Editorial Commentary

Molecular subtyping, tumor infiltration, and trimodal therapy for muscle-invasive bladder cancer: more questions than answers

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Trimodal therapy (TMT) is currently accepted as a suitable alternative to radical cystectomy (RC) with perioperative chemotherapy for muscle-invasive bladder cancer (MIBC). Recent publications demonstrated that survival outcomes are comparable when patients are appropriately selected (1,2).

Bladder-sparing treatments are being offered based on the patient's preference, clinical-pathological characteristics, and at the discretion of the treating physician (3,4). As the only randomized trial comparing RC *vs.* TMT failed due to poor accrual (5), novel biomarkers are needed to understand better which patients are ideal for each treatment.

Efstathiou and colleagues (6) recently published on genomic classification and immune/stromal infiltration signatures of the primary tumor and its association with survival after TMT (in comparison with RC) for MIBC. While others have been exploring this subject since 2014 in association with neoadjuvant chemotherapy (NAC) and RC (7,8), the influence of molecular and genetic profiling on bladder-preserving radiation-based therapies for MIBC has not been studied.

The primary endpoint of the study was the association between immune infiltration signatures and disease-specific survival (DSS) after TMT. Through whole transcriptome gene expression of pre-treatment tissue samples of 136 patients treated with TMT, the authors found that higher T-cell inflamed and IFN-gamma scores were associated with better DSS but not OS. In contrast, T-cell inflamed, and IFN-gamma scores were not associated with DSS or OS in patients treated with NAC + RC. On the other hand, higher stromal infiltration gene signature was significantly associated with worse DSS and OS in patients treated with NAC + RC but was not associated with outcome in patients treated with TMT.

Furthermore, the authors evaluated the impact on molecular subtypes (basal, claudin-low, luminal, and luminal-infiltrated) on outcome in patients treated with TMT. They did not find significant differences in complete response rates or survival across the molecular subtypes in patients treated with TMT. Due to the small sample size, repartition of the subtypes resulted in only a few claudin-low and luminal-infiltrated specimens (16 and 24, respectively). As such, no definitive conclusions can be made on the impact of molecular subtypes and radiation (RT) response.

Population selection

Although clinical and pathological differences between TMT and NAC + RC populations were evident, we believe this is consistent with the current practice as older patients and those with clinically organ-confined disease are being more commonly treated with TMT. Unfortunately, in the absence of a randomized trial, any attempt to compare TMT *vs.* surgery will always be limited by heterogeneity and selection bias.

For this analysis, the TMT population was taken from the vast Harvard experience previously published (3). Out of 475 patients, only 136 had a good quality of pre-treatment transcriptomes. Despite this substantial withdraw that could potentially lead to a selection bias, this subpopulation seemed to represent well the total cohort with no significant differences regarding age, gender, tumor stage, completeness of initial transurethral resection of bladder tumor (TURBT), and presence of hydronephrosis at diagnosis, as exposed later in an editorial reply by the authors (9).

It is essential to highlight that different protocols were used to treat those 136 TMT patients, and no information regarding dose, type of concurrent chemotherapy or use of adjuvant/NAC was reported. Moreover, since the endpoints of this study were DSS and OS, use of salvage radical cystectomy for residual disease or local recurrence should have been integrated into the analysis as the overall rate of salvage RC from the MGH group has been reported to be as high as 29% after 5 years of follow-up (3). Protocol variability and use of salvage radical cystectomy may compromise the power to assess the correlation between subtyping and immune/stromal signatures with TMT itself compared to the other treatments mentioned above.

A similar rationale applies to anal cancer. Currently, the first line treatment of squamous cell carcinoma of the anal canal is chemoradiation (10), and two randomized trials in the late 90s confirmed that adding 5 FU and mitomycin to radiotherapy for the locally advanced disease was beneficial in terms of local control and DSS. Overall survival, however, was not significantly improved (11,12) probably because patients with the persistent disease or late recurrences after chemoradiation were successfully rescued with radical salvage abdominoperineal resection and definitive colostomy, reducing overall survival outcomes differences between responders and non-responders (11).

Inflammation and response

Patients with higher T-cell inflamed and IFN-gamma expression had better DSS when treated with TMT while this was not true for NAC + RC. Based on these findings, the authors suggested that TMT would be a better treatment option for patients with higher immune activity MIBC.

It is known that, in general, more inflamed solid tumors have an intrinsically better prognosis as reported in a metanalysis published by Gooden *et al.* that showed a significant positive association between tumor-infiltrated lymphocytes (TIL) expression with both progression-free survival and OS (13). More recently, several publications are building up to this concept of a strong prognostic correlation between TIL's and ovarian, gastrointestinal, and hepatocellular cancers (14-16).

In the present study, while the authors provide a plausible hypothesis where the presence of immune cells before treatment may provide a more favorable environment in which RT can promote immune-mediated tumor cell killing, the lack of association between the immune signature and NAC + RC remains unanswered and contradictory to the current literature. In other words, saying that TMT is a better option considering only one population with a known better prognostic biomarker is premature. Perhaps it would be more interesting to investigate why having higher scores of inflammatory gene expression did not correlate with better outcomes after NAC + RC like it would be expected. Could NAC overcome the worse results of less inflammatory bladder tumors and turn their response to surgery similar to their more inflamed counterpart?

Stromal infiltration and response

Stromal tumor infiltration in this study was based on the expression of fibroblast and myofibroblast genes using a GenomeDx Laboratory signature. Little is known regarding tumor stromal infiltration and RT and this interesting association with NAC + RC, but not to TMT, reinforce the immune effect that radiotherapy has on tumor environment. Although we agree this is an adequate current strategy to quantify stromal infiltrates within the tumor, few studies evaluated this particular signature in bladder cancer as most were not specific to this disease (17).

A recent study by Mariathasan *et al.* with metastatic urothelial carcinoma reported on the role of TGF-β expression and poor response to anti-PDL-1, suggesting that stromal proliferation stimulated by TGF-β results in resistance to immunotherapy (18). Similarly, Liu *et al.* published on high mast cell stromal infiltration resulting in decreased rates of CD8 T-cells and a less activated immune state of MIBC, also associated with worse prognosis (19). Further work is needed to understand better if TMT can compensate for the negative effects of stromal infiltration and overcome the intrinsic worse prognosis of these patients, as suggested in this paper.

Conclusions and future directions

As baseline molecular and genetic characteristics of MIBC are continuously being studied, this will undoubtedly guide or change treatment in the future. Nevertheless, there is still a literature gap on what could be the main drivers of RT-resistance in MIBC and what changes after TMT in non-responders. All patients in this TMT cohort were systematically biopsied after treatment (3), and some certainly underwent salvage RC as mentioned above, but data on residual disease or late local recurrences and salvage therapy were not reported. Transcriptomes of persistent disease, recurrence, and salvage RC specimens could potentially identify immune environment changes after RT and help us better understand its dynamic effects on the disease.

The applicability of molecular subtyping in clinical practice is still limited. Current data is based on heterogeneous genetic classification methods; more importantly, its validation in larger populations and a prospective manner is necessary before we can classify molecular subtype individually (20). Regardless of its retrospective limitations, this was a well-designed study that will help set the ground for future work and generate a hypothesis on how immune environment and immune cell expression relates to clinical decisions in daily practice.

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Footnote

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

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