International Urology and Nephrology (2019) 51:1089–1099
https://doi.org/10.1007/s11255-019-02183-5

UROLOGY - REVIEW

Prediction of BCG responses in non-muscle-invasive bladder cancer in the era of novel immunotherapeutics

Aleksander Ślusarczyk[1] · Piotr Zapała[1] · Łukasz Zapała[1] · Tomasz Piecha[1] · Piotr Radziszewski[1]

Received: 21 March 2019 / Accepted: 26 May 2019 / Published online: 1 June 2019

Abstract

Bacillus Calmette–Guerin (BCG) instillations are considered as a gold standard of therapy in high- and intermediate-risk non-muscle-invasive bladder cancer (NMIBC). Unfortunately, up to 40% of patients might experience treatment failure and even 15% of patients initially diagnosed with NMIBC will progress to muscle-invasive disease. Since patients, who fail to respond to BCG, are at particular risk of progression, immediate radical cystectomy (RC) is currently recommended to provide cancer control. However, immunotherapy in NMIBC management still evolves. Immune checkpoint inhibitors emerge as new immunotherapeutics, which in the future might be combined with BCG and may serve as an alternative to radical cystectomy in patients, who failed to respond to BCG alone or are at particular a priori risk of BCG failure, especially if RC is not a safe option. Therefore, there is an urgent need to identify NMIBC patients that will not benefit from BCG therapy and demand radical cystectomy. In the following review, we attempt to focus on several clinical and molecular factors and demonstrate the efforts directed to unravel their significance in BCG-failure risk assessment.

Keywords BCG response · High-risk non-muscle-invasive bladder cancer · BCG failure · BCG-refractory bladder cancer

Introduction

Bladder cancer is the eleventh most commonly diagnosed cancer worldwide. Up to 75% of patients with bladder cancer present with the tumour not invading the muscle layer. Non-muscle-invasive bladder cancer (NMIBC) comprises cancer confined to mucosa (Ta and Tis) or to submucosa (T1). Transurethral resection of the bladder tumour (TURBT) and subsequent Bacillus Calmette–Guerin (BCG) instillations are considered as a gold standard of therapy in high- and intermediate-risk NMIBC (Fig. 1) [1, 2]. Unfortunately, clinical utility and oncological outcomes of BCG are diminished by the fact that up to 40% of patients might experience treatment failure and even 15% of patients initially diagnosed with NMIBC will progress to muscle-invasive disease [3, 4]. Patients with BCG failure can be assigned to relapse, refractory, resistant or intolerant group that are not prognostically equal [5]. BCG failure can be defined as progression to muscle-invasive disease (T2), recurrence of high-grade tumour, persistence of high-grade T1 tumour at 3 months or persistent presence of Tis or high-grade Ta in consecutive biopsies. Since patients, who fail to respond to BCG, are at particular risk of progression, immediate radical cystectomy (RC) is recommended to provide cancer control (Fig. 2). Radical cystectomy might be, however, associated with severe complications; thus, it can be considered as overtreatment in some individuals. Multiple efforts have been undertaken to avoid RC after BCG failure; however, none of them provided long-term disease control [6–9]. Although intravesical gemcitabine, taxanes and thermo-chemotherapy and combination of mitomycin C (MMC) with gemcitabine yielded with some promising responses, RC should be still regarded as the treatment of choice providing durable remission [10]. Also, a novel simulation model using platform for a virtual randomized trial demonstrated RC superiority to intravesical MMC in BCG refractory patients. In such in silico trial, RC has been shown to be more cost-effective and improve survival when compared to MMC [11]. Nevertheless, trials to implement electromotive drug administration of mitomycin C in BCG-refractory individuals demonstrated promising results and in particular groups (TaG3, T1G3) disease-free rates at 3-year follow-up reached over 70% [12].

✉ Piotr Zapała
zapala.piotrek@gmail.com

[1] Department of General, Oncological and Functional Urology, Medical University of Warsaw, Lindleya 4, 02-005 Warsaw, Poland

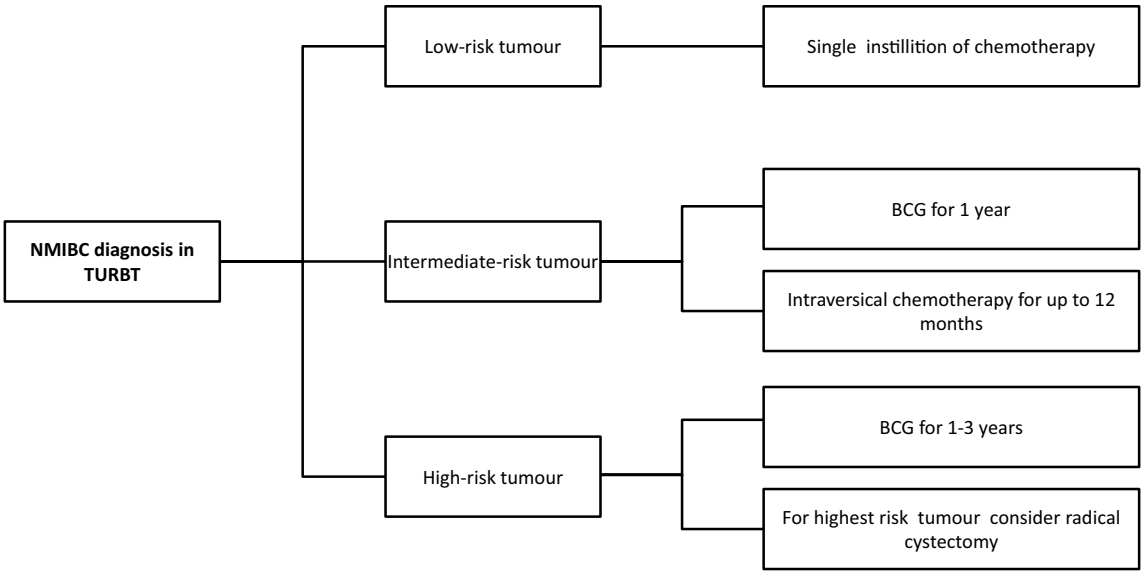

Fig. 1 Decision tree for non-muscle-invasive bladder cancer treatment. Asterisk: low-risk TaLG, high-risk T1 or CIS or HG tumour or multiple, recurrent and > 3 cm TaLG, intermediate-risk not included into low- and high-risk groups

Taking all of these into consideration, clinical decision on immediate RC must be patient-tailored. There is an urgent need to identify NMIBC patients that will not benefit from BCG therapy and demand radical cystectomy.

Immunotherapies under investigation in NMIBC

Importantly, immunotherapy in NMIBC management still evolves. Immune checkpoint inhibitors (ICI) emerge as new immunotherapeutics exhibiting efficacy in bladder cancer. Atezolizumab, durvalumab, avelumab, nivolumab and pembrolizumab have recently been FDA-approved for patients with metastatic and locally advanced bladder cancer. Aforementioned ICI are monoclonal antibodies, which block the interaction of PD-L1 with PD-1, which normally hampers anti-tumour T-cell response. Promising results of first clinical trials on checkpoint inhibitors in advanced bladder cancer delivered rationale to evaluate its efficacy in NMIBC. Multiple clinical trials are currently ongoing (Table 1). Atezolizumab, durvalumab, avelumab, nivolumab and pembrolizumab are investigated in the BCG-failure cohort (NCT03759496, NCT02625961, NCT02901548, NCT02792192, NCT03519256, NCT03892642, NCT02844816, NCT03711032). Noteworthy, atezolizumab and durvalumab are also evaluated in combination with BCG in the cohort of BCG-naïve patients (NCT03799835, NCT03528694). In the future, BCG combined with ICI may serve as an alternative to radical cystectomy in patients, who failed to respond to BCG alone or are at particular a priori risk of BCG failure, especially if RC is not a safe option. Therefore, clinical predictors, as well as easily available biomarkers of BCG failure, should be investigated to identify patients in whom intravesical BCG immunotherapy might not be sufficient in further cancer control.

Besides immune checkpoint inhibitors, a selection of vaccines, oncolytic viruses and exogenous cytokines have been recently investigated in NMIBC. PANVAC-VF is a cancer vaccine that contains virus producing carcinoembryonic antigen and mucin-1 (overexpressed in bladder cancer cells) and co-stimulatory molecules for T cells (NCT02015104). ALT-801 is an unique fusion protein that mediates interleukin-2 activation of T cells to cells with aberrant p53 (NCT01625260). Another emerging agent is a fusion protein known as ALT-803, which displays interleukin-15 superagonistic activity and leads to CD8+ T cells and effector NK cells activation (NCT03022825). Intravesical viral applications have also been introduced with CG0070 virus and adenovirus carrying interferon-α2b (rAd-IFN/Syn3) (NCT02365818, NCT02773849). The former one can enter tumour cell, induce its death and spread into adjacent cells, whereas the latter is responsible for IFNα-2b production, which in turn enhances immune response.

Current scoring systems in NMIBC outcome prediction

EORTC scoring system was developed to predict short- and long-term recurrence and progression of bladder cancer after analysis of 2596 patients data, who were diagnosed with superficial bladder cancer [13]. Since EORTC model was established on patients treated mostly with intravesical chemotherapy, it reveals inaccuracy of BCG-failure

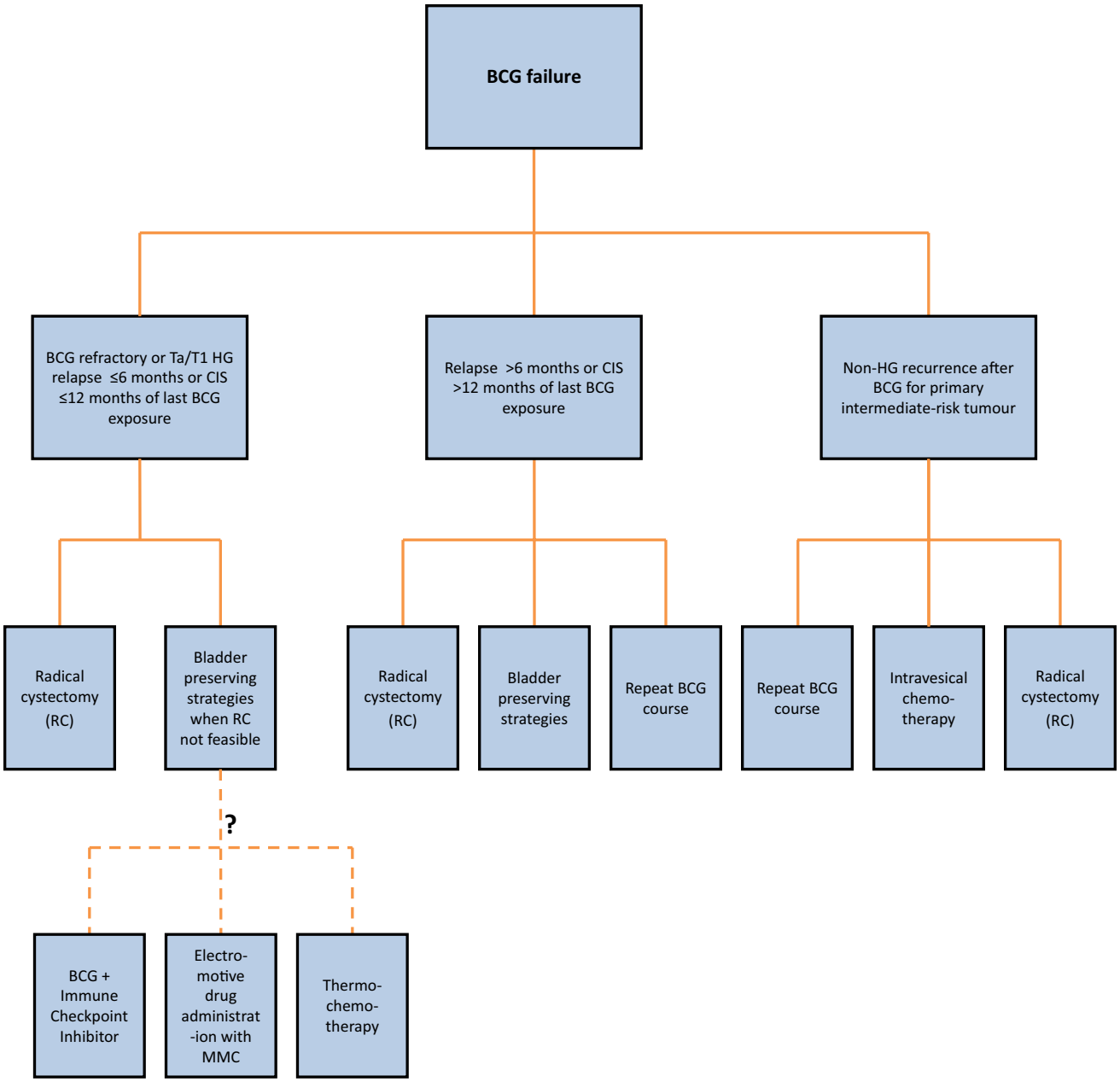

Fig. 2 Treatment decision tree for BCG-failure NMIBC. *HG* high grade, *MMC* mitomycin C, BCG refractory: T1G3/HG, non-muscle-invasive papillary tumour presence at 3 months or CIS or TaG3/HG presence at both 3 and 6 months or high-grade tumour appearance during BCG

prediction. Another tool used for NMIBC prognosis was established by CUETO, based on data from 1062 individuals treated with BCG [14]. Factors incorporated into EORTC and CUETO scoring models are summarized in Table 2. Not surprisingly, external validation of EORTC and CUETO models in BCG-treated cohort reveals noticeable accuracy advantage of CUETO tables; however, both tools tend to overestimate the risk of recurrence and progression in high-risk patients [15–17]. In the retrospective multicenter analysis, it was also reported that both EORTC and CUETO models reveal poor discrimination power for both disease recurrence and progression [15]. It can be partially explained by the different treatment guidelines at the time of both EORTC and CUETO studies when compared to current guidelines. Although CUETO was performed on patients managed with BCG instillations, the majority of patients received maximum 1-year maintenance. Thus, it is not created to predict response in high-risk patients treated with 3-year BCG maintenance, which is currently the treatment of choice [15].

Table 1 Ongoing clinical trials for high-risk NMIBC

Trial name	Description	End point	Status	Study population
NCT03759496[a]	Durvalumab intravesical	Maximum tolerated dose 6-month HGFR	Phase 2	BCG refractory
NCT02625961[a] (MK-3475-057/KEY-NOTE-057)	Pembrolizumab	3-year CRR and DFS	Phase 2	BCG-unresponsive
NCT03711032 (MK-3475-057/KEY-NOTE-057)	Pembrolizumab + BCG vs BCG	3.5-year CRR	Phase 3	High-risk NMIBC persistent or recurrent after BCG induction
NCT02901548[a]	Durvalumab	6-month CRR	Phase 2	BCG-refractory CIS
NCT03528694 POTOMAC	Durvalumab + BCG vs BCG	4-year DFS	Phase 3	BCG-naïve HG NMIBC
NCT03799835 ALBAN	Atezolizumab + BCG vs BCG	2-year RFS	Phase 3	BCG-naïve HG NMIBC
NCT02792192[a]	Atezolizumab + BCG vs BCG	Tolerability 6-month CRR	Phase 1 and 2	BCG-unresponsive
NCT03167151 PemBla	Pembrolizumab	Safety and tolerability	Phase 1 and 2	Recurrent intermediate-risk NMIBC
NCT03519256[a] CheckMate 9UT	Nivolumab + BMS-986205 ± BCG vs Nivolumab + BCG vs Nivolumab	5-year CRR	Phase 2	BCG-unresponsive
NCT03892642[a]	Avelumab + BCG	Freedom from DLT	Phase 1 and 2	BCG-unresponsive
NCT02844816[a]	Atezolizumab	18-month EFS	Phase 2	BCG-unresponsive
NCT03274284[a]	Cisplatin + conformal radiotherapy 55GY/20 fractions	2-year follow-up	Phase 4	BCG-failure T1HG NMIBC
NCT03636256	NanoDoce injection into tumour resection site + NanoDoce intravesical instillation	Safety and tolerability 6-month RFS	Phase 1 and 2	High-risk NMIBC and MIBC
NCT02982395[a]	Nanoxel®M vs MMC	1-year RFS	Phase 3	BCG refractory
NCT02009332[a]	Albumin-bound rapamycin nanoparticles (Nab-rapamycin, ABI-009)	Safety and tolerability	Phase 1 and 2	BCG- refractory or recurrent
NCT02773849[a]	INSTILADRIN	12-month CRR	Phase 3	BCG -unresponsive
NCT03335059[a]	Synergo® Radiofrequency-induced thermo-chemotherapy effect and MMC	3-month CRR	Phase 3	BCG -unresponsive CIS
NCT02015104[a]	BCG + PANVAC vs BCG alone	4–5-year DFS	Phase 3	At least one BCG course failure
NCT03022825[a]	BCG + ALT-803	2-year CRR, DFS	Phase 2	BCG-unresponsive
NCT01625260[a]	ALT-801 + gemcitabine	Safety and tolerability	Phase 1 and 2	BCG failure

[a]Ongoing trials in BCG failure; *BMS-986205* IDO-1 inhibitor, *MMC* mitomycin C, *NanoDoce* submicron particle docetaxel, *Nanoxel®M* ethanol-free docetaxel, *INSTILADRIN* non-replicating adenovirus vector harbouring the human IFN alpha2b gene, *PANVAC* recombinant virus vector vaccine, *ALT-803* interleukin-15 superagonist, *ALT-801* recombinant humanized TCR-IL-2 fusion protein, *HGRF* high-grade relapse free, *CRR* complete response rate, *DFS* disease-free survival, *RFS* recurrence-free survival, *PFS* progression-free survival, *DLT* dose-limiting toxicity, *EFS* event-free survival, *HG* high-grade, *MIBC* muscle-invasive bladder cancer

Data of 1812 BCG-treated patients from two EORTC randomized phase 3 trials revealed heterogeneity of high-risk NMIBC suggesting a need for an update of previous EORTC nomograms. Most crucial conclusion of the study is the fact that tumour stage and grade constitute strong progression and cancer-specific survival (CSS) predictors with T1HG representing most dangerous tumour type [18].

Histopathological factors serving as predictors of recurrence or progression have been extensively researched till date. What remains a challenge is further evaluation of clinical and molecular characteristics that can stratify risk in BCG-treated NMIBC. In the following review, we attempt to focus on the novel NMIBC prognostic factors and demonstrate the efforts directed to unravel their significance in BCG-failure risk assessment.

Table 2 Factors incorporated into EORTC and CUETO prediction model

EORTC scoring system	CUETO scoring system
T category (Ta, T1)	T category (Ta, T1)
Grade (G1, G2, G3)	Grade (G1, G2, G3)
Number of tumours (single, 2–7, ≥ 8)	Number of tumours (≤ 3, > 3)
Concomitant CIS (yes, no)	Concomitant CIS (yes, no)
Prior recurrence rate (primary, ≤ 1/year, > 1/year)	Recurrent tumour (yes, no)
Tumour size (< 3 cm, ≥ 3 cm)	Age (< 60, 60–70, > 70)
	Gender

Clinical factors

Gender

After initial denial of sex impact on BCG outcomes, several studies revealed female gender as associated with higher risk of disease recurrence in BCG-treated patients with high-grade T1 [19–21]. Female sex was confirmed as a significant predictor of NMIBC recurrence in the CUETO model [14, 22]. It has been proposed that carcinogenesis in the bladder may depend on sexual hormone receptors [23, 24]. Impact of sex-dependent immunomodulatory patterns cannot be ruled out since pathological specimens from NMIBC patients revealed higher T regulatory cells' counts in females [25]. A meta-analysis of 15,215 patients with high-grade T1 bladder cancer demonstrated that female sex was associated with higher risk of progression, but not with the risk of recurrence and disease-specific survival [26]. Explanation of gender disparity in bladder cancer epidemiology and response to treatment still remains elusive.

Age

It has been suggested that age over 70 years constitutes a negative prognostic factor of disease progression and overall survival in patients treated with BCG maintenance. It should be, however, noted that although diminished, BCG response remains still more effective than chemotherapy instillations in a subgroup of older patients [27]. Probably, older patients fail to respond to BCG due to attenuation of immune reactions, which progresses with age [28]. Another study demonstrated that despite similar initial response to BCG therapy in the cohort of patient over 70 years, late response on 5-year follow-up decreases with age [29]. At 2-year follow-up, the percentage of cancer-free patients was similar in different age groups; however, at 5-year follow-up, 27% of individuals over 70 years were cancer-free in comparison with 37% of younger than 70 years [29].

Overweight and obesity

It was retrospectively proven that overweight (HR 2.52) and obese (HR 2.52) patients with high-grade NMIBC (T1G3) are at higher risk of progression compared to patients with normal body mass [30]. Moreover, overweight (HR 4.00) and obesity (HR 5.33) were significantly associated with an increased risk of recurrence [30]. Such observations can be partially justified by higher levels of inflammatory cytokines (IL-6 and TNF-alfa) and lower level of adiponectin in patients with abundant adipose tissue [31]. Addition of BMI to the model comprising standard clinicopathological features increased its accuracy by 9.9 and 1.9 for recurrence and progression prediction, respectively [30]. Previous studies yielded observations that in general, obese patients with high-grade T1 bladder cancer are at higher risk of a worse outcome than non-obese counterparts [32]. Meta-analysis including 7193 patients showed that elevation of BMI by 1 kg/m^2 is associated with a 1.3% increased risk of bladder cancer recurrence (HR 1.01; 95% CI 1.01–1.02) [33].

Neutrophil to lymphocyte ratio

Prospective study on the cohort of 113 patients reported that preoperative neutrophil to lymphocyte ratio (NLR) is related to recurrence, especially in BCG-treated patients. In univariate Cox regression, NLR over 2.5 was associated with increased risk of recurrence in BCG group (HR 3.7). Moreover, addition of NLR to the EORTC scoring model might improve its predictive accuracy [34]. It was found that NLR is associated with shorter recurrence-free survival in both pre-stratified cohort of NMIBC patients and in the subset of BCG-treated ones (HR for recurrence 2.0 vs. 3.7, respectively). Interestingly, after stratification according to the treatment type, high prognostic significance of NLR has been maintained only in the BCG group [34]. Such observations may indicate that higher level of lymphocytes is indispensable to respond to BCG course. Lack of lymphocytes might lead to lesser infiltration of the bladder mucosa and poor response to BCG antigens [35]. Neutrophils, on the other hand, produce cytokines and proangiogenic factors (VEGF) which may support tumour growth [34]. NLR was also retrospectively evaluated in the cohort of patients with high-grade T1 bladder cancer. High preoperative NLR (> 3.0) was associated with female gender and residual T1HG/G3 on re-TURBT. NLR was independently associated with disease recurrence (HR 3.34), progression (HR 2.18) and cancer-specific survival (HR 1.65) [36]. The addition of NLR to a multivariable model that included age, gender, smoking status, tumour size, tumour multifocality, concomitant Tis and stage on re-TURBT improved its discrimination for RFS, PFS and CSS (accuracy benefit 6.9%, 1.8% and 1.7%, respectively) [36]. Another retrospective

study evaluating markers of systemic inflammatory response demonstrated that in the NMIBC baseline cohort as well as in the BCG-treated subgroup, both NLR over 2.5 and CRP over 5 mg/L were significantly associated with disease progression [37]. In the meta-analysis, the data collected from four studies, encompassing 599 patients, indicated that high preoperative NLR constitutes independent predictor of recurrence (four studies, pooled HR 2.31) and progression (three studies, pooled HR 2.54) in patients with T1 high-grade NMIBC [38].

Re-TURBT performance

In large multicenter retrospective study, it was reported that re-TURBT in patients with T1 HG and muscle layer in the specimen from primary TURBT does not improve recurrence, progression rate or overall survival [39]. Conversely, in patients with high-grade tumour without muscular layer in the specimen, re-TURBT revealed benefit in RFS, PFS and OS prolongation [39]. Moreover, due to significant percentage of residual disease in second resection in high-risk tumours, re-TURBT facilitates accurate staging in selected cases [40, 41].

Smoking status

Smoking intensity of more than 60 pack-years increased the risk of recurrence in the retrospective evaluation of 80 patients with NMIBC treated with BCG [42]. Moreover, continued smokers during the NMIBC treatment had 2.2 greater risk of recurrences than ex-smokers, who ceased smoking not more than a year before and not later than 3 month after diagnosis [43]. However, due to limited BCG-treated subgroup size, the influence of smoking on BCG response remains unclear [43]. Another retrospective study based on data from 623 patients failed to show any association between smoking status (current, ex-, never-smoker) and recurrence during BCG therapy. Ex-smokers were further stratified to those that ceased smoking 10 years before diagnosis, 0.1–10 years before and at diagnosis, but the subgroup analysis remained inconclusive concerning relation between smoking status and BCG outcome [44]. Another study indicated that risk of recurrence during BCG was higher in subgroup of current smokers (≥ 20 cigarettes per day) when compared to never and former smokers [45]. In the multivariable competing-risk analysis adjusted for major factors already proven to affect BCG response, such relationship was confirmed for current smokers (HR 1.44; 95% CI 1.01–2.04) [45]. It is suspected that immune responses are compromised by smoke, possibly due to reduced cytokine activity, impaired B-cell and T-cell responses [46]. Hypothesis that BCG efficacy is limited due to smoking remains controversial; thus, none particular recommendations for BCG protocol modification in smokers can be suggested.

Statins and anticoagulants

Evaluation of concomitant use of medication has not been evidenced to confound anti-tumour response during BCG therapy. Statins use was the subject of a large debate yielding controversial results [47]. In final conclusion based on large cohort studies, statins do not impair response to BCG therapy [48]. Another subject of discussion is the role of anticoagulants, which affect not only fibrin clot formation, but also fibronectin/BCG interaction. Importantly, fibronectin/BCG binding is necessary for BCG endocytosis to bladder cancer cells. Subsequently, BCG is recognized by immune cells infiltrating the tumour. In contrary to experimental studies in in vitro setting, clinical retrospective studies reveal no significant impact of anticoagulants on BCG failure [49]. On the cohort of 343 high-risk NMIBC patients, it has been found that antihypertensive, but not antidiabetic therapy, was associated with increased risk of tumour recurrence on BCG therapy (HR 1.45; 95% CI 1.069–1.962) [50].

Molecular factors

Urinary and blood cytokines

Cytokines mediate intercellular communication and due to their pleiotropic functions may reflect immune responses, inflammation or tumour invasion. It has been shown that the change of inducible cytokines in urine after BCG instillation might be used in identifying individuals with suboptimal response to the therapy. Based on the cohort of 130 patients, the nomogram was constructed using urinary cytokine levels (IL-2, IL-6, IL-8, IL-18, IL-1ra, TRAIL, IFN-γ, IL-12 [p70] and TNF-alfa) [51]. Final model reached 85.5% accuracy in prediction of the recurrence likelihood. However, multiple factors can confound cytokine profile assessment. This comprises lower urinary infection, systemic disease and intake of medication (e.g. immunomodulators) [51]. A number of studies evaluated the role of interleukin-2 in BCG response prediction. Earliest indicated that IL-2 mRNA level in peripheral blood mononuclear cells yielded a predictive value of 97% for remission [52]. Further studies demonstrated that patients with urinary IL-2 less than 27 pg/micromole creatinine measured after 5 BCG instillations were at significantly higher risk of recurrence than those with higher values. Collected evidence from multiple studies suggests that among cytokines urinary IL-2 levels might have the highest potency to become a biomarker of BCG response [53]. Another study demonstrated that high baseline urinary IL-8 predicts shorter time to tumour recurrence in

BCG-treated patients. Consequently, IL-8 level assessed in peripheral blood leukocytes can be associated with tumour recurrence in similar pattern [54]. The percentage change of IL-18 binding protein a, IL-23, IL-8 and CXCL10 measured after BCG initiation can be utilized in prediction of BCG failure [55]. Urinary IL-8 and IL-18 levels have shown association with disease-free survival (DFS) after BCG instillation [56].

In general, BCG therapy induces robust immunological response, which can be potentially assessed with the use of urinary cytokine levels. However, a pitfall may be the predictive value of cytokines' level, which might be diminished by false positive results caused by other pathologies, for instance asymptomatic bacteriuria, which is frequent, but does not constitute a contraindication to BCG instillation [57]. More efforts should be undertaken in order to optimize the time from BCG initiation (week of therapy) and last BCG instillation to urine collection and cytokine assessment. Studies often demonstrate own schedules of urinary cytokine measurement and divergent results due to the fact that cytokines' level changes over time after BCG instillation. Unfortunately, creation of reasonable prediction model based on cytokine profiles will remain a challenge, until the detailed mechanism of immune response towards BCG becomes clear.

FISH from urine cytology

Fluorescent in situ hybridization (FISH) can be successfully utilized to detect chromosomal anomalies in tumour cells obtained from urine samples. UroVysion FISH positivity (4 or more cells with polysomy on at least 2 chromosomes (3, 7, or 17) and/or at least 12 cells with a homozygous deletion for 9p21) predicts recurrence and progression on BCG therapy which has already been suggested as decision-aid in selection of individuals in whom alternative therapy should be considered [58]. The pre-BCG FISH test revealed a pooled sensitivity of 70%, specificity of 41% and area under the curve (AUC) of 0.60 for predicting recurrence [59]. Another multicenter prospective study demonstrated that FISH positivity at 3 months following BCG initiation is associated with 4.6 greater risk of recurrence. FISH sensitivity, specificity and accuracy in this series were 59, 84 and 77%, respectively [60].

Lymphocyte subsets and immunohistochemical biomarkers

Involvement of certain types and classes of lymphocytes is of major significance in the immunotherapy response. In series of 70 BCG-treated patients, abundance of both T regulatory cells (Tregs) and tumour-associated macrophages in resection specimen correlated with recurrence-free survival [25]. Immunosuppressive properties of tumour microenvironment attenuate adaptive response towards BCG antigens presented by cancer cells [25, 61]. High density of CD4+ and GATA3+ T cells, which are major population responsible for antigen recognition and stimulation of effector cells, has been demonstrated to associate with prolonged recurrence-free survival [62]. It has been also found that responders presented an increased productive Th1 immunity compared to non-responders [63]. Conversely, preexisting (before first BCG) Th2 intratumoural dominance predicted response to BCG as BCG intends to create Th1 response and therefore, only patients, who are yet to generate Th1 response may benefit. Immunohistochemistry markers demonstrated that the preexisting Th1 immunologic environment within the tumour was on the other hand associated with BCG failure [64]. Consequently, CD4+/CD8+ lymphocyte ratio in the primary specimen was suggested to predict BCG response [62]. Th1 and Th2 polarization can be reflected by the level of urinary cytokine levels. Namely, the former one associates with IL-2 excretion (its predictive value has been already summarized above) and the latter one with IL-10 production. Consequently, the ratio between IL-2 and IL-10 was found to have 83% sensitivity and 76% specificity in the recurrence prediction [65, 66].

Cell-cycle proteins including p53, RB and Ki-67 have also been suggested as potential immunohistochemical biomarkers. Although p53 and Ki67 relevance in disease progression has been already evidenced in meta-analysis [67, 68], their impact on recurrence and BCG response remains unclear [53]. RB expression was associated with decreased PFS and DFS, but the evidence is still limited [69]. In pT1 NMIBC, cytokeratin 20 expression was significantly associated with RFS in multivariate analysis (HR 5.89; 95% CI 1.44–24.15), whereas Ki-67 correlated significantly with PFS (HR 2.80; 95% CI 1.45–5.43). Furthermore, both CK20 and Ki-67 were significantly related to CSS in multivariate analysis [70]. Another immunohistochemical candidate marker is NEDD9 protein presenting 84.1% sensitivity and 80.9% specificity in predicting complete response to BCG [71]. Finally, PD-L1, which constitutes itself a therapeutic aim of targeted immunotherapy described before, can also be used in predicting BCG outcomes. In 334 pT1 NMIBC patients, PD-L1 mRNA levels have been associated with RFS (HR 0.48; 95% CI 0.31–0.72), PFS (HR 0.45; 95% CI 0.24–0.80) and CSS (HR 0.31; 95% CI, 0.13–0.67) [72]. High PD-L1 mRNA expression (≥ 33.8) constitutes a favourable prognostic factor and might be utilized to stratify patients to treatment groups (RC or immunotherapy) [72].

Molecular subtypes

Based on urothelial differentiation signature expression, bladder cancer might be divided into classes with

luminal- and basal-like characteristics [73, 74]. Small number of relevant studies indicates that the majority of NMIBC is luminal-like [73, 75, 76]. Based on the clustering of gene expression values, classification distinguishing three molecular classes was obtained. (About 9074 genes were analysed.) Classes 1 and 2 tumours revealed luminal features, whereas class 3 resembled basal phenotype. Moreover, tumours of high grade and stage, concomitant CIS and progression to muscle-invasive disease were more frequently observed in classes 2 and 3 than in class 1 [73]. Importantly, no significant difference in response to BCG was noticed between classes suggesting that tumour's transcriptional profile does not determine BCG response [73]. It has been also evidenced that in course of CIS progressing to muscle-invasive bladder cancer, expression of luminal markers decreased, whereas basal marker expression was enriched [77]. Prognostic value of molecular subtypes was initially evaluated in muscle-invasive bladder cancer, and its significance in NMIBC still remains uncertain and requires further investigation.

Conclusion

BCG failure in high-risk NMIBC remains enigmatic clinical phenomenon and significant therapeutic problem. Simultaneously, with advances in understanding of tumour microenvironment, emergence of novel therapies targeting immune response is observed. Early identification of individuals who are at particular risk of developing BCG resistance might aid selecting patients who could benefit most from combined treatment. Potentiation of BCG instillations with immune checkpoint inhibition might contribute to reducing overtreatment with radical cystectomy. Clinical trials evaluating such combination therapies are underway, but their exact clinical implementation in NMIBC remains unclear. Although histopathological features are currently the best predictors of recurrence and progression, an urgent need for new predictors of response to BCG remains apparent. Better understanding of BCG resistance pathophysiology might bring development of novel predictive models that can be easily utilized in planning patient-adjusted therapy.

Author contributions All authors listed on the manuscript have contributed significantly to the study. All authors have been involved in the writing of the manuscript at draft and any revision stages and contributed to the final version of the manuscript. All authors have read and approved the final version.

Funding The study was a non-profit, zero cost project. No funding has been obtained.

Compliance with ethical standards

Conflict of interest Piotr Zapała, Łukasz Zapała, Tomasz Piecha and Piotr Radziszewski declare that they participate in clinical trial assessing efficacy and safety of durvalumab and receive fees as clinical investigators from AstraZeneca on that account.

References

1. Malmstrom PU, Sylvester RJ, Crawford DE, Friedrich M, Krege S, Rintala E et al (2009) An individual patient data meta-analysis of the long-term outcome of randomised studies comparing intravesical mitomycin C versus bacillus Calmette–Guerin for non-muscle-invasive bladder cancer. Eur Urol 56(2):247–256
2. Sylvester RJ, Brausi MA, Kirkels WJ, Hoeltl W, Da Silva CF, Powell PH et al (2010) Long-term efficacy results of EORTC genito-urinary group randomized phase 3 study 30911 comparing intravesical instillations of epirubicin, bacillus Calmette–Guerin, and bacillus Calmette–Guerin plus isoniazid in patients with intermediate- and high-risk stage Ta T1 urothelial carcinoma of the bladder. Eur Urol 57(5):766–773
3. Zuiverloon TC, Zwarthoff EC (2016) Predicting response to intravesical bacillus Calmette–Guerin immunotherapy: are we moving forward? Eur Urol 69(2):201–202
4. Peyromaure M, Guerin F, Amsellem-Ouazana D, Saighi D, Debre B, Zerbib M (2003) Intravesical bacillus Calmette–Guerin therapy for stage T1 grade 3 transitional cell carcinoma of the bladder: recurrence, progression and survival in a study of 57 patients. J Urol 169(6):2110–2112
5. Herr HW, Milan TN, Dalbagni G (2015) BCG-refractory vs BCG-relapsing non-muscle-invasive bladder cancer: a prospective cohort outcomes study. Urol Oncol 33(3):108 e1–108 e4
6. Barlow L, McKiernan J, Sawczuk I, Benson M (2009) A single-institution experience with induction and maintenance intravesical docetaxel in the management of non-muscle-invasive bladder cancer refractory to bacille Calmette–Guerin therapy. BJU Int 104(8):1098–1102
7. Cockerill PA, Knoedler JJ, Frank I, Tarrell R, Karnes RJ (2016) Intravesical gemcitabine in combination with mitomycin C as salvage treatment in recurrent non-muscle-invasive bladder cancer. BJU Int 117(3):456–462
8. Di Lorenzo G, Perdona S, Damiano R, Faiella A, Cantiello F, Pignata S et al (2010) Gemcitabine versus bacille Calmette–Guerin after initial bacille Calmette–Guerin failure in non-muscle-invasive bladder cancer: a multicenter prospective randomized trial. Cancer 116(8):1893–1900
9. Nativ O, Witjes JA, Hendricksen K, Cohen M, Kedar D, Sidi A et al (2009) Combined thermo-chemotherapy for recurrent bladder cancer after bacillus Calmette–Guerin. J Urol 182(4):1313–1317
10. Yates DR, Brausi MA, Catto JW, Dalbagni G, Roupret M, Shariat SF et al (2012) Treatment options available for bacillus Calmette–Guerin failure in non-muscle-invasive bladder cancer. Eur Urol 62(6):1088–1096
11. Patel S, Dinh T, Noah-Vanhoucke J, Rengarajan B, Mayo K, Clark PE et al (2015) Novel simulation model of non-muscle invasive bladder cancer: a platform for a virtual randomized trial of conservative therapy vs. cystectomy in BCG refractory patients. Bladder Cancer 1(2):143–150
12. Racioppi M, Di Gianfrancesco L, Ragonese M, Palermo G, Sacco E, Bassi PF (2018) ElectroMotive drug administration (EMDA) of Mitomycin C as first-line salvage therapy in high risk "BCG failure" non muscle invasive bladder cancer: 3 years follow-up outcomes. BMC Cancer 18(1):1224

13. Sylvester RJ, van der Meijden AP, Oosterlinck W, Witjes JA, Bouffioux C, Denis L et al (2006) Predicting recurrence and progression in individual patients with stage Ta T1 bladder cancer using EORTC risk tables: a combined analysis of 2596 patients from seven EORTC trials. Eur Urol 49(3):466–475 **(discussion 75–77)**
14. Fernandez-Gomez J, Madero R, Solsona E, Unda M, Martinez-Pineiro L, Gonzalez M et al (2009) Predicting nonmuscle invasive bladder cancer recurrence and progression in patients treated with bacillus Calmette–Guerin: the CUETO scoring model. J Urol 182(5):2195–2203
15. Xylinas E, Kent M, Kluth L, Pycha A, Comploj E, Svatek RS et al (2013) Accuracy of the EORTC risk tables and of the CUETO scoring model to predict outcomes in non-muscle-invasive urothelial carcinoma of the bladder. Br J Cancer 109(6):1460–1466
16. Soukup V, Capoun O, Cohen D, Hernandez V, Burger M, Comperat E et al (2018) Risk stratification tools and prognostic models in non-muscle-invasive bladder cancer: a critical assessment from the European association of urology non-muscle-invasive bladder cancer guidelines panel. Eur Urol Focus. https://doi.org/10.1016/j.euf.2018.11.005
17. Holz S, Albisinni S, Gilsoul J, Pirson M, Duthie V, Quackels T et al (2017) Risk factor assessment in high-risk, bacillus Calmette–Guerin-treated, non-muscle-invasive bladder cancer. Res Rep Urol 9:195–202
18. Cambier S, Sylvester RJ, Collette L, Gontero P, Brausi MA, van Andel G et al (2016) EORTC nomograms and risk groups for predicting recurrence, progression, and disease-specific and overall survival in non-muscle-invasive stage Ta-T1 urothelial bladder cancer patients treated with 1–3 years of maintenance bacillus Calmette–Guerin. Eur Urol 69(1):60–69
19. Boorjian SA, Zhu F, Herr HW (2010) The effect of gender on response to bacillus Calmette–Guerin therapy for patients with non-muscle-invasive urothelial carcinoma of the bladder. BJU Int 106(3):357–361
20. Palou J, Sylvester RJ, Faba OR, Parada R, Pena JA, Algaba F et al (2012) Female gender and carcinoma in situ in the prostatic urethra are prognostic factors for recurrence, progression, and disease-specific mortality in T1G3 bladder cancer patients treated with bacillus Calmette–Guerin. Eur Urol 62(1):118–125
21. Kluth LA, Fajkovic H, Xylinas E, Crivelli JJ, Passoni N, Roupret M et al (2013) Female gender is associated with higher risk of disease recurrence in patients with primary T1 high-grade urothelial carcinoma of the bladder. World J Urol 31(5):1029–1036
22. Fernandez-Gomez J, Solsona E, Unda M, Martinez-Pineiro L, Gonzalez M, Hernandez R et al (2008) Prognostic factors in patients with non-muscle-invasive bladder cancer treated with bacillus Calmette–Guerin: multivariate analysis of data from four randomized CUETO trials. Eur Urol 53(5):992–1001
23. Gakis G, Stenzl A (2013) Gender-specific differences in muscle-invasive bladder cancer: the concept of sex steroid sensitivity. World J Urol 31(5):1059–1064
24. Tuygun C, Kankaya D, Imamoglu A, Sertcelik A, Zengin K, Oktay M et al (2011) Sex-specific hormone receptors in urothelial carcinomas of the human urinary bladder: a comparative analysis of clinicopathological features and survival outcomes according to receptor expression. Urol Oncol 29(1):43–51
25. Miyake M, Tatsumi Y, Gotoh D, Ohnishi S, Owari T, Iida K et al (2017) Regulatory T cells and tumor-associated macrophages in the tumor microenvironment in non-muscle invasive bladder cancer treated with intravesical bacille Calmette–Guerin: a long-term follow-up study of a Japanese cohort. Int J Mol Sci 18:E2186. https://doi.org/10.3390/ijms18102186
26. Martin-Doyle W, Leow JJ, Orsola A, Chang SL, Bellmunt J (2015) Improving selection criteria for early cystectomy in high-grade t1 bladder cancer: a meta-analysis of 15,215 patients. J Clin Oncol 33(6):643–650
27. Oddens JR, Sylvester RJ, Brausi MA, Kirkels WJ, van de Beek C, van Andel G et al (2014) The effect of age on the efficacy of maintenance bacillus Calmette–Guerin relative to maintenance epirubicin in patients with stage Ta T1 urothelial bladder cancer: results from EORTC genito-urinary group study 30911. Eur Urol 66(4):694–701
28. Nikolich-Zugich J (2018) The twilight of immunity: emerging concepts in aging of the immune system. Nat Immunol 19(1):10–19
29. Herr HW (2007) Age and outcome of superficial bladder cancer treated with bacille Calmette–Guerin therapy. Urology 70(1):65–68
30. Ferro M, Vartolomei MD, Russo GI, Cantiello F, Farhan ARA, Terracciano D et al (2019) An increased body mass index is associated with a worse prognosis in patients administered BCG immunotherapy for T1 bladder cancer. World J Urol. 37(3):507–514
31. Borges MD, Franca EL, Fujimori M, Silva SMC, de Marchi PGF, Deluque AL et al (2018) Relationship between proinflammatory cytokines/chemokines and adipokines in serum of young adults with obesity. Endocr Metab Immune Disord Drug Targets 18(3):260–267
32. Kluth LA, Xylinas E, Crivelli JJ, Passoni N, Comploj E, Pycha A et al (2013) Obesity is associated with worse outcomes in patients with T1 high grade urothelial carcinoma of the bladder. J Urol 190(2):480–486
33. Lin Y, Wang Y, Wu Q, Jin H, Ma G, Liu H et al (2018) Association between obesity and bladder cancer recurrence: a meta-analysis. Clin Chim Acta 480:41–46
34. Getzler I, Bahouth Z, Nativ O, Rubinstein J, Halachmi S (2018) Preoperative neutrophil to lymphocyte ratio improves recurrence prediction of non-muscle invasive bladder cancer. BMC Urol 18(1):90
35. Fridman WH, Pages F, Sautes-Fridman C, Galon J (2012) The immune contexture in human tumours: impact on clinical outcome. Nat Rev Cancer 12(4):298–306
36. Vartolomei MD, Ferro M, Cantiello F, Lucarelli G, Di Stasi S, Hurle R et al (2018) Validation of neutrophil-to-lymphocyte ratio in a multi-institutional cohort of patients with T1G3 non-muscle-invasive bladder cancer. Clin Genitourin Cancer 16(6):445–452
37. Mbeutcha A, Shariat SF, Rieken M, Rink M, Xylinas E, Seitz C et al (2016) Prognostic significance of markers of systemic inflammatory response in patients with non-muscle-invasive bladder cancer. Urol Oncol 34(11):483 e17–483 e24
38. Vartolomei MD, Porav-Hodade D, Ferro M, Mathieu R, Abufaraj M, Foerster B et al (2018) Prognostic role of pretreatment neutrophil-to-lymphocyte ratio (NLR) in patients with non-muscle-invasive bladder cancer (NMIBC): a systematic review and meta-analysis. Urol Oncol 36(9):389–399
39. Gontero P, Sylvester R, Pisano F, Joniau S, Oderda M, Serretta V et al (2016) The impact of re-transurethral resection on clinical outcomes in a large multicentre cohort of patients with T1 high-grade/Grade 3 bladder cancer treated with bacille Calmette–Guerin. BJU Int 118(1):44–52
40. Zapala P, Dybowski B, Poletajew S, Bialek L, Niewczas A, Radziszewski P (2018) Clinical rationale and safety of restaging transurethral resection in indication-stratified patients with high-risk non-muscle-invasive bladder cancer. World J Surg Oncol 16(1):6
41. Vasdev N, Dominguez-Escrig J, Paez E, Johnson MI, Durkan GC, Thorpe AC (2012) The impact of early re-resection in patients with pT1 high-grade non-muscle invasive bladder cancer. Ecancermedicalscience 6:269

42. Ajili F, Kourda N, Karay S, Darouiche A, Chebil M, Boubaker S (2013) Impact of smoking intensity on outcomes of patients with non muscle invasive bladder cancer treated by BCG immunotherapy. Ultrastruct Pathol 37(4):273–277
43. Chen CH, Shun CT, Huang KH, Huang CY, Tsai YC, Yu HJ et al (2007) Stopping smoking might reduce tumour recurrence in nonmuscle-invasive bladder cancer. BJU Int 100(2):281–286 **(discussion 6)**
44. Sfakianos JP, Shariat SF, Favaretto RL, Rioja J, Herr HW (2011) Impact of smoking on outcomes after intravesical bacillus Calmette–Guerin therapy for urothelial carcinoma not invading muscle of the bladder. BJU Int 108(4):526–530
45. Rink M, Xylinas E, Babjuk M, Pycha A, Karakiewicz PI, Novara G et al (2012) Smoking reduces the efficacy of intravesical bacillus Calmette–Guerin immunotherapy in non-muscle-invasive bladder cancer. Eur Urol 62(6):1204–1206
46. Sopori M (2002) Effects of cigarette smoke on the immune system. Nat Rev Immunol 2(5):372–377
47. Hoffmann P, Roumeguere T, Schulman C, van Velthoven R (2006) Use of statins and outcome of BCG treatment for bladder cancer. N Engl J Med 355(25):2705–2707
48. Kamat AM, Wu X (2007) Statins and the effect of BCG on bladder cancer. N Engl J Med. 356(12):1276 **(author reply-7)**
49. Lipsky MJ, Badalato GM, Motamedinia P, Hruby GW, McKiernan JM (2013) The effect of fibrin clot inhibitors on the immunomodulatory efficacy of bacillus Calmette–Guerin therapy for non-muscle-invasive bladder cancer. Urology 81(6):1273–1278
50. Dal Moro F, Bovo A, Crestani A, Vettor R, Gardiman MP, Zattoni F (2015) Effect of hypertension on outcomes of high-risk patients after BCG-treated bladder cancer: a single-institution long follow-up cohort study. Medicine (Baltimore) 94(9):e589
51. Kamat AM, Briggman J, Urbauer DL, Svatek R, Nogueras Gonzalez GM, Anderson R et al (2016) Cytokine panel for response to intravesical therapy (CyPRIT): nomogram of changes in urinary cytokine levels predicts patient response to bacillus Calmette–Guerin. Eur Urol 69(2):197–200
52. Kaempfer R, Gerez L, Farbstein H, Madar L, Hirschman O, Nussinovich R et al (1996) Prediction of response to treatment in superficial bladder carcinoma through pattern of interleukin-2 gene expression. J Clin Oncol 14(6):1778–1786
53. Zuiverloon TC, Nieuweboer AJ, Vekony H, Kirkels WJ, Bangma CH, Zwarthoff EC (2012) Markers predicting response to bacillus Calmette–Guerin immunotherapy in high-risk bladder cancer patients: a systematic review. Eur Urol 61(1):128–145
54. Qu K, Gu J, Ye Y, Williams SB, Dinney CP, Wu X et al (2017) High baseline levels of interleukin-8 in leukocytes and urine predict tumor recurrence in non-muscle invasive bladder cancer patients receiving bacillus Calmette–Guerin therapy: a long-term survival analysis. Oncoimmunology 6(2):e1265719
55. Salmasi A, Elashoff DA, Guo R, Upfill-Brown A, Rosser CJ, Rose JM et al (2018) Urinary cytokine profile to predict response to intravesical BCG with or without HS-410 therapy in patients with non-muscle invasive bladder cancer. Cancer Epidemiol Biomark Prev. https://doi.org/10.1158/1055-9965.EPI-18-0893
56. Thalmann GN, Sermier A, Rentsch C, Mohrle K, Cecchini MG, Studer UE (2000) Urinary Interleukin-8 and 18 predict the response of superficial bladder cancer to intravesical therapy with bacillus Calmette–Guerin. J Urol 164(6):2129–2133
57. Poletajew S, Zapala P, Radziszewski P (2017) Safety and efficacy of intravesical bacillus Calmette–Guerin immunotherapy in patients with non-muscle-invasive bladder cancer presenting with asymptomatic bacteriuria: a systematic review. Urol Int 99(1):1–5
58. Kamat AM, Willis DL, Dickstein RJ, Anderson R, Nogueras-Gonzalez G, Katz RL et al (2016) Novel fluorescence in situ hybridization-based definition of bacille Calmette–Guerin (BCG) failure for use in enhancing recruitment into clinical trials of intravesical therapies. BJU Int 117(5):754–760
59. Bao Y, Tu X, Chang T, Qiu S, Yang L, Geng J et al (2018) The role of fluorescence in situ hybridization to predict patient response to intravesical bacillus Calmette–Guerin therapy for bladder cancer: a diagnostic meta-analysis and systematic review. Medicine (Baltimore). 97(36):e12227
60. Liem E, Baard J, Cauberg ECC, Bus MTJ, de Bruin DM, Laguna Pes MP et al (2017) Fluorescence in situ hybridization as prognostic predictor of tumor recurrence during treatment with bacillus Calmette–Guerin therapy for intermediate- and high-risk non-muscle-invasive bladder cancer. Med Oncol 34(10):172
61. Ayari C, LaRue H, Hovington H, Decobert M, Harel F, Bergeron A et al (2009) Bladder tumor infiltrating mature dendritic cells and macrophages as predictors of response to bacillus Calmette–Guerin immunotherapy. Eur Urol 55(6):1386–1395
62. Pichler R, Fritz J, Zavadil C, Schafer G, Culig Z, Brunner A (2016) Tumor-infiltrating immune cell subpopulations influence the oncologic outcome after intravesical bacillus Calmette–Guerin therapy in bladder cancer. Oncotarget 7(26):39916–39930
63. Pichler R, Gruenbacher G, Culig Z, Brunner A, Fuchs D, Fritz J et al (2017) Intratumoral Th2 predisposition combines with an increased Th1 functional phenotype in clinical response to intravesical BCG in bladder cancer. Cancer Immunol Immunother 66(4):427–440
64. Nunez-Nateras R, Castle EP, Protheroe CA, Stanton ML, Ocal TI, Ferrigni EN et al (2014) Predicting response to bacillus Calmette–Guerin (BCG) in patients with carcinoma in situ of the bladder. Urol Oncol 32(1):e23–e30
65. Cai T, Mazzoli S, Meacci F, Tinacci G, Nesi G, Zini E et al (2007) Interleukin-6/10 ratio as a prognostic marker of recurrence in patients with intermediate risk urothelial bladder carcinoma. J Urol 178(5):1906–1911 **(discussion 11–12)**
66. Cai T, Nesi G, Mazzoli S, Meacci F, Tinacci G, Luciani LG et al (2012) Prediction of response to bacillus Calmette–Guerin treatment in non-muscle invasive bladder cancer patients through interleukin-6 and interleukin-10 ratio. Exp Ther Med 4(3):459–464
67. Du J, Wang SH, Yang Q, Chen QQ, Yao X (2016) p53 status correlates with the risk of progression in stage T1 bladder cancer: a meta-analysis. World J Surg Oncol 14:137
68. He Y, Wang N, Zhou X, Wang J, Ding Z, Chen X et al (2018) Prognostic value of ki67 in BCG-treated non-muscle invasive bladder cancer: a meta-analysis and systematic review. BMJ Open 8(4):e019635
69. Cormio L, Tolve I, Annese P, Saracino A, Zamparese R, Sanguedolce F et al (2009) Altered p53 and pRb expression is predictive of response to BCG treatment in T1G3 bladder cancer. Anticancer Res 29(10):4201–4204
70. Bertz S, Otto W, Denzinger S, Wieland WF, Burger M, Stohr R et al (2014) Combination of CK20 and Ki-67 immunostaining analysis predicts recurrence, progression, and cancer-specific survival in pT1 urothelial bladder cancer. Eur Urol 65(1):218–226
71. El-Babouly IM, Desoky EAE, El Sayed D, Ali MM, Harb OA, Ragab A et al (2018) The role of neural precursor cell-expressed developmentally down-regulated protein 9 in predicting bacillus Calmette–Guerin response in nonmuscle invasive bladder cancer. Urol Oncol 36(5):242 e9–242 e14
72. Breyer J, Wirtz RM, Otto W, Erben P, Worst TS, Stoehr R et al (2018) High PDL1 mRNA expression predicts better survival of stage pT1 non-muscle-invasive bladder cancer (NMIBC) patients. Cancer Immunol Immunother 67(3):403–412
73. Hedegaard J, Lamy P, Nordentoft I, Algaba F, Hoyer S, Ulhoi BP et al (2016) Comprehensive transcriptional analysis of early-stage urothelial carcinoma. Cancer Cell 30(1):27–42
74. Damrauer JS, Hoadley KA, Chism DD, Fan C, Tiganelli CJ, Wobker SE et al (2014) Intrinsic subtypes of high-grade bladder cancer

reflect the hallmarks of breast cancer biology. Proc Natl Acad Sci USA 111(8):3110–3115
75. Hurst CD, Alder O, Platt FM, Droop A, Stead LF, Burns JE et al (2017) Genomic subtypes of non-invasive bladder cancer with distinct metabolic profile and female gender bias in KDM6A mutation frequency. Cancer Cell 32(5):701 e7–715 e7
76. Kim D, Choi Y, Ireland J, Foreman O, Tam RN, Patel R et al (2016) Development and application of a microfluidics-based panel in the basal/luminal transcriptional characterization of archival bladder cancers. PLoS One 11(11):e0165856
77. Barth I, Schneider U, Grimm T, Karl A, Horst D, Gaisa NT et al (2018) Progression of urothelial carcinoma in situ of the urinary bladder: a switch from luminal to basal phenotype and related therapeutic implications. Virchows Arch 472(5):749–758