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Clinical states model for biomarkers in bladder cancer

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Abstract

Bladder cancer is a significant health care problem in the United States, with a high recurrence rate, the need for expensive continuous surveillance, and limited treatment options for patients with advanced disease. Research has contributed to an understanding of the molecular pathways involved in the development and progression of bladder cancer, and that understanding has led to the discovery of potentially diagnostic, predictive, and prognostic biomarkers. In this review, a clinical states model of bladder cancer is introduced and integrated into a paradigm for biomarker development. Biomarkers are systematically incorporated with predefined endpoints to aid in clinical management.

Keywords

bladder cancer; biomarkers; clinical management; chemotherapy

Bladder cancer overview

In the year 2000, the total expenditure for lower tract urothelial cancers in the United States was more than one billion dollars [1]. The National Cancer Institute estimates that there are over 600,000 individuals living with bladder cancer, and that over 14,000 will have died of this disease in 2008. The cost of bladder cancer per patient from diagnosis to death is the highest of all cancers [2].

Approximately 70% of newly diagnosed bladder cancer patients have “superficial disease” confined to the mucosa. This type of cancer requires lifelong monitoring by cystoscopy and frequent treatment. The remaining 30% of newly diagnosed patients have more advanced disease or progress to higher stages after treatment of superficial disease. Approximately half of those individuals with muscle-invasive bladder cancer develop metastasis within 5 years of surgery and succumb to the disease. Prognosis varies inversely with higher tumor stage and lymph node involvement. The 5- and 10-year survival rates for patients with lymph node involvement are 31% and 23%, respectively [3]. Combination platinum-based chemotherapy is the treatment of choice for patients with metastatic disease; however, median survival is only 15 months, with a 5-year survival rate of 15% [4].

The Advent of Biomarkers

Research has contributed to a detailed understanding of the molecular pathways involved in the development and progression of malignancies. The foundation was set with the

discovery of gain-of-function mutations in oncogenes and loss-of-function mutations in tumor suppressor genes [5]. Molecular alterations involved in tumorigenesis can potentially be used as biomarkers to aid in early disease detection, prediction of treatment response and disease progression, as well as determination of prognosis. Incorporating biomarkers into clinical practice offers the benefit of personalized cancer management and the potential for improved prognosis.

With the discovery of diagnostic, predictive, and prognostic biomarkers in bladder cancer, molecular markers have become an area of intense research, and the number of potential biomarkers in bladder cancer is steadily increasing, including the overexpression of mutated genes, cell-cycle related and apoptotic proteins, immunomodulators, chromosomal alterations including loss of heterozygosity (LOH), epigenetic markers, circulating tumor cells, whole genome-wide array signatures, and microRNAs. These biomarkers are being investigated in urine, blood, and tissue samples. For example, microarray gene expression profiling, which has historically been confined to tumor specimens, is being studied in the blood of cancer patients for the detection of gene expression patterns representing the cancer itself or a host's reaction to the tumor [6,7].

In this review, we examine the potential clinical applications of biomarkers derived from known genetic abnormalities and novel molecular profiling techniques for patients with bladder cancer. Most of these biomarkers are investigational and are undergoing further study, while some others have been approved by the US Food and Drug Administration (FDA) for clinical use. This review is limited to biomarkers that have either been independently validated or offer potential for future investigation. We also introduce a clinical states model for bladder cancer that aligns biomarker studies to the respective clinical states in order to aid in screening, prediction of treatment response, detection of recurrence, and determination of prognosis.

Clinical States Model

It is important to adopt a uniform method for developing and testing biomarkers in bladder cancer, a dynamic disease with a high incidence of recurrence, expensive clinical surveillance, and limited treatment options for patients with advanced disease. Here, we introduce a clinical states model for bladder cancer, adapted from prostate cancer [8]. Each clinical state segregates a discrete population of bladder cancer patients in which novel biomarkers can be systematically incorporated with predefined endpoints to aid in clinical management (Figure).

High-risk individuals

The most appropriate population of high-risk persons, who are likely to develop bladder cancer and for whom screening would be beneficial, is still being defined, but would likely include the following factors: age >65 years, history of tobacco use, other known toxic exposures, hematuria, incidental bladder mass on imaging, and family history of bladder cancer. In patients with no cancer diagnosis, the goal for biomarker development is to create a noninvasive, cost-effective screening test with sensitivity in a high-risk population.

Superficial bladder cancer

The clinical state of superficial bladder cancer includes Ta, Tis, and T1 tumors without lymph node or metastatic involvement (ie, N0-M0) using the TNM classification system. These individuals are managed with transurethral resection, often followed by intravesical therapy. Patients with superficial bladder cancer have a recurrence rate of 50%-70%, with 10%-15% of recurrent tumors progressing to muscle-invasive disease [9]. They undergo frequent surveillance with cystoscopy and urine cytology, the current gold standard for

evaluation and detection of recurrence. The National Comprehensive Cancer Network (NCCN) recommends that patients with superficial bladder cancer undergo surveillance cystoscopy every 3 months for the first 2 years, every 6 months for the next 2 years, then yearly thereafter. However, cystoscopy is an uncomfortable and invasive procedure associated with substantial costs. A noninvasive, low-cost biomarker that could detect recurrence or disease progression has the potential to decrease the frequency of cystoscopic surveillance as well as decrease health care costs and increase compliance with testing.

Muscle-invasive bladder cancer

Muscle-invasive bladder cancer (T2-4a, N0-M0) is managed with either surgery or radiation, frequently incorporating chemotherapy as well. Neoadjuvant cisplatin-based combination chemotherapy improves survival [10-13]. Patients with the potentially best long-term survival have either a complete pathologic response or are “downstaged” from muscle-invasive disease to noninvasive disease following chemotherapy [11,14]. Those patients who respond completely to chemotherapy cannot be identified without a radical cystectomy, but because these patients with a pathologic complete response have an excellent long-term survival, cystectomy should be avoided. Investigators have attempted to substitute clinical response (ie, a normal cystoscopy, negative biopsy, and normal CT scan) for pathological complete response. These attempts have been unsuccessful because one-third to one-half of patients with a clinical complete response have either persistent undetectable muscle-invasive or node-positive disease in the resected specimen at the time of cystectomy [9]. Biomarkers in this clinical state could serve several functions: predicting response to cisplatin-based neoadjuvant chemotherapy; diagnosing metastatic disease not seen on standard imaging; and predicting tumor sensitivity to chemotherapy in order to select those patients who would benefit as well as those who are not predicted to benefit from chemotherapy so that they may proceed directly to surgery.

Metastatic bladder cancer

Bladder cancer is one of the more chemosensitive neoplasms, with response proportions of over 50% to conventional cytotoxic regimens. However, despite a high rate of initial response, the median survival of patients with metastatic disease is only one year [15]. Metastatic bladder cancer represents a difficult clinical disease state to manage. Both prognostic biomarkers and biomarkers to select patients who would benefit most from targeted agents is an area of ongoing research [16]. Current studies are investigating new combinations of cytotoxic agents with improved side effect profiles, as well as novel agents targeting molecular pathways known to be active in bladder tumors.

Screening

Screening for bladder cancer in a high-risk population could decrease the death rate, a benefit underscored by Messing et al [17]. In their study, 1575 men of at least 50 years of age were enrolled from general medical clinics; their urine was repeatedly tested by a chemical reagent strip. Patients with hematuria underwent standard urologic evaluation including cystoscopy. Of the 1575 men screened, 258 (16%) were evaluated for hematuria; of these, 21 participants (8%) were diagnosed with bladder cancer. Men with bladder tumors detected by this hematuria screening were compared with a cohort of 509 men who had newly diagnosed bladder cancer without screening. There were fewer high-grade superficial or invasive bladder tumors in the screened men than in the unscreened men. No men died of bladder cancer in the screened cohort, whereas 20% of unscreened men died of bladder cancer at 14 years of follow-up.

With this improvement in outcome seen for hematuria screening, a number of newer methodologies have been investigated. Hemstreet et al [18] tested a biomarker profile of DNA ploidy, bladder tumor-associated antigen p300, and G-actin from voided urine in 1788 Chinese workers occupationally exposed to benzidine; and they compared these results with 373 nonexposed workers. Twenty-eight bladder cancers were diagnosed in exposed workers (87% of all bladder cancer cases in the cohort), and all of the tumors were clinically organ-confined. This study showed that screening a high-risk population using a cancer-specific biomarker may lead to detection at an early stage.

The cost effectiveness of screening for bladder cancer has been a controversial issue in the medical community. Lotan et al [19] studied the cost-effectiveness of bladder cancer screening in a high-risk population, and evaluated the life years saved. This study found that screening for bladder cancer in a population with a 4% incidence of bladder cancer resulted in a gain of 3.0 life years per 1000 subjects at a cost savings of \$101,000 for the population, assuming a 50% down-staging in the screened population from muscle-invasive to non-muscle-invasive disease. The model concluded that urine-based markers are cost effective in a high-risk population.

Identifying a biomarker that is low cost and highly sensitive is the holy grail of bladder cancer detection. Voided urine offers a unique, noninvasive, and easily accessible means of evaluating for bladder cancer-specific cells, proteins, RNA, and DNA. Although urine cytology is the standard approach for bladder-cancer detection, there is great variability in urine cytology sensitivity and specificity. A consistent limitation is its low sensitivity in low-grade tumors. A variety of proteins specific to bladder cancer development that are released into the urine have been studied in this disease state, including (NMP 22), bladder tumor antigen (BTA-Stat/TRAK), and survivin.

Urine Biomarkers

Nuclear matrix protein (NMP 22)—NMP 22 is part of the internal structural framework in the nucleus, and is an important regulator of mitosis. Its expression is increased in tumor cells; it is released into urine after cell death [20]. Two tests are available for NMP 22—a quantitative ELISA performed in a specialized laboratory and a qualitative point-of-care test, which is easily available and less expensive. The latter can be performed in a physician's office, and the results can be interpreted within 30 minutes. Both of these assays have been studied extensively. The NMP 22 enzyme-linked immunosorbent assay (ELISA) test has a sensitivity that ranges from 47%-100% [21,22]. This variability is a result of different cutoff limits in various studies, and primary versus recurrent tumor detection. The qualitative point-of-care NMP 22 test has a sensitivity of 40%-85% and a specificity of 77%-87% [23,24]. Most studies use NMP 22 to test for the recurrence of bladder cancer in combination with other modalities such as cystoscopy and cytology. Unfortunately, false positives with NMP 22 have been common in patients with benign bladder conditions [25], decreasing its potential as a screening biomarker for incidental bladder tumors. However, NMP 22 has been approved by the FDA for both the detection and monitoring of patients with known bladder cancer.

Bladder tumor antigen (BTA)—BTA measures complement factor H and complement H-related protein produced by bladder cancer in the urine. BTA-Stat is a qualitative point-of-care assay that can be performed in the office, while BTA-TRAK is a quantitative ELISA that must be sent to a specialized laboratory. Sensitivity of BTA-Stat ranges from 57%-82% and specificity ranges from 68%-93% [26-29]. BTA-TRAK has a range of sensitivity from 66%-77% and specificity range from 50%-75% [28,30,31]. Benign urologic conditions, such as benign prostatic hyperplasia, hematuria, urinary tract infections, and urolithiasis, lower

the specificity of BTA-Stat/TRAK [32], making these markers less ideal for screening. These tests have been approved by the FDA in monitoring for recurrent bladder cancer.

Survivin—Survivin is a highly expressed antiapoptotic protein that regulates cell death in the majority of human malignancies. The survivin gene is not expressed in normal tissue, but the high expression in cancer cells has been linked to accelerated relapses, refractory disease, and unfavorable outcome [33,34]. In one study, survivin was detected in the urine samples of all 46 patients with new or recurrent bladder cancer using a novel detection system and RT-PCR methodology. In that study, none of the healthy volunteers or patients with prostate, kidney, vaginal, or cervical cancer had detectable survivin in urine samples [35]. Weikert et al [36] evaluated survivin mRNA in the urine of bladder cancer patients, and reported that survivin outperformed urine cytology in sensitivity (69% compared with 31%) but with similar specificity (100% compared with 97%). Pu et al [37] evaluated urine survivin in 153 bladder cancer patients and 20 healthy volunteers, and found that overall sensitivity and specificity were 90% and 95%, respectively. In this study, the combined use of survivin, cytokeratin 20, and mucin 7 mRNA had the highest sensitivity for bladder cancer detection in voided urine [37]. The high sensitivity of urine survivin in these trials supports its extended evaluation in a high-risk population for bladder cancer screening.

Superficial Disease

Urine Biomarkers

Urine cytology—In addition to proteins, tumor cells are shed in the urine, underscoring the principal rationale for the use of urine cytology. Urine cytology is a highly specific indicator of disease, with sensitivity for the detection of high-grade bladder cancers but poor sensitivity for low-grade disease. It is also a subjective test, with variability in findings depending on the expertise of the pathologist. Urine markers that also detect features of tumor cells include ImmunoCyt, UroVysion, and Telomerase.

ImmunoCyt—ImmunoCyt is based on the visualization of carcinoembryonic antigen and sulfated mucin glycoproteins on bladder cancer cells using immunofluorescent labeled antibodies. The sensitivity of ImmunoCyt varies among studies, ranging between 60% and 100%; specificity ranging from 75% to 84% [38-42]. ImmunoCyt has been reported to be more sensitive than cytology in multiple studies, especially for low-grade, superficial disease [41]. A recent study by Mian et al [43] prospectively evaluated 942 patients who were being followed for superficial bladder cancer. The sensitivity of cytology increased from 8.3% for grade 1 tumors to 75% for grade 3 tumors. With ImmunoCyt, the sensitivity was 79% for grade 1 tumors and increased to 92% for grade 3 tumors. Optimal sensitivity was achieved by combining both cytology and ImmunoCyt, with 79% for grade 1 tumors and 99% for grade 3 tumors. Similar to cytology, ImmunoCyt has significant interobserver variability. ImmunoCyt has also been approved by the FDA to aid in the management of bladder cancer in conjunction with cytology and cystoscopy.

UroVysion—UroVysion is a composite of labeled DNA probes using fluorescent-in-situ hybridization (FISH) for alterations in chromosomes 3,7,17, and 9p21 identified in a given cell. The sensitivity of UroVysion ranges from 69%-74% and specificity ranges from 65%-95% [44-47]. Friedrish et al [46] reported that UroVysion had a better specificity than many other urine markers, but had lower sensitivity than NMP 22. An emerging problem relates to patients found to have positive FISH results but a negative or equivocal cystoscopy. Yoder et al [44] reported on 211 patients with a negative cystoscopy but a positive FISH result; 65% of these patients developed recurrent bladder cancer within 29 months. Thus, patients with a positive UroVysion test but a negative cystoscopy warrant

close surveillance. UroVysion has been approved by the FDA for use in conjunction with cystoscopy to monitor patients for bladder cancer recurrence.

Telomeres—Telomeres are repetitive DNA sequences that protect the end of chromosomes and maintain genomic stability. These DNA sequences are shortened with each successive cell division, leading to cellular senescence. Telomerase is an enzyme found in eukaryotic cells that adds telomeres at the end of chromosomes, therefore making a cell immortal [48]. Telomerase can be measured in exfoliated cells in urine by telomerase repeat amplification protocol (TRAP) or human telomerase reverse transcriptase polymerase chain reaction (RT-PCR) assays. The sensitivity of this assay varies from 70%-90%, with a specificity range from 60%-70%. Sanchini et al [49] reported that the TRAP assay was accurate in distinguishing men with bladder cancer from healthy age-matched controls. In the overall series, sensitivity was 90% and specificity was 88%. Limiting features of this assay are the high incidence of false positives in the setting of benign bladder conditions, and telomerase enzyme degradation in samples that are not processed in a timely manner [50].

Microsatellites—Microsatellites or simple sequence repeats (SSRs) are polymorphic repeating units of 1-6 base pairs in length, found in human DNA. Microsatellites can be amplified for identification by PCR and can be used as molecular markers. Microsatellite analysis (MSA) is a PCR analysis of DNA in exfoliated urine cells [51]. In bladder cancer, loss of heterozygosity (LOH) is the main microsatellite alteration found, with the majority of these located in chromosome 9. Disease progression is usually accompanied by increased chromosomal instability, frequently leading to additional numerical shifts of chromosomes 3p, 8p, 11p, 13q, 16q, and 17p [52-54]. Frigerio et al [55] prospectively analyzed LOH-analysis, UroVysion FISH, and conventional urine cytology on voided urine samples, and compared them with histological findings on consecutive transurethral biopsies. The highest sensitivity was obtained with the combination of LOH-assay and cytology, with 95% of high-grade tumors and 100% of invasive tumors detected using this combination. The authors suggested that surveillance could be modified by alternating cystoscopy with LOH plus cytology to reduce invasive interventions and consequently improve follow-up compliance. A study by Rouprêt et al [56] monitored the surveillance of 40 patients over 1 year with 9 microsatellite markers and the methylation status of 11 gene promoters, and found that microsatellite markers had better performance characteristics than promoter hypermethylation for detecting recurrence. Despite these early promising results in the detection of recurrence or progression of superficial bladder cancer, the MSA markers need to be standardized for this clinical setting.

Tissue Biomarkers

Ras signaling pathway—Low-grade noninvasive papillary tumors are characterized by mutations in the *HRAS* gene and fibroblast growth factor receptor 3 gene (*FGFR3*), indicating that receptor tyrosine kinase-Ras activation has an early and substantial role in bladder cancer tumorigenesis. Recent studies indicate that *HRAS* mutations occur in 10%-20% of bladder cancers [57]. Activation of Ras signaling also occurs as a result of overexpression of *HRAS* in the urothelium, which occurs in >50% of bladder cancers [58]. In addition to *HRAS* mutations and protein overexpression, constitutive activation of several receptor tyrosine kinases that occur upstream of RAS lead to activation of the Ras signaling pathway. *FGFR3* gene mutations are most notable in bladder cancer. As oncogenic activation of both *FGFR3* and Ras is predicted to result in stimulation of the mitogen-activated protein kinase (MAPK) pathway, it has been suggested that these are mutually exclusive events [59].

FGFR3—Alterations in *FGFR3* are seen in other malignancies, and it is both the overexpression and the mutational activation of *FGFR3* that contribute to tumor development [60,61]. In bladder cancer, *FGFR3* mutations are found predominantly in low-stage, low-grade disease, with a frequency of 50% to 67%. Mutations of *FGFR3* have been reported less frequently in invasive tumors [61-63]. The prognostic value of *FGFR3* mutations is not yet known. Hernández et al [63] prospectively evaluated 772 newly diagnosed patients with bladder tumors for a median follow-up of 62.6 months. *FGFR3* mutations were more common among neoplasms with low malignant potential (77%), Ta grade 1 (61%) and Ta grade 2 (58%) tumors. These findings support the impression that *FGFR3* mutations characterize a subgroup of bladder cancers with a good prognosis. A study by van Rhijn et al [64] found the recurrence rate per year of bladder cancer was 0.24 for *FGFR3* mutant tumors and 1.12 for tumors with a wild-type *FGFR3* gene. *FGFR3* mutation status was the strongest predictor of recurrence when compared with stage and grade, identifying patients with favorable disease characteristics. The authors suggested that the frequency of cystoscopic examinations may be reduced in patients with *FGFR3*-positive tumors. Lindgren et al [65] characterized 75 Ta and T1 bladder tumors by using gene expression profiles, mutational analyses of *FGFR3* and *TP53*, and LOH analyses of chromosome 9 and the *TP53* region on chromosome arm 17p. Grade 1 tumors had the highest frequency of *FGFR3* mutations and expression. *FGFR3* mutations may represent a tissue prognostic marker for patients with superficial bladder cancer.

Phosphatidylinositol 3-kinase (PIK3) pathway—Several oncogenes have been identified that activate the phosphatidylinositol 3-kinase (PIK3) pathway. An enzyme of this family encoded by *PIK3CA* locus is mutated in multiple tumors. Analysis of the sequence of exons 9 and 20 of *PIK3CA* in a panel of bladder tumors found that *PIK3CA* mutations are a common event that can occur early in bladder carcinogenesis in association with *FGFR3* mutations [66]. Interestingly, *PIK3CA* mutations are commonly associated with *FGFR3* mutations, and are more frequent in superficial tumors and less common in muscle-invasive disease. *PIK3CA* may constitute a novel prognostic tool, as well as a therapeutic target, in bladder cancer.

Muscle-invasive disease

Clinical trials have demonstrated that neoadjuvant cisplatin-based combination chemotherapy improves survival in patients with muscle-invasive bladder cancer, particularly for those achieving pT1 status after chemotherapy. Unfortunately, clinical assessment of chemotherapy response is inadequate, thus requiring a cystectomy. Biomarkers that can predict chemotherapy response and sensitivity to neoadjuvant chemotherapy are actively in development. Other biomarkers for the clinical state of muscle-invasive bladder cancer include those that may detect disease recurrence and progression, and determine prognosis.

Urine and Blood Biomarkers

TIMP-3—In the process of tumor formation, multiple genetic aberrations take place both in the genome and epigenome, such as DNA methylation. One epigenetic process involves methylation of tumor suppressor gene promoters, leading to gene silencing. Methylation markers may have potential in tumor prognosis. Hoque et al [67] examined urine sediment DNA for aberrant methylation of 9 genes, including tissue inhibitor of metalloproteinase-3 (TIMP-3), and found that the risk of death was statistically significant in patients with higher TIMP-3 methylation. Multivariate analysis subsequently revealed that TIMP-3 methylation was an independent prognostic factor for bladder cancer survival. In addition to urine, methylation markers can be studied in serum. One study used cell-free

serum DNA to examine the hypermethylation of prognostic genes (*adenomatosis polyposis coli*, *glutathione-s-transferase pi-1* and *tazarotene induced gene-1*), and found that this method provided valuable prognostic information [68].

E-cadherin—E-cadherin expression is associated with advanced-stage, high-grade cancers, and a higher probability of cancer progression and metastases. Cadherins are a class of type-1 transmembrane proteins, and play an important role in cell adhesion. Loss of E-cadherin function leads to dissociation of cells from coherent tissues, and generates invasiveness in tumor cells, suggesting that E-cadherin functions as an invasion-suppressor molecule [69]. Soluble forms of E-cadherin have been found in serum and urine, and may correlate with tumor biological behavior [70]. Shi et al [71] studied E-cadherin in 102 bladder tumors and 59 urine samples along with healthy controls, and demonstrated that E-cadherin correlates with grade and stage, but had no significant correlation with the size or number of the tumors.

Normal expression rate of E-cadherin is significantly higher in primary than in recurrent tumors. The level of urinary E-cadherin was higher in patients with bladder cancer than in normal controls or patients with benign disorders of the urinary system. Urinary E-cadherin levels were strongly correlated with tumor grade, and the levels were significantly higher in patients with recurrent tumors. These findings indicate that bladder cancer tissue expression and urinary levels of E-cadherin are closely associated with the biological behavior of bladder cancer. E-cadherin is not only an important adhesion molecule, but also a regulator of intracellular signaling cascades, including the Akt and p42/44MAPK pathways [72]. A recent study found that E-cadherin also seems to play a central role in modulation of epidermal growth factor receptor (EGFR) response in urothelial carcinoma to cetuximab therapy [73]. E-cadherin therefore has the potential to serve as a prognostic marker for bladder cancer and an immune modulator for targeted therapy.

Tissue Biomarkers

Many mechanisms of chemotherapy resistance in cancer have been suggested, but currently there are no validated markers to predict response. Functional resistance to chemotherapy directed against DNA can be achieved through re-establishing effective homologous recombination to repair double-strand breaks [74,75]. Increasingly, evidence suggests that the status of DNA repair mechanisms, specifically nucleotide excision repair and homologous recombination, impact a cancer cell's sensitivity to agents causing interstrand cross links, DNA adducts, and double-strand breaks. For cisplatin-based therapy, low levels of protein expression of excision repair cross complementation-1 (ERCC1) have been associated with improved survival following adjuvant therapy in lung cancer [76]. In patients with advanced lung cancer treated with the combination of gemcitabine and cisplatin, low expression of both ERCC1 and ribonucleotide reductase M1 (RRM1) was found to be associated with an improvement in median survival time [73,77]. Preliminary investigations in bladder cancer suggest that indicators of DNA repair mechanisms may similarly affect clinical response to cisplatin-based chemotherapy. Low mRNA expression of ERCC1 has been associated with improved survival (25.4 months vs 15.4 months) in a small number of patients treated with chemotherapy (n=57) [78,79]; mRNA levels of another DNA repair gene, BRCA1, showed a trend toward significance, but did not meet statistical significance [78]. However, BRCA1 mRNA expression has been associated with chemosensitivity and survival in lung and ovarian cancer patients [80,81]. A recently reported study analyzed BRCA1 mRNA expression by quantitative PCR in tumor biopsies obtained by transurethral resection from 51 patients with locally-advanced bladder cancer [82]. After undergoing chemotherapy, cystectomy was performed, and a close correlation was found between BRCA1 levels and pathological response. Low levels of BRCA1 were

shown to predict response to neoadjuvant cisplatin-based chemotherapy with a longer disease-free survival.

Chemotherapy resistance may be predicted by multiple rather than a few genes. The National Cancer Institute has used a panel of 60 diverse human cancer cell lines (the NCI-60) to screen >100,000 chemical compounds for anticancer activity. Lee and colleagues [83] evaluated expression microarray data for drug sensitivities in the NCI-60 cell lines, and developed an algorithm that predicts sensitivity and specificity to specific agents, termed “co-expression extrapolation” (COXEN). The authors showed that COXEN can accurately predict drug sensitivity of bladder cancer cell lines. They also used COXEN for *in silico* screening of 45,545 compounds, and identified an agent with activity against human bladder cancer. The COXEN algorithm not only has the potential for predicting therapeutic response of a tumor, but may also be useful in screening new therapies for activity.

CD8 lymphocytes—Immunologic interactions between the body and cancer cells are still being deciphered and are a subject of intense study. Recent research suggests an established host response to muscle-invasive bladder cancer. Tumor-infiltrating CD8 lymphocytes have been shown to correlate with prognosis among patients with muscle-invasive bladder cancer [84]. Sharma et al analyzed the presence of intratumoral CD8 T cells in bladder cancer tumors, and correlated the findings with clinical outcome. Patients whose muscle invasive and advanced bladder cancers had higher numbers of tumor-infiltrating CD8 lymphocytes experienced better disease-free survival and overall survival than did similarly staged patients with few intratumoral infiltrating CD8 lymphocytes. These observations are not limited to bladder cancer; similar associations have been reported in other epithelial tumors, including ovarian, colorectal, and renal cancers [85-88]. Tumor-infiltrating CD8 lymphocytes have the potential to serve as a prognostic biomarker in bladder cancer, and underscore the importance of cell-cell communication by the host immune system.

Metastatic Disease

The primary treatment for patients with metastatic bladder cancer is chemotherapy, with approximately 50% of patients responding to conventional cytotoxic regimens. Unfortunately, a minimum of 6-12 weeks of therapy are needed prior to assessment of treatment response with conventional radiographic imaging. Biomarkers that could predict tumor response to therapy either before or earlier in the treatment program would be of great benefit. Biomarkers that could also assist in identifying patients who would benefit from novel targeted agents in development would represent a major advance.

Blood Biomarkers

Circulating tumor cells—The presence of circulating tumor cells (CTCs) in the blood has been demonstrated to be a prognostic factor predicting a poor outcome in solid-tumor malignancies such as prostate, colon, and breast cancer [89-91]. Gallagher et al [92] studied CTCs from the peripheral blood of patients with metastatic bladder cancer, evaluating associations between CTC count and sites of metastatic disease. The authors found a significantly higher number of CTCs in patients with 2 sites of metastases compared to those with 1 site of metastases. Changes in the number of CTCs immediately after starting chemotherapy have particular significance in prostate cancer. Patients whose number of CTCs decline to <5 have a substantially longer survival than those without a CTC decline [93,94]. This information could potentially save patients from ineffective and toxic therapy, and allow for an earlier change to a more effective regimen. Similar studies are under way in bladder cancer.

Blood-based gene expression profiles—Blood-based markers are intrinsically more desirable than tissue markers because they are less invasive and allow for frequent sampling. Blood cells communicate with every tissue and organ in the body and interact closely with other cells, leading to changes in gene expression that result in the activation and suppression of hundreds of genes [95]. The development of gene expression profiles from blood samples of bladder cancer patients was first reported by Osman et al [6]. The authors found that the gene expression profiles of circulating mononuclear cells obtained from patients with bladder cancer were distinctly different from those observed in healthy patients without cancer; a 7-gene signature was identified with a sensitivity of 83% and a specificity of 93%. They also considered the possibility that the divergent hierarchical clustering of genes observed in the patients with bladder cancer was a general phenomenon associated with any cancer. Using hierarchical cluster analysis, they analyzed the genes from the blood of patients with bladder, testicular, and renal cancer, and identified a 524-gene set that discriminated the 3 tumors. Apolo et al [7] recently reported on 85 patients, comparing the gene expression profiles of patients with superficial bladder cancer, metastatic bladder cancer, and benign bladder pathology. The authors found that unsupervised clustering led to an almost complete separation of bladder cancer and benign patients, and a partial separation of superficial bladder cancer and metastatic patients. A subset of 600 genes (230 significantly overexpressed genes and 370 under-expressed genes) was strikingly different between metastatic and non-metastatic patients. A 12-gene signature was found that could differentiate bladder cancer patients with metastatic disease from those without metastatic disease, with a sensitivity of 74%, a specificity of 100%, and overall accuracy of 88%. Blood-based gene expression profiles are attractive novel biomarkers that are currently under development in bladder cancer.

Tissue Biomarkers

P53—Advanced bladder cancers are characterized by loss-of-function mutations in tumor suppressor genes. The most widely studied biomarker in bladder cancer has been p53, which regulates cell cycle activity. A meta-analysis reported by Malats et al [96] reviewed the ability of p53 to predict bladder cancer recurrence, progression, and mortality. One hundred sixty-eight publications from 117 studies and 10,026 patients were reviewed. High-grade tumors were seen in 76% of patients. The distribution of studies was as follows: 70 studies assessed recurrence; 42 progression; 62 mortality; 10 response to chemotherapy; and 4 response to radiotherapy. The final meta-analysis included only those estimates derived from Cox regression. Overall, the meta-analysis showed that changes in p53 were weakly predictive of recurrence, progression, and mortality in bladder cancer.

Gene expression profiling—Gene expression profiling has revolutionized prognostication and treatment outcome prediction. Gene expression profiling of primary tumors using microarrays and correlating signatures with clinical outcome is already used in breast and lung cancer [97-101], and is in development for bladder cancer. Modlich et al [102] used expression profiling by DNA microarray to identify genes underlying clinical heterogeneity of bladder cancer. The authors found several gene clusters that characterized invasive or superficial tumors. In superficial bladder tumors, there were increased mRNA levels of genes encoding transcription factors, protein synthesis and metabolism molecules, and some proteins involved in cell cycle progression and differentiation. In contrast, invasive bladder cancers upregulated transcripts for immune, extracellular matrix, adhesion, peritumoral stroma and muscle tissue components, proliferation, and cell-cycle controllers. Another study identified genetic signatures characteristic of aggressive clinical behavior in advanced bladder tumors. Hierarchical clustering classified normal urothelium, superficial, and invasive tumors with 82.2% accuracy, and stratified bladder tumors on the basis of clinical outcome [103]. Others studies have also identified expression profile signatures that

can distinguish early and late tumor stage in bladder cancer, and even tumor behavior and aggressiveness [104-108]. These findings support the value of gene expression profiles as prognostic markers in bladder cancer. One author studied the predictive potential of tumor gene expression profiles, and developed a 14-gene profile that separated muscle-invasive bladder cancer patients who responded to cisplatin-based chemotherapy and those who did not [109].

MicroRNAs—MicroRNAs (miRNA) are small, non-protein-coding RNAs that function as negative gene regulators controlling hundreds of gene targets [110]. They are novel biomarkers that are currently under intense investigation. microRNAs appear to be intrinsically involved in cellular regulation including cell proliferation and apoptosis [111-113], and in tumor development with the expression of oncogenic and tumor suppression genes [110,114-116]. Like gene expression profiles, miRNA can segregate tumors of different stages and biological behaviors in lung, breast, prostate, gastric, colon, and pancreatic cancer [117-122]. Tavazoie et al [116] identified 2 microRNAs, miR-335 and miR-126, which suppress metastases in human breast cancer by regulating genes that reduce overall tumor growth, proliferation, and cell invasion. Neely et al studied miRNA in bladder cancer tumors, and identified a miR-21:miR-205 expression ratio that has the ability to distinguish between invasive and noninvasive bladder tumors with the potential to identify superficial lesions at high risk for progression [123]. Further studies are needed to validate these miRNAs in human cancers; however, they offer potential value in determining prognosis.

Conclusion

Bladder cancer is a dynamic malignancy with several distinct disease states. A number of bladder cancer biomarkers can potentially be applied to the clinical management of patients with suspected or an actual diagnosis of bladder cancer. The incorporation of the bladder cancer clinical states model helps to define end points that may be utilized for biomarker development. For the clinical state of no bladder cancer diagnosis in a high-risk individual, potential urine biomarkers for screening include BTA-Stat/TRAK, NMP-22, and survivin. For patients with superficial disease, urinary biomarkers for the detection of recurrence or progression include BTA-Stat/TRAK, NMP-22, survivin, ImmunoCyt, UroVysion, telomerase, microsatellites, and DNA methylation. Tumor markers include *HRAS*, *FGFR3* and *PIK3CA* mutations. Biomarkers in muscle-invasive bladder cancer can be either predictive or prognostic, and include E-cadherin in urine and tissue, and ERCC1, BRCA-1, COXEN assay, and CD8 T cells in tumor tissue. In the clinical state of metastatic disease, biomarkers may be predictive and prognostic. They include gene expression profiles, CTCs in blood, gene expression profiles, microRNA, DNA repair enzymes, and infiltrating T cells in tumors.

Future perspectives

Biomarkers have the potential to dramatically change the way bladder cancer is managed. An increased understanding of bladder cancer biology has resulted in the discovery of new markers for multiple disease states. Many of these biomarkers are still in early development, but they will serve as powerful tools if they are found to be suitable for clinical practice. It is important for the research community to integrate these biomarkers into correlative clinical protocols in order to validate these markers within the clinical states model of bladder cancer.

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References

1. Whelan P. Bladder Cancer--Contemporary Dilemmas in Its Management. *European Urology*. 2008; 53(1):24–26. [PubMed: 17889428]
2. Botteman MF, Pashos CL, Redaelli A, Laskin B, Hauser R. The health economics of bladder cancer: a comprehensive review of the published literature. *Pharmacoeconomics*. 2003; 21(18):1315–1330. [PubMed: 14750899]
3. Stein JP, Lieskovsky G, Cote R, et al. Radical cystectomy in the treatment of invasive bladder cancer: long-term results in 1,054 patients. *J Clin Oncol*. 2001; 19(3):666–675. [PubMed: 11157016]
4. von der Maase H, Sengelov L, Roberts JT, et al. Long-term survival results of a randomized trial comparing gemcitabine plus cisplatin, with methotrexate, vinblastine, doxorubicin, plus cisplatin in patients with bladder cancer. *J Clin Oncol*. 2005; 23(21):4602–4608. [PubMed: 16034041]
5. Bishop, J.; Weinberg, RA. *Molecular Oncology*. Scientific American, Inc; New York: 1996.
6. Osman I, Bajorin DF, Sun TT, et al. Novel Blood Biomarkers of Human Urinary Bladder Cancer. *Clin Cancer Res*. 2006; 12(11):3374–3380. [PubMed: 16740760]
7. Apolo AB, Osman I, Shen R, Olshen AB, Bajorin DF. Peripheral-blood gene expression profiling in bladder cancer (BC) patients (pts). *Proc Am Soc Clin Oncol*. 2008; 26 Abstract 5076.
8. Scher HI, Heller G. Clinical states in prostate cancer: towards a dynamic model of disease progression. *Urology*. 2000; 55:323–327. [PubMed: 10699601]
9. Pagano F, Bassi P, Galetti TP, et al. Results of contemporary radical cystectomy for invasive bladder cancer: A clinicopathological study with an emphasis on the inadequacy of the tumor, nodes and metastases classification. *Journal of Urology*. 1991; 145(1):45–50. [PubMed: 1984097]
10. Neoadjuvant chemotherapy in invasive bladder cancer: a systematic review and meta-analysis. *Lancet*. 2003; 7(361):1927–1934.
11. Grossman HB, Natale RB, Tangen CM, et al. Neoadjuvant chemotherapy plus cystectomy compared with cystectomy alone for locally advanced bladder cancer. *New Eng J Med*. 2003; 349:859–866. [PubMed: 12944571]
12. Hall R. on behalf of the Intl Collaboration of Trialists of the MRC Advanced Bladder Cancer Group MCTU, London, UK. Updated results of a randomised controlled trial of neoadjuvant cisplatin (C), methotrexate (M) and vinblastine (V) chemotherapy for muscle-invasive bladder cancer (abstract 710). *Proc Am Soc Clin Oncol*. 2002; 21:178a.
13. Neoadjuvant chemotherapy in invasive bladder cancer: update of a systematic review and meta-analysis of individual patient data advanced bladder cancer (ABC) meta-analysis collaboration. *Eur Urol*. 2005; 48(2):202–206. [PubMed: 15939524]
14. Splinter TA, Scher HI, Denis L, et al. The prognostic value of the pathological response to combination chemotherapy before cystectomy in patients with invasive bladder cancer. *European Organization for Research on Treatment of Cancer- Genitourinary Group. J Urol*. 1992; 147:606–608. [PubMed: 1538438]
15. von der Maase H, Hansen SW, Roberts JT, et al. Gemcitabine and cisplatin versus methotrexate, vinblastine, doxorubicin, and cisplatin in advanced or metastatic bladder cancer: results of a large randomized multicenter, multinational multicenter, phase III study. *J Clin Oncol*. 2000; 18:3068–3077. [PubMed: 11001674]
16. Marrer E, D F. Biomarkers in oncology drug development: rescuers or troublemakers? *Expert Opinion on Drug Metabolism & Toxicology*. 2008; 4(11):1391–1402. [PubMed: 18950281]
17. Messing EM, Madeb R, Young T, et al. Long-term outcome of hematuria home screening for bladder cancer in men. *Cancer*. 2006; 107(9):2173–2179. [PubMed: 17029275]
18. Hemstreet GP III, Yin S, Ma Z, et al. Biomarker Risk Assessment and Bladder Cancer Detection in a Cohort Exposed to Benzidine. *J Natl Cancer Inst*. 2001; 93(6):427–436. [PubMed: 11259468]

19. Lotan Y, Svatek RS, Sagalowsky AI. Should we screen for bladder cancer in a high-risk population? *Cancer*. 2006; 107(5):982–990. [PubMed: 16862567]
20. Black PC, Brown GA, Dinney CP. Molecular markers of urothelial cancer and their use in the monitoring of superficial urothelial cancer. *J Clin Oncol*. 2006; 24(35):5528–5535. [PubMed: 17158538]
21. Lokeshwar VB, Habuchi T, Grossman HB, et al. Bladder tumor markers beyond cytology: International Consensus Panel on bladder tumor markers. *Urology*. 2005; 66(6, Supplement 1):35–63. [PubMed: 16399415]
22. Lokeshwar VB, Soloway MS. Current bladder tumor tests: Does their projected utility fulfill clinical necessity? *Journal of Urology*. 2001; 165(4):1067–1077. [PubMed: 11257640]
23. Grossman HB, Soloway M, Messing E, et al. Surveillance for Recurrent Bladder Cancer Using a Point-of-Care Proteomic Assay. 2006:299–305.
24. Kumar A, K R, Gupta NP. Comparison of NMP22 BladderChek Test and Urine Cytology for the Detection of Recurrent Bladder Cancer. *Jpn J Clin Oncol*. 2006; 36(3):172–175. [PubMed: 16520358]
25. Stampfer DS, Carpinito GA, Rodriguez-Villanueva J, et al. Evaluation of NMP22 in the detection of transitional cell carcinoma of the bladder. *The Journal of urology*. 1998; 159(2):394–398. [PubMed: 9649246]
26. Wiener HG, Mian C, Haitel A, Pycha A, Schatzl G, Marberger M. Can urine bound diagnostic tests replace cystoscopy in the management of bladder cancer? *Journal of Urology*. 1998; 159(6):1876–1880. [PubMed: 9598479]
27. Sarosdy MF, Hudson MA, Ellis WJ, et al. Improved detection of recurrent bladder cancer using the bard BTA stat test. *Urology*. 1997; 50(3):349–353. [PubMed: 9301696]
28. Heicappell R, Muller M, Fimmers R, Miller K. Qualitative determination of urinary human complement factor H-related protein (hcfHrp) in patients with bladder cancer, healthy controls, and patients with benign urologic disease. *Urologia internationalis*. 2000; 65(4):181–184. [PubMed: 11112865]
29. Pode D, S A, Wald M, Nativ O, Laufer M, Kaver I. Noninvasive detection of bladder cancer with the BTA Stat test. *The Journal of Urology*. 1999; 161(2):443–446. [PubMed: 9915422]
30. Ellis WJ, Blumenstein BA, Ishak LM, Enfield DL. Clinical evaluation of the BTA trak assay and comparison to voided urine cytology and the bard BTA test in patients with recurrent bladder tumors. *Urology*. 1997; 50(6):882–887. [PubMed: 9426718]
31. Thomas L, Leyh H, Marberger M, et al. Multicenter trial of the quantitative BTA TRAK assay in the detection of bladder cancer. *Clinical Chemistry*. 1999; 45(4):472–477. [PubMed: 10102906]
32. Alvarez A, Lokeshwar VB. Bladder cancer biomarkers: current developments and future implementation. *Current opinion in urology*. 2007; 17(5):341–346. [PubMed: 17762628]
33. Salz W, Eisenberg D, Plescia J, et al. A Survivin Gene Signature Predicts Aggressive Tumor Behavior. *Cancer Research*. 2005; 65(9):3531–3534. [PubMed: 15867343]
34. Altieri DC. Validating survivin as a cancer therapeutic target. *Nat Rev Cancer*. 2003; 3(1):46–54. [PubMed: 12509766]
35. Smith SD, Wheeler MA, Plescia J, Colberg JW, Weiss RM, Altieri DC. Urine Detection of Survivin and Diagnosis of Bladder Cancer. *JAMA*. 2001; 285(3):324–328. [PubMed: 11176843]
36. Weikert S, C F, Schrader M, Krause H, Miller K, Müller M. Quantitative analysis of survivin mRNA expression in urine and tumor tissue of bladder cancer patients and its potential relevance for disease detection and prognosis. *Int J Cancer*. 2005; 116(1):100–104. [PubMed: 15761870]
37. Pu XY, Wang ZP, Chen YR, Wang XH, Wu YL, Wang HP. The value of combined use of survivin, cytokeratin 20 and mucin 7 mRNA for bladder cancer detection in voided urine. *Journal of cancer research and clinical oncology*. 2008; 134(6):659–665. [PubMed: 18026991]
38. Lodde M, Mian C, Negri G, et al. Role of uCyt+ in the detection and surveillance of urothelial carcinoma. *Urology*. 2003; 61(1):243–247. [PubMed: 12559316]
39. Pfister C, Chautard D, Devonec M, et al. Immunocyt Test Improves the Diagnostic Accuracy of Urinary Cytology: Results of a French Multicenter Study. *The Journal of Urology*. 2003; 169(3):921–924. [PubMed: 12576813]

40. Hautmann S, Toma M, Gomez MFL, et al. Immunocyt and the HA-HAase Urine Tests for the Detection of Bladder Cancer: A Side-by-Side Comparison. *European Urology*. 2004; 46(4):466–471. [PubMed: 15363562]
41. Messing EM, Teot L, Korman H, et al. Performance of urine test in patients monitored for recurrence of bladder cancer: a multicenter study in the United States. *Journal of Urology*. 2005; 174(4 Pt 1):1238–1241. [PubMed: 16145377]
42. Mian C, Pycha A, Wiener H, Haitel A, Lodde M, Marberger M. Immunocyt: A new tool for detecting transitional cell cancer of the urinary tract. *Journal of Urology*. 1999; 161(5):1486–1489. [PubMed: 10210378]
43. Mian C, M K, Comploj E, Lodde M, Berner L, Lusuardi L, Palermo S, Vittadello F, Pycha A. uCyt +/ImmunoCyt in the detection of recurrent urothelial carcinoma: an update on 1991 analyses. *Cancer*. 2006; 108(1):60–65. [PubMed: 16411183]
44. Yoder BJ, Skacel M, Hedgepeth R, et al. Reflex UroVysion testing of bladder cancer surveillance patients with equivocal or negative urine cytology: a prospective study with focus on the natural history of anticipatory positive findings. *American journal of clinical pathology*. 2007; 127(2): 295–301. [PubMed: 17210520]
45. Sarosdy MF, Schellhammer P, Bokinsky G, et al. Clinical Evaluation of a Multi-target Fluorescent in Situ Hybridization Assay for Detection of Bladder Cancer. *The Journal of Urology*. 2002; 168(5):1950–1954. [PubMed: 12394683]
46. Friedrich MG, Toma AH, Pantel K, Weisenberger DJ, Noldus J, Huland H. Comparison of multitarget fluorescence in situ hybridization in urine with other noninvasive tests for detecting bladder cancer. *BJU International*. 2003; 92(9):911–914. [PubMed: 14632845]
47. Laudadio J, Keane TE, Reeves HM, Savage SJ, Hoda RS, Lage Daynna JM, Wolff J. Fluorescence in situ hybridization for detecting transitional cell carcinoma: implications for clinical practice. *BJU International*. 2005; 96(9):1280–1285. [PubMed: 16287445]
48. Zakian VA. Life and Cancer Without Telomerase. *Cell*. 1997; 91(1):1–3. [PubMed: 9335327]
49. Sanchini MA, Gunelli R, Nanni O, et al. Relevance of Urine Telomerase in the Diagnosis of Bladder Cancer. *JAMA*. 2005; 294(16):2052–2056. [PubMed: 16249419]
50. Liu BCS, Loughlin KR. Telomerase in human bladder cancer. *Urologic Clinics of North America*. 2000; 27(1):115–123. [PubMed: 10696250]
51. Mao L, Schoenberg MP, Scicchitano M, et al. Molecular detection of primary bladder cancer by microsatellite analysis. *Science*. 1996; 271(5249):659–662. [PubMed: 8571131]
52. Fearon ER, Feinberg AP, Hamilton SH, Vogelstein B. Loss of genes on the short arm of chromosome 11 in bladder cancer. *Nature*. 1985; 318(6044):377–380. [PubMed: 2999610]
53. Yoon DS, Li L, Zhang RD, et al. Genetic mapping and DNA sequence-based analysis of deleted regions on chromosome 16 involved in progression of bladder cancer from occult preneoplastic conditions to invasive disease. *Oncogene*. 2001; 20(36):5005–5014. [PubMed: 11526485]
54. Presti JC, Reuter VE, Galan T, Fair WR, Cordon-Cardo C. Molecular genetic alterations in superficial and locally advanced human bladder cancer. *Cancer Res*. 1991; 51:5405–5409. [PubMed: 1680549]
55. Frigerio S, Padberg BC, Strebel RT, Lenggenhager DM, Messthaler A, Abdou MT, Moch H, Zimmermann DR. Improved detection of bladder carcinoma cells in voided urine by standardized microsatellite analysis. *Int J Cancer*. 2007; 121(2):329–338. [PubMed: 17373664]
56. Rouprêt M, H V, Yates DR, Comperat E, Catto JW, Meuth M, Lackmichi A, Ricci S, Lacave R, Gattegno B, Richard F, Hamdy FC, Cussenot O. A comparison of the performance of microsatellite and methylation urine analysis for predicting the recurrence of urothelial cell carcinoma, and definition of a set of markers by Bayesian network analysis. *BJU International*. 2008; 101(11):1448–1453. [PubMed: 18325051]
57. Knowles MA. Role of FGFR3 in urothelial cell carcinoma: biomarker and potential therapeutic target. *World J Urol*. 2007
58. Wu XR. Urothelial tumorigenesis: a tale of divergent pathways. *Nat Rev Cancer*. 2005; 5(9):713–725. [PubMed: 16110317]

59. Jebar AH, Hurst CD, Tomlinson DC, Johnston C, Taylor CF, Knowles MA. FGFR3 and Ras gene mutations are mutually exclusive genetic events in urothelial cell carcinoma. *Oncogene*. 2005; 24(33):5218–5225. [PubMed: 15897885]
60. Black PC, Agarwal PK, Dinney CP. Targeted therapies in bladder cancer--an update. *Urol Oncol*. 2007; 25(5):433–438. [PubMed: 17826665]
61. Tomlinson DC, Baldo O, Harnden P, Knowles MA. FGFR3 protein expression and its relationship to mutation status and prognostic variables in bladder cancer. *J Pathol*. 2007; 213(1):91–98. [PubMed: 17668422]
62. van Rhijn BW, Vis AN, van der Kwast TH, et al. Molecular grading of urothelial cell carcinoma with fibroblast growth factor receptor 3 and MIB-1 is superior to pathologic grade for the prediction of clinical outcome. *J Clin Oncol*. 2003; 21(10):1912–1921. [PubMed: 12743143]
63. Hernandez S, Lopez-Knowles E, Lloreta J, et al. Prospective study of FGFR3 mutations as a prognostic factor in nonmuscle invasive urothelial bladder carcinomas. *J Clin Oncol*. 2006; 24(22):3664–3671. [PubMed: 16877735]
64. van Rhijn BWG, Lurkin I, Radvanyi F, Kirkels WJ, van der Kwast TH, Zwarthoff EC. The Fibroblast Growth Factor Receptor 3 (FGFR3) Mutation Is a Strong Indicator of Superficial Bladder Cancer with Low Recurrence Rate. *Cancer Research*. 2001; 61(4):1265–1268. [PubMed: 11245416]
65. Lindgren D, Liedberg F, Andersson A, et al. Molecular characterization of early-stage bladder carcinomas by expression profiles, FGFR3 mutation status, and loss of 9q. *Oncogene*. 2006; 25(18):2685–2696. [PubMed: 16532037]
66. Lopez-Knowles E, Hernandez S, Malats N, et al. PIK3CA Mutations Are an Early Genetic Alteration Associated with FGFR3 Mutations in Superficial Papillary Bladder Tumors. *Cancer Research*. 2006; 66(15):7401–7404. [PubMed: 16885334]
67. Hoque MO, Begum S, Brait M, et al. Tissue Inhibitor of Metalloproteinases-3 Promoter Methylation is an Independent Prognostic Factor for Bladder Cancer. *The Journal of Urology*. 2008; 179(2):743–747. [PubMed: 18082200]
68. Ellinger J, El Kassem N, Heukamp LC, et al. Hypermethylation of Cell-Free Serum DNA Indicates Worse Outcome in Patients With Bladder Cancer. *The Journal of Urology*. 2008; 179(1):346–352. [PubMed: 18006010]
69. Vleminckx K, Vakaet L, Mareel M, Fiers W, Van Roy F. Genetic manipulation of E-cadherin expression by epithelial tumor cells reveals an invasion suppressor role. *Cell*. 1991; 66(1):107–119. [PubMed: 2070412]
70. Durkan B, Mellon. The impact of transurethral resection of bladder tumour on serum levels of soluble E-cadherin. *BJU International*. 1999; 83(4):424–428. [PubMed: 10210565]
71. Shi B, Laudon V, Yu S, Dong D, Zhu Y, Xu Z. E-cadherin tissue expression and urinary soluble forms of E-cadherin in patients with bladder transitional cell carcinoma. *Urologia internationalis*. 2008; 81(3):320–324. [PubMed: 18931551]
72. Pece S, Gutkind JS. Signaling from E-cadherins to the MAPK Pathway by the Recruitment and Activation of Epidermal Growth Factor Receptors upon Cell-Cell Contact Formation. *J Biol Chem*. 2000; 275(52):41227–41233. [PubMed: 10969083]
73. Black PC, Brown GA, Inamoto T, et al. Sensitivity to Epidermal Growth Factor Receptor Inhibitor Requires E-Cadherin Expression in Urothelial Carcinoma Cells. *Clin Cancer Res*. 2008; 14(5):1478–1486. [PubMed: 18316572]
74. Edwards SL, Brough R, Lord CJ, et al. Resistance to therapy caused by intragenic deletion in BRCA2. *Nature*. 2008; 451(7182):1111–1115. [PubMed: 18264088]
75. Sakai W, Swisher EM, Karlan BY, et al. Secondary mutations as a mechanism of cisplatin resistance in BRCA2-mutated cancers. *Nature*. 2008; 451(7182):1116–1120. [PubMed: 18264087]
76. Olausson KA, Dunant A, Fouret P, et al. DNA Repair by ERCC1 in Non-Small-Cell Lung Cancer and Cisplatin-Based Adjuvant Chemotherapy. *N Engl J Med*. 2006; 355(10):983–991. [PubMed: 16957145]
77. Ceppi P, Volante M, Novello S, et al. ERCC1 and RRM1 gene expressions but not EGFR are predictive of shorter survival in advanced non-small-cell lung cancer treated with cisplatin and gemcitabine. *Ann Oncol*. 2006; 17(12):1818–1825. [PubMed: 16980606]

78. 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):522–528. [PubMed: 17229776]
79. Kim K, D I, Yi S, Lim D, Ji S, Park M, Park S, Kwon G, Lim H. ERCC1 expression in advanced bladder cancer patients receiving gemcitabine/cisplatin chemotherapy. *Proc Am Soc Clin Oncol (GU ASCO).* 2009 Abstract 269.
80. Taron M, Rosell R, Felip E, et al. BRCA1 mRNA expression levels as an indicator of chemoresistance in lung cancer. *Human molecular genetics.* 2004; 13(20):2443–2449. [PubMed: 15317748]
81. Quinn JE, James CR, Stewart GE, et al. BRCA1 mRNA Expression Levels Predict for Overall Survival in Ovarian Cancer after Chemotherapy. *Clin Cancer Res.* 2007; 13(24):7413–7420. [PubMed: 18094425]
82. Font A, Taron M, Costa C, et al. BRCA1 mRNA expression in patients (p) with bladder cancer treated with neoadjuvant cisplatin-based chemotherapy. *Proc Am Soc Clin Oncol.* 2008; 26(15_suppl):5021.
83. 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):13086–13091. [PubMed: 17666531]
84. Sharma P, Shen Y, Wen S, et al. CD8 tumor-infiltrating lymphocytes are predictive of survival in muscle-invasive urothelial carcinoma. *Proc Natl Acad Sci U S A.* 2007; 104(10):3967–3972. [PubMed: 17360461]
85. Zhang L, Conejo-Garcia JR, Katsaros D, et al. Intratumoral T Cells, Recurrence, and Survival in Epithelial Ovarian Cancer. *N Engl J Med.* 2003; 348(3):203–213. [PubMed: 12529460]
86. Naito Y, Saito K, Shiiba K, et al. CD8+ T Cells Infiltrated within Cancer Cell Nests as a Prognostic Factor in Human Colorectal Cancer. *Cancer Res.* 1998; 58(16):3491–3494. [PubMed: 9721846]
87. Galon J, Costes A, Sanchez-Cabo F, et al. Type, Density, and Location of Immune Cells Within Human Colorectal Tumors Predict Clinical Outcome. *Science.* 2006; 313(5795):1960–1964. [PubMed: 17008531]
88. Nakano O, Sato M, Naito Y, et al. Proliferative Activity of Intratumoral CD8+ T-Lymphocytes As a Prognostic Factor in Human Renal Cell Carcinoma: Clinicopathologic Demonstration of Antitumor Immunity. *Cancer Res.* 2001; 61(13):5132–5136. [PubMed: 11431351]
89. Danila DC, Heller G, Gignac GA, et al. Circulating Tumor Cell Number and Prognosis in Progressive Castration-Resistant Prostate Cancer. *Clin Cancer Res.* 2007; 13(23):7053–7058. [PubMed: 18056182]
90. Sastre J, Maestro ML, Puente J, et al. Circulating tumor cells in colorectal cancer: correlation with clinical and pathological variables. *Ann Oncol.* 2008; 19(5):935–938. [PubMed: 18212090]
91. Cristofanilli M, Budd GT, Ellis MJ, et al. Circulating tumor cells, disease progression, and survival in metastatic breast cancer. *N Engl J Med.* 2004; 351(8):781–791. [PubMed: 15317891]
92. Gallagher DJ, Milowsky MI, Ishill N, et al. Detection of circulating tumor cells in patients with urothelial cancer. *Ann Oncol.* 2008 mdn627.
93. Wu C, Hao H, Li L, et al. Preliminary investigation of the clinical significance of detecting circulating tumor cells enriched from lung cancer patients. *J Thorac Oncol.* 2009; 4(1):30–36. [PubMed: 19096303]
94. de Bono JS, Scher HI, Montgomery RB, et al. Circulating Tumor Cells Predict Survival Benefit from Treatment in Metastatic Castration-Resistant Prostate Cancer. *Clin Cancer Res.* 2008; 14(19):6302–6309. [PubMed: 18829513]
95. Liew CC, Ma J, Tang HC, Zheng R, Dempsey AA. The peripheral blood transcriptome dynamically reflects system wide biology: a potential diagnostic tool. *J Lab Clin Med.* 2006; 147(3):126–132. [PubMed: 16503242]
96. Malats N, Bustos A, Nascimento CM, et al. P53 as a prognostic marker for bladder cancer: a meta-analysis and review. *The Lancet Oncology.* 2005; 6(9):678–686. [PubMed: 16129368]

97. Choi NN, Son DSDS, Lee JJ, et al. The signature from messenger RNA expression profiling can predict lymph node metastasis with high accuracy for non-small cell lung cancer. *Journal of Thoracic Oncology*. 2006; 1(7):622–628. [PubMed: 17409928]
98. Dressman HK, Hans C, Bild A, et al. Gene Expression Profiles of Multiple Breast Cancer Phenotypes and Response to Neoadjuvant Chemotherapy. *Clin Cancer Res*. 2006; 12(3):819–826. [PubMed: 16467094]
99. Chen HY, Yu SL, Chen CH, et al. A Five-Gene Signature and Clinical Outcome in Non-Small-Cell Lung Cancer. *N Engl J Med*. 2007; 356(1):11–20. [PubMed: 17202451]
100. Cronin M, Sangli C, Liu ML, et al. Analytical validation of the Oncotype DX genomic diagnostic test for recurrence prognosis and therapeutic response prediction in node-negative, estrogen receptor-positive breast cancer. *Clinical chemistry*. 2007; 53(6):1084–1091. [PubMed: 17463177]
101. Rouzier R, Rajan R, Wagner P, et al. Microtubule-associated protein tau: a marker of paclitaxel sensitivity in breast cancer. *Proc Natl Acad Sci U S A*. 2005; 102(23):8315–8320. [PubMed: 15914550]
102. Modlich O, Prisack HB, Pitschke G, et al. Identifying Superficial, Muscle-Invasive, and Metastasizing Transitional Cell Carcinoma of the Bladder: Use of cDNA Array Analysis of Gene Expression Profiles. *Clin Cancer Res*. 2004; 10(10):3410–3421. [PubMed: 15161696]
103. Sanchez-Carbayo M, Socci ND, Lozano J, Saint F, Cordon-Cardo C. Defining molecular profiles of poor outcome in patients with invasive bladder cancer using oligonucleotide microarrays. *J Clin Oncol*. 2006; 24(5):778–789. [PubMed: 16432078]
104. Dyrskjot L, Thykjaer T, Kruhoffer M, et al. Identifying distinct classes of bladder carcinoma using microarrays. *Nature genetics*. 2003; 33(1):90–96. [PubMed: 12469123]
105. Dyrskjot L, Kruhoffer M, Thykjaer T, et al. Gene expression in the urinary bladder: a common carcinoma in situ gene expression signature exists disregarding histopathological classification. *Cancer research*. 2004; 64(11):4040–4048. [PubMed: 15173019]
106. Elsamman E, Fukumori T, Ewis AA, et al. Differences in gene expression between noninvasive and invasive transitional cell carcinoma of the human bladder using complementary deoxyribonucleic acid microarray: preliminary results. *Urologic oncology*. 2006; 24(2):109–115. [PubMed: 16520272]
107. Sanchez-Carbayo M, Socci ND, Charytonowicz E, et al. Molecular profiling of bladder cancer using cDNA microarrays: defining histogenesis and biological phenotypes. *Cancer Res*. 2002; 62(23):6973–6980. [PubMed: 12460915]
108. Blaveri E, Simko JP, Korkola JE, et al. Bladder cancer outcome and subtype classification by gene expression. *Clin Cancer Res*. 2005; 11(11):4044–4055. [PubMed: 15930339]
109. Takata R, Katagiri T, Kanehira M, et al. Predicting response to methotrexate, vinblastine, doxorubicin, and cisplatin neoadjuvant chemotherapy for bladder cancers through genome-wide gene expression profiling. *Clin Cancer Res*. 2005; 11(7):2625–2636. [PubMed: 15814643]
110. Esquela-Kerscher A, Slack FJ. Oncomirs - microRNAs with a role in cancer. *Nat Rev Cancer*. 2006; 6(4):259–269. [PubMed: 16557279]
111. Hayashita Y, Osada H, Tatematsu Y, et al. A polycistronic microRNA cluster, miR-17-92, is overexpressed in human lung cancers and enhances cell proliferation. *Cancer Res*. 2005; 65(21):9628–9632. [PubMed: 16266980]
112. Si ML, Zhu S, Wu H, Lu Z, Wu F, Mo YY. miR-21-mediated tumor growth. *Oncogene*. 2007; 26(19):2799–2803. [PubMed: 17072344]
113. Chan JA, Krichevsky AM, Kosik KS. MicroRNA-21 is an antiapoptotic factor in human glioblastoma cells. *Cancer research*. 2005; 65(14):6029–6033. [PubMed: 16024602]
114. Kent OA, Mendell JT. A small piece in the cancer puzzle: microRNAs as tumor suppressors and oncogenes. *Oncogene*. 2006; 25(46):6188–6196. [PubMed: 17028598]
115. Calin GA, Croce CM. MicroRNA signatures in human cancers. *Nat Rev Cancer*. 2006; 6(11):857–866. [PubMed: 17060945]
116. Tavazoie SF, Alarcon C, Oskarsson T, et al. Endogenous human microRNAs that suppress breast cancer metastasis. *Nature*. 2008; 451(7175):147–152. [PubMed: 18185580]

117. Yanaihara N, Caplen N, Bowman E, et al. Unique microRNA molecular profiles in lung cancer diagnosis and prognosis. *Cancer cell*. 2006; 9(3):189–198. [PubMed: 16530703]
118. Lu J, Getz G, Miska EA, et al. MicroRNA expression profiles classify human cancers. *Nature*. 2005; 435(7043):834–838. [PubMed: 15944708]
119. Iorio MV, Ferracin M, Liu CG, et al. MicroRNA gene expression deregulation in human breast cancer. *Cancer Res*. 2005; 65(16):7065–7070. [PubMed: 16103053]
120. Porkka KP, Pfeiffer MJ, Waltering KK, Vessella RL, Tammela TL, Visakorpi T. MicroRNA expression profiling in prostate cancer. *Cancer research*. 2007; 67(13):6130–6135. [PubMed: 17616669]
121. Volinia S, Calin GA, Liu CG, et al. A microRNA expression signature of human solid tumors defines cancer gene targets. *Proc Natl Acad Sci U S A*. 2006; 103(7):2257–2261. [PubMed: 16461460]
122. Bloomston M, Frankel WL, Petrocca F, et al. MicroRNA expression patterns to differentiate pancreatic adenocarcinoma from normal pancreas and chronic pancreatitis. *Jama*. 2007; 297(17):1901–1908. [PubMed: 17473300]
123. Neely LA, Rieger-Christ KM, Neto BS, et al. A microRNA expression ratio defining the invasive phenotype in bladder tumors. *Urologic Oncology: Seminars and Original Investigations*. In Press, Corrected Proof.

Figure. A dynamic clinical states model in bladder cancer (Adapted from Scher et al[8])

Table 1
Goals of biomarkers in bladder cancer

Clinical State	Biomarker Objectives
Initial bladder evaluation	Screening
(no cancer diagnosis)	Diagnosis
Superficial disease	Recurrence
	Progression
	Prognosis
Invasive disease	Recurrence
	Progression
	Prognosis
	Prediction
Metastatic disease	Prognosis
	Prediction

Table 2
Biomarkers by clinical states in bladder cancer, techniques and objectives

Source	Biomarkers	Technique	Objectives
CLINICAL STATE: Initial Bladder Evaluation			
Urine	BTA Stat	Dipstick colorimetric antigen-antibody reaction for human complement factor H-related protein	Screening and diagnosis
	BTA TRAK	Sandwich ELISA for human complement factor H-related protein Sandwich ELISA for nuclear mitotic apparatus protein	Screening and diagnosis
	NMP22	RT-PCR and dot blot assay using an antibody for survivin protein	Screening and diagnosis
	Survivin		Screening and diagnosis
CLINICAL STATE: Superficial Bladder Cancer			
Urine	BTA Stat	Dipstick colorimetric antigen-antibody reaction for human complement factor H-related protein	Recurrence or progression
	BTA TRAK	Sandwich ELISA for human complement factor H-related protein Sandwich ELISA for nuclear mitotic apparatus protein	Recurrence or progression
	NMP22	RT-PCR and dot blot assay using an antibody for survivin protein	Recurrence or progression
	Survivin	IHC to CEA antigen and mucins	Recurrence or progression
	ImmunoCyt	FISH of exfoliated cells for alterations in chromosomes 3,7,17, and 9p21 RT-PCR and TRAP assay	Recurrence or progression
	UroVysion	Genomic DNA PCR of Chromosome markers	Recurrence or progression
	Telomerase	RT-PCR	Recurrence or Progression
	Microsatellite	IHC or RT-PCR	Recurrence or Progression
	DNA methylation		Prognosis
Tissue	HRAS, FGFR3, PIK3CA		Prognosis
CLINICAL STATE: Muscle Invasive Bladder Cancer			
Urine	E-cadherin	ELISA	Prognosis
Blood	DNA methylation	RT-PCR	Prognosis
Tissue	E-cadherin	RT-PCR	Prognosis
	ERCC1	RT-PCR	Predictive
	BRCA-1	RT-PCR	Predictive
	COXEN assay	Microarrays profiling and algorithm	Predictive
	CD8 T cells	IHC	Prognosis
CLINICAL STATE: Metastatic Bladder Cancer			

Source	Biomarkers	Technique	Objectives
Blood	Gene expression profile	Microarrays profiling	Prognosis
	CTCs	CellSearch system or immunofluorescence	Prognosis
Tissue	Gene expression profile	Microarray profiling	Prognosis
	MicroRNA	miRNA microarray profiling	Prognosis
	P53	IHC	Prognosis
	E-cadherin	RT-PCR	Prognosis
	ERCC1	RT-PCR	Predictive
	BRCA-1	RT-PCR	Predictive
	COXEN assay	Microarrays profiling and algorithm	Predictive
	CD8 T cells	IHC	Prognosis

Abbreviations: BTA (bladder tumor antigen); TRAP (telomeric repeat amplification protocol assay); NMP (Nuclear Matrix Protein); ELISA (enzyme-linked Immunosorbent assay); RT-PCR (reverse transcription polymerase chain reaction); IHC (immunohistochemistry); CEA (carcinoembryonic antigen); HRAS (Harvey rat sarcoma viral oncogene homolog); FGFR (Fibroblast Growth Factor Receptor); PIK3CA (Phosphatidylinositol 3-kinase, catalytic, alpha polypeptide); ERCC(excision repair cross-complementation group); BRCA(breast cancer); CTC (circulating tumor cell); COXEN (COeXpression Extrapolation)