

New Insights into the Diagnosis, Molecular Taxonomy, and Treatment of Bladder Cancer

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Received: 11 February 2021; **Accepted:** 4 March 2021

Abstract

This review aims to emphasize new insights into the diagnosis, classification, and therapy of bladder cancer (BC). Bladder cancer is a heterogeneous, complex disease on a morphological, molecular, diagnostic, and prognostic level. Cancer stage is still the most important attribute for prognosis and treatment, while early detection with optimal and rapid individual therapeutic and surveillance approach is crucial. The vast majority of patients have a superficial, non-muscle-invasive tumor associated with a good prognosis after resection and adjuvant intravesical maintenance immuno or chemotherapy if needed. On the other hand, muscle-invasive bladder cancer is a highly aggressive disease with high morbidity and mortality. However, it has become a model for oncology success over the last five years with many available targeted therapeutic modalities. Metastatic BC is now amenable to multimodal treatment combining cystectomy and neoadjuvant chemotherapy and immunotherapy and is a target for precision medicine. **Conclusion.** A new molecular taxonomy for bladder cancer has been proposed and provided insight into BC's carcinogenesis, with some possible effects on therapy decisions. However, this classification is still not applicable in routine clinical practice. It opens new questions regarding the interplay between tumor genetic signature, intratumoral heterogeneity, therapy implications, and tumor progression.

Key Words: Bladder Cancer Pathology ■ Bladder Cancer Therapy ■ Bladder Cancer Genetics.

Introduction

Bladder cancer (BC) accounts for 3% of global cancer with higher frequency in the developed countries. According to the GLOBCAN data in 2020, the incidence was 9.5 (men) and 2.4 (women) per 100 000 with mortality rates of 1.9/100 000. It is the fourth most common cancer in men, 11th in women, and the ninth most common cause of cancer deaths in Europe. Sixty-five years is the median age at diagnosis, and the average 5-year survival is about 75% in developed countries. Five-year sur-

vival for metastatic disease is up to 5%. Smoking is the strongest risk factor related to BC. Schistosomiasis infection with persistent chronic inflammation in Africa and the Middle East is a critical tropical pathogen in BC carcinogenesis (1, 2).

Although BC is a heterogeneous disease in many ways, which is confirmed with new techniques such as next-generation sequencing, at the morphological level, urothelial carcinoma of the usual subtype (UC) comprises 90% of BC. Among these, up to 85% of patients will have disease confined to the mucosa (non-invasive BC; pTa) or

submucosa (non-muscle invasive BC; pT1), which were formerly called “superficial” bladder cancer. Muscle invasive BC (\geq pT2) is a high-grade, aggressive disease, which requires early detection with optimal and rapid therapeutic and surveillance approaches (1, 3).

For the sake of clarity, in this review, we will use the following terminology: NMIBC (non-muscle invasive BC) and MIBC (muscle-invasive BC). Whereas the tumor stage primarily determines BC’s prognosis and therapy, other aspects of this malignancy also have considerable clinical significance. These clinically important data on the diagnosis, classification, and therapy of BC will be discussed in this review.

Clinical Presentation, Screening, and Diagnosis

The most common symptom of bladder tumors (recorded in up to 85% of BC) is painless hematuria. Macrohematuria is usually associated with higher-stage disease. BC may also present with lower urinary tract symptoms (hesitancy, poor and intermittent stream, straining, prolonged micturition, incomplete bladder emptying, dribbling, frequency, urge incontinence, and nocturia, lower urinary tract symptoms and especially irritative voiding, commonly seen with in situ carcinoma (CIS) (4).

After excluding urinary tract infection, clinical examination of the abdomen, external genitalia, urethra, and prostate is required. Ultrasound (US) of the kidneys and bladder, followed by cystoscopy using a flexible, fiberoptic cystoscope, is standard of care. In case of negative cystoscopy findings, further steps are urine cytology, computed tomography (CT) or urography/intravenous urography if CT is not available (5). Positive urine cytology is a sign of UC anywhere in the urinary tract. However, negative cytology does not exclude its presence since the false-negative rate is up to 20% in high-grade UC. In high-grade tumors, urine cytology with cystoscopy has high sensitivity, up to 84%. In low-grade tumors, sensitivity is very low, up to 16%. Although cytological interpretation is user-

dependent, the test’s specificity can be up to 90% in experienced centers (5, 6). In 2016 the Paris Working Group redefined the diagnostic categories for urine cytology, suggesting the diagnostic reports to be classified into the following diagnostic categories: a) Negative for high-grade urothelial carcinoma (Negative); b) Atypical urothelial cells (AUC); c) Suspicious for high-grade urothelial carcinoma (Suspicious); d) High-grade urothelial carcinoma (HGUC); and e) Low-grade urothelial neoplasia (LGUN) (7).

In the last decade, urine and cytology samples are being adopted as promising and suitable sources to develop non-invasive, accurate, and cost-beneficial tests to diagnose and monitor BC patients, particularly for early low-grade tumors (8). Such tests include panels of markers related to gene expression and epigenetic changes such as DNA methylation patterns and post-translational histone modifications. In addition to cellular DNA or RNA, in urine samples, cell-free DNA (cfDNA), referring to degraded tumor DNA fragments, is valuable for detecting genetic and epigenetic alterations (9). The most useful panels for BC are those searching for *TERT* promoter mutations and *FGFR3* mutations. Some studies have shown these changes months before the clinical manifestation of BC (10). In addition to cfDNA assays for urine, some promising plasma cfDNA diagnostic platforms show good detection of genetic changes in patients with NMIBC and invasive and metastatic disease (11).

Imaging of Bladder Cancer

In the initial diagnosis of BC, imaging plays an important role. The US is the first-line evaluation for patients with hematuria due to its availability. It can be used for staging, particularly in patients with renal insufficiency or contrast allergy; however, it may underestimate the local depth of invasion. Newer US contrast involving microbubbles has enabled the developing of a novel imaging technique called contrast-enhanced ultrasound (CEUS), which is promising in predicting the grade of BC and T-stage. US is still the most im-

portant imaging method for the initial evaluation of hematuria and follow-up of early-stage NMIBC after resection (12).

Computed tomography is the primary imaging modality for assessing the extent of the tumor. The National Comprehensive Cancer Network's current recommendations for the staging of MIBC include CT of the chest, abdomen, and pelvis. Magnetic resonance imaging (MRI) of the abdomen and pelvis with additional non-contrast chest CT in patients with contrast allergies is required (13). Recent improvements in CT cancer imaging, including multidetector acquisition with higher image quality and radiation exposure reduction, have solidified its role as the primary imaging modality, despite the rise and availability of more complex and modern methods. Studies show that CT's specificity and sensitivity in bladder cancer detection are 79-89% and 91-94%, respectively (14, 15). One of the main diagnostic goals is to assess extravesical transmural spread. CT urography has almost wholly replaced intravenous urography for the diagnosis and surveillance of localized bladder carcinoma. However, it lacks the resolution to be used in primary tumor staging as it cannot distinguish between different layers of the bladder wall, and it can miss lesions smaller than 1 cm in size (16, 17). If CT is used to analyze and follow-up changes after transurethral resection of bladder cancer, its accuracy can be further reduced due to inflammatory changes, which can be mistaken for BC (17, 18).

Despite its relatively low cost, rapid turnover, wide availability, and new low dose protocols, CT is still less advantageous than MRI or PET scan for local and distal lymph node involvement, with specificity ranging between 68-100% (16). A new imaging approach called dual-energy CT (DECT) uses software to merge 2 CT scans and create a split-dose CT urography in which 1/3 of the total contrast dose is given 8 minutes before the scan and the other 2/3 of the dose 2 minutes before the scan. With additional subtraction of contrast from initial CT scans, a virtual non-contrast CT is also recreated. The results in an artificially created triple-phase exam (non-contrast, venous, and

delayed urographic) are completed in one scan acquisition at 1/3 of the radiation dose since the delay non-contrast pass is not needed. The main benefit is reduced radiation exposure (17). When compared to MRI, CT is faster and more cost-effective. Downsides include ionizing radiation, high interobserver variability, and the inability to differentiate the bladder's muscle layers and distinguish T1 from T2 disease. Specificity and sensitivity of CT imaging are low for extravesical extension of locally advanced BC and small metastatic lesions, compared with MRI (17-20).

MRI is used for preoperative staging in T2 and advanced disease and staging after cystoscopy. Transurethral resection (TUR) is considered the most accurate technique for staging invasive and non-muscle invasive tumors. However, it can still underestimate this cancer by 42%. MRI provides extensive soft-tissue resolution with the ability to detect T3 and T4 diseases. MRI is superior to CT in distinguishing T2a from T2b stage. Diffusion-weighted MRI are shown to be an excellent tool for differentiating benign and malignant bladder lesions, tumor staging, and assessment after chemo-radiotherapy treatment (19, 20).

Multiparametric MRI (mpMRI) is a new combination of MRI sequences composed of T1W-MRI, T2W-MRI, and functional MRI methods, including DCE-MRI and DW-MRI. It showed potential for detection, and staging assessment, particularly for assessing muscular invasion depth (21). MRI-PET was approved in 2011. It combines the advantages of two complex scans providing superior sensitivity and specificity for bladder cancer detection and characterization; however, there are not enough clearly defined and large prospective studies to validate these findings (22).

Histopathological Diagnosis

Histological confirmation of BC diagnosis is based on TUR sample analysis in most cases. The most common histological subtype is urothelial carcinoma, constituting approximately 90% of all bladder cancers (in some institutions, the term transitional cell carcinoma is still used). The diagnosis is

Table 1. Bladder Cancer Histological Types*

Urothelial Carcinoma, Pure or Mixed With Other Type
Squamous differentiation
Glandular differentiaton
Sarcomatoid differentiaton
Trophoblastic differentiation
Nested variant
Micropapillary variant
Microcystic variant
Lymphoepitelioma like carcinoma
Giant cell variant
Clear cell variant
Lipid cell variant
Neuroendocrine (Carcinoid, Small cell/large cell carcinoma)

*Moch et al. WHO classification of tumours of the urinary system and male genital organs, 2016.

based on architectural and cytological characteristics. Architecturally, BC shows papillary, infiltrative-solid, or mixed growth patterns (3) (Table 1).

Grade

In NMIBC, grade is still the most important finding for therapy and follow-up decisions. In the WHO 2004 classification, a two-tiered grading system was recommended and confirmed in the WHO 2016 classification of UC (3). Low-grade

UC is characterized by papillary architecture and distinct but low-grade cytologic abnormality, with increased crowding and layering of the atypical cells, which are relatively uniform in size and without significant nuclear pleomorphism. Mitoses are mostly rare but sometimes easily visible and placed at the basal tumor layers (3, 23) (Figure 1).

High-grade UC shows prominent architectural and cytologic abnormalities with anastomosing papillae and confluence on low-power examination. Cells show dyscohesion, nuclear pleomorphism and anaplasia, prominent nucleoli, and irregularly clustered disorganized cells. Mitotic figures are numerous and atypical and occupy the full thickness of the epithelial layer. Frequently in situ urothelial carcinoma is found close to high-grade UC (3, 23).

A three-tiered grading system is still used in some institutions (Grade 1: Well-differentiated; Grade 2: Moderately differentiated; and Grade 3: Poorly differentiated UC) but is mostly abandoned due to low interobserver concordance. To convert to a two-tiered system, grade I and II are defined as low grade and grade III as high grade. The grade is of particular importance in NMIBC due to differences in the therapeutic approach. With rare exceptions, MIBC are high-grade tumors (23, 24) (Figure 1).

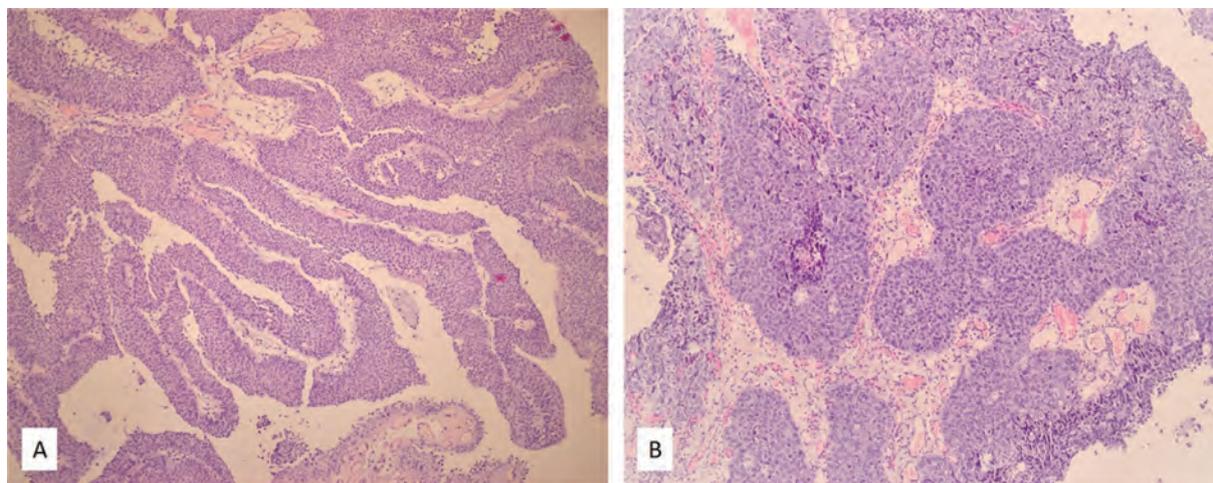


Figure 1. A. Low grade papillary urothelial carcinoma, $\times 100$; B. High-grade urothelial carcinoma, $\times 100$ (Hematoxylin and Eosin stain).

Staging

Tumor-node-metastasis (TNM) staging system is used in histopathological reports for exophytic and endophytic growth patterns. The tumor stage is determined by the depth of tumor invasion into the bladder wall's layers, whose anatomy and histology are variable and can sometimes be confusing even for pathologists. Despite some limitations in sample adequacy and provided data in TUR specimens, it is still obligatory to determine the depth of invasion, defined as the highest pT stage in a given case. Clinical TNM and staging include not only pTNM but also other diagnostic findings (25).

In NMIBC, it is most important to determine the basal lamina's integrity and distinguish between CIS and non-invasive UC. The 2017 American Joint Committee on Cancer TNM recommended pT1 substaging and put the cut-off for microinvasion at 0.5 mm. In the invasive growth pattern (invasion through basal lamina), the absence or presence of muscularis propria and its invasion is of the highest importance. It assigns a pT2 stage category and is an indicator of TUR adequacy. In MIBC, the stage is still the most important prognostic factor. In TUR specimens, muscularis propria may be mimicked by hyperplastic muscle bundles in the lamina propria, leading to overstaging. It is crucial to distinguish these two muscle types morphologically and immunohistochemically. All MIBC tumors are classified as pT2 tumors when confined to the bladder and pT3 tumors when the perivesical fat invasion is found (24-26).

It may be difficult to demarcate the irregular muscularis propria at the perivesical soft tissue junction in cystectomy specimens. Studies have shown significantly poorer outcomes in pT3b compared with pT3a tumors. Proper gross assessment of perivesical soft tissue invasion is of utmost importance for the proper staging of pT3 tumors (25, 26).

Molecular Classification of Urothelial Carcinoma

All malignant tumors are composed of different cell clones, which harbor different genetic makeup

and gene expression changes, including molecular characteristics between primary and metastatic tumors (27). UC is well known for its heterogeneity at the morphological and molecular levels, with various subclones developing during tumor progression, metastatic spread, and therapy-induced changes. Identification of lethal tumor subclones and their molecular signature is crucial for precision medicine and patients' survival with MIBC and metastatic BC. In BC, it is also essential to identify NMIBC with the potential for aggressive behavior and progression to MIBC (28, 29).

The main drivers in UC carcinogenesis are changes in DNA. Comprehensive wide genome multiplatform analyses by the TCGA (The Cancer Genome Atlas) group showed various DNA mutations in UC patients. It provided a strong base for future classification of UC based on molecular taxonomy (30). DNA changes included mutations in multiple genes involved in cell-cycle regulation, chromatin remodeling, kinase receptor signaling, transcription, and DNA repair. These findings are in line with melanoma and lung cancer profiles, which are malignancies with most genetic alterations. The most frequently mutated gene in UC was *TP53*, found in half of the samples, and it was mutually exclusive with the amplification/overexpression of mouse double minute 2 homolog (*MDM2*). Another frequently mutated gene was mixed-lineage leukemia 2 (*MLL2*), essential for chromatin remodeling and epigenetic regulation. *RB1* mutation was mutually exclusive with *CDKN2A* deletion. Recurrent hotspot mutations in the *TERT* promoter regions are also common in UC regardless of grade, stage, or histological subtype. Other mutated genes essential for cell proliferation and differentiation were FAT atypical cadherin 1 (*FAT1*), CREB-binding protein (*CREBBP*), *ERBB2/HER2*, spectrin alpha non-erythrocytic 1 (*SPTAN1*), hotspot activating receptor tyrosine kinases mutations, and gene fusions of *FGFR3*, *PIK3CA*, lysine (K)-specific methyltransferase 2C (*KMT2C*), ataxia-telangiectasia mutation (*ATM*), and lysine (K)-specific methyltransferase 2A 700 Arch (*KMT2A*) (30, 31).

Only a few alterations are retained from primary tumors in respective metastases. Molecular/genetic changes are more expressed over time and due to therapy. Early tumor forms in UC are characterized by *FGFR3*, *AFDN*, and *H3F3A* mutations, which are not found in invasive subclones. Mutations in *KDM6A*, *TP53*, *PIK3CA*, and *FGFR3* genes are also characteristics of primary UC clones, while *TP53*, *MLL3*, *FBXW7*, and *SETD2* mutations are more commonly seen in metastatic clones (29). Most experts agree that high tumor mutation burden (TMB) reflects frequent mutations and their accumulation over time in bladder cancer. MIBC has TMB >7 mutations per Mb and changes in genomic and transcriptional levels, which are not easy to frame. The DNA-editing enzyme apolipoprotein B mRNA catalytic polypeptide-like (APOBEC3) family is believed to be responsible for high TMB in UC. Chemotherapy may affect APOBEC3 expression, further influencing the genetic signature in UC clones (32, 33). Several molecular subclassification systems have been proposed based on different gene expression. These efforts may be limited by intratumoral heterogeneity, which may be seen morphologically in distinct BC subtypes within the same tumor (31-35). Recently blood and/or urine-based liquid biopsy platforms have been rapidly developing and may be a useful tool for capturing the fast changes in BC's molecular signatures (34). Therefore, a new classification of UC based on histopathological findings and molecular characteristics is needed and proposed during the last five years.

There is strong evidence for the existence of two pathways of bladder carcinogenesis. The first pathway comprises 80% of NMIBC with papillary architecture and precursor lesions in the form of urothelial dysplasia (36). These are locally recurrent tumors without risk of invasive growth. The second pathway is related to urothelial carcinoma in situ and shows high-grade tumor characteristics with infiltrative growth (MIBC). Up to 15% of low-grade papillary tumors progress to high-grade lesions and invasive carcinomas with time.

Urothelial dysplasia and low-grade papillary tumors are characterized by activating fibroblast

growth factor receptor 3 (*FGFR3*) mutations, which activate the *RAS* gene (36). In situ UC shows inactivation of *TP53* and *RB1* pathways with SV40 large T antigen. Activation of the phosphoinositide 3-kinase/protein kinase B (PI3K/AKT/mTOR) pathway, resulting from deletions or mutations of tumor suppressor genes, promotes invasive growth. Additionally, loss of phosphatase/tenascin homolog (*PTEN*) is well known to be associated with invasive growth and high-grade tumors, which presumes PI3K/AKT/mTOR pathway as the driver of the invasive phenotype. Downregulation of *TP53* and *RB1* is essential in urothelial carcinoma's invasive phenotype (35-37).

An important question is which cells harbor the mentioned mutations, and are the cell of origin for BC development? The multilayered urothelium comprises three cell types; basal cells, which sit on the basal membrane, an intermediate cell layer, and umbrella surface cells. These cells express different cell membrane markers, which may be an essential clue to track the tumor cell of origin. In UC, this cell of origin is believed to come from the basal cell layer (37, 38).

Non-Muscle Invasive Bladder Cancer

The molecular diversity of UC is responsible for the different clinical behavior, progression, and response to conventional and targeted therapies. Different studies are currently trying to gather all the morphological, molecular, and clinical information needed to define the molecular subgroups of UC to simplify therapy selection and improve clinical response and prognosis of the disease. In 2012, Sjö Dahl et al. (38) analyzed gene expression profiles of NMIBC. They described three major molecular subtypes: urothelial-like (which expressed *FGFR3* and cyclin D1 and showed loss of 9p21), genomically unstable, which expressed Forkhead box M1 [*FOXM1*], with loss of *RB1*, and basal/squamous cell carcinoma-like (which expressed cytokeratins CK5 and CK14). The authors showed prognostic differences: urothelial-like had a good prognosis, genomically unstable intermediate prognosis, and basal-like showed the worst outcome. For the first

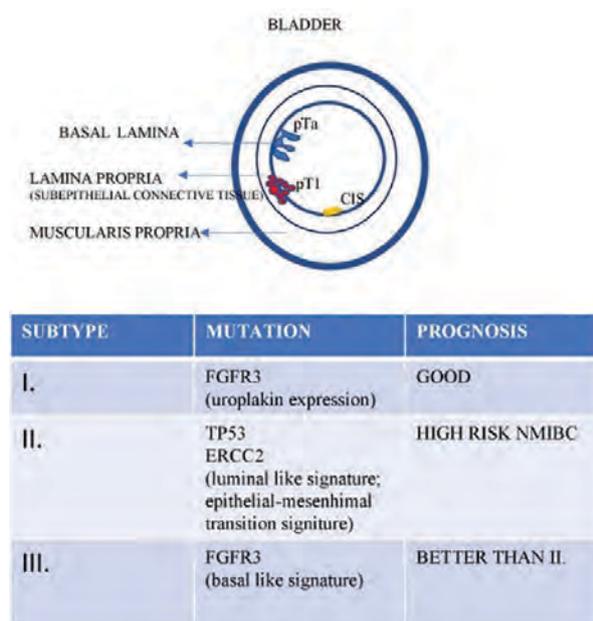


Figure 2. NMIBC molecular subgroups. FGFR3 – Fibroblast growth factor receptor 3, ERCC2 – Excision repair cross complementing repair 2.

time, an immunohistochemical staining panel distinguishing those subtypes was described (39). A comprehensive transcriptional analysis was done, and finally, three different molecular subgroups of NMIBC were found. The first group harbors FGFR3 mutation, expresses uroplakins, and is correlated with a good prognosis. The second group shows luminal-like differentiation with TP53 and ERCC2 mutations and is associated with high-risk NMIBC. The third group harbors FGFR3 mutations and expresses KRT5 and KRT15 as markers of undifferentiated or basal cells (40, 41) (Figure 2).

Muscle Invasive Bladder Cancer

Different molecular subclassifications for MIBC were described as well. Guo et al. (31), based on whole-genome mRNA expression, proposed three subtypes of MIBC: basal, luminal, and p53-like. An immunohistochemical profile was proposed to differentiate these three groups. The basal subtype morphologically showed squamous or sarcomatoid differentiation and expressed CK5/6, CK14, and p63. The luminal subtype was characterized by uroplakins, CK18, CK20, GATA-3 expression, pap-

illary architecture, FGFR3 mutation, and ERBB2 amplification. p53-like was described as a subtype of luminal tumors resistant to chemotherapy. The basal subtype showed aggressive behavior with an excellent response to cisplatin-based therapy (31). The following year, Robertson et al. (30) proposed five distinct molecular subtypes based on clinicopathological findings and mRNA expression. These included luminal-papillary, luminal-infiltrated, luminal, basal squamous, and neuronal. Luminal-papillary was related to the FGFR3 pathway (overexpression, amplification, or mutation), papillary architecture, low-grade morphology, and low progression risk. The luminal infiltrating showed strong stromal reaction and myofibroblastic proliferation with dense intratumoral and peritumoral lymphocytic infiltration and revealed the importance of the microenvironment for tumor growth and progression. In this subtype, immune checkpoint markers were highly expressed [programmed death ligand-1 (PD-L1), cytotoxic T lymphocyte-associated protein (CTLA-4)], tagging this subtype as a right candidate for immunotherapy with immune checkpoint inhibitors. The basal/squamous subtype showed squamous differentiation and expression of basal markers (CD44, CK5, CK6A, CK14) as well as transglutaminase 1 (TGM1), desmocollin 3 (DSC3), PI3, and occasionally immune checkpoint markers. This subtype also showed a good response to both cisplatin-based chemotherapy and immune checkpoint therapy. The neuronal subtype was characterized by neuroendocrine marker expression but did not always show morphological features of neuroendocrine tumors, and it was correlated with the worst clinical outcome (36, 42). MIBC with luminal features is likely to progress from pre-existing superficial papillary tumors, while basal tumors develop from flat in situ lesions.

Despite the plurality of molecular taxonomies of UC, the most comprehensive one seems to be presented by the Lund University group and the TCGA group. Recently, the Bladder Cancer Molecular Taxonomy Group issued its recommendations based on cohorts and studies proposing different classifications trying to assimilate and harmonize

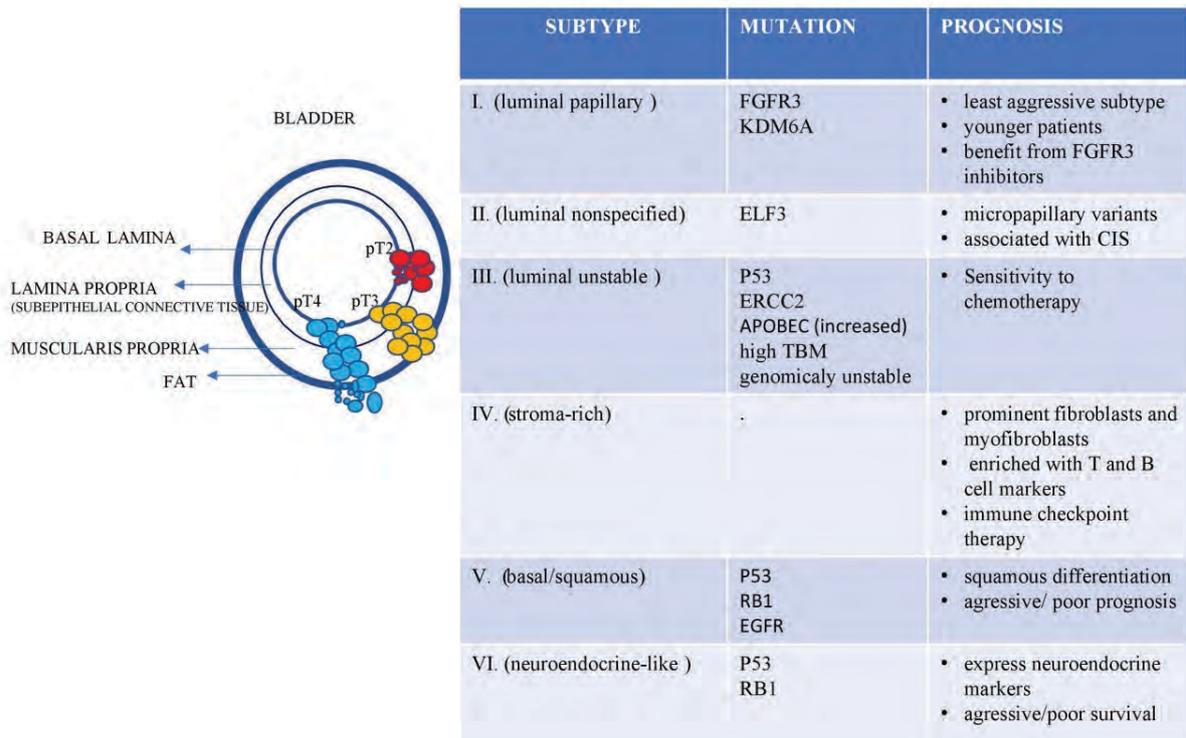


Figure 3. MIBC molecular subgroups. FGFR3 – Fibroblast growth factor receptor 3, KDM6A – Lysine demethylase 6A, ELF3 – ETS like transcription factor 3, ERCC2- excision repair cross-complementing repair 2, APOBEC3 – Apolipoprotein B mRNA catalytic polypeptide-like enzyme, RB1 – RB transcriptional corepressor 1.

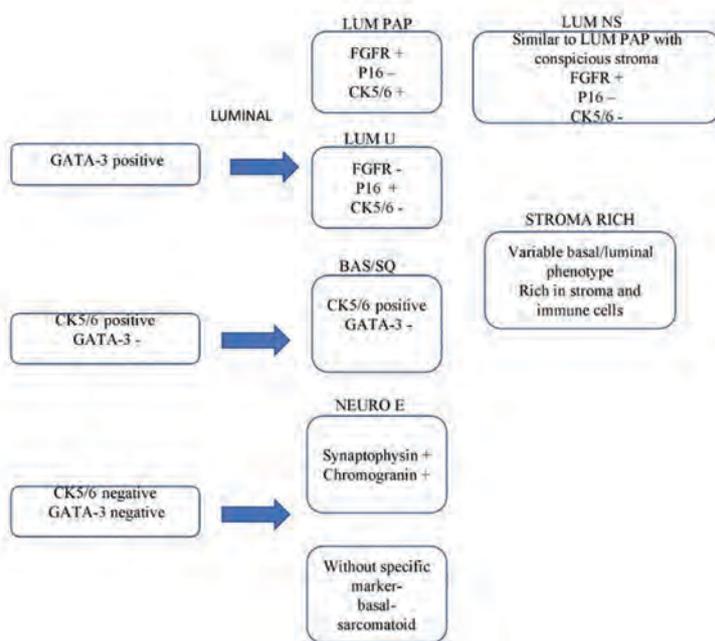


Figure 4. Proposed immunohistochemical panel for bladder cancer subtyping. LumPap – luminal papillary, LumNS – luminal nonspecified, LumU – luminal unstable, Ba/Sq – basal/squamous, and Neuro E – neuroendocrine-like.

the terminology for these subtypes and provide a robust classification system of clinical relevance. Their proposed consensus classification includes six molecular types: luminal papillary (LumP), luminal nonspecified (LumNS), luminal unstable (LumU), stroma-rich, basal/squamous (Ba/Sq), and neuroendocrine-like (NE-like). All luminal tumors show urothelial differentiation, *FGFR3* genetic changes, and active *PPARG* and *GATA3* regulons. LumP shows the least aggressive behavior and frequently harbors *TP53* wild type. LumNS is strongly related to micropapillary morphology and CIS. LumU tumors are characterized by enrichment in genomic instability and mutations in the genes encoding

for the APOBEC protein family and the highest levels of *TP53* and *ERCC2* mutations, associated with sensitivity to chemotherapeutic agents (41-44). Stroma-rich tumors show high expression of endothelial and myofibroblastic gene signatures, T, and B cell markers. Ba/Sq and NE-like subtypes were confirmed as very aggressive with the worst prognosis (45) (Figure 3). To ease every-day histopathological evaluation of BC and enable the best molecular classification in routine pathologists' work, immunohistochemical algorithms for molecular BC subtyping have been proposed (Figure 4) (43).

Molecular subtypes and BC classification are based on different underlying oncogenic mechanisms, genetic and epigenetic alterations, changes in the microenvironment and non-tumor cells, infiltration by immune cells, histologic patterns, and clinical outcomes. Still, some unresolved questions about different histological subtypes of BC are an ongoing topic of research and discussion. Currently, there is not enough evidence about the connection between molecular features and chemotherapy response.

Therapy

Non-Muscle Invasive Bladder Cancer

NMIBC of low-grade is subject to monitoring and early detection protocols, which can be improved with the above-mentioned multigene assays from urine to detect recurrent tumors and search for markers of NMIBC with increased risk of progression into the invasive high-grade tumor (8). NMIBC of high-grade is treated with locally applied chemotherapy (intravesical chemotherapy), which is very effective, together with TUR, in reducing a local recurrence. Mitomycin C, epirubicin, thiotepa, gemcitabine, and doxorubicin are the most commonly used cytotoxic agents (46). BC has been for decades the paradigm of immune-responsive disease. For NMIBC, the standard treatment is still local instillation of *Bacillus Calmette-Guérin* (BCG), which acts as an immunomodulatory agent eliciting a cell-mediated immune response. This

therapy reduces recurrence and progression. It is a good maintenance therapy for patients at intermediate risk and high risk of NMIBC (47).

Muscle Invasive Bladder Cancer

Radical cystectomy with pelvic lymph node dissection as the sole treatment modality offers a chance for a cure only in a minority of patients with MIBC. Occult distant metastases are common even in patients that present with localized MIBC. Moreover, after radical surgery, 50% of patients experience metastatic relapse with a median time to distant failure around one year post-cystectomy. Strategies undertaken to address such high distant failure rates include the use of perioperative chemotherapy. While perioperative chemotherapy implies the use of chemotherapy before or after cystectomy, several distinct features make neoadjuvant chemotherapy specifically an appealing option for the curative treatment of MIBC (48, 49).

Neoadjuvant chemotherapy (NAC) (3 cycles of methotrexate, vinblastine, doxorubicin, cisplatin (MVAC)) followed by radical cystectomy can confront a lower burden micrometastatic disease. The in-vivo therapeutic effect of chemotherapy is observed, and before tolerate chemotherapy better before surgery (decline in performance status, deterioration in kidney function, postoperative morbidity). In patients who respond to NAC, downstaging is possible, optimally resulting in pathologic complete response and clear surgical margins. Despite the level I evidence, NAC's uptake was relatively weak across different healthcare settings and hardly reached 25% of eligible MIBC patients (50-52). There are several possible explanations. Up to 50% of patients with MIBC have significant renal function impairment, which precludes the use of cisplatin-based chemotherapy (kidney filtration rate <60 mg/minute/1.73 m² threshold), which leaves them out of the window of opportunity to benefit from NAC (53). Moreover, MIBC patients are often older, frail, and have a high comorbidities burden (primarily cardiovascular, including heart failure). Around half of all patients with MIBC are ineligible to receive cisplatin (54) from the outset.

Apart from eligibility issues, there is a fear of delaying cystectomy due to toxic chemotherapy given to frail patients, which is truly effective only in the minority of patients (25-35% of patients experience tumor downstaging or pathologic complete response). A delay in cystectomy beyond 12 weeks was associated with inferior survival outcomes only when no NAC was given. However, most of the neoadjuvant regimens can be completed within this period, causing no surgery delay. Reassuringly, there was no difference in radical cystectomy rates between the patients randomized to NAC and patients treated with radical cystectomy alone in landmark SWOG 8710 randomized trial (82% vs. 81%) (55, 56).

Standard-dose MVAC regimen has significant toxicity. A novel approach to shorten the duration of treatment and decrease toxicity is the development of a dose-dense (ddMVAC) 2-week regimen with the support of granulocyte-colony-stimulating factors. The observed complete pathologic response after 3 or 4 cycles of ddMVAC is 26% and 38%, respectively. The time from initiation of NAC to cystectomy was well within the optimal 12 weeks window (9.7 weeks). The most common toxicity was manageable myelosuppression and mucositis, with no severe and life-threatening side effects and no cystectomy cancellation (56-58). NAC is standard of care for patients with MIBC fit for cisplatin and is supported by European Association of Urology and National Comprehensive Cancer Network guidelines (50, 51).

Adjuvant chemotherapy for high-risk bladder cancer is based on an actual assessment of pathological risk factors after radical surgery to tailor treatment based on the individualized risk of relapse. This approach would overcome NAC's main shortcoming: unselective treatment with high toxicity in a difficult-to-treat population with a small margin of benefit. However, only a few patients with high-risk pathological features following cystectomy (node-positive patients, pT3-4 disease, positive surgical margins, and extracapsular extension) can receive cisplatin-based adjuvant chemotherapy. This is secondary to several reasons: radical cystectomy is a major and highly morbid

surgical procedure with a long recovery and many hospital re-admissions; frailty, poor kidney function, and malnutrition are significant problems that frequently preclude timely receipt of chemotherapy (58, 59).

Treatment of metastatic disease Metastatic bladder cancer is an incurable disease, and current data still support cisplatin-based combination chemotherapy as a standard approach for patients who can tolerate cisplatin. The expected response rate with first-line cisplatin combinations is in the range of 40-60%, with a median survival of 13-16 months (60). Available chemotherapy regimens in metastatic settings include standard MVAC, gemcitabine-cisplatin, and ddMVAC, which are considered standard first-line treatment options for metastatic bladder cancer (61). It was mentioned above how in NMIBC, the use of intravesical BCG activates the immune response. In the era of tumor molecular insights, the discovery of a high tumor somatic mutation load in BC, typical for environmentally caused cancers, as well as a peritumoral cell response, the use of immune checkpoint inhibitor (ICI) therapy became an option in metastatic BC (62). Although it came relatively late in bladder cancer therapy, the first report of the prognostic role of programmed death (PD) PD-1/PD-L1 blockade was published in 2007 by Sharma et al. (63). Phase I testing of the activity of anti-PD-L1 in metastatic bladder cancer was published in 2014, which completely transformed the therapeutic landscape of BC. Over the last few years, five ICI agents were approved for second-line treatment of advanced BC after prior platinum-based chemotherapy progression. Those agents include atezolizumab, pembrolizumab, nivolumab, durvalumab, and avelumab. Two ICI agents were approved for first-line treatment of advanced bladder cancer in patients ineligible for cisplatin with positive PD-L1 status (atezolizumab, pembrolizumab) (64-67). Dual ICI is also being tested as an upfront treatment for advanced urothelial cancer. Current data indicate that avelumab maintenance will become the standard option following induction chemotherapy in patients with advanced disease (68). Recently the selective tyrosine kinase inhibi-

tor erdafitinib has obtained FDA approval to treat *FGFR3* mutated metastatic UC resistant to first-line chemotherapy (15-20% of the cases). Erdafitinib represents the first approved targeted therapy for BC (69, 70). Although BC has been considered a grim disease in the not-so-distant past, it has now become a model for oncology success with many available therapeutic options over the last five years. Several ongoing clinical trials will have definitive results that will define the role and optimal use of ICI and targeted treatments, hopefully further revolutionizing advanced bladder cancer management.

Biomarkers of Response to Chemotherapy

The main drawback of chemotherapy is its unselective nature associated with an absence of validated response biomarkers. More precisely, only a minority of patients exhibit clinical benefit while exposed to toxic treatment. To overcome these issues, an effort has been made to develop single gene-based assays, gene expression, and transcriptome panels to characterize MIBC molecularly. These efforts aim to develop both prognostic and predictive biomarkers, as was previously mentioned. Using gene expression patterns, investigators could dichotomize MIBC in basal and luminal cancers, similar to previous breast cancer efforts, which had a significant clinical impact.

Currently, six biologically relevant consensus molecular classes have been described: luminal papillary, luminal nonspecified, luminal unstable, stroma-rich, basal/squamous, and neuroendocrine-like. The use of immunohistochemistry to reveal the molecular profile is an appealing strategy, given its potential applicability in routine pathology practice. However, this approach is not clinically validated (39-45). Despite some progress in unraveling the molecular complexities of MIBC over the last decade, there is still no readily available molecular biomarker of neoadjuvant chemotherapy response. Moreover, clinicians should be extremely cautious when making clinical decisions based on presumed molecular subtypes. Data supporting neoadjuvant chemotherapy sensitivity in

basal tumors are retrospective and require prospective validation (49, 50).

Conclusions

Bladder cancer is a genetically heterogeneous disease. Recent advances have uncovered some aspects of bladder cancer development and progression, which have led to a unified molecular classification of this disease. It was hoped these efforts would lead to clinically relevant subtyping of bladder cancer similar to breast cancer. This goal, however, is yet to be achieved. Some expected benefits include a selection of patients for chemotherapy and immunotherapy. Immunotherapy is an emerging treatment modality in advanced bladder cancer and soon is expected to become the standard of care. In summary, the outlook for bladder cancer patients is substantially improving, with new theranostic and therapeutic options expected to become available in the following years.

Conflict of Interest: The authors declare that they have no conflict of interest.

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