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European Association of Urology

Platinum Priority – Review – Bladder Cancer
Editorial by XXX on pp. x-y of this issue

Predicting Response to Intravesical Bacillus Calmette-Guérin Immunotherapy: Are We There Yet? A Systematic Review

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Article info

Article history:
Accepted October 2, 2017

Associate Editor:
Giacomo Novara

Keywords:
Bladder cancer
Immunotherapy
BCG
Response prediction
Personalized therapy

Abstract

Context: Bacillus Calmette-Guérin (BCG) is currently the most effective intravesical therapy for nonmuscle invasive bladder cancer, reducing not only recurrence rates but also preventing progression and reducing deaths. However, response rates to BCG vary widely and are dependent on a multitude of factors.
Objective: To review existing data on clinical, pathologic, immune, and molecular markers that allow prediction of BCG response.
Evidence acquisition: PubMed and MEDLINE search of English language literature was conducted from its inception to July 2017 using appropriate search terms. Following systematic literature review and analysis of data, consensus voting was used to generate the content of this review.
Evidence synthesis: As seen in the EORTC and CUETO risk nomograms, clinicopathologic features, especially tumor stage and grade, are the most effective predictors of BCG response. Data are less robust with regards to the association of response with age, female sex, recurrent tumors, multiplicity of tumors, and the presence of carcinoma in situ. Single biomarkers, such as tumor p53 and urinary interleukin-2 expression, have had limited success in predicting BCG response, possibly due to the multifaceted nature of the generated immune response. More comprehensive biomarker panels (eg, urinary cytokines), have a more robust correlation with response, as do patterns of urinary cytologic fluorescent in-situ hybridization examination. Gene expression data correlate with disease progression, but studies examining potential associations with BCG response are limited.
Conclusions: Currently, the best predictors of BCG response are clinicopathologic features such as tumor grade and stage. Panels of urinary cytokines, as well as fluorescent in-situ hybridization patterns of cytologic anomalies, appear to be promising biomarkers. The complexity of the immune response to BCG and the heterogeneity of bladder

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https://doi.org/10.1016/j.eururo.2017.10.003

Please cite this article in press as: Kamat AM, et al. Predicting Response to Intravesical Bacillus Calmette-Guérin Immunotherapy: Are We There Yet? A Systematic Review. Eur Urol (2017), https://doi.org/10.1016/j.eururo.2017.10.003

cancer suggest that future studies should amalgamate measures reflecting innate immune response and tumor/stromal gene expression before these can be adopted for clinical use.

Patient summary: Bacillus Calmette-Guérin (BCG) immunotherapy is an effective treatment for many patients with nonmuscle invasive bladder cancer. An individual's response to BCG can be predicted by using various features of the cancer. In the future, predictive markers using molecular measures of the tumor type and the immune response to BCG may allow us to precisely know an individual's likely outcome after BCG treatment.

1. Introduction

For more than 4 decades, intravesical Bacillus Calmette-Guérin (BCG) has been the most effective intravesical therapy for nonmuscle invasive bladder cancer (NMIBC) [1]. Despite its success, recurrence rates range from 32.6% to 42.1% and progression rates from 9.5% to 13.4% [2,3]. Patients who develop disease progression often have compromised survival due to the delay in curative therapy (eg, radical cystectomy or trimodal therapy) [4]; even recurrent, nonprogressive disease imposes heavy financial burdens on health care systems and is associated with morbidity for patients. Thus, clinically applicable tools to predict disease recurrence and progression are much needed. In this review, we summarize the published evidence on markers of response to intravesical immunotherapy with BCG.

2. Evidence acquisition

PubMed and MEDLINE search of the English language literature was conducted from its inception to July 2017 using terms: "non-muscle invasive bladder cancer," "bladder cancer," "BCG," "immunotherapy," "cytokine," "interleukin," "immune response," "recurrence," "progression," "survival," "molecular marker," "prognosis," "single nucleotide polymorphism," "gene signature," and "immune signature." Reference lists in pertinent articles were reviewed to augment source material. Full texts of selected studies (eg, review articles) [5,6] relevant to this manuscript were reviewed. Evidence was collated and condensed by the first and second authors and a summary document circulated to all coauthors for consensus on "definitely useful" and "probably useful" in predicting response to BCG. Evidence not robust enough to be classified into the above categories was placed into the "emerging strategies" category (Table 1).

3. Evidence synthesis

3.1. *Definitely useful*

3.1.1. *Clinicopathologic features*

The first attempts to predict response to BCG centered on clinicopathologic features. Recognizing that a combination of factors would be most accurate, two comprehensive efforts were put forth by the European Organization for Research and Treatment of Cancer (EORTC) and Club Urologico Espano de Tratamiento Oncologico (CUETO) groups. In a pooled cohort of 1062 patients treated with BCG, the CUETO group identified female sex (hazard ratio [HR]: 1.71), recurrent tumors (HR: 1.9), tumor multiplicity (HR: 1.1–1.7), and presence of carcinoma in situ (CIS; HR: 1.54) to predict recurrence, and recurrent tumor (HR: 1.62), high-grade tumors (HR: 5.64), T1 tumors (HR: 2.15), and recurrence on 3-mo endoscopic examination (HR: 4.6) to predict progression to muscle invasive bladder cancer (MIBC) [3]. Based on the analysis, a scoring system was constructed categorizing patients into four risk groups each for recurrence (C-index: 0.64) and progression (C-index: 0.69–0.70) [7].

While useful, one weakness of the CUETO data was the group's nonstandard maintenance BCG protocol of six fortnightly treatments after induction wherein patients only received 5–6 mo of maintenance therapy. In contrast, a subsequent EORTC nomogram was formulated with data extracted from 1812 patients who received 1–3 yr of maintenance therapy in accordance with the widely used

Table 1 – Consensus classification of factors as "definitely useful" and "probably useful" in predicting response. Evidence not robust enough to be classified is listed as "Emerging strategies"

Definitely useful		Probably useful	Emerging strategies
Before treatment		**Before treatment**	**Before treatment**
Clinicopathologic features (level of evidence)		Tumor molecular biomarkers[a]	Molecular subtypes
Grade	(+++)	Host genomic signature[b]	
Stage	(+++)		
Recurrent tumors	(++)	**During treatment**	
Multiplicity	(++)	Clinical immune response measures	
CIS	(+)	Urinary cytokines (eg CyPRIT)	
Female sex	(+)	Cell-mediated immunity markers	
Age	(+)	Immunologic milieu (Th1 vs Th2)	
During and after treatment			
FISH pattern on cytologic examination			

CIS = carcinoma in situ; FISH = fluorescent in-situ hybridization; Th = T helper.
[a] See Table 2.
[b] See Table 3.

Southwest Oncology Group protocol [2]. The EORTC model was additionally enhanced by more granular data on recurrence rates and tumor multiplicity. In this model, early recurrence (ie, at the postinduction evaluation) was predicted by prior recurrence rate >1/yr, tumor multiplicity (≥4 tumors), and grade (C-index: 0.65–0.67). Similarly, late recurrence was predicted by prior recurrence rate >1/yr and tumor multiplicity (C-index: 0.56–0.59). For disease progression, tumor grade and T stage were significant predictors (C-index: 0.64–0.72) [2].

Notably, both models identified recurrent tumors and multiplicity as predictors of recurrence, and high tumor grade and stage as risk factors for progression. This was not surprising since evidence for a predictive value of tumor stage and grade abound. As evidence for the poor prognosis associated with these two factors, progression rates ranged as high as 17.1–21% in patients with stage cT1 high-grade tumors initially treated with BCG [8–10].

By contrast, the association between recurrent tumors and multiplicity with response to BCG is not as clear. Although recurrence rates are more frequent in patients with prior history of bladder cancer in some studies [11], others have reported contradictory results [12,13]. Similarly, while some studies have demonstrated an association between tumor multifocality and post-BCG recurrence [14], others have not [11–13]. However, our consensus is that these factors are indeed important, since many negative studies were underpowered or did not use maintenance BCG according to today's standards.

The presence of CIS has been extensively examined as a predictor of BCG response. In two cohorts treated only with induction BCG, concomitant CIS was found to be a predictor of shorter progression-free survival (PFS) and cancer-specific survival (CSS) [15,16]. The effect was especially pronounced in patients with T1 lesions [15]. This was subsequently corroborated in a cohort treated with induction and maintenance therapy [17].

Female sex has been reported as a poor prognostic factor [10] in studies outside the CUETO report and appears to be supported by different levels of urinary cytokines detected after BCG treatment in women [18]. This is in line with the hypothesis that hormone status may affect carcinogenesis in the bladder. However, some large retrospective series have not found differences in response to BCG between the two sexes [19].

Age is another host factor purported to play a role in BCG responsiveness. Presumably, poor outcomes are due to waning immune response, attenuating the effect of BCG therapy [3,20]. A subset analysis of the BCG plus interferon (IFN)-α phase 2 study revealed that patients over 80 had the poorest recurrence-free survival (RFS) [20] and age over 80 yr was an independent predictor of recurrence (HR: 1.56). Another study suggested that although patients >70 yr did not have inferior initial response to BCG, more older patients recurred on long term follow-up [14]. Furthermore, age was also found to be an independent predictor for progression by the CUETO group [3]. Analysis of the prospective EORTC 30911 study recapitulated the poor prognostic effect of age on RFS, PFS, and CSS [21]; however, it was seen that even in older patients (>70 yr), BCG was more effective than epirubicin. Thus, old age appears to be just as prognostic as it is predictive, and applies to all intravesical therapies.

We would like to caution the reader that it is critical to differentiate between prognostic factors and those that predict BCG response. Although some variables are associated with higher likelihood of BCG failure, they may reflect poor overall prognosis, rather than the lack of benefit from BCG. In this light, many abovementioned risk factors for BCG failure, such as patient age, recurrence rate, tumor stage, grade, and concomitant CIS, have also been linked to poor overall prognosis from NMIBC [8,9]. The same must also be considered for the molecular markers described below. Additionally, many of the studies considered heretofore have not been conducted in the era when re-transurethral resection, or optical enhanced cystoscopy, was performed routinely.

3.1.2. *Fluorescent in situ hybridization on tumor cells*

Urinary fluorescent in situ hybridization (FISH; Urovysion, Abbott Molecular, Des Plaines. IL, USA), a molecular cytogenetic test used to detect chromosomal abnormalities, was initially developed for bladder cancer detection and surveillance. While its use in this area is diminishing by virtue of its ability to anticipate tumor formation [22], FISH is a valuable clinical tool for predicting failure after BCG. A positive FISH result after BCG induction confers increased risk of recurrence (3–5 fold) and progression (5–13 fold), depending on timing of FISH positivity. For example, in one study, at the 3-mo time point, patients with a positive FISH result had a 58% risk of recurrence compared with 15% with a negative result ($p < 0.001$). For disease progression, the incidence was 25% with a positive FISH compared with 7% with a negative result ($p < 0.013$) [23]. Since many patients who have a positive FISH test have no visible tumor at the time of assessment but subsequently develop recurrence in 6–24 mo, this phenomenon has been categorized as a "molecular failure" and such patients are encouraged to enroll into clinical trials for salvage therapies [24].

3.2. *Probably useful*

3.2.1. *Tumor molecular biomarkers*

New understandings in molecular carcinogenesis and more powerful diagnostic platforms have ushered in a new era of personalized medicine. Molecular biomarkers have been identified and successfully used to predict treatment efficacy, but the same caveats of prognostic versus predictive significance exist.

p53, a cell cycle regulator, had been studied most extensively. Immunohistochemical (IHC) p53 overexpression, while not predictive for recurrence, was found to correlate with progression [25–27]. However, it was unknown whether p53 overexpression on IHC correlated with actual loss of function. For example, when levels of p21, a downstream effector of p53 [28] were measured, recurrence rates correlated not with p53, but instead with p21 levels. Variations in p53 quantification methods and

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Table 2 – Biomarkers related to BCG response

Authors, yr	Biomarker	Biomarker function	No. of patients	Significance
Esuvarantahn et al. 2007 [81]	RB	Cell cycle regulator	47	Underexpression may be predictive of non-response to BCG+IFN-α
Cormoio et al. 2010 [82]	RB	Cell cycle regulator	27	Under- or overexpression may predict recurrence and progression
Hausladen et al. 2003 [83]	Survivin	Apoptosis inhibitor	25	Detection in urine 1 month after therapy predicts recurrence
Okamura et al. 1998 [84]	bcl-2	Apoptosis inhibitor	79	Lower bcl-2 in patients with recurrent disease
Serdar et al. 2005 [85]	e-cadherin	Cell adhesion molecule	61	Abnormal expression associated with cancer recurrence, progression and disease specific survival
Palou et al. 2009 [86]	ezrin	Cell adhesion molecule	92	Ezrin membrane expression <20% associated with increased progression and shorter survival
Lima et al. 2013 [87]	sialyl-Tn and sialyl-6-T	Cell adhesion molecule	94	High expression leads to effective BCG treatment
Blanchet et al. 2001 [88]	Ki-67	Proliferation index	70	Ki-67 index of 13 or higher predicted progression
Langel et al. 2016 [89]	FGFR3	Growth factor	11	Down regulation of FGFR3 predicts good response

BCG = bacillus Calmette-Guérin; FGFR = fibroblast growth factor receptors; IFN = interferon.

arbitrary thresholds used in the different studies make it difficult to compare results. A landmark meta-analysis found considerable differences in the technical aspects of p53 evaluation, study design, patient selection, and consequently the yielded results [29].

In addition to p53, a multitude of other molecules have been assessed as potential predictors for BCG response (Table 2). These include cell cycle regulators (RB), apoptosis inhibitors (survivin, bcl-2), cell adhesion molecules (E-cadherin ezrin), and markers of proliferation (Ki-67). However, studies of these biomarkers all suffer from the same shortfalls pertaining to the p53 studies: nonstandard methods of measurement, subjective readouts, arbitrary cutoffs, small study populations, differences in patient selection, and lack of validation. It is our consensus that the molecular heterogeneity of bladder cancer, coupled with the multifaceted immunologic effects unleashed by BCG, make it unlikely that response can be predicted with individual molecular biomarkers.

3.2.2. *Clinical immune response*

Interest in measuring the innate immune response of patients to BCG is not new. As skin reactivity to purified protein derivative (PPD) is the gold standard to detect antituberculin immunity, many have postulated that it can detect pre-existing BCG-specific immunity and predict for improved antitumor response to BCG. In one recent study, when patients were stratified according to their pre-BCG PPD status, median recurrence-free survival was 25 mo in the PPD-negative group and was not reached in the PPD-positive group ($p < 0.05$) [30]. However, other studies have not found similar correlation [31,32].

A corollary is the use of treatment side effects to predict response since it has been reported that patients developing fever during treatment have significantly lower recurrence rates [32]. An analysis of the EORTC 30911 results also indicated improved response rates in patients with significant side effects [33]. However, this could be due to the fact that responding patients continue on BCG longer, and thus have more side effects. Indeed, a separate analysis of the same study comparing patients developing symptoms within 6 mo of treatment with those who did not failed to find any difference in RFS.

3.2.3. *Immune cell response*

Another way to measure the efficacy of BCG-induced immunity is to quantify the infiltrating immune cell response after BCG therapy. A higher level of leukocyturia following BCG induction is associated with improved response to BCG [34] as well as increased self-reported adverse events [31,32]. Within this initial proinflammatory response, polymorphonuclear cells are implicated as effector cells of cytotoxicity, specifically through the production of tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) [35].

Several studies have suggested that molecules associated with antigen presentation may increase either on tumor cells or associated immune cells. For example, heat shock protein 90—thought to contribute to antigen presentation and the recruitment of various downstream effector cells—has been associated with response to BCG in one study (patients with <40% of tumor cells expressing heat shock protein 90 failed to respond to BCG) [36]. A greater increase in the expression of major histocompatibility complex class I after BCG treatment has also been found to predict longer RFS [37], with a suggestion that expression levels on the tumor cell are most relevant [38]. Similarly, bladder cancer cells have also been described to display other antigen presenting molecules such as major histocompatibility complex class II (usually restricted to immune cells) and immune signaling molecules such as ICAM1 [39,40]. However, it must be noted that the expression of antigen presentation molecules is influenced by IFN levels. Thus, they will be upregulated during BCG therapy, which stimulates IFN release. Whether these molecules actually contribute to tumor antigen presentation remains to be determined.

Adding to the confusion, how professional antigen-presenting cells contribute to the antitumor effects of BCG is unclear. Dendritic cells are “sentinels” of the immune system, acquiring and processing antigens and are capable of activating natural killer (NK) cells and γδ cells upon

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exposure to BCG in vitro [41]. However, their role in the BCG response remains largely undefined; immature dendritic cells have been detected more frequently in the urine of BCG responders [42], but high levels of mature, tumor infiltrating dendritic cells have also been shown to predict treatment failure [43].

The role of macrophages in the response to BCG is complicated by their potential dual roles—they can induce cytotoxicity but also can promote tumorigenesis [44]. Subtyping of macrophages has been proposed to distinguish those participating in T helper 1 (Th1) response leading to tumor cell killing (M1), and those involved in Th2 response thought to stimulate cancer growth (M2). Along these lines, two independent reports found that higher numbers of tumor infiltrating CD68+ tumor-associated macrophages predicted higher recurrence rates after BCG [43,45], and hypothesized that these cells are part of the inflammatory circuit that may promote tumor progression.

Regardless of the type of antigen-presenting cells, antigen presentation leads to activation of effector cells such as lymphocytes and NK cells. Early studies demonstrated that BCG-induced antitumor activity was lost in athymic nude mice (lacking lymphocytes), supporting that lymphocytes are necessary for BCG-mediated immunity [46]. Subsequently, multiple IHC studies in immunocompetent patients demonstrated an increase in the number of CD4+ Th cells after BCG treatment [40,47] and the number of CD4+ T cells (HR: 0.13, $p = 0.025$) and CD4/CD8 ratio (HR: 0.03, $p = 0.001$) in pretreatment tumors as significant predictors of response [48]. However, subtypes of T lymphocytes with contradictory influences on immune response exist. In particular, tumor immune escape has been attributed to the recruitment of CD4+CD25^{hi}FOXP3+ T regulatory cells [49]. In the aforementioned study, RFS was found to be decreased in patients with CD25^{hi} or FOXP3+ T lymphocytes in pre-treatment tumor samples [48].

NK cell, are also crucial for BCG induced tumor cytotoxicity [50]. Interactions between NK cells and tumor ligands have been put forward as potential predictors of BCG response. In a small study, synthetic mimics of NK cells' natural cytotoxicity receptors were incubated with tumor specimens collected prior to BCG treatment [51]; IHC revealed higher levels of interaction in patients responding to BCG therapy compared to those with recurrences.

Adding even more complexity to this multifaceted BCG response, we must consider the immunologic milieu surrounding the tumor microenvironment. BCG induces Th1-polarized immune responses consisting of specific inflammatory cytokines (eg, IFN-γ, interleukin [IL]-12, and tumor necrosis factor [TNF]-α) [52]. The Th1 response includes priming of CD8+ cytotoxic T-cells with tumor antigen, while the Th2 response is characterized by increased angiogenesis and inhibition of cell-mediated antitumor immunity. It is hypothesized that BCG is effective only when the tumor microenvironment converts from Th2 to Th1, and has no effect on microenvironments already polarized to Th1. Interestingly, this was indeed shown to be the case in one study of CIS patients [52] where IHC measurements of eosinophil infiltration and degranulation (Th2-polarized), as well as the ratio of GATA-3+ (Th2-polarized) to T-bet+ (Th1-polarized) lymphocytes were found to be significant predictors of BCG response. Combining these three markers, the authors created a Th2 signature proposed to predict treatment response. Another study independently validated this predictive value of the GATA-3+/Tbet+ ratio and also used increased urinary Th1 markers during treatment to enhance predictive power [53].

Validation of the above measurements is hindered by inherent limitations in the evaluation of cell-mediated immunity (appropriate tissue procurement and sample processing as well as interpretation). Moreover, the inflammatory response generated by BCG treatment precludes tissue sampling for real-time evaluation of the dynamic immune response during the treatment period.

3.2.4. *Urinary cytokines*

Throughout the post-BCG immune response, cytokines are responsible for downstream effector cell recruitment, differentiation of the immunologic microenvironment, and direct tumor cytotoxicity. With increasing understanding of their functions, cytokine profiles can be analyzed to assess efficacy of BCG-induced cytotoxic response and can be detected in the urine within 1–4 wk from the start of treatment [54].

IL-2 expression has been most extensively studied. A canonical Th-1 cytokine, IL-2 is secreted by CD4+ T cells upon activation and stimulates cytotoxic CD8+ lymphocyte proliferation, macrophage activation, and delayed type hypersensitivity. Multiple groups have reported higher IL-2 levels in the urine collected from BCG responders compared to nonresponders [55] and IL-2 levels peak earlier than IL-10 levels (Th2 cytokine) in responders [6,56]. From this, one may hypothesize that benefit derived from BCG therapy is confined within a limited window early on when the Th1 response (marked by high IL-2 levels) predominates and that patients with predominantly Th2 responses during induction may not gain additional benefit from maintenance.

Since a robust Th2 response (an indirect indicator of a weak Th1 response) can foretell an unfavorable outcome after BCG therapy, it was put forth that high urinary IL-10 expression, a surrogate for Th2 responses, would predict treatment failure. However, this has not been the case in multiple studies [6,18,56]. Given the complex interactions between cytokines, investigators have evaluated ratios between Th1/Th2 markers with success; for example, the ratio between IL-6 and IL-10 was found to have 83% sensitivity and 76% specificity in predicting recurrence after BCG [57]. This was later validated on a larger cohort of 72 patients with high risk NMIBC [58].

In addition to studying the Th1/Th2 immunologic microenvironment, one can predict BCG response based on IL-8, a promoter of the initial polymorphonuclear cell-driven proinflammatory response; higher levels of urinary IL-8 levels, as well as IL-18 (an inducer of IL-8) after BCG therapy significantly correlated with longer CSS [59]. The predictive value of IL-8 was subsequently validated in an

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independent cohort. Other urinary cytokines identified to be potential predictors of BCG therapy include TNF-α [60], IL-12 [53], and TRAIL [35].

Due to the complexity of the immune response to BCG, no single cytokine or biomarker is likely to be definitively predictive of a positive or negative response. Kamat and colleagues [61] prospectively tested the hypothesis that a panel of urinary cytokines can accurately assess the multifaceted immune response generated by intravesical BCG. In a prospective study of 125 patients, urine was collected at various time points and multiple cytokines assessed. Various time point and ratio combinations were studied using computational analysis. After extensive modelling, it was found that the inducible levels of cytokines at the last induction (sixth) BCG instillation—calculated as the difference from preinstillation levels to postinstillation levels (4 h after BCG)—was most predictive of response. The number of cytokines required was then drilled down to the minimum required to retain predictive power. A nomogram (CyPRIT, Cytokine Panel for Response to Intravesical Therapy) using a panel of nine cytokines (IL-2, IL-6, IL-8, IL-18, IL-1ra, TRAIL, IFN-γ, IL-12 [p70], and TNF-α) was found to have an accuracy of 85.5% in predicting response to BCG (95% CI: 77.9–93.1%; Fig. 1). Efforts to validate the use of CyPRIT are currently underway.

Notwithstanding its promise, there are potential pitfalls with using urinary cytokine levels. Since urinary cytokine production may reflect local inflammatory responses, at minimum, urinary tract infections need to be excluded prior to analyzing urinary cytokine levels. Also, since urinary cytokine levels may be altered by systemic processes, whether the changes in cytokine levels can accurately reflect the magnitude of local immune response in patients receiving systemic immunomodulators is unknown [62].

3.2.5. *Host genomic signature*

An interesting avenue of response prediction is to parse out responders based on genomic variations affecting key genes implicated in BCG induced inflammatory pathways. There is currently a plethora of studies showing such correlation. For example, single nucleotide polymorphisms studies have shown that a variant genotype in *IL-6* is associated with increased risk of recurrence with BCG [63]. This was postulated to attenuated production of IFN-γ due to insufficient IL-6, thus leading to a suboptimal Th1 response. Using a similar rationale, others have identified variant polymorphisms in genes encoding several cytokines (*IL17*, *IL-2*, and *TNF-α*), chemokines (*MCP-1*) as well as effector molecules (TRAIL receptor) to be associated with increased recurrence after BCG [64]; adding these genomic signatures to key clinicopathologic features, the authors constructed a risk score achieving an area under the curve of 82%. Additional genes linked to outcomes following BCG treatment include those involved in detoxification (hGPX1)

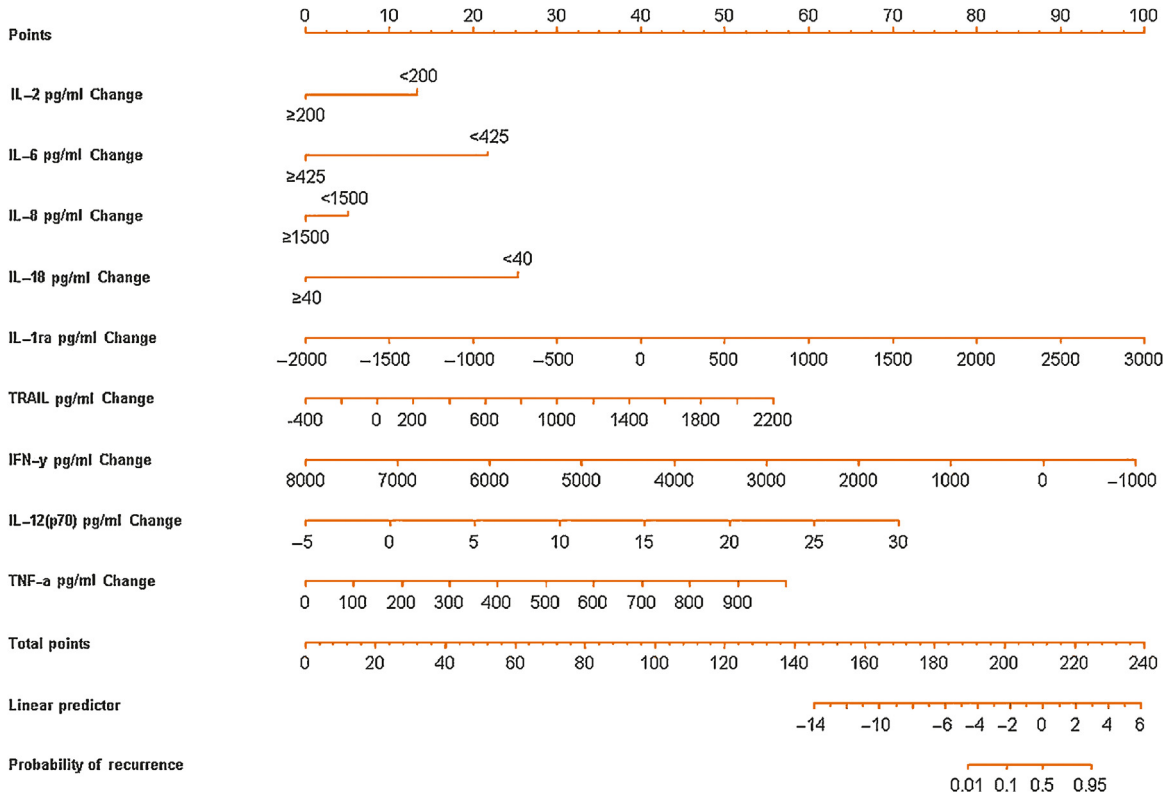

Fig. 1 – CyPRIT Nomogram for calculating the risk of recurrence using changes in urinary cytokine levels from immediately before to 4 h after instillation of BCG at the last dose of induction (ie, at 6 wk).
BCG = bacillus Calmette-Guérin; CyPRIT = Cytokine Panel for Response to Intravesical Therapy; IFN = interferon; IL = interleukin; TNF = tumor necrosis factor.
Adapted from Kamat et al [61].

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Table 3 – Genetic variants related to BCG response

Authors, yr	Genetic variant	Gene function	No. of patients	Significance
Leibovici et al. 2005 [63]	IL-6	Host immune response	519	Related to tumor recurrence in patients treated with induction + maintenance
Ahirwar et al. 2008 [90]	IL-6	Host immune response	136	Genotype C/C associated with low recurrence rates
Lima et al. 2015 [64]	Multiple genes	Host immune response	204	Predictive risk score developed from multi-gene panel to determine outcomes after BCG
Zhao et al. 2004 [91]	Glutathione peroxidase 1	Detoxification	224	Wild type had higher recurrence after BCG
Gu et al. 2005 [66]	Multiple genes	Nucleotide excision repair	288	Genetic variants had higher recurrence rates
Jaiswal et al. 2012 [92]	Survivin	Apoptosis inhibitor	200	Variant of Survivin-31 G>C associated with reduced risk of recurrence
Chen et al. 2010 [93]	Multiple genes	Sonic Hedgehog pathway	419	GLI3 wildtype variants significantly predicted for lower recurrence

BCG = bacillus Calmette-Guérin; IL = interleukin.

[65], nucleotide excision repair [66], and regulation of macrophage susceptibility to intracellular mycobacterial growth (*NRAMP1*) [65] (Table 3).

Polymorphisms impairing cellular DNA damage repair (DDR) have counter intuitively been associated with better outcomes after BCG treatment [66]. In line with recent findings correlating DDR mutations and response to checkpoint inhibitor therapy, impaired DDR may lead to higher mutational burden and neoantigens that ultimately provoke a stronger immune response. In a recent study of high-risk NMIBC patients, a higher total mutation burden was found in patients who responded to intravesical therapy compared to those who did not [67]. It is likely that further studies will show that tumor mutational burden is likely both predictive and prognostic.

Finally, methylation profiles in several panels of genes have been examined and correlated with RFS, PFS, and CSS after BCG therapy [68]. Of these, methylation status of several tumor suppressor genes, including *MSH6* and *THBS1*, may hold promise.

Overall, the association between genotypic differences and phenotypic response to BCG warrants further validation. Moreover, as most studies on gene polymorphisms were performed in homogeneous ethnic and/or geographic populations, it is still unknown whether these associations can be extended to the global population of NMIBC patients at large.

3.2.6. *Concurrent medication use*

In addition to the variables mentioned, concurrent use of certain medications may affect outcomes after BCG treatment. Three classes of drugs have been investigated in this setting: antibiotics, statins, and anticoagulants. Hypothesizing that concurrent use of isoniazid may reduce adverse events during treatment, a large randomized trial conducted by the EORTC showed no difference in efficacy with combination therapy [69]. Unfortunately, the incidence of adverse events was not reduced. In a much-publicized report in *New England Journal of Medicine* the outcomes in 19 patients concomitantly using statin during BCG treatment was compared with 65 who did not [70]. The statin group had higher cancer progression rates, leading to the recommendation that statin use should be stopped. This report was immediately rebutted by a larger study which clearly showed no deleterious effect of statin use on response to BCG [71]. Oral anticoagulants are postulated to impede the fibronectin mediated attachment and internalization of BCG by urothelial cells [72], with resultant impairment of antitumor activity. In large cohort studies, conflicting results emerge; while patients on warfarin had increased risk of tumor recurrence and progression to surgery, those on aspirin had decreased risk [73]. The exact mechanism by which these medications affect BCG function requires further elucidation.

3.3. *Emerging strategies*

Despite the plethora of studies summarized herein, no consensus existed on what constituted BCG “failure” until recent expert consensus on BCG unresponsive disease [74,75]. Consequently, it is difficult to interpret results across studies due to their different definitions and endpoints as well as variability in treatment protocols and patient selection. Future investigations on predictors of BCG efficacy are encouraged to adhere to the consensus definitions of BCG unresponsiveness and the standard Southwest Oncology Group maintenance treatment protocol.

Notwithstanding these constraints, exciting novel methods of predicting BCG response have emerged with the advent of new molecular platforms and our increasing understanding of mechanism of action of BCG. The use of next-generation sequencing for the comprehensive molecular characterization of bladder cancer has not only shed light on tumor biology, but also provided clues for molecular mechanisms of treatment success and failure. In regards to chemotherapy for MIBC, therapy-driven clonal evolution leading to chemoresistance [76] has been demonstrated. Furthermore, somatic mutations in DDR genes also appear to confer cisplatin-based chemosensitivity [77] and molecular subtyping of MIBC has been linked to different phenotypic responses after neoadjuvant chemotherapy [78]. Similarly, three molecular subtypes of NMIBC have been proposed based; however, no distinct recurrence

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or progression patterns have been identified pertaining to BCG treatment [79].

Finally, recent advances in the understanding of tumor immunology are shedding light on the problem of predicting response to BCG. High expression of programmed death-ligand 1 (PD-L1; a T-cell inhibitory molecule) in BCG granulomata is predictive of BCG failure [80]. In addition to its predictive power, PD-L1 induced T-cell anergy has also been identified as an actionable immunotherapeutic target. Trials combining therapy with PD-L1 inhibitor and BCG for high-risk NMIBC patients are underway (NCT02792192).

4. Conclusions

Although previous efforts to predict BCG responsiveness have largely been mixed, much has been learned. Clinicopathologic features are prognostic as well as predictive for BCG response. Efforts at using single biomarkers to predict BCG response are inherently compromised due to BCG's multifaceted mechanism of action. Thus, the best predictors of response to intravesical immunotherapy currently available are grade and stage of tumors, recurrence pattern of prior tumors, nomograms such as the EORTC and CUETO tables, panels of urinary cytokines, and FISH patterns of urine cytologic examination. Moving forward, the complexity of the immune response generated by BCG will necessitate amalgamation of large amount of data from multiplex platforms, measure of innate immune response, and tumor gene expression before these can be adopted for clinical use. Equipped with maturing genomic characterization platforms and knowledge accumulated on cancer immunotherapy in general, we are approaching the cusp of understanding the molecular mechanisms driving BCG-induced tumor kill and thus predicting its response.

Author contributions: Ashish M. Kamat had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

Study concept and design: Kamat, Li.
Acquisition of data: Kamat, Li.
Analysis and interpretation of data: Kamat, Li, O'Donnell, Black, Roupret, Catto, Comperat, Ingersoll, Witjes, McConkey, Witjes.
Drafting of the manuscript: Kamat, Li.
Critical revision of the manuscript for important intellectual content: Kamat, Li, O'Donnell, Black, Roupret, Catto, Comperat, Ingersoll, Witjes, McConkey, Witjes.
Statistical analysis: Kamat, Li.
Obtaining funding: Kamat.
Administrative, technical, or material support: Kamat.
Supervision: Kamat.
Other: None.

Financial disclosures: Ashish M. Kamat certifies that all conflicts of interest, including specific financial interests and relationships and affiliations relevant to the subject matter or materials discussed in the manuscript (eg, employment/affiliation, grants or funding, consultancies, honoraria, stock ownership or options, expert testimony, royalties, or patents filed, received, or pending), are the following: Kamat: advisory board for Sanofi Pasteur, Merck, Medac, RESEARCH FUNDINg FKD & Abbott Molecular, patent holder (with UT MD Anderson Cancer Center) for CyPRIT assay; Black: Grant funding and shared patent with GenomDx; Roupret: Honorarium from Sanofi Pasteur, Medac consultant; Witjes: Advisor of Sanofi Pasteur.

Funding/Support and role of the sponsor: None.

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