Published in final edited form as:

Curr Opin Urol. 2011 September ; 21(5): 420–427. doi:10.1097/MOU.0b013e32834956d6.

Biomarkers for prognosis and treatment selection in advanced bladder cancer patients

Yuanbin Ru¹, Garrett M. Dancik¹, and Dan Theodorescu^{1,2,3,*}

¹Department of Surgery, University of Colorado, Aurora, Colorado, USA

²Department of Pharmacology, University of Colorado, Aurora, Colorado, USA

³University of Colorado Comprehensive Cancer Center, Aurora, Colorado, USA

Abstract

Purpose of review—Patients with locally “advanced” or muscle invasive bladder cancer have higher mortality rates than patients with non-muscle invasive (“superficial”) bladder cancer. Biomarkers can stratify clinical outcomes and thus promise to more accurately prognosticate and thus help assign patients to the appropriate treatments. The aim of this review is to summarize biomarker developments in the past year and to discuss their implications in prognosis and treatment selection in locally advanced bladder cancer.

Recent findings—Prognostic biomarkers for bladder cancer are identified at the DNA, RNA and/or protein levels. Some are new markers; others are established markers with new roles in bladder cancer. Markers can report on the risk of disease recurrence or metastasis, or treatment responsiveness and thus are useful in determining “who to treat” and “what to treat with”.

Summary—The list of biomarkers for prognosis and treatment selection for advanced bladder cancer is growing. For most, their clinical relevance is unclear due to their lack of validation in external datasets. MicroRNAs and new techniques including next-generation sequencing offer additional opportunities for biomarker discovery, validation, and clinical applications.

Keywords

biomarker; prognosis; treatment selection; advanced bladder cancer

Introduction

There were approximately 386,300 new cases and 150,200 deaths from bladder cancer in 2008 globally [1]. Among all cancers in men, bladder cancer ranks as seventh and ninth in the number of new cases and deaths, respectively [1], and is also the most expensive cancer to treat [2]. There are two major biological pathways in human bladder tumor development, leading to two major subtypes of bladder cancer: superficial/non-muscle-invasive (NMIBC) and advanced/muscle-invasive (MIBC) [3]. The advanced, MI disease accounts for 20%–30% of newly diagnosed cases [4]. At presentation, the 5-year survival rate of MIBC patients in the United States is approximately 50% [5]. Biomarkers can provide detailed molecular insights into progression and metastasis and their clinical use can lead to more accurate and patient-specific prognosis and surveillance. In addition, the use of biomarkers may improve the quality of life of patients by limiting aggressive standard treatments to only

*Corresponding author: Departments of Surgery and Pharmacology and University of Colorado Comprehensive Cancer Center, Aurora, CO 80045, Tel: 303-724-7135, Fax: 303-724-3162, dan.theodorescu@ucdenver.edu.

The authors declare no conflict of interest.

those that benefit [6, 7]. In this review, we summarize biomarker developments within the past year that are related to prognosis and treatment selection of locally advanced (MI) and metastatic bladder cancer patients. Many important bladder cancer biomarkers fall outside the scope of this article but fortunately have been reviewed elsewhere [8, 9, 10, 11].

Prognostic biomarkers that identify high-risk patients

A large number of prognostic biomarkers in advanced bladder cancer have been studied during the past year. These include new markers as well as some established ones (*p53*, mTOR pathway genes etc.) with new implications for prognosis and treatment. This section reviews biomarkers that are associated with patient outcome, such as progression, survival and development of metastasis (Table 1). They are grouped according to their molecular type DNA, RNA, or protein. These prognostic biomarkers identify high-risk patients without informing which particular treatment to use. Biomarkers that offer implications in selecting and optimizing specific treatments are discussed in the next section “Biomarkers for treatment selection”.

Nucleic Acid Biomarkers

Human 8-oxoguanine DNA glycosylase 1 (hOGG1) is one of the enzymes responsible for DNA base excision repair [29]. Genotyping of polymorphisms within *hOGG1* codon 326 in blood samples and primary tissues from more than 110 MIBC patients revealed that patients with the genotype Cys326Cys had higher progression-free survival (PFS) rates than those with Cys326Ser and Ser326Ser genotypes [12, 13]. Other biomarkers have been detected at the RNA level. Cadherins are a family of transmembrane glycoproteins mediating cell-cell adhesion [30]. Change in cell-cell adhesion results in altered signaling pathways and plays a role in tumor progression [30]. In 30 MIBC patients receiving cystectomy (surgical removal of all or part of the bladder), either up-regulation of *N-cadherin* (N+, $p = 0.0064$) or down-regulation of *E-cadherin* (E-, $p = 0.0017$) was associated with shorter overall survival [14]. Patients with both N+ and E- had the lowest overall survival rate ($p = 0.0015$) [14]. However, a conflicting study that measured N-cadherin levels in 92 MIBC patients after surgery found that high *N-cadherin* mRNA levels correlated with better disease-specific survival (DSS) rates ($p = 0.02$) [15]. mRNA expression of another biomarker *Galectin-3*, encoding a carbohydrate-binding protein, was profiled in 72 MIBC cystectomy samples [16]. Patients with decreased galectin-3 mRNA levels showed better overall survival than those with higher levels ($p = 0.03$).

Protein Biomarkers

Most tissue prognostic biomarkers are measured by Western blot or immunohistochemical staining. In a population of 266 MIBC patients, individuals with positive immunostaining of cyclo-oxygenase-2 (COX-2) have higher DSS ($p = 0.006$) and recurrence-free survival ($p = 0.003$) rates than those with negative staining [17]. However, another study of 46 MIBC patients undergoing cystectomy failed to show statistically significant association between COX-2 positive staining and survival [18] but did find a negative correlation between COX-2 protein levels and the extent of lymph node metastasis ($p = 0.008$) [18]. In contrast to the result in MIBC patients [17], lower COX-2 protein expression in individuals with squamous cell carcinomas had higher recurrence-free (hazard ratio (HR) = 2.1, $p = 0.031$) and DSS (HR = 2.3, $p = 0.046$) rates [19]. D-type cyclins are also prognostic markers in advanced bladder cancer. Higher protein expression of cyclin D1 ($p = 0.023$) and D2 ($p = 0.042$) and lower levels of D3 ($p = 0.032$) correlate with better DSS rates in a population of 57 MIBC patients [20].

Immunohistochemical staining of tumors from 132 cystectomies for mammalian target of rapamycin (mTOR) pathway genes (*PTEN*, *c-myc*, *p27*, *phosAkt*, and *phosS6*) showed their down-regulation in MIBC tumors compared with benign urothelium [21]. *phosS6* and *c-myc* independently predicted DSS outcome ($p = 0.03$) and progression ($p = 0.02$), respectively [21]. In MIBC patients undergoing radical cystectomy, low or no protein expression of second mitochondria-derived activator of caspase (Smac/DIABLO) was associated with reduced 5-year DSS rates [22]. Positive immunohistochemical staining of p53 is associated with decreased recurrence-free and disease-specific survival rates in 152 squamous cell carcinoma patients receiving radical cystectomy ($p < 0.05$ for both survival rates) [23], where most tumors were invasive. The same associations of positive p53 staining with low DSS and recurrence-free survival rates were also observed in 692 advanced bladder cancer patients treated with radical cystectomy and lymphadenectomy ($HR \geq 1.65$ and $p < 0.001$ for both survival rates) [25].

Ribonucleotide reductase subunit M1 (RRM1), a prognostic biomarker in non-small cell lung cancer (NSCLC) [31], is also prognostic in younger (aged ≥ 70 years), but not older (aged < 70 years), MIBC patients treated by radical cystectomy [26]. In younger patients, patients with high RRM1 protein levels had a longer median overall survival time than patients with low RRM1 levels (10.6 vs. 2.3 years). In older patients, there was no difference in median overall survival between patients with high and low RRM1 expression (1.6 years in both groups).

Biomarkers of metastatic risk after radical cystectomy

More than 50% of the invasive high-grade tumors metastasize despite definitive local therapy with only 6% of metastatic patients surviving at five years [5, 32]. Advanced bladder cancer patients with a high risk of metastasis may benefit from biomarkers for earlier detection and thus earlier systemic therapy along with definitive local therapy. Using DNA microarray data, a 20-gene model for predicting node-positive disease in node-negative patients at the time of cystectomy was developed from two training cohorts and prospectively validated on a set of patients enrolled in a phase 3 clinical trial [27**]. It is important to note that this is the first predictive multigene mRNA expression model that has been validated in a prospectively collected cohort. The use of COX-2 expression, as mentioned above [18], and a relative risk score from this 20-gene model would identify high-risk patients of node metastasis. Karyopherin- $\alpha 2$ (KPNA2), an importin family member, is another biomarker of metastatic risk [28]. In 377 MIBC patients that underwent radical cystectomy, patients with KPNA2-positive tumors detected by immunohistochemistry had a lower overall survival rate ($p = 0.03$) and a higher risk of visceral metastasis ($p = 0.04$) than those with KPNA2-negative tumors. Interestingly, NMIBC patients with KPNA2-positive tumors have a higher risk of progression than KPNA2-negative patients [28].

Multiplexing Biomarkers to Improve Prognostic Accuracy

Combinations of biomarkers offer the potential advantage over individual biomarkers of providing more accurate prognoses for advanced bladder cancer patients. The aforementioned 20-gene model is one example [27**]. In another example, down-regulation of each one of the four cell cycle regulators (p53, pRB, p21 and p27) or at least three of the four markers is associated with higher recurrence-free and disease-specific survival rates (all $p \leq 0.001$) [24*]. In a multivariate model for either disease recurrence or DSS that considers age, gender, stage, grade, lymph node status, adjuvant chemotherapy, and other clinical parameters, the addition of immunostaining results for any of the four genes individually did not increase the accuracy of the model. Interestingly, adding results of all four cell cycle regulators together to the model modestly but significantly increases predictive accuracy of

disease recurrence from 66% to 70% ($p < 0.001$) and of DSS from 67% to 71% ($p < 0.001$) in all pT3N0, pT4N0 and node-positive patients and in each patient subgroup [24*].

Biomarkers for treatment selection

One method for maximizing treatment efficacy in advanced bladder cancer patients is by using standard treatment approaches only in patients that benefit, sparing the remainder the morbidity of unnecessary treatment. For example, integrating neoadjuvant chemotherapy with high-quality cystectomy improves overall survival in MIBC patients [6, 7]. However while a few patients benefit from this approach, most patients do not benefit from neoadjuvant treatment. Therefore, the use of biomarkers to choose the best treatment for a particular patient population, and vice versa, to choose patients that are most likely to benefit from a given treatment offers significant promise (Table 2). In addition, biomarker-based selection of individuals in clinical trials can lead to the approval of drugs that are effective in these subpopulations but that would have otherwise not been approved in the unselected population. A good example is crizotinib, an anaplastic lymphoma kinase (ALK) inhibitor that targets *ML4-ALK* translocation which occurs in only 5% of NSCLC patients [40].

Biomarkers for selection of cystectomy vs. bladder-preserving chemoradiotherapy

Radical radiotherapy and chemoradiation therapy are bladder-preserving alternatives to radical cystectomy in MIBC patients. Although no randomized trials have compared the two populations, MIBC patients treated by cystectomy showed no significant difference from radiotherapy patients in overall, disease-specific, and recurrence-free survival rates [41] and no difference from chemoradiation therapy patients in overall survival rate [42] at the population level. However, biomarkers can in principle, identify subpopulations for which bladder-preserving therapy or cystectomy is more beneficial. The MRE11-RAD50-NBS1 (MRN) complex is essential for detecting and repairing DNA double-strand breaks [43] caused by radiotherapy. Meiotic recombination 11 (MRE11), one of the key proteins in the MRN complex, is positively correlated with DSS in patients treated with radiotherapy but not in cystectomy patients [33**]. Specifically, individuals with high expression levels of *MRE11* had a significantly better 3-year DSS rate when receiving radiotherapy compared to cystectomy (70% vs. 54%, $p = 0.021$) [33**]. Therefore, patients with high *MRE11* expression are likely to benefit more from radical radiotherapy than from cystectomy. Another study showed that, using immunohistochemistry, 86% (12/14) of ERCC1 negative MIBC patients had complete response to chemoradiation therapy while 75% (6/8) of ERCC1 positive patients did not ($p = 0.008$) [35]. This finding indicates that advanced bladder cancer patients with low ERCC1 level will likely benefit most from chemoradiotherapy and those with high levels are advised to choose cystectomy.

Biomarkers predicting response to neoadjuvant chemotherapy before cystectomy

A meta-analysis of 3005 invasive bladder cancer patients from 11 randomized controlled trials showed that cisplatin-based neoadjuvant chemotherapy, in combination with local treatment (cystectomy or radiotherapy), has 5% and 9% absolute improvements in 5-year overall and disease-free survival, respectively, when compared with local treatment alone [44]. Given this limited efficacy leading to delay of definitive treatment, neoadjuvant chemotherapy has not been widely adopted in the community [45, 46]. Low expression levels of the *breast cancer susceptibility gene 1 (BRCA1)* were associated with more favorable response to cisplatin-based chemotherapy in ovarian cancer and NSCLC patients [47]. Similarly in advanced bladder cancer, patients with low BRCA1 mRNA levels were more sensitive to cisplatin-based neoadjuvant chemotherapy [34]. The median survival and

5-year survival rates are 168 months and 64% in patients with low BRCA1 levels, compared with 34 months and 12% in others with high BRCA1 levels ($p = 0.002$) [34].

Biomarkers of response to platinum-based adjuvant chemotherapy

Two genes, *multidrug resistance gene 1* (*MDR1*) and *excision repair cross-complementing 1* (*ERCC1*), are prognostic markers in advanced bladder cancer patients treated by platinum-based adjuvant chemotherapy [36, 37]. Low expression levels of *MDR1*, encoding a membrane-associated and ATP-dependent drug efflux pump, is associated with high PFS (HR = 0.28, $p = 0.002$, 2-year progression rate 25% vs. 65%) and overall survival (HR = 0.25, $p = 0.0006$, 5-year survival rate 62% vs. 23%) rates in locally advanced bladder cancer patients receiving cisplatin-based adjuvant chemotherapy [36]. Low expression of *ERCC1*, one of the DNA repair genes, is predictive of prolonged PFS (HR = 0.52, $p = 0.03$, 5-year progression 45% vs. 70%) [36] and overall survival ($p = 0.03$, median survival 25 vs. 15 months) [37] in locally advanced and prolonged PFS in metastatic bladder cancer ($p = 0.03$, median survival 10.6 vs. 8.4 months) [38] after cisplatin-based chemotherapy. These results suggest that advanced bladder cancer patients with low *MDR1* and/or *ERCC1* level will be sensitive to cisplatin-based adjuvant chemotherapy and are likely to benefit, whereas other patients are likely to be less sensitive. Alternative treatments may therefore be preferred for these patients.

Biomarkers of response to targeted agents

Higher mRNA levels of *endothelin-1* (*ET-1*) and *ET-1 receptor A* (*ET_AR*) were observed in MIBC, rather than in NMIBC patients in three cohorts, and were associated with lower DSS rates in two of the cohorts with follow-up information (all $p \leq 0.01$) [39**]. This was confirmed in a fourth patient cohort, in which high ET-1 protein levels were associated with worse DSS rates in both MIBC ($p = 0.047$) and NMIBC patients ($p < 0.0005$) [39**]. Blocking *ET_AR* suppresses metastasis [48], but not tumor growth in bladder cancer xenograft models [49]. It has been shown in cancer cells and mouse models that ET-1 and *ET_AR* play a more important role in metastatic lung colonization than in established tumor growth [39**]. These findings suggest that the time to treat metastatic bladder cancer is critical: inhibitors of ET-1 and *ET_AR* will likely be more effective when used before the cancer spreads rather than after metastatic cancer is established.

The future of biomarkers in bladder cancer prognosis and treatment selection

Although therapeutic treatment options for advanced bladder cancer patients are currently limited, treatments including targeted therapies based on biomarkers are promising. Clinical trials are ongoing for the mitotic inhibitor Paclitaxel, with or without Trastuzumab (Herceptin), which targets the receptor tyrosine kinase HER2 (RTOG-0524); Erlotinib, which inhibits the epidermal growth factor receptor (EGFR) and Polyphenon E (UCLA-0301091-02); and bevacizumab (Avastin®) and sorafenib, which target the vascular endothelial growth factor (VEGF) pathway (NCI-05-C-0022). More targeted therapeutic agents will be available as more biomarkers are discovered. In the future, we expect that it will become possible to measure all known biomarkers in bladder cancer patients and determine what drugs and treatments are best for each individual.

Currently, only a small portion of the biomarkers discussed in this review have been validated in independent datasets (Tables 1 and 2). A study testing published prognostic mRNA based gene signatures for survival found that none could be validated on external data, either in combined NMI and MI tumors or MI tumors alone [50*]. Furthermore, the published gene signatures for survival performed no better than randomly selected genes in

external datasets, suggesting that genes which correlate with survival are difficult to identify and may vary between different patient populations and treatment regimens [50*]. Importantly, validating the prognostic biomarkers in multiple independent patient cohorts is essential for ensuring their clinical utility.

Methods that help facilitate the translation of biomarkers from bench to bedside are also important. An algorithm called CO-eXpression ExtrapolationN (COXEN) was developed to predict response to chemotherapeutic agents in bladder, breast, and ovarian cancer patients based on drug response in cell lines [51, 52], and for *in silico* drug discovery [51, 53]. The idea behind COXEN is that specialized biomarkers that show similar co-regulation between patient tumors and cell lines indicate translatability between cell line and tumor drug sensitivity. Approaches like COXEN can narrow down candidate drugs, while increasing their chance of success, in early clinical research.

In addition, applying next-generation sequencing to biomarker discovery in bladder cancer, as already reported in leukemia, melanoma, lung and breast cancers [54], offers direct measurement of gene expression and additional information of mutation, methylation, single-nucleotide polymorphism, and genomic rearrangement. A genome-wide association study has identified loci associated with bladder cancer risk [55] and has the potential for broader application. Epigenetic biomarkers and some non-protein-coding genes, microRNAs in particular, can also be used in prognostication as shown previously [56, 57]. Finally, identification of multiple biomarkers in the same biological pathway can provide evidence that the pathway is important in cancer and is therefore a promising target for treatment.

Conclusion

Biomarker discovery in advanced bladder cancer has progressed in recent years with a large number of prognostic biomarkers identified in the past year. Some of these markers are measured at the DNA level, such as polymorphisms of *hOGG1*. Biomarkers such as *N-cadherin* have been profiled at the RNA level. Most of the prognostic biomarkers, p53 and other cell cycle regulators for example, are protein markers that are measured by immunohistochemistry and/or Western blot. Quantifying biomarkers at multiple levels and integrating multiple biomarkers for prognostication lead to more accurate predictions. Some of the prognostic biomarkers, due to their correlation with particular biological processes (metastasis for example) and time-specific processes (metastatic lung colonization, etc.), are useful for identifying high-risk patients and the optimal time of treatment, respectively. Other biomarkers are associated with the effectiveness of a treatment and thus are appropriate for treatment selection. However, the clinical relevance of most of the biomarkers is unclear due to the lack of independent validation in additional datasets. Approaches that facilitate the translation from bench to bedside, such as COXEN, are also important. Non-coding RNAs, epigenetics, and techniques including next-generation sequencing and genome-wide association studies offer additional opportunities in biomarker discovery, validation, and clinical application.

Acknowledgments

This work was supported in part by National Institutes of Health grants CA075115 and CA143971 to DT.

References

1. Jemal A, Bray F, Center MM, et al. Global cancer statistics. *CA Cancer J Clin*. 2011; 61(2):69–90. [PubMed: 21296855]

2. Botteman MF, Pashos CL, Redaelli A, et al. The health economics of bladder cancer: a comprehensive review of the published literature. *Pharmacoeconomics*. 2003; 21(18):1315–30. [PubMed: 14750899]
3. McConkey DJ, Lee S, Choi W, et al. Molecular genetics of bladder cancer: Emerging mechanisms of tumor initiation and progression. *Urol Oncol*. 2010; 28(4):429–40. [PubMed: 20610280]
4. Jacobs BL, Lee CT, Montie JE. Bladder cancer in 2010: how far have we come? *CA Cancer J Clin*. 2010; 60(4):244–72. [PubMed: 20566675]
5. Jemal A, Siegel R, Xu J, Ward E. Cancer statistics, 2010. *CA Cancer J Clin*. 2010; 60(5):277–300. [PubMed: 20610543]
6. Feifer AH, Taylor JM, Tarin TV, Herr HW. Maximizing Cure for Muscle-Invasive Bladder Cancer: Integration of Surgery and Chemotherapy. *Eur Urol*. 2011
7. Stenzl A, Cowan NC, De Santis M, et al. Treatment of Muscle-invasive and Metastatic Bladder Cancer: Update of the EAU Guidelines. *Eur Urol*. 2011
8. Proctor I, Stoeber K, Williams GH. Biomarkers in bladder cancer. *Histopathology*. 2010; 57(1):1–13. [PubMed: 20579130]
9. Van Tilborg AA, Bangma CH, Zwarthoff EC. Bladder cancer biomarkers and their role in surveillance and screening. *Int J Urol*. 2009; 16(1):23–30. [PubMed: 19120523]
10. Lintula S, Hotakainen K. Developing biomarkers for improved diagnosis and treatment outcome monitoring of bladder cancer. *Expert Opin Biol Ther*. 2010; 10(8):1169–80. [PubMed: 20446896]
11. Bryan RT, Zeegers MP, James ND, et al. Biomarkers in bladder cancer. *BJU Int*. 2010; 105(5):608–13. [PubMed: 19793380]
12. Ha YS, Yan C, Kim IY, et al. Tissue hOGG1 Genotype Predicts Bladder Cancer Prognosis: A Novel Approach Using a Peptide Nucleic Acid Clamping Method. *Ann Surg Oncol*. 2010
13. Kim EJ, Yan C, Ha YS, et al. Analysis of hOGG1 genotype as a prognostic marker for muscle invasive bladder cancer: A novel approach using peptide nucleic acid-mediated, real-time PCR clamping. *Urol Oncol*. 2010
14. Wallerand H, Cai Y, Wainberg ZA, et al. Phospho-Akt pathway activation and inhibition depends on N-cadherin or phospho-EGFR expression in invasive human bladder cancer cell lines. *Urol Oncol*. 2010; 28(2):180–8. [PubMed: 19070520]
15. Jager T, Becker M, Eisenhardt A, et al. The prognostic value of cadherin switch in bladder cancer. *Oncol Rep*. 2010; 23(4):1125–32. [PubMed: 20204300]
16. Canesin G, Gonzalez-Peramato P, Palou J, et al. Galectin-3 expression is associated with bladder cancer progression and clinical outcome. *Tumour Biol*. 2010; 31(4):277–85. [PubMed: 20401558]
17. Aziz A, Lessard A, Moore K, et al. Improved cancer specific-survival in patients with carcinoma invading bladder muscle expressing cyclo-oxygenase-2. *BJU Int*. 2010
18. Naruse K, Yamada Y, Nakamura K, et al. Potential of molecular targeted therapy of HER-2 and Cox-2 for invasive transitional cell carcinoma of the urinary bladder. *Oncol Rep*. 2010; 23(6):1577–83. [PubMed: 20428812]
19. Youssef RF, Shariat SF, Kapur P, et al. Prognostic value of cyclooxygenase-2 expression in squamous cell carcinoma of the bladder. *J Urol*. 2011; 185(3):1112–7. [PubMed: 21255800]
20. Levidou G, Saetta AA, Karlou M, et al. D-type cyclins in superficial and muscle-invasive bladder urothelial carcinoma: correlation with clinicopathological data and prognostic significance. *J Cancer Res Clin Oncol*. 2010; 136(10):1563–71. [PubMed: 20174823]
21. Schultz L, Albadine R, Hicks J, et al. Expression status and prognostic significance of mammalian target of rapamycin pathway members in urothelial carcinoma of urinary bladder after cystectomy. *Cancer*. 2010; 116(23):5517–26. [PubMed: 20939013]
22. Mizutani Y, Katsuoka Y, Bonavida B. Prognostic significance of second mitochondria-derived activator of caspase (Smac/DIABLO) expression in bladder cancer and target for therapy. *Int J Oncol*. 2010; 37(2):503–8. [PubMed: 20596678]
23. Youssef RF, Shariat SF, Kapur P, et al. Expression of cell cycle-related molecular markers in patients treated with radical cystectomy for squamous cell carcinoma of the bladder. *Hum Pathol*. 2011; 42(3):347–55. [PubMed: 21111452]

- 24*. Shariat SF, Chade DC, Karakiewicz PI, et al. Combination of multiple molecular markers can improve prognostication in patients with locally advanced and lymph node positive bladder cancer. *J Urol.* 2010; 183(1):68–75. This article demonstrated the advantage of multiple over single biomarkers in cancer prognosis. [PubMed: 19913255]
25. Shariat SF, Bolenz C, Karakiewicz PI, et al. p53 expression in patients with advanced urothelial cancer of the urinary bladder. *BJU Int.* 2010; 105(4):489–95. [PubMed: 19659466]
26. Harshman LC, Bepler G, Zheng Z, et al. Ribonucleotide reductase subunit M1 expression in resectable, muscle-invasive urothelial cancer correlates with survival in younger patients. *BJU Int.* 2010; 106(11):1805–11. [PubMed: 20438561]
- 27**. Smith SC, Baras AS, Dancik G, et al. A 20-gene model for molecular nodal staging of bladder cancer: development and prospective assessment. *Lancet Oncol.* 2011; 12(2):137–43. One of the few studies that validated gene signatures in a prospective dataset. The paper described a 20-gene model to predict risk of node metastasis after cystectomy in bladder cancer patients. The model was developed from two patient cohorts and validated in a third cohort. Patients with predicted high risk are good candidates for neoadjuvant chemotherapy. [PubMed: 21256081]
28. Jensen JB, Munksgaard PP, Sorensen CM, et al. High Expression of Karyopherin-alpha2 Defines Poor Prognosis in Non-Muscle-Invasive Bladder Cancer and in Patients with Invasive Bladder Cancer Undergoing Radical Cystectomy. *Eur Urol.* 2011
29. Friedberg EC. How nucleotide excision repair protects against cancer. *Nat Rev Cancer.* 2001; 1(1):22–33. [PubMed: 11900249]
30. Cavallaro U, Christofori G. Cell adhesion and signalling by cadherins and Ig-CAMs in cancer. *Nat Rev Cancer.* 2004; 4(2):118–32. [PubMed: 14964308]
31. Zheng Z, Chen T, Li X, et al. DNA synthesis and repair genes RRM1 and ERCC1 in lung cancer. *N Engl J Med.* 2007; 356(8):800–8. [PubMed: 17314339]
32. Wu XR. Urothelial tumorigenesis: a tale of divergent pathways. *Nat Rev Cancer.* 2005; 5(9):713–25. [PubMed: 16110317]
- 33**. Choudhury A, Nelson LD, Teo MT, et al. MRE11 expression is predictive of cause-specific survival following radical radiotherapy for muscle-invasive bladder cancer. *Cancer Res.* 2010; 70(18):7017–26. The study suggests that MIBC patients with high MRE11 protein levels are likely to benefit more from radical radiotherapy than from cystectomy. The association of high MRE11 expression with good DSS is tested and validated in two separate radiotherapy cohorts but is lacking in a cystectomy cohort. High MRE11 expression associated with an absolute increase of 16% DSS rate in radiotherapy patients over cystectomy patients. [PubMed: 20843819]
34. Font A, Taron M, Gago JL, et al. BRCA1 mRNA expression and outcome to neoadjuvant cisplatin-based chemotherapy in bladder cancer. *Ann Oncol.* 2011; 22(1):139–44. [PubMed: 20603439]
35. Kawashima A, Nakayama M, Kakuta Y, et al. Excision repair cross-complementing group 1 (ERCC1) may predict the efficacy of chemoradiotherapy for bladder cancer. *Clin Cancer Res.* 2010
36. Hoffmann AC, Wild P, Leicht C, et al. MDR1 and ERCC1 expression predict outcome of patients with locally advanced bladder cancer receiving adjuvant chemotherapy. *Neoplasia.* 2010; 12(8):628–36. [PubMed: 20689757]
37. Bellmunt J, Paz-Ares L, Cuello M, et al. Gene expression of ERCC1 as a novel prognostic marker in advanced bladder cancer patients receiving cisplatin-based chemotherapy. *Ann Oncol.* 2007; 18(3):522–8. [PubMed: 17229776]
38. Kim KH, Do IG, Kim HS, et al. Excision repair cross-complementation group 1 (ERCC1) expression in advanced urothelial carcinoma patients receiving cisplatin-based chemotherapy. *APMIS.* 2010; 118(12):941–8. [PubMed: 21091775]
- 39**. Said N, Smith S, Sanchez-Carbayo M, Theodorescu D. Tumor endothelin-1 enhances metastatic colonization of the lung in mouse xenograft models of bladder cancer. *J Clin Invest.* 2011; 121(1):132–47. Association of high ET-1 and ETAR expression with decreased DSS was confirmed in multiple patient cohorts. In cancer cells and mouse models, ET-1 and ETAR are essential for metastatic lung colonization but less important in established tumor growth,

suggesting inhibitors of ET-1 and ETAR as adjuvant therapeutic agents before metastasis. [PubMed: 21183790]

40. Gerber DE, Minna JD. ALK inhibition for non-small cell lung cancer: from discovery to therapy in record time. *Cancer Cell*. 2010; 18(6):548–51. [PubMed: 21156280]
41. Kotwal S, Choudhury A, Johnston C, et al. Similar treatment outcomes for radical cystectomy and radical radiotherapy in invasive bladder cancer treated at a United Kingdom specialist treatment center. *Int J Radiat Oncol Biol Phys*. 2008; 70(2):456–63. [PubMed: 17904301]
42. Rene NJ, Cury FB, Souhami L. Conservative treatment of invasive bladder cancer. *Curr Oncol*. 2009; 16(4):36–47. [PubMed: 19672423]
43. Lamarche BJ, Orazio NI, Weitzman MD. The MRN complex in double-strand break repair and telomere maintenance. *FEBS Lett*. 2010; 584(17):3682–95. [PubMed: 20655309]
44. Advanced bladder cancer (ABC) meta-analysis collaboration. Neoadjuvant chemotherapy in invasive bladder cancer: update of a systematic review and meta-analysis of individual patient data. *Eur Urol*. 2005; 48(2):202–5. [PubMed: 15939524]
45. Hussain MH, Wood DP, Bajorin DF, et al. Bladder cancer: narrowing the gap between evidence and practice. *J Clin Oncol*. 2009; 27(34):5680–4. [PubMed: 19858384]
46. Sanchez-Ortiz RF, Huang WC, Mick R, et al. An interval longer than 12 weeks between the diagnosis of muscle invasion and cystectomy is associated with worse outcome in bladder carcinoma. *J Urol*. 2003; 169(1):110–5. [PubMed: 12478115]
47. Stordal B, Davey R. A systematic review of genes involved in the inverse resistance relationship between cisplatin and paclitaxel chemotherapy: role of BRCA1. *Curr Cancer Drug Targets*. 2009; 9(3):354–65. [PubMed: 19442054]
48. Titus B, Frierson HF Jr, Conaway M, et al. Endothelin axis is a target of the lung metastasis suppressor gene RhoGDI2. *Cancer Res*. 2005; 65(16):7320–7. [PubMed: 16103083]
49. Herrmann E, Tiemann A, Eltze E, et al. Endothelin-A-receptor antagonism with atrasentan exhibits limited activity on the KU-19-19 bladder cancer cell line in a mouse model. *J Cancer Res Clin Oncol*. 2009; 135(10):1455–62. [PubMed: 19430814]
- 50*. Lauss M, Ringner M, Hoglund M. Prediction of stage, grade, and survival in bladder cancer using genome-wide expression data: a validation study. *Clin Cancer Res*. 2010; 16(17):4421–33. This study found that of six published survival gene signatures, none could be validated in external data, suggesting the importance of independent validation in biomarker discovery. [PubMed: 20736328]
51. Lee JK, Havaleshko DM, Cho H, et al. A strategy for predicting the chemosensitivity of human cancers and its application to drug discovery. *Proc Natl Acad Sci U S A*. 2007; 104(32):13086–91. [PubMed: 17666531]
52. Williams PD, Cheon S, Havaleshko DM, et al. Concordant gene expression signatures predict clinical outcomes of cancer patients undergoing systemic therapy. *Cancer Res*. 2009; 69(21):8302–9. [PubMed: 19843853]
53. Smith SC, Havaleshko DM, Moon K, et al. Use of yeast chemigenomics and COXEN informatics in preclinical evaluation of anticancer agents. *Neoplasia*. 2011; 13(1):72–80. [PubMed: 21253455]
54. Roukos DH. Trastuzumab and beyond: sequencing cancer genomes and predicting molecular networks. *Pharmacogenomics J*. 2011; 11(2):81–92. [PubMed: 20975737]
55. Rothman N, Garcia-Closas M, Chatterjee N, et al. A multi-stage genome-wide association study of bladder cancer identifies multiple susceptibility loci. *Nat Genet*. 2010; 42(11):978–84. [PubMed: 20972438]
56. Kim YK, Kim WJ. Epigenetic markers as promising prognosticators for bladder cancer. *Int J Urol*. 2009; 16(1):17–22. [PubMed: 18721202]
57. Dyrskjot L, Ostensfeld MS, Bramsen JB, et al. Genomic profiling of microRNAs in bladder cancer: miR-129 is associated with poor outcome and promotes cell death in vitro. *Cancer Res*. 2009; 69(11):4851–60. [PubMed: 19487295]

Key points

1. Prognostic biomarkers, usually measured at DNA, RNA, and/or protein levels, are associated with progression, recurrence, metastasis and survival in advanced bladder cancer patients.
2. Biomarkers have the potential to allow practitioners to choose the best treatment for a specific patient population, and vice versa, to choose patients that are most likely to benefit from a given treatment, and to decide the best time to treat.
3. Biomarkers must be validated in external patient datasets, ideally from tissues obtained in prospective trials, to determine their clinical relevance.
4. Approaches like COXEN could facilitate the translation of biomarkers in drug discovery from cell lines to patients to identify sensitive and resistant individuals.
5. Non-coding RNAs, epigenetics, next-generation sequencing and genome-wide association studies provide additional opportunities in biomarker discovery, validation, and clinical application.

Table 1

Biomarkers for prognosis in advanced bladder cancer: recent discoveries

Biomarker	Detection level	Detection method	Sample population	Independent validation	Outcome	Biomarker status indicating good outcome	Reference
hOGG1	DNA (codon 326 polymorphisms)	Peptide nucleic acid-mediated real-time PCR clamping	117 MIBC tumors	No	PFS	Cys326Cys	[12]
	DNA (codon 326 polymorphisms)	Peptide nucleic acid-mediated real-time PCR clamping	113 MIBC blood samples	No	PFS, DSS	Cys326Cys	[13]
E-cadherin and N-cadherin [*]	mRNA	DNA microarray	30 MIBC patients undergoing cystectomy	No	OS	Low N-cadherin and/or high E-cadherin	[14]
N-cadherin [*]	mRNA	Quantitative RT-PCR	92 MIBC tumors	No	DSS	High expression	[15]
Galectin-3	mRNA	DNA microarray	72 MIBC cystectomy samples	No	OS	Low expression	[16]
COX-2 [*]	Protein	Immunohistochemical staining	266 MIBC patients	No	DSS, recurrence-free survival	High expression	[17]
			46 MIBC patients	No	The extent of node metastasis	High expression	[18]
			152 squamous cell carcinomas (142 MI)	No	Recurrence-free survival, DSS	Low expression	[19]
cyclin D1	Protein	Immunohistochemical staining	57 MIBC tumors	No	DSS	High expression	[20]
cyclin D2	Protein	Immunohistochemical staining	57 MIBC tumors	No	DSS	High expression	[20]
cyclin D3	Protein	Immunohistochemical staining	57 MIBC tumors	No	DSS	Low expression	[20]
c-myc	Protein	Immunohistochemical staining	132 (27 NMIBC and 105 MIBC) cystectomies	No	Progression	High expression	[21]
phosS6	Protein	Western blot	132 (27 NMIBC and 105 MIBC) cystectomies	No	DSS	High expression	[21]
SmaC/DIABLO	Protein	Immunohistochemical staining	84 tumor samples	No	DSS	High expression	[22]
p53	Protein	Immunohistochemical staining	152 squamous cell carcinomas (142 MI)	No	Recurrence-free survival, DSS	Low expression	[23]
p53, pRB, p21, or p27 (individual)	Protein	Immunohistochemical staining	692 pT3-4N0 or pTanyNpositive tumors	No	Recurrence-free survival, DSS	Low expression	[24*, 25]

Biomarker	Detection level	Detection method	Sample population	Independent validation	Outcome	Biomarker status indicating good outcome	Reference
p53, pRB, p21, and p27 (combined)	Protein	Immunohistochemical staining	692 pT3-4N0 or pTanyNpositive tumors	No	Recurrence-free survival, DSS	< 2 markers with high expression	[24*]
RRM1	Protein	Immunofluorescent staining	84 MIBC cystectomy samples	No	OS	High expression and ages <70 years (p = 0.001)	[26]
A 20-gene model	mRNA	DNA microarray	Two training cohorts (n=90 & 66); one validation cohort (n=185); almost all are MIBC patients	Yes	Risk of node metastasis	Low relative risk score	[27**]
KPNA2	Protein	Immunohistochemical staining	377 MIBC patients receiving radical cystectomy	No	OS, visceral metastasis	Low expression	[28]

Abbreviations: DSS, disease-specific survival; OS, overall survival; PFS, progression-free survival.

* Conflicting results from different studies.

Table 2
Biomarkers for treatment selection in advanced bladder cancer: recent discoveries

Biomarker	Detection level	Detection method	Sample population	Independent validation	Outcome	Biomarker status good outcome	Implications for treatment selection	Reference
MRE11	Protein	Immunohistochemical staining	86 and 93 radical radiotherapy patients in test and validation cohorts, respectively; 88 cystectomy patients in the third cohort	Yes	DSS	High expression	Patients with high MRE11 expression are likely to benefit more from radical radiotherapy than from cystectomy	[33**]
BRCA1	mRNA	Quantitative real-time RT-PCR	57 locally advanced bladder cancer patients treated with neoadjuvant chemotherapy	No	OS	Low expression	Patients with lower BRCA1 expression would have more favorable response to neoadjuvant cisplatin-based chemotherapy	[34]
ERCC1	Protein	Immunohistochemical staining	22 MIBC patients treated by chemoradiation therapy	No	Response to chemoradiation therapy	Low expression	Patients with lower ERCC1 level are likely to benefit more from chemoradiotherapy	[35]
	mRNA	Quantitative real-time RT-PCR	108 patients with locally advanced bladder cancer receiving adjuvant chemotherapy	No	PFS	Low expression	Patients with low MDR1 and/or ERCC1 level will likely benefit more than other patients from cisplatin-based adjuvant chemotherapy	[36]
			57 advanced and metastatic patients receiving adjuvant chemotherapy	No	OS	Low expression		[37]
			89 patients with advanced or recurrent disease	No	PFS	Low expression		[38]
MDR1	mRNA	Quantitative real-time RT-PCR	108 patients with locally advanced bladder cancer	No	PFS, OS	Low expression		[36]
ET-1 and ET _A R	mRNA	DNA microarray	Cohort 1 = 26 NMIBC & 65 MIBC patients; cohort 2 = 103 NMIBC & 62 MIBC patients	Yes	DSS	Low expression	Inhibitors of ET-1 and ET _A R as adjuvant therapeutic agents before metastasis	[39**]
	Protein	Immunohistochemical staining	Cohort 3 = 102 MIBC patients	Yes	DSS	Low expression		

Abbreviations: DSS, disease-specific survival; OS, overall survival; PFS, progression-free survival.