Need for a personalized approach for muscle invasive bladder cancer: role of tumor biology in response to neoadjuvant chemotherapy

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Bladder cancer (BC) is the 2nd most common genitourinary malignancy with an annual occurrence of 81,190 new cases and 17,240 deaths (1). The majority of patients are diagnosed with superficial BC [non-muscle invasive bladder cancer (NMIBC)] and are managed by a non-systemic therapy approach, which includes transurethral resection (TUR) of tumor with or without intravesical treatment depending on the grade of tumor, depth of invasion and presence of carcinoma in situ. However, BC with muscle invasive disease (MIBC) requires use of cisplatin-based neoadjuvant chemotherapy (NAC) followed by radical cystectomy (RC) or bladder sparing approach with systemic chemotherapy plus definitive radiation therapy. Cisplatin-based NAC in MIBC seems to achieve a pathological complete response in about 40% of patients (2), however a majority of patients tend to have residual disease making them high risk for recurrence of cancer locally or distantly. This highlights a need for identifying biomarkers that predict resistance to cisplatin-based NAC.

Amongst MIBC patients, NAC responders are thought to be molecularly different than those who are non-responders (3). The variability in response of MIBC to cisplatin-based NAC may be related to the underlying tumor heterogeneity. Several groups have classified BC into molecular subtypes (3-6). Lund University Group was amongst the first to classify BC based on gene expression using both MIBC and NMIBC (4). Markers of cell cycle regulation including *Rb1*, *p16* (*CDKN2A*), and basal/squamous differentiation were used to create 5 major molecular subtypes-urobasal A, genomically unstable, urobasal B, squamous cell carcinoma (SCC)-like and immune infiltrated type. The prognosis in this cohort differed by molecular subtypes. Urobasal B and the SCC-like subtypes showed the worst disease specific survival whereas the genomically unstable and the immune infiltrated subtypes showed intermediate prognosis and urobasal A subtype showed the best prognosis. Urobasal A subtype was overwhelmingly NMIBC whereas the remaining groups included many MIBC tumors, particularly the Urobasal B, SCC-like, and infiltrated types.

Choi *et al.*, reported three distinct molecular subtypes of BC using whole genome mRNA expression profiling on 73 TUR bladder tumor specimens: basal, luminal and p53-like (3). Basal tumors frequently had squamous differentiation, appeared driven by the transcription factor p63, and had the worst prognosis. Luminal tumors showed strong expression of peroxisome proliferator activator receptor (PPAR) pathway activation as well as higher

occurrence of activating fibroblast growth factor-3 (FGFR3) mutations, making it a focus for targeted therapeutic approaches. The p53-like group was enriched in stromal markers. While several luminal and basal cancers responded to NAC (methotrexate, vinblastine, doxorubicin and cisplatin), the p53-like subtype appeared resistant. However, this data needs further validation and the results make one question about the heterogeneity of the underlying cancer that could play a major role in response to NAC.

The Cancer Genome Atlas (TCGA) classified the MIBC into 4 expression clusters, clusters I–IV (6,7): Clusters I, II are considered luminal subtypes, whereas clusters III, IV are basal subtypes (8) (the updated TCGA study defines groups differently (9), but we adhere to this older terminology for the sake of comparison). Per TCGA (7), Cluster I frequently had papillary histology and FGFR3 dysregulation; Cluster I and II had features similar to the luminal A breast cancer and expressed HER2 (*ERBB2*). However, cluster II was similar to the p-53 like tumors. Cluster III had tumors with predominant squamous histology and was similar to SCC-like subtype described by Lund university and it was thought to be very similar to lung squamous cell, head and neck squamous cell and basal-like breast cancer. Cluster IV was similar to cluster III but in addition had features of the surrounding stroma and muscle and expressed immune markers. Further research in Cluster IV tumors has demonstrated decreased expression of PPAR-γ and GATA3, and significantly increased expression of IFN, antigen presentation pathway genes, MHC class II genes as well as genes involved in T-cell cytolytic activity (10). Immunotherapy clinical trial has shown that the basal subtypes are more enriched with programmed death-ligand 1 (PD-L1) expression when compared to luminal subtypes but this did not correlate with response (11). All TCGA subtypes had responded to PD-L1 inhibitor, atezolizumab, but the highest objective responses were observed in cluster II subtype. Cluster II subtype contained high expression of CD8+ effector T cells, with decent PD-L1 expression, which could have translated into better immune response in this cohort. In contrast cluster I had low expression of PD-L1 and low expression of CD8+ effector T cells. Basal clusters III and IV had high PD-L1 immune and tumor cell expression and in addition had high CD8+ effector T cell expression but still had poor response to atezolizumab when compared to cluster II, suggesting the possibility of underlying immunosuppressive activity in basal subtype that could avert the immune response. Similarly, Mariathasan *et al.* showed genomically unstable

tumors (per Lund Classification), which partly overlap with luminal tumors, demonstrated superior response to checkpoints inhibitors (12). Hence the landscape between luminal and basal subtypes could be further affected by some additional underlying immunobiological factors than what we presently know and could dictate the response to immunotherapy.

Seiler *et al.*, performed molecular subtyping of TURBT specimens on 343 pre-NAC MIBC patients (13). This was a retrospective analysis where the researchers assembled data for discovery and validation cohorts and used a single-sample classifier to assign MIBC to one of the 4 subtypes based on previous molecular classifications, specifically basal, claudin-low, luminal, and luminal-infiltrated. They compared these molecular subtypes with survival based on presence or absence of NAC. While NAC improved survival substantially in patients with basal tumors, those with claudin low tumors did poorly with or without NAC, and those with luminal tumors did best irrespective of treatment. However, given the retrospective nature of the study, it could only provide the preliminary evidence to develop the rationale for doing future studies but a lot of research is still desired to define the clinical utility of MIBC molecular classification.

BC is often considered heterogeneous and this has been well recognized based on presence of different histologies in the same tumor type in a single patient—such as mixed histology of urothelial and squamous. Warrick *et al.*, recently demonstrated the concept of intratumoral molecular heterogeneity in BC patients (14). They assigned the 83 histologically variant BC tumors into molecular subtypes based on Lund university classification: urothelial like, genomically unstable, basal squamous, mesenchymal-like, neuroendocrine-like subtypes. In their cohort, 39% with multiple histologies demonstrated molecular heterogeneity. This was seen predominantly in basal squamous subtypes, which co-occurred with either urothelial-like or genomically unstable tumors. However, they did not observe co-occurrence of genomically unstable and urothelial-like subtypes. This information is helpful to understand the evolution of BC into variant histology. It also highlights the fact that MIBC could have molecular heterogeneity thus one has to be cautious in using the different molecular classification when guiding treatment for these patients.

Most of the previously published work has been focused on developing molecular subtypes for MIBC at the time of diagnosis i.e., pre-NAC samples. Liu *et al.*, in 2017, had

demonstrated in a small cohort of MIBC patients that, tumor heterogeneity in post-NAC tumors was associated with poor overall survival, and alterations in cell-cycle and immune checkpoint regulation genes (15). Definition of molecular subtypes in residual tumor post-NAC is certainly an unmet need. It is important to understand the residual tumor's signature post cisplatin-based treatment, as it could have a direct impact on rates of recurrence and thus the overall survival.

Seiler *et al.*, recently reported results from a study highlighting the importance of underlying biology of MIBC that do not respond to cisplatin-based NAC (16). The researchers examined retrospectively gene expression from 134 BC patients who had residual disease post NAC and correlated with clinical characteristics; in addition, 21 non-cancerous scar tissue samples were collected from patients with complete pathological responders (pT0N0) post NAC. They matched the gene expression in 116 tumor samples between two time points, pre (TUR specimen) and post NAC (RC specimen) in each patient who had residual disease (16). Four consensus clusters (CC) were demonstrated in post NAC cohort who had residual disease-CC1-basal, CC2-luminal, CC3-immune subtype, CC4-scar like subtype. CC3 had the highest T cell infiltration, lacking basal and luminal markers whereas CC4 had gene expression of scarring/wound healing. In their retrospective analyses of cohort from pre-immunotherapy era, the authors showed that CC4 had the best prognosis (16).

Seiler *et al.*, did a phenomenal work in assembling paired tumor tissue, pre and post NAC and clinical information on patients from 7 institutions with MIBC who got 3 cycles of cisplatin-based NAC and then correlated their clinical outcome with whole transcriptome analyses for gene expression and immunohistochemistry (16). Amongst the MIBC patients who had paired samples, around 64% of patients had >pT2 disease and about 40% were node positive. The authors harmonized the classification strategy to define molecular subtypes, by incorporating the previously described molecular subtypes-Genomic Subtyping Classifier (GSC), TCGA, University of North Carolina (UNC) Lund University and MD Anderson (MDA) group (3-6,13,17). Patients with basal or luminal subtypes had favorable prognosis compared to claudin low or luminal infiltrated tumors for both pre and post-NAC (16). However, MDA p53-like and Lund infiltrated showed better prognosis in post-NAC analyzed samples. Post-NAC molecular subtypes differed from pre-NAC subtypes, with post-NAC samples consisting of

fewer luminal subtypes but more of basal, p53, infiltrated subtypes (16).

Seiler *et al.*'s study comparing pre and post NAC samples in MIBC is an important step in identifying the possible molecular signature for cisplatin resistance and could pave the way for future clinical studies. Based on their observations, the authors had concluded that the molecular subtype could change from baseline after exposure to cisplatin. This is rather not a surprising finding as we have long suspected that cisplatin-based chemotherapy could alter the tumor microenvironment and mutational signature (15). The authors classified the cisplatin resistant tumors (BC patients who had residual tumors post-NAC) into 4 molecular subtypes, CC1–CC4. The CC1 and CC3 subtypes had higher expressions of immune associated genes (*CTLA4*, *MPEG1* and *CD27*) when compared to CC2, CC4. In addition, CC1 and CC3 also had higher expression of immune suppressor genes PD-L1, PD-L2, suggesting these subtypes could respond well to check point inhibitor. CC2 had higher expression of PPAR-gamma and lower immune expression, suggesting immune deprived subtype. The study showed that 34% of cases displayed a significant change in molecular subtypes between pre and post NAC with loss of luminal and basal markers and gain of tumor infiltration by CC3-immune subtype. Of those tumors that lacked immune infiltration, 32% became immune infiltrated post NAC.

This study had a number of limitations, including the retrospective nature of the study without standardized specimen collection across the 7 participating institutions, lack of follow-up, limited overall sample size for a biomarker study and the absence of a validation cohort. It is also possible that the difference in gene expression could be attributed to tumor heterogeneity, i.e., different sites of tumor being analyzed for TUR specimen when compared to the RC sample, since some sites of tumor could easily be missed during TUR at the time of diagnosis. This study was also conducted in the pre-immunotherapy era, and if repeated in today's setting may show a different outcome, such as, the molecular subtypes with high expression of immune markers could get a durable response to checkpoint inhibitor, translating into better survival. In spite of the intriguing results from Seiler's study, we are still very far from utilizing these findings in our clinic. It is imperative to note that biomarker results need to be validated in an independent cohort, more so in a prospective fashion before being used for therapeutic decision-making tool in real time. At present some prospective clinical studies are incorporating DNA damaging repair genes, as a predictive

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biomarker of response to cisplatin-based NAC and the results of these may help identify some important gaps in understanding the landscape of MIBC.

It would be important to acknowledge that translating the use of molecular subtypes in selecting patients for NAC in MIBC would be very challenging. Currently for all MIBC patients, cisplatin-based NAC is the accepted standard of care, as it is known to improve overall survival. Hence, conducting any future prospective clinical study would need withholding NAC from some patients whose signature denotes poor response to NAC. This would be a daunting task, as we would first need to establish safety of such an intervention even before we can demonstrate survival benefit based on molecular subtypes in a prospective fashion. In addition, a very large sample size would be required to obtain meaningful results for a prospective biomarker study in MIBC. One could argue to develop a bladder genome diagnostic model that incorporates a gene expression score, similar to the oncotype recurrence score in breast cancer, to have low, intermediate and high tertiles reflecting the benefit from NAC. Nevertheless, the study by Seiler is vital because it not only focuses on NAC's response but more importantly on the molecular signature post NAC for patients who had residual disease after RC. Post-NAC tumor's molecular subtype could help direct the use of adjuvant or salvage therapy in these MIBC patients and could play a crucial role in improving the overall outcome for these patients. One proposed clinical trial design could be in adjuvant setting, classifying patients post NAC followed by RC with residual disease (pT1–T4 or node positive M0) into CC1–CC4; CC1 subtype to get adjuvant immunotherapy combination, CC3 subtype to receive adjuvant checkpoint inhibitor, whereas CC2 patients with FGFR alterations could get adjuvant FGFR inhibitor, and CC4 subtype could be observed with no adjuvant treatment. However, in the present era of immunotherapy, where many studies are evaluating the efficacy of checkpoint inhibitors in neoadjuvant setting either alone or in combination with NAC in MIBC, we envision that in future, there may be a shift in the standard of care from NAC to NAC+ immune checkpoint inhibitor. Therefore, conducting any future prospective studies incorporating the molecular subtypes alone, as a predictive biomarker for either evaluating response to NAC or need for adjuvant therapy would not be enough. In spite of these potential hurdles, one should continue to explore the identification of biomarkers in order to spare some patients from unnecessary toxicity to NAC and devise a more personalized care approach for all MIBC patients. We truly believe that the availability of modern sequencing technology and immunotherapy could help develop a more precise predictive biomarker panel for neoadjuvant therapy paving the way for a brighter tomorrow for all BC patients.

Acknowledgements

Footnote

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

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