Predictive Biomarkers in the Management of Bladder Cancer: Perspectives in an Evolving Therapeutic Landscape

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Abstract

Bladder cancer (BC) is a heterogeneous disease with prognosis and therapeutic strategies highly dependent on tumor grade and stage. Predictive biomarkers of therapeutic response have been studied to guide selection of intravesical and/or systemic therapy. A predictive biomarker is measured before the start of treatment and provides information on the likelihood of response to a specific therapy. Many candidate predictive biomarkers for BC have been identified, but few have been rigorously validated or distinguished from simply having treatment-agnostic prognostic capacity. Identifying predictive biomarkers tailored to therapeutic mechanism of action has considerable implications for the sequencing of therapies, as well as bladder preservation strategies in advanced disease states. We evaluate predictive tissue-based, urine-based, and serum-based biomarkers across the spectrum of non–muscle-invasive and muscle-invasive BC and preview predictive biomarkers for emerging targeted therapies.

Introduction

Biomarker development has undergone an evolution over the years, with increasing focus on predictive biomarkers in the field of bladder cancer. A predictive biomarker is measured before the start of treatment and provides information on the likelihood of response to a specific therapy. While many candidate predictive biomarkers for bladder cancer (BC) therapeutic response have been proposed, few have had their predictive value compared with non-treated cohorts to distinguish them from simply having prognostic capacity.

Predictive biomarkers have utility throughout the spectrum of disease in BC, from aiding diagnosis to guiding initial therapy selection, and even to prompting timely abandonment of ineffective treatment in lieu of definitive surgical management, radiotherapy, or other systemic therapy. Our understanding of specific therapeutic mechanisms of action is key to designing predictive markers that offer insight into innate tumor biology and therapeutic susceptibility. Measuring the predicted or elicited response to local or systemic therapies provides an opportunity to tailor biomarker development to specific therapies.

Key Words

Bladder cancer, predictive biomarker, urine biomarker, tissue biomarker, circulating tumor DNA, molecular subtype, immunohistochemistry

Competing Interests

None declared.

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Abbreviations

BC bladder cancer
BCG Bacillus Calmette-Guérin
ctDNA circulating tumor DNA
NAC neoadjuvant chemotherapy
NMIBC non–muscle-invasive bladder cancer
MIBC muscle-invasive bladder cancer
RFS recurrence-free survival
TAM tumor-associated macrophage
TMB tumor mutational burden
TME tissue microenvironment
TURBT transurethral resection of bladder tumor

In this narrative review, we discuss predictive BC tissue-based, urine-based, and serum-based markers (Figure 1), identify current limitations and unmet needs, and define the evolution of biomarker development in the landscape of targeted therapies. We summarize the current state of predictive biomarkers for both NMIBC and MIBC using a non-systematic review of published literature and provide expert opinion on the accuracy and clinical applicability of emerging biomarkers.

Predictive Tissue Biomarkers

Non–muscle-invasive bladder cancer

The most extensively studied tissue-based molecular markers predictive of therapeutic response to intravesical therapy for non–muscle-invasive BC (NMIBC) to date are p53 and Ki67, both potent cell cycle regulators. Dysregulation of the tumor suppressor p53 has been correlated with BC progression, but not recurrence, following BCG therapy[1–3]. However, a prospective study failed to validate p53 as a predictive biomarker[4]. Ki67, a nuclear protein indicative of cell proliferation, has predictive ability for intravesical therapies. The expression of Ki67 has been correlated with recurrence following BCG[5] and both recurrence and progression following intravesical chemotherapy[6].

FIGURE 1.

Biomarker source material and analysis for NMIBC and MIBC

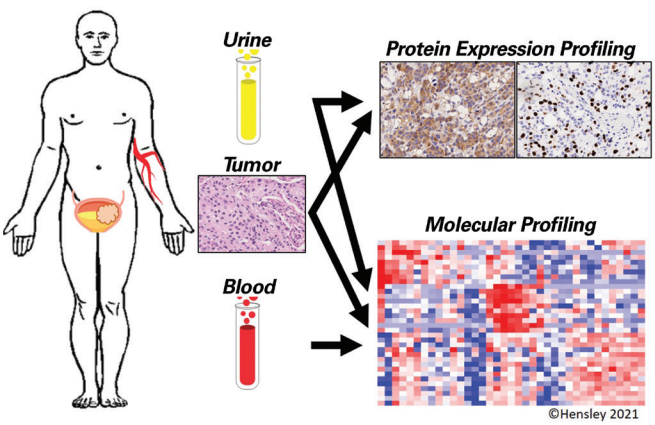

Table 1 summarizes other molecular biomarkers demonstrated to be predictive of BCG response. These include cell cycle regulators, apoptosis inhibitors, cell adhesion molecules, and proliferative markers[7]. The majority of these biomarkers have been evaluated only retrospectively in small single-center cohorts with non-standardized methods of measurement and without external validation, thus limiting our ability to derive definitive conclusions about their utility as predictive biomarkers.

Predictive biomarker panels have also been recently evaluated following intravesical BCG. A subgroup analysis of 2 large Nordic randomized trials performed by Malmström et al. analyzing expression of ezrin, CK20, and Ki67 failed to show a correlation between biomarker expression and recurrence or progression following BCG therapy[8]. Similarly, Park et al. evaluated the altered expression of 7 potential biomarkers (p53, pRb, PTEN, Ki67, p27, FGFR3, and CD9) and found no predictive value for recurrence or progression among high-grade T1 tumors treated with BCG[9]. These conflicting data underscore the need for comprehensive validation studies.

Functional mutations in DNA mismatch repair genes have also been implicated in predicting therapeutic response in BC. Sanguedolce et al. demonstrated that *MutL homologue 1*(*MLH1*) was an independent predictor of progression-free survival among patients treated with adequate BCG[10]. In patients who received BCG, polymorphisms within DNA repair pathways have been associated with recurrence-free survival (RFS)[11]. Furthermore, tumor mutational burden has recently been shown to correlate with response to intravesical therapy[12]. These data were corroborated through a comprehensive gene analysis on index non–muscle-invasive tumors, with increased mutational burden noted among high-grade NMIBC[13]. Specifically, *ARID1A* mutations were predictive of shorter RFS in patients treated with BCG; however, no association was noted between RFS and other analyzed markers, including *TP53, MDM2, ERBB2,* and *FGFR3* following BCG therapy[13].

Damrauer et al. recently performed RNA-based profiling to identify a novel expression signature of an inflamed tumor microenvironment (TME) which was predictive of BCG response[14]. Notably, molecular subtyping and immune checkpoint gene expression were not predictive of treatment response. However, a pre-treatment TME enriched with CD25+ regulatory T cells and tumor-associated macrophages and decreased

TABLE 1.

Predictive urine biomarkers for BCG therapeutic response in patients with NMIBC

Reference	Year	Marker	Study Population[a]	Number of patients	Detection Method(s)	Results
Palou et al.[5]	2009	Ezrin, Ki67	HG T1	92	IHC	*Low ezrin expression (< 20%) associated with increased progression among patients receiving induction BCG (P = 0.031)*[b] *Differential expression of Ki67 in patients with early recurrence following induction BCG (P = 0.015)*[b]
Zachos et al.[83]	2009	Telomerase reverse transcriptase (hTERT)	HG T1 NMIBC	30	IHC	hTERT nucleolar staining in >75% of cells was associated with worse RFS following induction BCG (relative risk of recurrence 8.85 [95% CI 1.9–41.6])[b]
Cormio et al. [84]	2010	pRb	HG T1	27	IHC	Altered pRb expression was predictor of recurrence (P = 0.037) and progression (P = 0.018) in patients treated with adequate BCG[c]
Alvarez-Mugica et al.[85]	2010	Myopodin methylation	HG T1 NMIBC	170	Methylation analysis	Among patients treated with adequate BCG, myopodin methylation associated with increased recurrence rate (P = 0.011) and progression (P = 0.030)[b]
Shirotake et al.[86]	2011	Angiotensin II Type 1 Receptor (AT1R)	NMIBC	79	IHC	Strong AT1R expression associated with worse 1-year RFS following BCG(P = 0.0012)[b]
Lima et al.[87]	2013	sialyl-Tn (sTn), sialyl-6-T(s6T)	High-risk NMIBC	94	IHC	High sTn and s6T expression was associated with BCG response (P = 0.024 and P < 0.0001) and with increased RFS (P = 0.001)[c]
Sen et al.[88]	2014	Nestin	HG T1	63	IHC	Recurrence rates were higher in nestin(+) compared to nestin(-) among patients receiving induction BCG (60.6% versus 30%, P = 0.014)[c]
Cheng et al.[89]	2015	E2F4	NMIBC	188	RNA sequencing	Treatment with BCG in E2F4 score > 0 patients associated with improved progression-free survival (P = 0.06)
Raspollini et al.[90]	2016	P16, galectin-3, CD44, CD138, E-cadherin, survivin, HYAL-1, topoisomerase-liα	HG T1 (≤ 3cm)	92	IHC	TOPO-2α predicted DFS (P = 0.029), surviving predicted PFS (P = 0.020), surviving and E-cad predicted OS (P = 0.006, 0.030)[b]

[a]All studies listed were analyses of retrospective cohorts. [b]Multivariate analysis. [c]Univariate analysis.

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TABLE 1.

Predictive urine biomarkers for BCG therapeutic response in patients with NMIBC, *Cont'd*

Reference	Year	Marker	Study Population[a]	Number of patients	Detection Method(s)	Results
Meeks et al.[12]	2016	Cancer-associated gene panel	High-risk NMIBC	25	DNA sequencing	Increased total mutational burden associated with IO response between non-progressors, progressors and metastatic tumors (15, 10.1, 5.1 mutations/MB, respectively; $P = 0.02$)[c]
Pietzak et al.[13]	2017	ARID1A	NMIBC	65	DNA sequencing	ARID1A mutations associated with shorter RFS after BCG (HR 3.14, $P = 0.002$)[b]
Sanguedolce et al.[10]	2018	MLH1	HG T1	67	IHC	MLH1 expression was an independent predictor of PFS among patients treated with adequate BCG[b]
Mano et al.[91]	2018	HSP 60 HSP 70 HSP 90	HG T1	54	IHC	HSP70 associated with lower risk of recurrence (HR 0.29, P = 0.003) and progression (HR 0.33, $P = 0.045$), HSP 60 associated with higher risk of progression (HR 3.96, $P = 0.012$) among patients treated with at least induction BCG[b]
Shao et al.[92]	2021	Next generation sequencing	Intermediate or high-risk NMIBC	58	DNA sequencing	NEB, FGFR1, and SDHC were independent predictors of recurrence following BCG ($P = 0.001$, $P = 0.004$, and $P = 0.017$, respectively)[b]

[a]*All studies listed were analyses of retrospective cohorts.* [b]*Multivariate analysis.* [c]*Univariate analysis.*

density of Th2-predominant CD4+ T cells was predictive of poor RFS following BCG therapy[15]. Lim et al. also noted that TME-tissues from BCG-responders was enriched with active CD8+PD-1(-) T cells and non-regulatory CD4+FOXP3(-) T cells, whereas the TMEs of non-responders were characterized by increased levels of exhausted CD8+PD-1(+) T cells[16].

Among patients with carcinoma in situ (CIS) treated with induction BCG, lower tumor-associated macrophage (TAM) density was associated with improved recurrence-free survival compared with those with higher TAM density[17]. Furthermore, when subsets of TAMs (M1 and M2) were analyzed, a low density of M1-TAM and high density of M2-TAM were predictors of worse disease-specific survival among patients treated with BCG[18]. Other components of the complex immune response to BCG therapy that have been predictive of favorable treatment response include increased expression of major histocompatibility complex 1[19], low level of infiltration by tumor-infiltrating dendritic cells[20], and increased levels of natural killer cell receptor ligands[21]. Taken together, these data indicate that the TME likely plays an important role in modulating the BCG therapeutic response and serves as a promising predictive biomarker target.

While there is a paucity of investigation into predictive biomarkers for individual intravesical chemotherapeutic agents in NMIBC, single-institution series suggest high FOXM1 expression[22] and tumors with high proliferation index (as measured by Ki67) achieve favorable responses to mitomycin C[6,23].

Muscle-invasive bladder cancer

Cisplatin-based neoadjuvant chemotherapy (NAC) before radical cystectomy confers an overall survival benefit for patients with MIBC[24]. Genomic interrogation revealed that a significant proportion of MIBCs harbor mutations in DNA damage repair (DDR) genes. DDR pathways play a critical role in the cellular

response to platinum-based chemotherapy, providing a rationale for their use as predictive biomarkers.

ERCC2 encodes a DNA helicase that plays a central role in the nucleotide excision repair pathway, repairing DNA cross-linking caused by genotoxic agents such as platinum chemotherapies. Van Allen et al. performed whole-exome sequencing on pre-treatment tumor and germline DNA from 50 patients with MIBC receiving cisplatin-based NAC before cystectomy[25]. *ERCC2* was the only significantly mutated gene enriched in cisplatin-responders compared with non-responders. This finding was mechanistically confirmed in vitro, as expression of wild-type *ERCC2* in an *ERCC2*-deficient BC cell line rescued cisplatin sensitivity. Using an independent MIBC cohort, Liu et al. found *ERCC2* alterations in 8/20 responders to chemotherapy (40%) versus 2/28 non-responders (7%) ($P = 0.010$, OR 8.3 [95% CI 1.4 to 91.4])[26]. In a subsequent phase II trial of neoadjuvant dose-dense gemcitabine and cisplatin in patients with MIBC, the presence of one or more alterations in a panel of 29 DDR genes, including *ERCC2*, was associated with chemosensitivity (positive predictive value for <pT2N0 of 89%)[27].

In addition to *ERCC2*, alterations in *ATM*, *Rb1* and *FANCC* have also been shown to be predictive of response to cisplatin-based NAC[28]. Plimack et al. showed that alteration in 1 or more of these 3 DNA repair genes was able to predict pathologic complete response to NAC. Additionally, missense mutations in the receptor tyrosine kinase *ERBB2* (*HER2*) were enriched in patients with a complete response to platinum-based NAC (24% of complete responders compared with 0% in non-responders)[29]. As a result of these studies, several trials are investigating the ability of predictive markers to triage patients for bladder sparing after a favorable clinical response to cisplatin-based NAC (**Table 2**). Recent studies, however, have found no correlation between *ERCC2* mutation status and treatment response[30,31], but have instead identified other DDR biomarkers associated with response (eg, *BRCA2*), highlighting a high level of tumor heterogeneity and inter-cohort variability.

Large-scale expression and sequencing analysis have subtyped BCs based on genomic RNA expression or specific genomic alterations[32,33]. Choi et al. reported that the basal subtype, with enriched expression of genes in the p63 pathway, responded favorably to neoadjuvant MVAC; in contrast, patients with "p53-like tumors," with activated wild-type p53 gene expression signatures, were consistently chemoresistant. These findings were largely recapitulated through tumor profiling of patients in a phase II trial of dose-dense neoadjuvant MVAC and bevacizumab before cystectomy in which patients with basal tumors exhibited favorable survival but similar pathologic response rates[34]. Similarly, a large multicenter study that performed whole transcriptome profiling on transurethral resection of bladder tumor (TURBT) specimens from 343 patients with MIBC[35] concluded that patients with basal subtype tumors derived the most improvement in overall survival compared with those who had surgery alone, whereas patients with luminal tumors had similarly favorable outcomes irrespective of NAC use.

A multiomics approach to identifying molecular markers of cisplatin sensitivity in the neoadjuvant or first-line settings was recently reported[30]. Contrary to the findings of the aforementioned reports, patients with basal/squamous gene expression subtypes responded poorly to cisplatin-based chemotherapy, while patients who had tumors with immune cell infiltration and high PD-1 protein expression exhibited a favorable therapeutic response. Notable differences in cohort selection and primary outcomes measurements may account for differing conclusions on platinum-sensitivity of basal tumors[36]. While Seiler et al.[35] primarily compared survival outcomes in a NAC cohort with those of the TGCA cohorts, managed with variable adjuvant chemotherapeutic regimens, Taber et al.[30] measured therapeutic response through pathologic downstaging and response on imaging in NAC and first-line metastatic patients, respectively. Taken together, these data indicate that more research is needed in this setting to determine the predictive value of expression subtypes.

The role of molecular subtypes as predictive biomarkers for response to neoadjuvant immunotherapy have also been assessed. In the PURE-01 study evaluating the efficacy of neoadjuvant pembrolizumab, basal tumors had better response rates than non-basal tumors[37]. Tumor immunophenotypes have also been correlated with response to the anti-PD-L1 agent atezolizumab[38]. Favorable response was associated with the CD8+ T-cell phenotypes and high tumor mutational burden, while so-called "immune desert" tumors and those with high transforming growth factor β (TGFβ) signaling in the TME exhibited therapeutic resistance.

Despite promising results, molecular subtyping using whole transcriptome RNA expression profiling has yet to be adopted into clinical practice. This is primarily due to the technical difficulty of handling and profiling RNA as well as the lack of definitive evidence from clinical trials specifically designed for measuring biomarker accuracy. However, studies have suggested that immunohistochemistry, which already has clinical applications, may be used to classify BC into molecular subtypes, thus facilitating their use in clinical practice[39].

Several PD-1/PD-L1 immune checkpoint inhibitors (ICI) have been approved for cisplatin-ineligible patients and those with locally advanced or metastatic

TABLE 2.

Clinical trials investigating tissue-based biomarkers predictive of neoadjuvant therapy response in MIBC

NCT	Trial Name	Study Population	Biomarker(s) Under Investigation	Intervention	Primary Endpoints
02710734	Risk Enabled Therapy After Initiating Neoadjuvant Chemotherapy for Bladder Cancer (RETAIN)	cT2-3 N0 bladder cancer	Sequenced pre-NAC TURBT specimens for DDR mutations	TUR followed by accelerated MVAC[a]; patients with complete clinical response and DDR mutation managed with bladder sparing, others treated with intravesical chemotherapy, radiation therapy or radical cystectomy	Metastasis-free survival (MFS) at 2 years
03609216	Alliance A031701	cT2-3 N0 bladder cancer	Sequenced pre-NAC TURBT specimens for DDR mutations	TUR followed by gemcitabine/cisplatin; patients with DDR mutation and pathologic response (≤ycT1) managed with bladder sparing; others treated with chemoradiation or radical cystectomy	Event-free survival (MFS) at 3 years
03558087	HCRN GU 16-257	cT2-4 N0 bladder cancer	Sequenced pre-NAC TURBT specimens for DDR mutations and tumor mutational burden	TUR followed by gemcitabine/cisplatin/nivolumab; patients with complete clinical response managed with bladder sparing and maintenance novilumab, others treated with radical cystectomy	(1) complete clinical response rate (2) ability of complete clinical response to predict 2-year metastasis-free survival
02177695	SWOG 1314	cT2-4 N0 bladder cancer	Co-expression extrapolation (COXEN) gene expression algorithm	TUR followed by neoadjuvant dose-dose MVAC[a] or gemcitabine/cisplatin prior to radical cystectomy	Assess whether COXEN profile is (1) prognostic of pT0 rate or ≤pT1 at cystectomy and (2) a predictive factor between chemotherapy regimens

[a]*methotrexate, vinblastine, doxorubicin, cisplatin*

BC[40–46]. The indication has since expanded, with several groups investigating the use of PD-1/PD-L1 inhibitors in the neoadjuvant setting. In the PURE-01 study, 50 patients with MIBC received 3 cycles of the PD-1 inhibitor pembrolizumab followed by radical cystectomy[47]. Pathologic complete response (pT0) was observed in 40% of PD-L1 positive patients (≥ 10% combined positive score) compared with only 16% of PD-L1 negative patients. Furthermore, a greater pathologic response to pembrolizumab was seen in patients with higher tumor mutational burden (TMB >15 mutations/MB). In contrast to the PURE-01 study and trials in the metastatic setting, the neoadjuvant ABACUS study of the PD-L1 inhibitor atezolizumab was unable to show a significant association between PD-L1 expression (either on tumor cells or infiltrating cells) and therapeutic response[48]. The lack of standardization of PD-L1 assessment, such as different antibodies, thresholds for PD-L1 positivity, and immune cell quantification, is likely to contribute to its limited predictive ability in BC. Bandini et al. recently constructed a probability calculator incorporating 2 biomarkers (PD-L1 expression and TMB) and baseline clinical T stage to predict pathologic complete response after pembrolizumab[49]. This predictive model performed well with a concordance index of 0.77 (95% CI 0.68 to 0.86), highlighting the complexity of the tumor-immune interaction and utility of predictive biomarker panels compared with single markers alone.

Predictive Urine Biomarkers

Non–muscle-invasive bladder cancer

Urine is a uniquely qualified biomarker source material, as it is readily available, easily collected, and has direct tumor contact. Urine-based biomarkers have been primarily studied for purposes of diagnosis and surveillance of BC, with relatively few having sufficient accuracy to predict therapeutic response. The true predictive biomarker capacity of the urine-based markers mentioned herein remain largely uncharacterized as many were studied exclusively in treated populations or measured as an elicited response after intravesical therapy.

BCG has proven efficacy in reducing recurrence and progression in intermediate and high-risk non–muscle-invasive BC (NMIBC)[50]. Reliable biomarkers predictive of BCG therapeutic response could have tremendous implications in sequencing of therapy for NMIBC. Unfortunately, given the relative non-specific mechanism of action and elicited immune response by BCG, clinicopathologic factors such as tumor stage, grade, size, presence or absence of CIS, tumor focality and recurrence history remain the most reliable predictors of BCG therapeutic response[51].

The rationale for several candidate preclinical biomarkers have employed the mechanism of BCG therapeutic response[52]. Interleukin (IL)-8 is one of the first cytokines with induced expression after BCG therapy. In a pilot study of 20 patients, high levels of IL-8 expression measured 6 hours after BCG instillation had lower rates of recurrence and progression[53]. Additionally, failure to induce expression of IL-2 and IL-18 after BCG has been associated with poor BCG therapeutic response[54].

Because BCG immunogenicity is complex and non-specific, single candidate markers alone may be unreliable predictive tools. The Cytokine Panel for Response to Intravesical Therapy (CyPRIT) nomogram was generated from expression profiling of 9 inducible urinary cytokines (IL-2, IL-6, IL-8, IL-18, IL-1ra, TRAIL, IFN-γ, IL-12[p70], and TNF-α) in 130 patients with NMIBC at the MD Anderson Cancer Center using an enzyme-linked immunosorbent assay (ELISA) at baseline and at specified time points throughout BCG therapy[55]. This nomogram predicted the likelihood of recurrence with 85.5% accuracy. Additionally, baseline levels of pro-tumorigenic cytokines were profiled pre-treatment. Indeed, expression of IL-8 in urine was associated with recurrence in BCG-treated patients, with patients who had higher baseline urinary IL-8 levels experiencing a 4-fold increased risk of tumor recurrence[56]. Interestingly, higher baseline levels of IL-8 expression in peripheral blood leukocytes similarly correlated with disease recurrence, suggesting a role for this cytokine as a systemic marker for BCG immunogenicity and therapeutic response. Contrasting these results to the aforementioned studies indicating that induced urinary IL-8 expression after BCG instillation is a marker of disease recurrence and progression, these data highlight the complexity of baseline and elicited immune states, as well as the stability and kinetics of cytokine profiling, in determining their potential as predictive biomarkers.

The Oncuria urine-based assay measures the expression of cancer-associated markers in voided urine specimens[57]. Using urine samples from the CyPRIT cohort, investigators found that pre-treatment concentrations of MMP9, VEGFA, CA9, SDC1, PAI1, APOE, A1AT, ANG, and MMP10 were increased in subjects with disease recurrence. A predictive model of treatment outcomes reached an area under the receiver operating curve of 0.89 (95% CI 0.80 to 0.99), with a test sensitivity of 81.8% and a specificity of 84.9%.

While not specifically FDA approved for this indication, the fluorescence in situ hybridisation (FISH) assay, which detects aneuploidy in chromosomes 3, 7, and 17 and loss of the 9p21 locus in voided urine samples (UroVysion), has been studied in the context of BCG therapeutic response. In a study of 37 patients primarily receiving BCG for NMIBC, 100% of patients with a positive post-treatment UroVysion FISH developed tumor recurrence[58]. The predictive capacity of positive post-treatment UroVysion was independently confirmed in several studies with variable adjuvant intravesical agents for NMIBC[59–62].

A subset of "molecular BCG failure" patients based on mid-treatment persistence of a positive FISH assay was subsequently defined. In a study of 126 patients, those with a positive FISH test during therapy were 3 to 5 times more likely to develop recurrence and 5 to 13 times more likely to progress compared with patients with negative mid-treatment FISH[63]. This was subsequently validated in an independent, multicenter trial where FISH was predictive of recurrence and/or progression events at baseline (HR 2.59; 95% CI 1.42 to 4.73), before the sixth induction instillation (HR 1.94; 95% CI 1.04 to 3.59) and at 3-month follow-up (HR 3.22; 95% CI 1.65 to 6.27)[64]. Defined as positive FISH at 6 weeks and 3 months after induction BCG in the setting of a negative cystoscopic evaluation, this molecular failure denotes a group at high risk of stage progression if managed with further BCG therapy, and who should be considered for enrolment into clinical trials or timely cystectomy[65].

Muscle-invasive bladder cancer

There currently exist no urine-based biomarkers to reliably predict therapeutic response in MIBC. However, broad genomic expression and mutational profiling of molecular targets of novel therapeutic agents, including

monoclonal antibodies and antibody-drug conjugates, have emerging rationale. For example, UroSEEK is a urine-based molecular assay which detects alterations in 11 commonly mutated genes which are druggable targets: TERT, FGFR3, PIK3CA, TP53, HRAS, KRAS, ERBB2, CDKN2A, MET, MLL, and VHL[66].

As sequencing technology becomes more refined, urine-based genetic material, including exfoliated tumor cells, cell-free DNA, and exosomes may prove feasible sources for molecular subtyping and further predictive biomarker development for MIBC.

Predictive Serum Biomarkers

Serum biomarkers for BC remain an area of active research. These liquid biopsy tests may have a role in cancer risk stratification, characterization of tumor molecular signatures, and predicting response to systemic treatment, as well as for cancer surveillance. To date, these tests have remained proof-of-concept in preclinical studies but have emerging clinical relevance to guide treatment decisions.

Circulating tumor cells (CTC) represent one of the first studied serum biomarkers. While they have a poor sensitivity of 35% for the detection of BC due to their scarcity in circulating blood, the presence of CTCs has been associated with higher histological stage, grade, lymph node involvement, and presence of metastatic disease[67,68]. In the pre-radical cystectomy setting, CTCs have been shown to predict poor oncological outcomes, independent of clinicopathological variables[69].

Serum RNA markers such as long non-coding RNAs (IncRNAs) and microRNAs (miRNA) have been reported to have prognostic value. Zhang et al. reported that high serum UBC1 expression was associated with lower NMIBC RFS ($P = 0.01$) [70]. In a systematic review and meta-analysis of 26 studies, 6 miRNA (miR-21, miR-143, miR-155, miR-214, and miR-222) were identified as being predictive of early disease recurrence and progression[71].

More recently, there have been rapid advancements in circulating tumor DNA (ctDNA). Developments in deep-sequencing technology have allowed for the reliable identification of double strand DNA fragments as small as 150 bp. Birkenkamp-Demtröder et al. developed personalized ctDNA assays based on NGS of tumor tissue. They report that ctDNA was present even in NMIBC patients, and the presence of higher levels of ctDNA was associated with subsequent disease progression and metastasis[72]. In patients undergoing radical cystectomy, ctDNA predicted oncological outcomes in several settings[31]. Patients positive for ctDNA at diagnosis before NAC had a higher 12-month recurrence rate (42% versus 0%)[31]. Similarly, following NAC, patients positive for ctDNA had a higher rate of 12-month disease recurrence (75% versus 7%) than did ctDNA negative patients[31]. Additionally, in the surveillance setting, ctDNA had a median lead time of 96 days over radiological imaging[31]. This lead time of ctDNA detection before radiologic or symptomatic clinical detection is allowing investigators to define a "biochemical relapse" to guide timely initiation of first-line atezolizumab after RC in a clinical trial setting (NCT04138628). The role of ctDNA as a predictive biomarker for atezolizumab has also been reported in a study where patients with ctDNA positivity had a significantly improved overall survival compared with the observational arm (HR: 0.59; 95% CI 0.41 to 0.79)[73].

Unmet Needs in Biomarker Development

Characteristics for the ideal biomarker predictive of therapeutic response vary considerably by disease stage. For NMIBC, BCG is the gold standard intravesical treatment because of its efficacy, favorable cost, and tolerability. To date, biomarkers predictive of response to BCG have primarily focused on identifying early non-responders in an effort to transition them to off-label salvage intravesical chemotherapy options or timely radical cystectomy. However, in the era of BCG shortages and emerging intravesical and systemic therapies available in earlier disease states, we would benefit from predictive markers that could guide initial therapeutic response. With emphasis on bladder preserving strategies, it will become equally important to identify predictive biomarkers of salvage intravesical and systemic therapies after BCG failure.

Recent molecular classification of NMIBC has correlated candidate molecular subtypes to innate sensitivity and resistance to BCG therapy, and provided therapeutic rationale for upfront use of FGFR inhibitors, ICIs, or intravesical chemotherapy[74]. Lastly, favorable results have recently been reported in the Phase III trial of intravesical nadofaragene firadenovec (rAd-IFNa/Syn3) for BCG unresponsive NMIBC[75]. These investigators are validating a serum-based adenoviral antibody titer assay to evaluate immunogenicity of the gene therapy and corresponding therapeutic response[76].

While guidelines support the role of NAC before radical cystectomy for MIBC, there remains a role for risk-stratified NAC patient selection. Clinicopathologic risk factors have been implemented in predicting response to cisplatin-based NAC[77,78], but efforts are underway to profile tissue-based biomarkers for this purpose. The Southwest Oncology Group (SWOG) 1314 trial prospectively profiled the ability of the COXEN tissue-based genetic classifier to predict complete pathologic response to cisplatin-based NAC (**Table 2**)[79].

Recently reported results indicate limited predictive capacity of the genomic classier for individual treatment response, underscoring the importance of prospective validation of predictive markers in the clinical trial setting. Our group, among others, has also been involved in profiling immunohistochemical signatures in TURBT specimens predictive of response to NAC to improve appropriate stratification of patients for NAC or upfront cystectomy[80,81]. Lastly, biomarkers could significantly aid in the ability to accurately predict and assess a complete clinical response to NAC in those electing for bladder perseveration, a concept with supporting clinical data[82] and currently being evaluated in 2 randomized trials (RETAIN, Alliance A031701; **Table 2**).

Conclusions

There are no currently FDA-approved predictive biomarkers for therapies in BC–neither for NMIBC nor for MIBC. The lack of clinically available predictive biomarkers is likely multifactorial, including difficulties in profiling intratumoral heterogeneity and dynamic cellular processes (ie, epithelial-mesenchymal transition, cell growth and proliferation, immune response) as well as the lack of "fit for purpose" profiling of biomarkers with mechanistic rationale. The ever-evolving armamentarium of therapeutic options further emphasizes unique unmet needs for predictive biomarker development. By convention, we have relied on non-specific markers of therapeutic response to drugs with non-specific targets—ie, cytokine expression profiling as a litmus for induced immunogenicity after intravesical BCG or DNA mismatch repair genetic alterations to predict response to cytotoxic chemotherapies. With the growth of novel therapeutic modalities with specific targets, including monoclonal antibodies, antibody-drug conjugates, and gene therapies, we expect that biomarkers which are highly specific for, and even proprietary to, the proposed mechanism of action of individual therapies are on the horizon.

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