Editorial

Next-generation sequencing in non-muscle-invasive bladder cancer—a step towards personalized medicine for a superficial bladder tumor

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Urothelial bladder cancer is the most common cancer of the urinary system. Exposure to toxins and carcinogens from urine plays an essential role in the pathogenesis of this disease. Non-muscle-invasive bladder cancer (NMIBC) constitutes around three-quarters of the disease burden of bladder cancer. Treated with non-systemic therapy, it has the potential to progress in about a quarter of patients, causing fatal disease in approximately one out of every ten patients. These factors create the need for further refinement of prognostic and predictive genomic markers as published by Pietzak *et al.* in June 2017 2014, "Next-generation sequencing of nonmuscle-invasive bladder cancer reveals potential biomarkers and rational therapeutic targets" (1).

Standard treatment of NMIBC is relatively limited and can be distilled to two main approaches dictated by tumor grade. Low-grade Ta tumor treatment is dominated by complete transurethral resection (TUR) of all visible bladder tumor and the appropriate surrounding muscle tissue (2). Resected tissue is biopsied for tumor characterization, and risk stratification is applied as a predictive test for continued treatment (3). Treatment often ends with resection and in some cases single intravesical instillation of mitomycin if the excised tumor is characterized as low-grade Ta (4). If the resected tumor is high-grade T1 or Tis, treatment entails intravesical therapy with *bacillus Calmette-Guerin* (*BCG*) for a 6-week induction. This may be followed by a one to 3-year period of maintenance BCG treatment (5). Failure of BCG leads to other less effective or more aggressive treatment options such as intravesical chemotherapy, radiation, and cystectomy (6).

There are limitations to both major treatment approaches of NMIBC. Tumor size, stage and grade, multifocality, history of recurrence, and the presence of carcinoma in situ (CIS) are clinical determinants of progression. The initial tumor characterization greatly influences the treatment approach. Rieken *et al.* found that, when applying different classification strategies, 37.9% of analyzed patients could have been classified into a higher recurrence risk group while 11.8% of patients may have belonged to a higher progression risk group (7). Treatment selection based on under-diagnosed disease may result in higher rates of recurrence. To avoid pitfalls from inaccurate grade and stage the most recent recommendations released by European Association of Urology (EAU) include a second TUR in select cases (8). Despite BCG instillation being effective in a majority of patients with high-grade disease, some patients develop BCG toxicity and/or unresponsiveness during ongoing treatment for high-grade NMIBC. A 2003 study found 73.7% of patients receiving intravesical BCG reported some form of toxicity while 30.0% of said patients discontinued treatment due to side effects (9). Patients who receive sufficient intravesical BCG treatment, as per current recommendations, are considered BCG unresponsive if the disease does not respond, if additional low-grade tumors appear, or if the patient later relapses with high-grade NMIBC (10). Intravesical chemotherapy choices

exist but are limited in efficacy. BCG unresponsive patients are left with little to no recourse, other than radiation or cystectomy, without the existence of targeted therapy for NMIBC. No standard biomarkers currently exist to predict the likelihood of BCG failure.

Enormous strides in the understanding of the molecular basis and therapeutic strategies for cancer have been realized by the joint efforts of the National Cancer Institute (NCI) and National Human Genome Research Institute (NHGRI) in creating the Cancer Genome Atlas (TCGA). The Cancer Genome Atlas program has successfully provided a comprehensive library of genomic alterations found in the 33 most commonly diagnosed and lethal cancers, allowing targeted approaches for genomic characterization of tumors by next generation sequencing. Moreover, transcriptomic profiling provides a basis to create predictive and prognostic categories, identifying actionable pathways in tumors.

In 2014, the first comprehensive characterization of muscle-invasive bladder cancer by TCGA using 131 MIBC tumors was published (11). Several known and previously unknown genomic alterations were identified. Bladder cancer was noted to have the highest frequency of chromatin remodeling genomic alterations among all tumors studied under TCGA. In an update in 2017, the role of epigenetics in bladder cancer was further consolidated with epigenetic genetic alterations noted in 78% of the muscle-invasive urothelial bladder cancers in this expanded cohort of 412 patients (12).

In 2014, Liu *et al.* demonstrated success in transcriptomic profiling of high and low-grade formalin-fixed-paraffin–embedded NMIBC tissue with an acceptable degree of concordance with the fresh frozen tissue sample. Not only did this open the possibility of bringing transcriptome analysis to archived tissue, but also offered a peek into the molecular differences between high and low-grade NMIBC. A greater degree of genomic heterogeneity was noted in high-grade tumors (13). Patschan *et al.* have further characterized 167 T1 urothelial bladder cancers into three prognostic categories—urobasal, genomically unstable and squamous-cell-carcinoma-like, with the latter two carrying a worse prognosis (14). In a separate, larger, multi-center study, comprehensive transcriptomic analysis and unsupervised clustering of 460 NMIBC once again revealed three separate classes of NMIBC comparable to the previously described urobasal, genomically unstable and squamous-cell-carcinoma-like classes. High risk tumors identified by both of these molecularly defined classification systems are concordant with the high-risk tumors identified by the clinical European Organization for Research and Treatment of Cancer (EORTC) risk scores (15).

New treatment options for NMIBC are being pursued in clinical trials, but with limited attempts to address predictive genomic therapeutic targets. Many of the NMIBC clinical trials registered with the National Institutes of Health (NIH) in the last ten years apply different variations on traditional NMIBC treatments, testing less toxic or more effective analogs of chemotherapeutic agents, administering intravesical BCG on different schedules and in combination with various chemotherapy agents, or checking existing non-specific chemotherapies for efficacy (16). An exciting approach being actively investigated in the setting of BCG failure is the use of immune checkpoint inhibitors. Immune checkpoint inhibition with anti PD-1/PD-L1 has proven efficacy in the metastatic disease setting and the experience gained in this setting can enrich the approach used for the treatment of NMIBC (17,18). A small but growing number of recent trials are attempting to narrow the focus of treatment. Multiple ongoing phase I and II trials are helping immunotherapy gain traction as an indirect method of targeting predictive biomarkers in NMIBC (19,20). Two such trials are testing modified versions of cytokines interluekin-2 and interleukin-15, in combination with the traditional approaches, which indirectly target known NMIBC predictive biomarkers p53 overexpression and major histocompatibility complex molecules, respectively (21,22). In a more directly predictive approach, the compound ABI-009 is being tested for its ability to inhibit the mammalian target of rapamycin (mTOR) function in the hopes of further downstream inhibition of vascular endothelial growth factor (VEGF); both mTOR and VEGF have been implicated in angiogenesis in bladder cancer (23).

With next-generation sequencing of clinically annotated 105 treatment naïve NMIBC tumors, Pietzak *et al.* begin to address the gap in the knowledge base of targeted therapy for NMIBC and offer up the solution of next-generation sequencing as a tool for identifying potentially targetable as well as prognostic biomarkers (1). In this study of NMIBC tumors, a broad range of low and high-grade tumors, ranging from TIS to Ta and T1 stages, were included. As previously known, *TERT* promoter mutations (73%) and chromatin remodelling gene mutations (69%) were found to be most prevalent in the sequenced tumors irrespective of stage and grade suggesting a role of these in early pathogenesis of the disease. Alterations of *FGFR3* (49%) and *ERBB2* were present in 57% of the tumors and were mutually exclusive. *FGFR3* alterations were more commonly

prevalent in the low-grade tumors at a frequency of 83%, *ERBB2* alterations were present in high-grade tumors only. In all, the receptor tyrosine kinase/phosphatidylinositol 3-kinase pathway alterations were present in 79% (83/105) of NMIBC tumors, with *PIK3CA* mutations being present in 26% of the tumors.

47% of the NMIBC tumors had alterations involving *TP53* or the cell cycle pathway. These alterations were associated with higher grades and stages of the disease. Similarly, DNA damage repair (DDR) gene mutations were more prevalent in high-grade NMIBC (30%) than low-grade disease (4%). The tumor mutational burden (TMB) correlated with the presence of DDR defect and was higher in the high-grade tumors with DDR gene mutations. *ERCC2* mutations (17%) were the most commonly prevalent DDR gene mutations. Targeted therapies are available for the majority of genomic alterations in NMIBC. Targeted therapy directed towards the fibroblast growth factor receptor (FGFR) pathway has shown promise in metastatic urothelial carcinoma (24). This therapy may be especially useful in both high and low-grade NMIBC.

The comparable prevalence of DDR gene alterations and the degree of TMB in the high grade NMIBC cohort to muscle-invasive disease indicates a close molecular resemblance of high-grade NMIBC to MIBC. Moreover, the experience gained from the use of DDR gene alterations and TMB in predicting response to treatment in more advanced disease states can be applied in NMIBC.

Within the main mutated gene set, the authors identified a significant subgroup of mutated chromatin modifying genes present in 69% of all sequenced NMIBC tumors, notably *KDM6A* and *ARID1A*. Of the seven mutated chromatin remodeling genes found in the study, *ARID1A* is of particular interest for its ability to be both prognostic and predictive. Only *ARID1A* mutations were shown to be statistically significant, across multiple comparisons for tumor recurrence, after a 6-week induction course of BCG in high-grade NMIBC, making it a prime target for developing novel therapeutics.

When paired with hallmark therapeutic targets such as TP53 pathway mutations, cell cycle regulator mutations, and DDR pathway mutations, it seems rational that the noted chromatin remodelling gene mutations are clear path forward as impactful predictive biomarkers.

The use of targeted therapy in localized disease for which systemic therapy is not a standard treatment approach is a relatively novel concept. Usually, the efficacy of targeted therapies involves measurement of response rates using measurable disease and progression-free survival. Localized disease, the bulk of which is surgically resected, does not lend itself to measurement. Unless preventive in nature, targeted therapy would not be able to show any putative benefit. However, bulky tumors that are difficult to surgically resect may shrink with targeted therapy, allowing symptomatic relief and easier resection.

Moreover, when curative treatment options exist, would exposure to anti-proliferative targeted therapy merely temporarily suppress tumor growth in the bladder lining, delay definitive treatment, and diminish the possibility of cure? Is it possible that the tumor may evolve into a more aggressive disease form (epithelial-mesenchymal transformation), being coerced to survive the exposure to the targeted therapy? Indeed, FGFR pathway activation is significantly more prevalent in low-grade disease but is a promising target for systemic therapy in platinum-resistant metastatic disease, indicating a significant contribution to the disease by other additional genomic alterations.

Considering the lack of established targeted genomic therapeutic options and the limited number of clinical trials addressing rational therapeutic targets, any patient suffering from high risk NMIBC can assume a poor prognostic outlook of their disease. A paradigm shift in NMIBC patients with *ARID1A* loss towards a more aggressive treatment approach may be warranted. Since the risk of BCG failure is high, efforts may be directed towards targeted therapy in combination with BCG. Alternatively, an approach similar to muscle-invasive bladder cancer treatment may be warranted.

Next-generation sequencing can be a powerful tool to discover predictive biomarkers which, when directly addressed, can lead to improved overall disease prognosis. In combination with previously established clinical risk factors and molecular risk categories, the genomic alterations identified by next-generation sequencing can add a higher degree of sophistication to prediction of disease course and treatment response. Personalized comprehensive evaluation of risk of disease recurrence, progression, and relapse, and tailored targeted therapy in NMIBC is a future possibility. The matter of prioritizing the genomic target(s) in a disease with multiple genomic drivers needs further thought.

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Footnote

Conflicts of Interest: The authors have no conflicts of interest to declare.

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