Competing interests
The author declares no competing interests.

Molecular biomarkers in urothelial carcinoma of the bladder: are we there yet?

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Abstract | The unprecedented advances in cancer genetics and genomics are rapidly affecting the clinical management of solid tumors. Molecular diagnostics are now an integral part of routine clinical management for patients with lung, colon, and breast cancer. In sharp contrast, molecular biomarkers have been largely excluded from current management algorithms for urologic malignancies. The need for new treatment options that can improve upon the modest outcomes currently associated with muscle-invasive bladder cancer is evident, and validated prognostic molecular biomarkers that can help clinicians to identify patients in need of early, aggressive management are lacking. Robust predictive biomarkers that are able to forecast and stratify responses to emerging targeted therapies are also needed.

Introduction
Accumulating molecular genetic evidence supports the existence of two broadly distinct pathogenetic pathways for bladder cancer development that parallel the contrasting biological and clinical phenotypes of non-muscle-invasive (superficial) and muscle-invasive urothelial carcinoma. Whereas the majority of invasive urothelial carcinomas are likely to originate from the progression of dysplasia to flat carcinoma in situ (CIS) and high-grade noninvasive lesions, superficial urothelial lesions are thought to develop from benign urothelium through a process of urothelial hyperplasia (Figure 1). A small percentage (10–15%) of all noninvasive lesions will progress to muscle-invasive disease, and genetic instability is crucial to this progression.1–4

Alterations in the Ras–MAPK and PI3K–AKT–mTOR pathways are largely responsible for promoting cell growth in urothelial neoplasia. Activating mutations in the genes encoding the Ras subfamily lead to activation of MAPK and PI3K pathways. Mo et al.1 have proposed a model to explain the biomolecular process underlying early oncogenesis in noninvasive papillary urothelial neoplasms (Figure 2). In this model, Ras promotes survival and angiogenesis by activating the PI3K–AKT–mTOR and JAK–STAT pathways. Inactivation of the tumor suppressor gene PTEN—an mTOR pathway inhibitor—can also lead to activation of the AKT proteins and the JAK–STAT pathway. Alternatively, FGFR3 mutations can trigger Ras activation, leading to a similar downstream signal transduction. PIK3CA and FGFR3 mutations generally occur together, and there is evidence to suggest these mutations have an additive oncogenic effect.

Emerging biomarkers in urothelial cancer
As our understanding of the molecular pathways involved in urothelial oncogenesis expands, advances in molecular prognostication, theranostics, and targeted therapy for bladder cancer sharply gain momentum.6–24 Prognostic parameters that can accurately predict progression of non-muscle-invasive bladder cancer (NMIBC) are actively sought in order to identify patients in need of vigilant surveillance and aggressive treatment. The former is especially pertinent, as surveillance imposes a substantial financial burden and reduction in quality of life for patients with bladder cancer. In the USA, bladder cancer costs more to treat per patient than any other single solid tumor, with an estimated annual cost of $3 billion.25 Given the poor outcomes currently associated with muscle-invasive disease (overall survival rate of 60% or less), markers that can improve prognostic accuracy in this group of patients are urgently needed.25

Chromosomal alterations and FISH assays
Alterations in chromosome 9 are the earliest genetic changes thought to occur in both of the divergent pathways of bladder cancer development. They are responsible for providing the necessary milieu of genetic instability that allows genetic defects to accumulate.4 Several additional structural and numerical somatic chromosomal alterations are also common in bladder cancer. Chromosome 3q, 7p and 17q gains and 9p21 deletions (p16 locus) are of particular interest, given their potential diagnostic and prognostic value.26,27

Based on these numerical chromosomal alterations, a fluorescence in situ hybridization (FISH) cytogenetic urine assay has been developed, and this is now commercially available and widely used in clinical management.28 Although it was initially only approved by the FDA for the surveillance of patients with previously diagnosed bladder cancer, the test has subsequently
A 'molecular grade' (based on genomics)

The main genetic alterations underlying muscle-invasive bladder cancer involve tumor suppressor genes encoding proteins that regulate cell cycle and apoptosis pathways, including TP53, CDKN2A, CDKN1A, CDKN1B, and RB1. Delineation of the molecular pathways involved in bladder oncogenesis has fueled the quest for much-needed prognostic biomarkers, new targeted therapies, and markers of therapy response.

A 'molecular grade' (based on FGF3 and MIB1 expression) and a multigene fluorescence in situ hybridization (FISH) assay have both achieved sufficient validation to be included in prospective multi-institutional trials.

A panel of multiple cell cycle control markers—TP53, CDKN1B, CDKN1A, CDKN2A, CCND1, and RB1—offers superior prognostic power over single marker approaches, and merits further rigorous prospective validation.

Figure 1 | The divergent molecular pathways of oncogenesis in non-muscle-invasive and muscle-invasive bladder cancers. Genetic alterations are depicted for key stages of disease progression. The main alterations underlying noninvasive low-grade urothelial carcinoma development involve FGFR3, HRAS, and mTOR pathway member genes. By contrast, alterations leading to progression to noninvasive high-grade and muscle-invasive urothelial carcinoma involve the tumor suppressor genes TP53 and RB1, as well as several tumor microenvironment alterations. Abbreviations: AKT, v-akt murine thymoma viral oncogene; CIS, carcinoma in situ; COX-2, cyclooxygenase 2; E-cad, cadherin 1 (E-cadherin); EGFR, epidermal growth factor receptor; FGFR3, fibroblast growth factor receptor 3; HG URCa, noninvasive high-grade urothelial carcinoma; HRAS, Ha-ras Harvey rat sarcoma viral oncogene homolog; IL-8, interleukin 8; LAMC2, laminin subunit gamma 2; LG URCa, noninvasive low-grade urothelial carcinoma; MMP, matrix metalloproteinase; p53, tumor protein p53; PI3K, phosphoinositide-3-kinase; pRb1, retinoblastoma-like protein 1; THBS1, thrombospondin 1; URCa, urothelial carcinoma of urinary bladder; VEGF, vascular endothelial growth factor.

Key points
- Molecular genetic evidence supports the existence of two distinct pathogenetic pathways for bladder cancer development, corresponding to two distinct biological and clinical phenotypes: non-muscle-invasive and muscle-invasive urothelial carcinoma.
- Disruption to the PI3K–AKT–mTOR pathway and alterations in the tyrosine kinase receptor gene FGFR3 and the oncogene HRAS are associated with non-muscle-invasive bladder cancer.
- The main genetic alterations underlying muscle-invasive bladder cancer involve tumor suppressor genes encoding proteins that regulate cell cycle and apoptosis pathways, including TP53, CDKN2A, CCND1, CDKN1B, and RB1.
- Delineation of the molecular pathways involved in bladder oncogenesis has fueled the quest for much-needed prognostic biomarkers, new targeted therapies, and markers of therapy response.
- A ‘molecular grade’ (based on FGF3 and MIB1 expression) and a multigene fluorescence in situ hybridization (FISH) assay have both achieved sufficient validation to be included in prospective multi-institutional trials.
- A panel of multiple cell cycle control markers—TP53, CDKN1B, CDKN1A, CDKN2A, CCND1, and RB1—offers superior prognostic power over single marker approaches, and merits further rigorous prospective validation.

Receptor tyrosine kinases and PCR-based assays
A significant proportion of NMIBCs, particularly pTa and pT1 tumors, recur following transurethral resection of the bladder (TURB). A minority of cases will progress to high-grade carcinoma and, ultimately, muscle-invasive disease. Genetic instability is pivotal to this progression, and three primary genetic alterations that have been consistently associated with the pathogenesis pathway of NMIBC affect the receptor tyrosine kinase (RTK) genes FGFR3, HRAS, and PIK3CA. Studies have indicated that the potential prognostic value of evaluating expression levels of RTK genes such as FGFR3, EGFR, and PIK3CA expression status, as markers of early recurrence during surveillance. Both Zuiverloon et al. and Miyake et al. independently developed sensitive PCR-based assays for detecting FGFR3 mutations in voided urine. A positive urine sample, as determined by the assay developed by Zuiverloon’s group, was associated with concomitant or future recurrence in 81% of NMIBC patients. An even higher positive predictive value of 90% was achieved in

by cystoscopy up to 29 months later. Such encouraging results indicate great potential for FISH assays in the early detection of bladder cancer and in the allocation of rigorous, frequent follow-up cystoscopy appointments to patients who are at increased risk of progression.

In addition, several studies have pointed to a potential prognostic role of multitarget FISH analysis. Maffezini et al. were able to demonstrate that low-risk FISH-positive patients, with 9p21 loss or chromosome 3 abnormalities, experienced a higher rate of recurrence than patients with negative FISH results. The recurrence rate was even greater in high-risk FISH-positive patients with abnormalities in chromosome 7 or 17. Kawauchi et al. (using bladder washings) and Kruger et al. (using formalin-fixed paraffin-embedded [FFPE] transurethral biopsy samples) independently showed that loss of 9p21 predicted recurrence, but not progression, in patients with NMIBC. Furthermore, both Savic et al. and Whitson et al. demonstrated that urine cytology and FISH analysis on post–BCG bladder washings are predictive of BCG therapy failure in patients with NMIBC. This promising prognostic role for multitarget FISH awaits a prospective randomized trial before it can be integrated into clinical practice algorithms. Clear guidelines for interpreting and testing performance parameters, particularly in terms of interobserver reproducibility, are also needed.
patients with consecutive FGFR3-positive urine samples. Similarly, Miyake et al. were able to detect FGFR3 mutations in 53% of their 45 patients and found their assay to be superior to cytology (78% versus 0%) for detecting post-TURB recurrence in patients harboring FGFR3 mutations in primary NMIBC tumors.

Kompier et al. have developed a multiplex PCR assay for performing analysis of mutational ‘hot spots’ within HRAS, KRAS, NRAS, FGFR3, and PIK3CA in FFPE TURB samples. They found evidence of a mutation in at least one of these sites in up to 88% of low-grade NMIBC tumors. Hernandez et al. observed that FGFR3 mutations were more common among neoplasms with low malignant potential (present in 77% of samples), TaG1 tumors (61%), and TaG2 tumors (58%) than among TaG3 (34%) and T1G3 tumors (17%). Multivariate analysis showed that these mutations were associated with an increased risk of recurrence in patients with NMIBC.

In addition, several studies have suggested a negative prognostic role for ERBB2 amplification or overexpression in MIBC. Using multivariate analysis, Bolenz et al. found that patients harboring tumors with ERBB2 overexpression were twice as likely to experience recurrence, and to die from their cancer, than patients with ERBB2-negative tumors.

Cell cycle regulators
The pathogenesis pathway for MIBC primarily involves alterations in the tumor suppressor genes responsible for cell cycle control, including TP53, P16, and RB1. Progression of NMIBC to higher-grade muscle-invasive disease is also due to alterations in TP53 and RB1 (Figures 1 and 3). Early studies by Sarkis et al. found TP53 alterations to be strong independent predictors of disease progression in patients with NMIBC, MIBC, and CIS. Recent studies have supported these findings by showing an independent role of TP53 alteration in predicting disease-free survival (DFS) and disease-specific survival (DSS) in patients with pT1 and pT2 tumors who have undergone cystectomy.

Other cell cycle regulators, such as cyclin D3, cyclin D1, cyclin-dependent kinase inhibitor 2A (also known as p16), CDK-interacting protein 1 (also known as p21), and cyclin-dependent kinase inhibitor 1B (also known as p27) have also been evaluated as prognosticators for NMIBC. Lopez-Beltran et al. identified roles for the overexpression of cyclin D3 and cyclin D1 in predicting progression in patients with pT1 and pT2 tumors. However, their findings were contradicted by a subsequent report from Shariat and colleagues, emphasizing the need for further validation in large multi-institutional patient cohorts.
Prognostic tests that assess the expression levels of multiple cell cycle regulators are emerging for the management of both NMIBC and MIBC.\textsuperscript{58–60,70–72,85} Shariat \textit{et al.}\textsuperscript{71} found that patients with NMIBC and alterations in \textit{TP53}, \textit{CDKN1A}, \textit{RBL1}, and \textit{CDKN1B} had a significantly reduced DFS compared with patients with alterations in only three of these markers. This trend continued and the negative predictive effect decreased with decreasing number of altered markers. The same group later found that combining \textit{TP53}, \textit{CDKN1B}, and \textit{MKI67} expression assessment on PT1 radical cystectomy specimens further improved the accuracy of DFS and DSS predictions.\textsuperscript{70}

Similarly, Chatterjee \textit{et al.}\textsuperscript{16} demonstrated synergism in the prognostic power of assessing multiple molecular markers—\textit{TP53}, \textit{RBL1}, and \textit{CDKN1A}—in patients undergoing cystectomy for MIBC. The superiority of a multimarker approach over the current single-marker approaches certainly merits further assessment.\textsuperscript{70–75,70–72,75}

Assuming that additional large prospective trials confirm these promising findings, a multimarker approach could soon be integrated into the standard of care for bladder cancer management.

**Hypermethylation and MSP-PCR**

Epigenetic analysis is gaining momentum as a non-invasive diagnostic tool for the screening and surveillance of patients with bladder cancer, and has also shown potential as a prognostic tool.\textsuperscript{73–86} In an early study by Catto and colleagues,\textsuperscript{66} 11 CpG promoter islands were analyzed for hypermethylation using methylation-specific PCR (MSP-PCR) on samples from 116 bladder tumors and 164 upper urinary tract tumors. Promoter methylation was found in 86% of all tumors, was noticeably more common in upper tract tumors than in bladder cancer samples, and was generally associated with advanced tumor stage and higher rates of tumor progression and mortality. Importantly, multivariate analysis of the data established an association between methylation at the \textit{RASSF1A} and \textit{DAPK1} gene promoters and disease progression that occurred independently of tumor stage and grade.

Following on from this study, the same group\textsuperscript{86} used quantitative MSP-PCR on 17 candidate gene promoters to identify five genes associated with disease progression: \textit{RASSF1A}, \textit{CDH1}, \textit{TNFRSF25}, \textit{EDNRB}, and \textit{APC}. Multivariate analysis revealed that the overall degree of methylation was more significantly associated with subsequent progression and mortality than with tumor stage. An epigenetic predictive model, developed using artificial intelligence techniques, was able to predict the likelihood and timing of progression with 97% specificity and 75% sensitivity. The authors developed a neuro-fuzzy model to examine the importance of methylation at the various gene promoters. Two modeling strategies were used to rank the extent of each individual promoter’s influence upon the predictive accuracy of the model: a “leave-one-out” approach removed each variable in turn from the model, and a “selectivity” approach spanned each variable from minimum to maximum while fixing all other variables within the model to nominal values. By comparing this ranking to the methylation profile of the tumor, the neuro-fuzzy model was able to predict the presence and timing of disease progression.

In another study of the diagnostic role of promoter hypermethylation, Lin \textit{et al.}\textsuperscript{74} used MSP-PCR to determine the methylation status of 4 genes—\textit{CDH1}, \textit{CDKN2A}, \textit{CDK2AP2}, and \textit{RASSF1A}—in DNA taken from the primary tumors and urine sediment of 57 individuals with bladder cancer. Hypermethylation analysis of \textit{CDH1}, \textit{CDK2AP2}, or \textit{RASSF1A} in urine sediment DNA detected 85% of superficial and low-grade bladder tumors, 79% of high-grade tumors, and 75% of invasive bladder cancers. The study highlighted the great potential of this test in detecting NMIBC. A similar diagnostic role for methylation status was found by Cabello \textit{et al.}\textsuperscript{76} using a novel technology—methylation-specific multiplex ligation-dependent probe amplification (MS-MLPA)—to analyze the methylation status of 25 tumor-suppressor genes thought to have a role in bladder cancer oncogenesis, including \textit{PTEN}, \textit{CD44}, \textit{WT1}, \textit{GSTP1}, \textit{BRCA1}, \textit{BRCA2}, \textit{RBL1}, \textit{TP53}, \textit{TP73}, \textit{RARB}, \textit{VHL}, \textit{ESR1}, \textit{PAX5}, \textit{CDKN2A}, and \textit{PAX6}. The authors found that \textit{BRCA1}, \textit{WT1}, and \textit{RARB} were the most frequently methylated sites, with receiver operating characteristic (ROC) curve analyses revealing significant diagnostic accuracy. In addition to potential roles in diagnosing and
prognosing bladder cancer, promoter hypermethylation is providing insights into the process underlying bladder cancer oncogenesis. Research led by Dudziec\textsuperscript{29} has shown an association between the hypermethylation of CpG islands and ‘shores’ around specific mitrons and microRNAs, and bladder cancer phenotype.

**Genetic profiling and array analysis**

Several gene expression studies have identified sets of differentially expressed genes with potential roles in diagnosing bladder cancer and predicting recurrence and progression.\textsuperscript{1,7,9,21,23,80,87–91} A landmark study by Sanchez-Carbayo et al.\textsuperscript{7} used oligonucleotide arrays to analyze the transcription profiles of 105 patients with bladder cancer. Hierarchical clustering and supervised algorithms were used to stratify bladder tumors based on stage, nodal metastases, and overall survival. Predictive algorithms based on levels of differentially expressed proteins in both non-muscle-invasive and invasive tumors were found to be 89% accurate for tumor staging. In terms of predicting overall survival, accuracies of 82% (for the entire cohort) and 90% (for MIBC patients only) were reported. A genetic profile, built using 174 probes, was able to identify patients with positive lymph node status and poor survival. Furthermore, two independent Global Test runs—which measure the influence of a group of genes associated with lymph node metastases on overall survival status—confirmed a robust association between genetic profile, lymph node metastases, and overall survival. Synuclein, which encodes the ligand of the cannabinoid or synuclein receptor, was selected as one of the top-ranking genes for predicting lymph node invasion and overall survival. Immunohistochemical analyses of tissue arrays confirmed a significant association between synuclein expression, tumor staging, and clinical outcomes that occurred independently of clinical/pathologic parameters.

Birkhahn et al.\textsuperscript{21} attempted to identify genes that are predictive of recurrence and progression in patients with pTa bladder tumors. They adopted a quantitative pathway-specific approach by checking the expression of 24 key genes involved in pathways relevant to NMIBC oncogenesis (such as cell cycle, apoptosis, and angiogenesis) using real-time PCR on tumor biopsy samples taken at initial patient presentation. They showed that CCND3 expression is highly sensitive and moderately specific for predicting recurrence (97% and 63%, respectively), HRAS, E2F1, BIRC5, and KDR expression levels were all independently predictive of progression, and multivariate analysis of combined HRAS, VEGFR2, and VEGFA expression status predicted progression with an impressive 81% sensitivity and 94% specificity.

Mengual et al.\textsuperscript{23} performed gene expression analyses on 341 urine samples from patients with NMIBC and MIBC and 235 controls using TaqMan* arrays (Applied Biosystems, Carlsbad, CA, USA). A panel of 12 differentially expressed genes—ANXA10, AHNAK2, CTSE, CRH, IGF2, KLF9, KRT20, MAGEA3, POSTN, PPP1R14D, SLC1A6, and TERT—obtained from comparing the gene expression signatures of urine samples from patients with and without urothelial carcinoma demonstrated very high sensitivity and specificity for identifying bladder cancer (98% and 99%, respectively). The addition of two other genes that are differentially expressed between NMIBC and MIBC (ASAM and MCM10) enabled accurate prediction of tumor aggressiveness (79% sensitivity and 92% specificity). This signature was validated in voided urine samples and shown to maintain high levels of accuracy.

In an integrated genetic–epigenetic approach, Serizawa et al.\textsuperscript{80} screened for mutations in 6 genes (FGFR3, PIK3CA, TP53, HRAS, NRAS, and KRAS) and quantitatively assessed the promoter methylation status of 11 additional genes (APC, ARF, DBC1, INK4A, RARB, RASSF1A, SFRP1, SFRP2, SFRP4, SFRP5, and WIFI1) in NMIBC tumor biopsies and corresponding urine samples from 118 patients and 33 controls. A total of 95 oncogenic mutations and 189 hypermethylation events were detected. Assessing FGFR3 mutations in combination with the methylation status of three markers (APC, RASSF1A, and SFRP2) provided sensitivities of 90% in tumors and 62% in urine, with 100% specificity for both. Evaluation of the complete panel of markers provided sensitivities of 93% and 70% in biopsy and urine samples, respectively.

Selecting patients for neoadjuvant chemotherapy on the basis of their risk of node-positive disease could benefit high-risk patients and spare low-risk patients from the associated toxic effects and delay to cystectomy. Smith et al.\textsuperscript{92} recently reported a 20-gene expression model that is able to predict pathological nodal status independently of standard clinicopathologic prognostic criteria. They tested this model using primary tumor tissue samples from three independent cohorts of patients with no clinical evidence of nodal metastasis; initially in two separate cohorts of 90 and 66 patients and then in a phase III trial involving 185 patients.

**Molecular grading and signatures**

Van Rhijn et al.\textsuperscript{93} proposed a molecular grade parameter, based on a combination of FGFR3 gene mutation status and MIB1 labeling index, as an alternative to pathologic grading for NMIBC. The same group recently validated this parameter\textsuperscript{42} using the European Organization for Research and Treatment of Cancer (EORTC) NMIBC risk calculator,\textsuperscript{44} which generates a weighted score from six variables (WHO 1973 grade, stage, presence of CIS, multiplicity, size, and prior recurrence rate). The molecular grade was found to be more reproducible than the pathologic grade (89% versus 41–74% reproducibility). FGFR3 mutations correlated with favorable disease parameters, whereas increased MIB1 expression was frequently associated with pT1 tumors, high-grade disease, and high EORTC risk scores. Molecular grade held independent significance for predicting progression and DSS, and the addition of molecular grade to the EORTC model for progression increased the predictive accuracy from 74.9% to 81.7%\textsuperscript{93}.

In another study, Lindgren et al.\textsuperscript{95} suggested that a combined molecular and histopathological classification
of bladder cancer might prove more powerful in predicting outcomes and stratifying treatment. The authors combined gene expression analysis, whole genome array comparative genomic hybridization (CGH) analysis, and mutational analysis of FGFR3, PIK3CA, KRAS, HRAS, NRAS, TP53, CDKN2A, and TSC1 to identify two intrinsic molecular signatures (MS1 and MS2). Genomic instability was the most distinguishing genomic feature of the MS2 signature, which occurred independently of TP53 and MDM2 alterations. Genetic signatures were validated by two independent data sets that successfully classified urothelial carcinomas into low-grade or high-grade NMIBC and MIBC tumors with high precision and sensitivity. Furthermore, a gene expression signature that independently predicts metastasis and DFS was also defined. These data clearly support the role of molecular grading as a complement to standard pathologic grading.

Tumor proliferation indices

Tumor proliferation index, calculated from either KI67 or MIB1 expression levels, has consistently been shown to be an effective prognosticator for bladder cancer. MIB1 tumor proliferation index has a role in determining the prognosis of NMIBC as a component of the molecular grade parameter proposed by Van Rhijn et al. An independent prognostic role for KI67 tumor proliferation index has also been shown, with KI67 indices calculated from NMIBC TURB biopsy samples predicting progression-free survival (PFS) and DSS. A prognostic role for tumor proliferation index has also been established for MIBC. The Bladder Consortium Multi-Institutional Trial (which included 7 institutions and 713 patients) was able to confirm a prognostic role for KI67 tumor proliferation index has also been shown, with KI67 indices calculated from NMIBC TURB biopsy samples predicting progression-free survival (PFS) and DSS.

Ploidy and morphometric analysis

Several reports have suggested an independent prognostic role for ploidy and S phase analysis in the management of NMIBC. Ploidy analysis can be performed by flow cytometry or automated image cytometry (ICM) and can be applied to urine cytology specimens, as well as biopsy supernatant and disaggregated TURB FFPE specimens. In one of the largest studies assessing DNA ploidy in NMIBC tumors (377 in the test set and 156 in the validation set), Ali-Et-Dein et al. found that stage, DNA ploidy, tumor multiplicity, history of recurrence, tumor configuration, and type of adjuvant therapy can all independently predict recurrence. Recurrence (3 months after initial treatment), grade, and DNA ploidy were also effective predictors of progression to muscle-invasive disease. Baak et al. supported these findings by showing that ploidy status and S phase kinetics measured by ICM are strong independent predictors of recurrence and progression in patients with pTa and pT1 tumors. Although the data collected so far are encouraging, ploidy analysis still awaits large prospective randomized trials before ICM or flow cytometry techniques can be introduced into management algorithms for patients with NMIBC.

Other emerging potential biomarkers

Another genre of biomarkers with encouraging, but less robust, data regarding their potential prognostic power in bladder cancer management are tumor microenvironment markers, including the cell adhesion markers E-cadherin and N-cadherin, and angiogenesis modulators such as the hypoxia-inducible factors HIF1A and HIF2, vascular endothelial growth factor (VEGF), carbonic anhydrase IX protein (CAIX), and thrombospondin-1. miTOR pathway markers have also been shown to have a potential prognostic role in bladder cancer, and the mitotic serine-threonine kinase Aurora-A—which has an integral role in cell proliferation—has also been investigated in this setting. Finally, microRNA profile alterations have shown promise within this field and are likely to be heavily investigated in the future as noninvasive markers for diagnosing and prognosing patients with bladder cancer.

Bladder cancer biomarkers in targeted therapy

Key players in the Ras–MAPK and PI3K–AKT–mTOR pathways, as well as the angiogenesis pathway of the tumor microenvironment, offer promising opportunities for new targeted treatments of bladder cancer. The RTKs, in particular, have been a focus for investigation. In a multicenter phase II trial, 44 patients with advanced metastatic bladder cancer testing positive for ERBB2 (also known as HER2) were treated with a combination of carboplatin, paclitaxel, gemcitabine, and trastuzumab (a humanized monoclonal antibody against ERBB2). Approximately 70% of treated patients demonstrated a partial (59%) or complete (11%) response, with a median overall survival of 14.1 months. Improved response rates were observed in patients with 3+ ERBB2 expression scores and ERBB2 gene amplification (determined by IHC and FISH respectively) than in patients with 2+ ERBB2 expression scores and FISH-negative tumors. Interestingly, in contrast to the strong correlation between ERBB2 gene amplification and 3+ ERBB2 HIC expression scores typically observed in breast cancer patients, ERBB2 overexpression was not commonly associated with ERBB2 gene amplification in bladder cancer.

An ongoing randomized phase II trial has been set up to evaluate the role of cetuximab, an anti-EGFR recombinant humanized murine monoclonal antibody. Patients with metastatic, locally recurrent, or nonresectable disease are being treated with standard gemcitabine and carboplatin (GC) chemotherapy with or without cetuximab. By blocking EGF binding to the extracellular EGFR domain, cetuximab inhibits the downstream signal transduction pathway, accounting for
its antiproliferative activity in solid tumors. In bladder cancer cells, an additional antiangiogenic effect could also at play.137,140 However, the phase II Cancer and Leukemia Group B trial, which investigated the role of gefitinib (a small molecule inhibitor of EGFR) in patients with advanced bladder cancer found no survival or time to progression advantage for combination therapy over GC therapy alone.139,140

Lapatinib—a tyrosine kinase inhibitor (TKI) that targets both EGFR and ERBB2—is currently used in combination therapy for ERBB2-positive breast cancer.141 The results of a phase II single-arm trial suggest a therapeutic advantage of lapatinib in patients with EGFR-positive and ERBB2-positive bladder tumors.142 A phase II/III randomized trial is currently underway to investigate whether lapatinib could be used as maintenance therapy in patients who demonstrate an objective response to first-line chemotherapy and test positive for either EGFR or ERBB2 using IHC or FISH analysis.

The role of multitarget TKIs in bladder cancer has also been investigated, with mixed results. Although phase II trials of sorafenib (an inhibitor of RAF1, BRAF, PDGFRB, KDR, and FLT4) have failed to show a significant objective response, sunitinib has shown promise in a recent phase II trial (n = 77) at Memorial Sloan Kettering Cancer Center.121 A clinical benefit was observed in almost one-third of these patients. A subsequent randomized double-blind phase II trial is underway to investigate the efficacy of sunitinib in delaying tumor progression in patients with a positive initial response to standard chemotherapy.142

In an attempt to target the dependence of bladder tumors on angiogenesis, monoclonal antibodies and small-molecule inhibitors of angiogenesis are currently under investigation for patients with advanced disease. A potential role has been proposed for bevacizumab, a recombinant humanized monoclonal anti-VEGF antibody, as a first-line combination therapy with GC in patients with metastatic bladder cancer.143 A phase II study found that two-thirds of patients demonstrated an objective response, with 14% showing complete response—albeit with significant treatment-related toxicity.143 The CALGB phase III randomized trial is now underway to assess the effect of this combination therapy on inhibiting angiogenesis in metastatic urothelial carcinoma.123 Another phase II trial is currently investigating the benefits of administering bevacizumab in combination with M-VAC (methotrexate, vinblastine, adriamycin, and cisplatin) chemotherapy.144

Given the recent evidence to suggest the importance of mTOR pathway alterations in bladder cancer, a phase II trial is underway to evaluate the potential effects of treatment with everolimus—a sirolimus-derived inhibitor of the mTOR pathway—that is currently used in the treatment of renal cell cancer—in patients with advanced bladder cancer.113–115

**Conclusions**

As our understanding of the complex molecular mechanisms involved in bladder cancer development deepens, our approaches to the diagnosis and management of this disease continue to evolve. In the near future, the current clinicopathology-based prognostic approaches for predicting progression in NMIBC136,145–147 (Box 1) will likely be supplemented by a molecular-guided approach, based on molecular biomarkers (Box 2). Given the advantages in expected cost and turnaround time associated with next-generation sequencing technology, a genomic approach to providing noninvasive diagnostic and predictive tools should be actively pursued.

Evidently, a rigorous validation process must precede the incorporation of novel molecular biomarkers into
clinical management. The results of initial retrospective studies will first need to be confirmed and validated in large independent cohorts, followed by well-controlled, multi-institutional randomized prospective trials. A lack of these trials has hindered the clinical utilization of several promising biomarkers to date.\textsuperscript{35,36} For new biomarkers to be considered for integration into clinical practice, these trials should demonstrate a clear benefit over existing management algorithms.\textsuperscript{5,4,14} Several targeted therapies are now under investigation that combine standard chemotherapy agents and novel biomarkers—either as first-line treatments or as maintenance therapy to prolong response in patients with advanced bladder cancer. Exciting challenges and opportunities await investigators with a special interest in bladder cancer, and clinical investigations will likely bring about much-needed progress in the management of patients with this disease.

\textbf{Review criteria}

A systematic review of the literature was performed to identify English language full-text manuscripts published between 1990 and 2011. Cited articles were found by searching PubMed using the terms “molecular”, “bladder cancer”, “prognostic”, “theranostic”, and “targeted therapy”. Reference lists of included papers were also checked for further relevant articles. Larger studies with statistical vigor were given priority.


van Rhijn, B. W. et al. Molecular grading of urothelial cell carcinoma with fibroblast growth factor receptor 3 mutation.


