Non-Muscle-Invasive Bladder Cancer

Subjects: Oncology | Urology & Nephrology

Contributor: Alessandro Audisio

Non-muscle-invasive bladder cancer (NMIBC) is characterized by a high rate of cure, but also by a non-negligible probability of recurrence and risk progression to muscle-invasive disease. NMIBC management requires a proper local resection and staging, followed by a risk-based treatment with intravesical agents. For many years, the current gold standard treatment for patients with intermediate or high-risk disease is transurethral resection of the bladder (TURB) followed by intravesical bacillus Calmette–Guérin (BCG) instillations. Unfortunately, in about half of high-risk patients, intravesical BCG treatment fails and NMIBC persists or recurs early. While radical cystectomy remains the gold standard for these patients, new therapeutic targets are being individuated and studied. Radical cystectomy in fact can provide an excellent long-term disease control, but can deeply interfere with quality of life. In particular, the enhanced immune checkpoints expression shown in BCG-unresponsive patients and the activity of immune checkpoints inhibitors (ICIs) in advanced bladder cancer provided the rationale for testing ICIs in NMIBC. Recently, pembrolizumab has shown promising activity in BCG-unresponsive NMIBC patients, obtaining FDA approval. Meanwhile multiple novel drugs with alternative mechanisms of action have proven to be safe and effective in NMIBC treatment and others are under investigation.

non-muscle-invasive bladder cancer | BGC-unresponsive | immunotherapy | immune-checkpoint inhibitors | pembrolizumab

1. Immunotherapy in NMIBC: From BCG to the New Horizons of ICIs

1.1. BCG Administration Drives an Antitumour Innate and Adaptative Immune Response in NMIBC

BCG is a live attenuated strain of Mycobacterium bovis, its activity on NMIBC was firstly demonstrated by Morales and colleagues in 1976 [1]. The BCG mechanism of action is still not completely understood; however, it is known that BCG exposure of urothelium and bladder-resident macrophages elicits an inflammatory and immune response against tumoral cells [2][3][4]. The presumed mechanism is explicated in **Figure 1.**

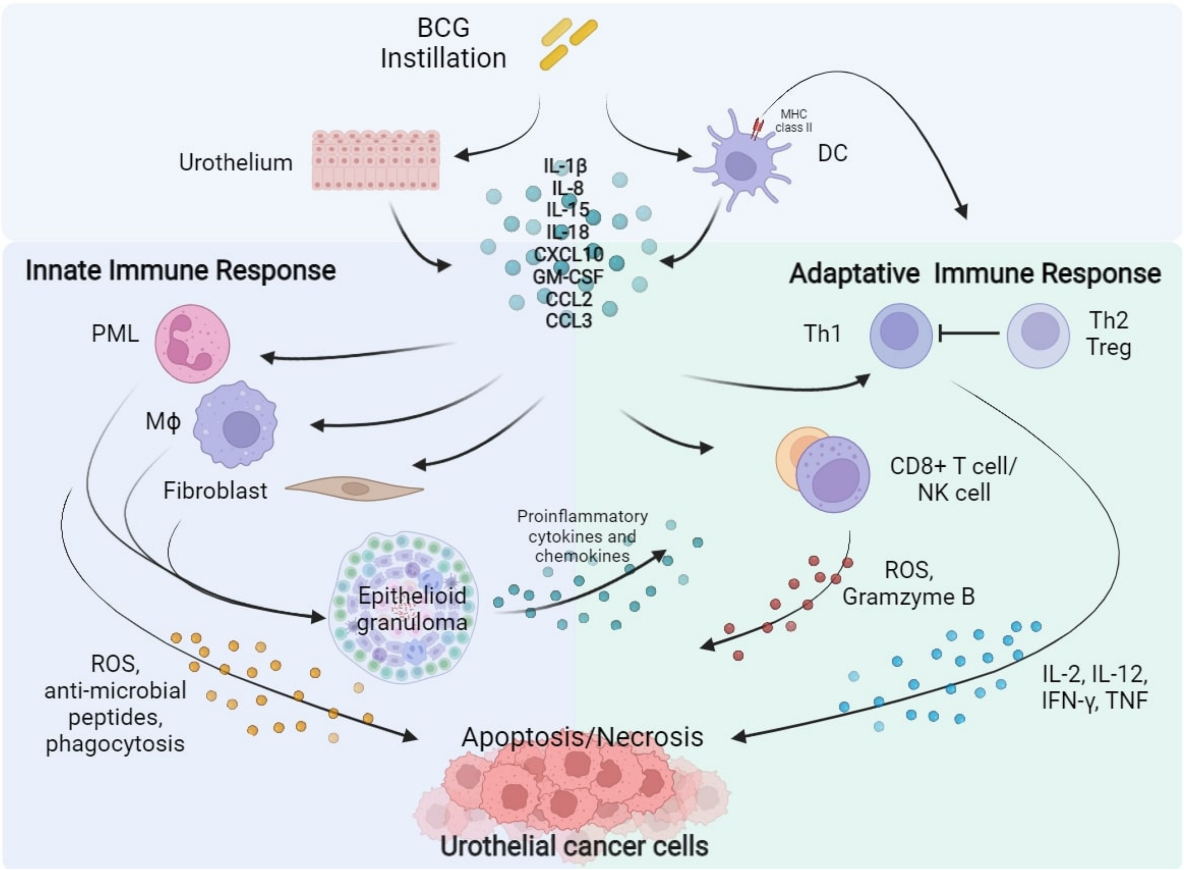

Figure 1. BCG instillation elicits both innate and adaptative immune response against urothelial cancer cells. BCG, Bacillus Calmette–Guérin; DC, Dendritic Cell; IL, Interleukin; CXCL10, C-X-C motif Chemokine Ligand 10; GM-CSF, Granulocyte-Macrophage Colony-Stimulating Factor; CCL2, C-C Motif Chemokine Ligand 2; CCL3, C-C Motif Chemokine Ligand 3; PML, Polymorphonuclear Leukocytes; Th, Helper T cell; Treg, Regulatory T cell; Mϕ, Macrophage; CD8, Cluster of Differentiation 8; NK, Natural Killer; ROS, Reactive Oxygen Species; IFN-γ, Interferon-γ; TNF, Tumour Necrosis Factor.

The activation of antigen-presenting cells (APC) and urothelial cells following BCG internalization induces the release of several cytokines as Interleukin (IL)-1b, IL-8, IL-15, IL-18 and chemokine as CXC motif chemokine ligand 10 (CXCL10), Granulocyte-Macrophage Colony-Stimulating Factor (GM-CSF), CC-motif Chemokine ligand 2 (CCL2), and CCL3 activating the innate and adaptive immune response [5]. Local innate immunity activation leads to recruitment of macrophages, granulocytes, fibroblasts, dendritic cells, and lymphocytes, which form typical epithelioid and gigantocellular granulomas. In addition, neutrophils, Cluster of differentiation 8 (CD8) + T cells, and Natural Killer (NK) cells may have a direct antitumour effect, inducing the production of reactive oxygen species (ROS), antimicrobial enzymes and pro-apoptotic factors [6][7][8]. Concurrently, BCG causes the expression of the major histocompatibility complex (MHC) class II presenting BCG antigens on APC and urothelial cells driving the activation of adaptative immunity [9][10]. A prominent T Helper (TH) 1 cells-mediated immune response, associated with the secretion of IL-2, IL-12, Interferon (IFN)-γ, and Tumour Necrosis Factor (TNF) correlates with a response to BCG instillation. On the contrary, TH2 cells activation, characterized by the releasing of IL-4, IL-5, IL-6, and IL-10, is associated with an immunosuppressive microenvironment enrich of T regulatory cells (Treg), which is associated to a BCG-unresponsive state [2][11].

Several randomized controlled studies and large meta-analysis have clearly demonstrated that intravesical BCG after TURB, administered with an induction schedule of 6 weekly instillations, followed by additional maintenance every 3 to 6 months over 1 to 3 years is significantly superior compared to TURB alone or TURB followed by intravesical chemotherapy in NMIBC recurrence prevention. BCG treatment provides an high rate of CR in both patients with high-risk papillary tumours and with CIS and lowers tumour progression risk, representing the standard treatment for these patients [12].

1.2. PD-L1 and PD-1 Expression Is Associated to BCG Immune-Resistance

The main resistance mechanism to BCG treatment is linked to an intrinsic or an acquired immune resistance. The interaction of PD-1, expressed by T cells, with its ligand Programmed Death-Ligand 1 (PD-L1), normally expressed by a subset of macrophages and inducible on activated T, B and NK cells, endothelial cells, and other non-malignant cells in an inflammatory milieu is a major immune checkpoint pathway involved in immune homeostasis, down-regulating T cell response in case of chronic antigen exposure. Cancer-related overexpression of PD-L1 lets cancer cells to evade immune response, inducing T cell anergy. The use of PD-1 or PD-L1-directed mAb, can prevent their interaction and restore T cell activity against cancer cells [13][14].

Kates and colleagues showed in an analysis on tissue microarrays of paired pre- and post-BCG bladder samples that BCG-unresponders patients had in 25–30% of cases a pre-treatment enrichment of PD-L1 + cells, high density of CD8+ T cells, and lacked of CD4+ T cells. On the contrary, PD-L1 expression was nearly absent among BCG responders [15]. Pierconti et al. confirmed these results. PD-L1 expression in tumour cells and in immune cells was higher in BCG-unresponsive CIS patients than in BCG-responders, suggesting that PD-L1 expression could help to identify CIS that would fail BCG therapy [16]. In addition, BCG treatment could enhance PD-L1 and PD-1 expression. Hashizume et al. observed that PD-L1 expression levels increased after BCG. Similarly, Fukumoto et al., testing PD-1 staining in a cohort of NMIBC treated with BCG, found that PD-1 expression was superior in BCG-unresponsive tumors compared with pretreatment tumors from the same patients, hypothesizing that BCG could induce this immune checkpoint. Furthermore, PD-1 expression was correlated with worse clinical outcomes [17][18]. BCG instillation seems to induce the expression of PD-L1 in tumour and inflammatory cells trough the induction of CD8+ T cells, which are the major responsible of IFN-γ production [17]. Chevalier and colleagues reported an increasing number of PD-L1-expressing CD4+ T cells (PD-L1+ Tregs) in BCG-resistant patients [19], while Copland et al. demonstrated that BCG treatment causes the up-regulation of PD-L1 expression on APCs inducing the secretion of some cytokines as Il-6, IL-10, leading to STAT3 phosphorylation and ultimately PD-L1 expression [20].

1.3. ICIs for the Treatment of Advanced Urothelial Cancer

The high tumoral mutational burden (TMB) of urothelial cancer, similar to melanoma and non-small-cell lung cancer, and the expression of immune checkpoint PD-1 and PD-L1 both by immune cells and microenvironmental cells constitute the biological rational for the activity of ICIs in bladder cancer [21][22]. Nowadays ICIs represent the standard second-line therapy in patients with advanced or metastatic urothelial cancer who progressed on first-line platinum-based chemotherapy. Pembrolizumab, an anti-PD-1 mAb, according to results of phase III trial

KEYNOTE-045 is the preferred option [23][24]. First-line immunotherapy does not provide a statistical significant survival benefit compared to platinum-based chemotherapy, even when it was given in association; however, avelumab, an anti-PD-L1 monoclonal IgG, as maintenance in patients who did not have disease progression with first-line chemotherapy, gets the approval on the basis of JAVELIN Bladder 100 [24][25]. Moreover, nivolumab, another PD-1 mAb was recently granted FDA approval for the adjuvant treatment of patients with urothelial carcinoma who are at high risk of recurrence after RC on the basis of results of CheckMate-274, and several trials are investigating the role of ICIs in neoadjuvant and perioperative setting [24][26]. Clinical or biological markers predictive of response are still lacking; however, PD-L1 expression and elevated TMB status seem to be correlated with an increased response rate [27].

1.4. ICIs Activity in BCG-Unresponsive NMIBC

The enhanced immune checkpoint expression shown in BCG-unresponsive patients and the efficacy of ICIs in advanced BC represented the rationale for testing them in NMIBC. Pembrolizumab was investigated in the phase II KEYNOTE-057 trial (**Table 1**). In the cohort A of the study, intravenous pembrolizumab was administered for up to 24 months in patients with BCG-unresponsive CIS patients, who resulted ineligible or declines RC. After a median follow-up of 36.4 months, 41% of patients (95% CI 30.7–51.1%) achieved a CR assessed by cystoscopy and urine cytology. Eleven of 39 patients with CR (28%) were disease-free at data cut-off analysis. Results of the study cohort B, which enrolled patients with BCG-unresponsive NMIBC without CIS, have not been published yet. Safety profile was consistent with other studies testing pembrolizumab; serious treatment-related adverse events (G3 or G4 according to World Health Organization, WHO) were rare [28]. On the basis of these results, in January 2020, FDA approved pembrolizumab for the treatment of patients with BCG-unresponsive CIS who are ineligible for or who decline RC [28].

Table 1. Key positive clinical trials enrolling patients with BCG-unresponsive NMIBC.

Agent/ Target	NCT/ Acronym	Phase	Primary Endpoint	Patients Enrolled	Median Follow Up	Results
Pembrolizumab * ICI Anti-PD1 IgG4/kappa	NCT02625961 KEYNOTE-057 [28]	II	CRR of high-risk NMIBC	Cohort A (CIS): 101 pts Cohort B (Non-CIS): 47 pts	36.4 mos.	Cohort A: 41% (39 out of 96 pts, 95% CI 30.7–51.1%)
Atezolizumab ICI Anti-PD-L1 IgG1	NCT02844816 SWOG S1605 [29]	II	CRR at 25 weeks in CIS-cohort	CIS cohort: 70 pts pre-planned Non-CIS cohort: 65 pts pre-planned	NR	CIS cohort: 27% (20 out of 74 pts, 95% CI NR)
Nadofaragene firadenovec rAd-IFNa2b/Syn3	NCT02773849 [30]	III	CRR at 12 mos. in CIS-cohort	CIS-cohort: 107 pts Non-CIS cohort: 50 pts	19.7 mos.	CIS-cohort: 53.4% (55 out of 103 patients,

Agent/ Target	NCT/ Acronym	Phase	Primary Endpoint	Patients Enrolled	Median Follow Up	Results
						95% CI 43.3–63.3%)
Oportuzumab Monatox EpCAM scFv linked to ETA	NCT02449239 [31]	III	CRR in CIS-cohort	126 pts CIS-cohort: 89 pts [32]	NR	CIS-cohort: 40% (95% CI NR)

ients with was given %; 95% CI nts (29%; 90% CI 22–36%) were free of recurrence or progression at 18 months, the percentage of event-free survival was greater in non-CIS patients than in CIS patients. The treatment was globally well tolerated. Serious grade adverse events occurred in 17% of patients and there were two treatment-related deaths [29].

* FDA approved. NCT, Number Clinical Trial; ICI, Immune Checkpoint Inhibitor; PD1, Programmed cell Death protein-1; IgG, Immunoglobulin G; CRR, Complete Response Rate; NMIBC, Non-Muscle-Invasive Bladder Cancer; CIS, Carcinoma In Situ; mos., months; pts, patients; PD-L1, Programmed death-Ligand 1; NR, Not reported; rAd-IFNa2b, non-replicating recombinant adenovirus type 5 (Ad5)-vector encoding the interferon alpha-2b; EpCAM, Epithelial Cell Adhesion Molecule; scFv, single-chain Fragment variable; ETA, Pseudomonas exotoxin A; BCG, Bacillus Calmette–Guérin.

Several clinical trials are now ongoing testing different ICIs in BCG-unresponsive NMIBC (**Table 2**). Durvalumab, an anti-PD-L1 Immunoglobulin G1 (IgG1) mAb, camrelizumab, an anti PD-1 ICI, and HX008 (pucotenlimab), a new recombinant anti-PD-1 monoclonal IgG4 are being tested as monotherapy respectively in NCT04738630, NCT04706598, NCT03759496. ADAPT-BLADDER study (NCT03317158) is investigating durvalumab activity in association with radiotherapy, while PREVERT trial (NCT03950362) activity of avelumab. Durvalumab is, furthermore, being evaluated in association with an anti-CTLA4 mAb, tremelimumab in RIDEAU study (NCT05120622). SunRISe-1 study (NCT04640623) endpoints are to assess the efficacy and safety of TAR-200, an intravesical gemcitabine-delivery system, in association with an anti PD-1 mAb, cetrelimab, or of these two drugs alone in BCG-unresponsive high-risk NMIBC. NCT04164082 trial investigates the combination of pembrolizumab and gemcitabine.

Table 2. Ongoing clinical trials testing ICIs (in bold font) alone or in combination in NMIBC.

NCT/Acronym	Status	Phase	Drug(s)	Control	Primary Endpoints
(a) BCG-unresponsive or BCG-intolerant NMIBC					
NCT05120622 Rideau	Recruiting	1, 2	**Durvalumab, tremelimumab**	—	TRAEs, MTD
NCT04738630	Recruiting	2	**HX008** (Pucotenlimab)	—	CRR, EFS
NCT04706598	Recruiting	1, 2	**Camrelizumab**	—	MTD, RFS
NCT04640623 SunRISe-1	Recruiting	2	TAR-200, **Cetrelimab**	TAR-200 or Cetrelimab	CRR
NCT04387461 CORE-001	Recruiting	2	CG0070, **Pembrolizumab**	—	CRR
NCT04164082	Recruiting	2	**Pembrolizumab**, gemcitabine	—	CRR in CIS subpopulation, EFS

NCT/Acronym	Status	Phase	Drug(s)	Control	Primary Endpoints
NCT03950362 PREVERT	Not yet recruiting	2	**Avelumab**, RDT	—	RFS
NCT03759496	Recruiting	2	**Durvalumab**	—	MTD, RFS
NCT03519256 CheckMate 9UT	Active, not recruiting	2	**Nivolumab**, BMS-986205 (Linrodostat mesylate)	Nivolumab	CRR, DoR
NCT03317158 ADAPT-BLADDER	Recruiting	1, 2	**Durvalumab**, RDT	—	RP2D, RFS
NCT04149574 CheckMate 7G8	Recruiting	3	**Nivolumab**, BCG	BCG	EFS
NCT04106115 DURANCE	Not yet recruiting	1, 2	**Durvalumab**, S-488210/S-488211 vaccine	—	DLT, DFSR
NCT03892642 ABC Trial	Active, not recruiting	1, 2	**Avelumab**, BCG	—	DLT
			(b) BCG-naïve NMIBC		
NCT04922047 TACBIN-01	Recruiting	1, 2	**Tislelizumab**, BCG	—	DLT
NCT04730232	Recruiting	2	**Tislelizumab**, nab-paclitaxel	—	CRR
NCT04165317 * CREST	Recruiting	3	**Sasanlimab**, BCG	BCG	EFS, CRR
NCT03799835 ALBAN	Recruiting	3	**Atezolizumab**, 1y BCG	BCG	RFS
NCT03711032 * KEYNOTE-676	Recruiting	3	**Pembrolizumab**, BCG	BCG	CRR, EFS
NCT03528694 POTOMAC	Active, not recruiting	3	**Durvalumab**, BCG	BCG	DFS

ression of
s that are
CG-naïve
s a phase
MIBC after

BCG induction. CheckMate 7G8 (NCT04149574) and POTOMAC (NCT03528694) studies testing respectively nivolumab and durvalumab have a similar design. NCT03892642 is a phase I/II trial planned to evaluate BCG in association with avelumab as induction treatment. The primary endpoint of the phase I of the trial was the completion of a full induction course. The primary endpoint was reached, the combination of BCG with an ICI was reported to be safe and well tolerated, and phase II is still ongoing [33]. The NCT04730232 study is testing tislelizumab in association with nab-paclitaxel chemotherapy, while in the DURANCE trial (NCT04106115), it is in combination with S-488210/S-488211, a cancer multi-peptide vaccine able to stimulate a cytotoxic T cell (CTL) response against urothelial cancer cells [34].

(a) Enrolling patients with BCG-unresponsive or BCG-intolerant NMIBC. (b) Enrolling patients with BCG-naïve NMIBC. * Enrolling patient with either BCG-unresponsive or BCG-naïve NMIBC. NCT, Number Clinical Trial; BCG, Bacillus Calmette-Guérin; NMIBC, Non-Muscle-Invasive Bladder Cancer; TRAEs, Treatment-Related Adverse Events; MTD, Maximum Tolerated Dose; CRR, Complete Response Rate; EFS, Event-Free Survival; RFS, Recurrence-Free Survival; CIS, Carcinoma In Situ; RP2D, Recommended phase 2 dose; DLT, Dose-Limiting Toxicity.

2. Alternative Targets: The Way to Develop New Effective Drugs

The deep improvement in the knowledge regarding the biological mechanisms responsible for neoplastic cells progression and BCG resistance mechanisms has led to identification of new targets (**Figure 2**); consequently, several innovative agents were developed and are now under investigation in the treatment of NMIBC (**Table 3**).

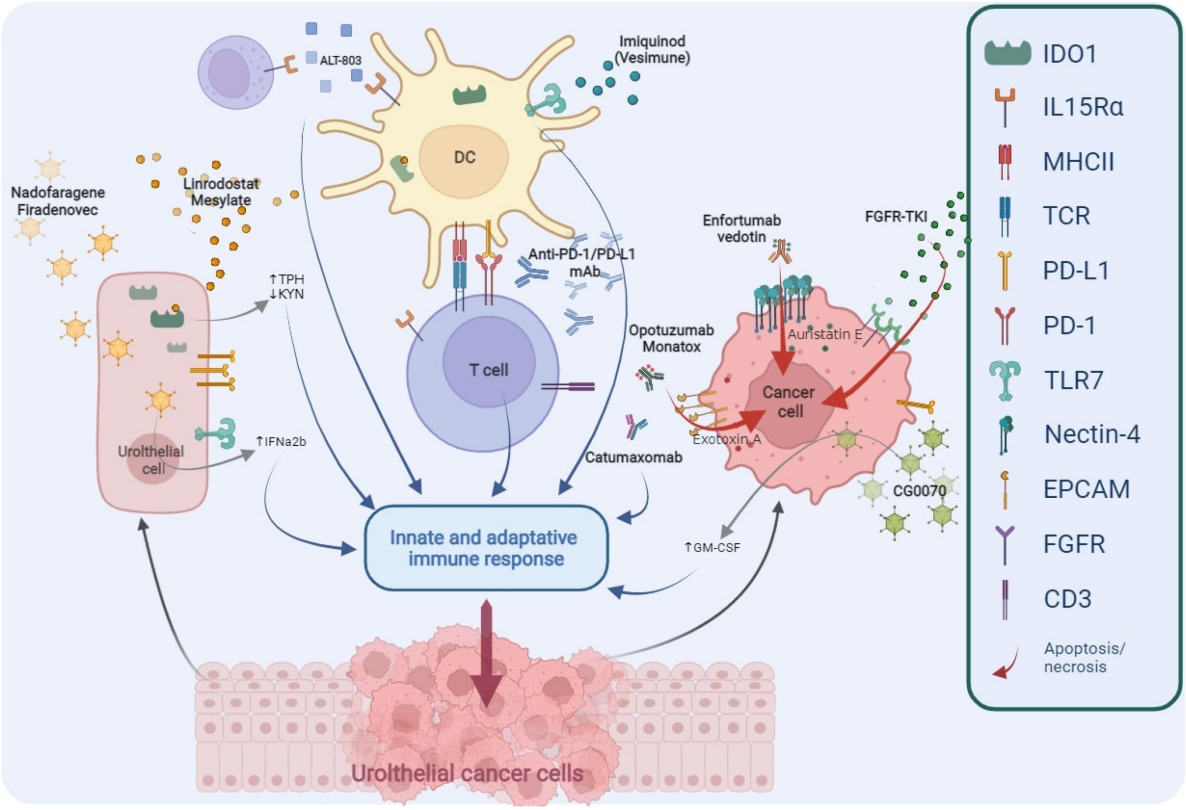

Figure 2. Main targets of novel drugs being investigated in BCG-unresponsive NMIBC. TPH, Tryptophan; KYN, Kynurenine; IFNα2b, Interferon α2b; DC, Dendritic Cell; PD1, Programmed cell Death protein 1; PD-L1, Programmed Death-Ligand 1; mAb, monoclonal Antibody; GM-CSF, Granulocyte-Macrophage Colony-Stimulating Factor; FGFR, Fibroblast Growth Factor Receptor; TKI, Tyrosine Kinase Inhibitors; IDO1, Indoleamine 2,3-Dioxygenase 1; IL-15Rα, Interleukin-15 receptor α; MHCII, Major Histocompatibility Complex Class II; TCR, T Cell Receptor; TLR7, Toll-like Receptor 7; EpCAM, Epithelial Cell Adhesion Molecule; CD3, Cluster of Differentiation 3.

Table 3. Clinical trials testing novel or emerging drugs (in bold font) alone or in combination in NMIBC.

NCT/Acronym	Status	Phase	Drug(s)	Target or Mechanism	Primary Endpoints
(a) BCG-unresponsive or BCG-intolerant NMIBC					
NCT05014139	Not yet recruiting	1	**Enfortumab Vedotin**	ADC against Nectin-4	TRAEs, DLT
NCT04917809	Not yet	2	**Erdafitinib**	FGFR-TKI	ORR

NCT/Acronym	Status	Phase	Drug(s)	Target or Mechanism	Primary Endpoints
	recruiting				
NCT04799847	Not yet recruiting	1, 2	**Catumaxomab**	Bispecific (anti-EpCAM, anti-CD3) Ab	DLT, TRAEs
NCT04498702	Completed	2	**APL-1202**	MetAP2 inhibitor	RFR
NCT04452591 BOND-003	Recruiting	3	**CG0070**	Oncolytic adenovirus	CRR
NCT04172675	Recruiting	2	**Erdafitinib** vs. gemcitabine/MMC	FGFR-TKI	RFS
NCT03914794	Recruiting	2	**Pemigatinib**	FGFR1-3-TKI	CRR
NCT03022825 QUILT-3.032	Recruiting	2, 3	BCG, **ALT-803**	IL-15 superagonist	CRR, DFR
NCT02009332	Completed	1, 2	**Nab-sirolimus**, gemcitabine	mTOR inhibitor	DLT, CRR
NCT01731652	Completed	2	**Vesimune**	TLR-7 agonist	CRR
NCT02371447	Active, not recruiting	1, 2	**VPM1002BC**	Modified BCG	DLT, RFR
			(b) BCG-naïve NMIBC		
NCT04736394 ASCERTAIN	Not yet recruiting	3	**APL-1202** vs. epirubicin	MetAP2 inhibitor	EFS
NCT02138734	Recruiting	1, 2	**ALT-803**, BCG	IL-15 superagonist	CRR, DFS

(a) Enrolling patients with BCG-unresponsive or BCG-intolerant NMIBC. (b) Enrolling patients with BCG-naïve NMIBC. NCT, Number Clinical Trial; BCG, Bacillus Calmette–Guérin; NMIBC, Non-Muscle-Invasive Bladder Cancer; ADC, Antibody-Drug Conjugate; TRAEs, treatment-related Adverse Events; DLT, Dose-Limiting Toxicity; MMC, Mitomycin C; FGFR, Fibroblast Growth Factor-receptor; TKI, Tyrosine Kinase Inhibitor; ORR, Objective Response Rate; EpCAM, Epithelial Cell Adhesion Molecule; CD3, Cluster of Differentiation 3; Ab, Antibody; MetAP2, Methionyl Aminopeptidase 2; RFR, Recurrence-free Rate; CRR, Complete Response Rate; RFS, Recurrence-Free Survival; IL-15, Interleukin-15; DFR, Disease-Free Rate; mTOR, Mammalian Target of Rapamycin; TLR-7, Toll-Like Receptor 7; EFS, Event-Free Survival.

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