4 T-cells, CD8 T-cells, and natural killer cells because of MDSCs from bladder cancer itself suppresses CD4 T-cells, CD8 T-cells, and NK cells. MDSCs can attract CXCL8 (IL-8) and CCL22 which is related with poor prognosis of bladder cancer [42]. Regulatory T-cells (Treg) cells were found higher levels in the peripheral blood of patients with bladder carcinoma, while Th17 cells were enriched in bladder carcinoma tissue. Polyfunctional effector cytokines



**Figure 2.** Schematic diagram on the mechanism of CTLA-4 signals and Immune checkpoint inhibitors (ICIs). CTLA-4: cytotoxic T lymphocyte-associated antigen-4; TCR: T cell receptor; MHC: major histocompatibility complex.

such as TNF- $\alpha$  and IFN- $\gamma$  were highly expressed in these Th17 cells in bladder tumor tissue. These evidence suggests that tumor-infiltrating Th17 cells might be functional effector T cells which directly work on the tumor cells. Some cytokines such as IL-17A, IL-17F, IL-21, IL-22, and CCL20 secreted from Th17 cells can promote the proliferation of malignant cells and induce angiogenic constituents via stimulating fibroblasts to upregulate vascular endothelial growth factor (VEGF), resulting in tumor neovascularization [43, 44].

Natural Killer (NK) cells are regarded as cytotoxic lymphocytes which are members of the innate immune system. In these cells, the antigen specificity of T and B cells are lacking but can recognize cells with downregulated human leukocyte antigen (HLA) class I and upregulated markers of cellular stress, such as MICA, MICB, ULBP-1, and the polio virus receptor. Due to such molecular properties on the cell surface suggests that NK cells are important components of both antiviral defense and tumor immunosurveillance [41]. A study by Concepció et al. demonstrate that CD226 expression on peripheral blood NK cells improved immunological stratification in intermediate-risk T1-T4 bladder cancer patients [45]. Another molecular biomarker Siglec-7 in blood, urine, and tumors from patients with bladder cancer are found related with poor clinical outcomes and maybe involved in the regulation of antitumor immunity mediated by NK cells in bladder cancer [46].

PD-1 and PD-L1 mediate the anti-tumor effect of the CD4<sup>+</sup> T cells and CD8<sup>+</sup> T cells [47]. PD-1 expressed in antigen-presenting cells (APC) can combine PD-L1 expressed in T cells, which can have an effect to the control of T cells [48]. CD4<sup>+</sup> T cells (especial Th1) was regarded as a helper role in the progress of anti-tumor by CD8<sup>+</sup> T cells after recognition of tumor antigens presented by MHC class I. CD8<sup>+</sup> T cells and CD4<sup>+</sup> T cells commonly suggest there were good prognosis in most human cancers while the role of CD4<sup>+</sup>, CD8<sup>+</sup>, double positive (DP) T-cells is lacking [49]. There was high expression of PD-1 of the receptor for PD-L1 in the bladder tumor tissue with a lot of CD8 T-cells infiltrating, which suggest an exhausted phenotype feature under antigenic stimulation. CD8<sup>+</sup> T cell response is suppressed by immunosuppressive cytokines in multiple immune population

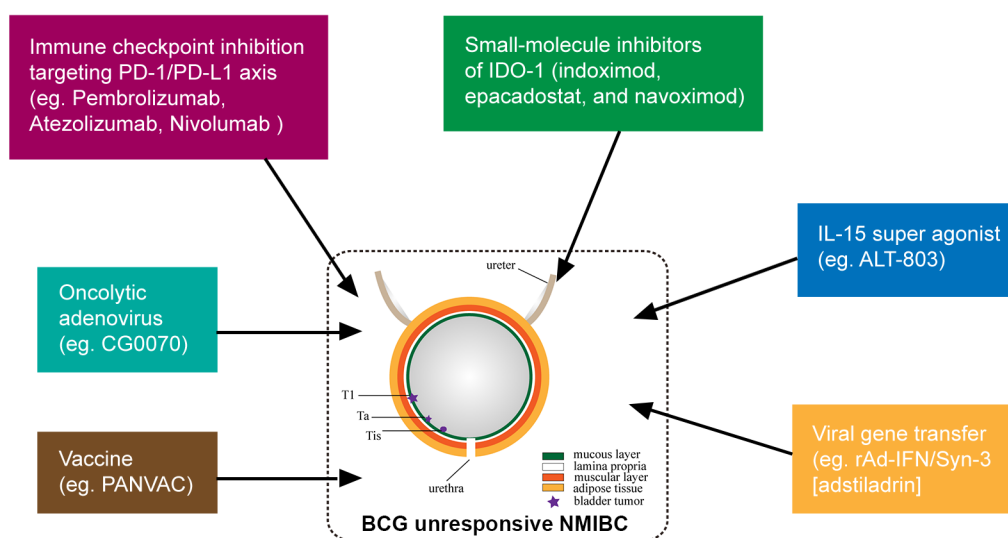
and there is a lack of IFN- $\gamma$  and IL-12 in the immunosuppressive environment of the tumor [50]. In addition, PGE2 play an important role in cancer progression, cancer-related immune inflammation and immune evasion, which is secreted from bladder carcinoma tissues due to the high expression of the inducible inflammatory enzyme COX2 [48].

#### PD-1/PD-L1 signals

The background of immunotherapy relies to treat both BCG-naïve and BCG-failure patients, new optional strategy that immune checkpoint blockade emerged, which is the most popular programmed death (PD-1) inhibitory receptor expressed by T lymphocytes [36, 48, 51-53]. Combination of PD-1 receptor and its ligand PD-L1 can suppress the T-cell receptor on T-cell activation [47]. However, cancer cells in human body can prevent themselves from attacking of T cells via the PD-L1 expression. To inhibit the effect of combination of PD1 and PD-L1 is a new strategy to generate anti-tumor response. Immune checkpoint inhibitors (ICIs) are PD-1 and PD-L1 monoclonal antibodies that have been developed to enable T-lymphocyte activation against tumor cells via inhibit the PD1-PD-L1 pathway. On the bladder treatment option, PD-L1 checkpoint inhibitors were reported demonstrating clinical effect since 2014 and was approved by FDA for clinical treatment later that year [54]. Because increased expression of PD-1 and PD-L1 in high-grade NMIBC, the anti-tumor effect of PD-1-PD-L1 inhibitor in high-grade NMIBC is more obvious compared to low grade NMIBC [55-57].

There are few ICIs approved and are in use in the clinic for bladder cancer [58-61]. In 2016, Atezolizumab as the first PD-L1 inhibitor was approved to treat patients with locally advanced or metastatic bladder cancer who progressed on or after platinum-based chemotherapy or have progressed within 12 months of neoadjuvant or adjuvant treatment with a platinum-containing chemotherapy [58]. Next year, Atezolizumab was approved to treat bladder cancer patients with locally advanced or metastatic disease and who are not eligible for cisplatin-containing therapy [58].

Recently, Pembrolizumab (KEYNOTE-057), Atezolizumab (SWOG S1605), Nivolumab, and Durvalumab were used in the



**Figure 3. Current used novel immunotherapy approaches for non-muscle invasive bladder cancer (NMIBC) in clinical trials or clinic.**

clinical studies on the patients with bladder cancer after BCG failure [62-64]. The single-arm, multicenter phase II trial of KEYNOTE-057 reported that there were three-month complete response rate of 41%, and a median response time of 16.2 months in patients treated with intravenous pembrolizumab 200 mg every three weeks for two years. Complete response case of the primary endpoint is considered as a cystoscopy without evidence of disease and negative cytology. There were 12.7% of 96 patients with Grade 3/4 adverse effects and 8.8% patients abandon treatment due to toxicity. Therefore, pembrolizumab is considered to be a new alternative to cystectomy post BCG failure approved by the FDA in 2020 [65, 66].

In another single-arm multicenter phase II trial of Atezolizumab (SWOG S1605), patients with high-risk BCG-refractory bladder tumors was treated with atezolizumab 1200 mg every three weeks for one year. The primary endpoint is complete response rate at 24 weeks as defined by biopsy, which is stricter compared to trial of KEYNOTE-057. It reported that the complete response rate at three months is 42% nearly with pembrolizumab study. Only 6% of the patients dropped out of the study as a result of adverse events, with 17% of grade 3/4 treatment-related adverse events [62, 67, 68].

A current randomized-controlled trial with 700 participants, CheckMate 7G8 of Nivolumab was used in the patients with high-risk recurrences within 24 months following completion of BCG treatment, excluding BCG unresponsive tumors. Patients with BCG-refractory carcinoma in situ were treated with BCG induction and maintenance for three years plus Nivolumab 480 mg every four weeks for two years. In this trial, the primary end-point is event-free survival (defined as time to recurrence, progression or death) [62, 67]. The results are still awaited for the trial.

CTLA-4 (cytotoxic T-lymphocyte-associated protein 4) function as activation of co-inhibitory receptors of T cells as the immune escape similar to PD-1. Ipilimumab and tremelimumab are combinations with anti-PD(L)1 and anti-CTLA-4 inhibitors which are currently ongoing in clinical trials [37]. Overall, the use of ICIs for BCG failure bladder cancer are promising alternative treatment options which require additional studies.

#### Other emerging immunotherapeutic agents

#### Vaccine therapy

PANVAC is a recombinant poxviral vaccine expressing tumor associated antigens carcinoembryonic antigen and mucin-1, along with 3 costimulatory molecules. ALT-803 is a recombinant fusion protein with enhanced IL-15 activity which is important for the development and activation of natural killer and CD-8 cells [69, 70]. Two phase II studies are conducted to identify the effect of PANVAC and ALT-803 on high-grade BCG-unresponsive bladder cancer and patients who refuse or are unfit for radical cystectomy (NCT02015104 and NCT01625260) [71, 72].

#### III-Gene therapy

rAd-IFN $\alpha$ /Syn3 is non-replicating adenovirus vector with the human IFN  $\alpha$ 2b gene. Intravesical administration of the rAd-IFN results in transduction of the virus into the epithelial cell lining in the bladder, leading to the synthesis and expression of large amount of IFN  $\alpha$ 2b protein allowing for high and durable IFN $\alpha$  in urine as a result of IFN  $\alpha$ 2b gene incorporating into the cellular DNA [73]. Until now, two studies (NCT01687244 and NCT02773849) were ongoing on patients with high-grade BCG-refractory or relapsed NMIBC by intravesical rAd-IFN $\alpha$ /Syn3 [74, 75].

CG-0070 which is an oncolytic adenovirus that expresses the immune stimulatory cytokine GM-CSF being administered intravesically as a single-arm intervention in an open-label, phase III study (NCT02365818) [13, 76, 77].

#### Small-molecule inhibitors of IDO1

As a result of demonstrating the role in tryptophan catabolism, indoleamine 2, 3-dioxygenase (IDO), an enzyme of interest in immuno-oncology has shown immunosuppressive effects [78]. IDO inhibitors were developed to work on the suppression of CD8 $^{+}$  T effector cells and NK cells as well as increased activity of CD4 $^{+}$  T regulatory cells (Treg) and MDSC [79]. The first developed IDO inhibitors are indoximod, epacadostat, and navoximod [79]. Higher IDO expression levels were found in bladder cancer compared



to the noncancerous tissue highlighting their important role in bladder cancer [80]. Currently, there were several IDO inhibitors undergoing a clinical evaluation which are now in phase II clinical trials [81]. IDO inhibitors in combination with other ICIs are currently applied in clinical trials of bladder cancer [82]. **Figure 3** shows that currently used novel immunotherapy approaches for NMIBC in clinical trials.

## Conclusion

The introduction and improvement therapeutic method on BCG unresponsive NMIBC as alternative radical cystectomy is still challenging. BCG and traditional chemotherapy are current standard of care treatment option for NMIBC, however it seems that immune checkpoint inhibitors (ICIs) are the most promising agents for BCG unresponsive NMIBC in the future [83]. Meantime, vaccine therapy and III-Gene therapy are emerging immunotherapeutic agents.

## Acknowledgements

None.

## Ethical policy

All procedures performed in this study were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. Informed consent was obtained from all individual participants included in the study. Approval from institutional ethical committee was taken.

## Availability of data and materials

All data generated or analysed during this study are included in this publication.

## Author contributions

PZ conceptualised, designed the study and was responsible for the writing of the original draft. PZ and YD reviewed, edited, and approved the final manuscript.

## Competing interests

The authors declare no conflict of interest.

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