

Spatial Architecture of the Tumor Microenvironment in Immune Checkpoint Inhibitor–Induced Hyper Progressive Bladder Cancer

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Commentary

Muscle invasive bladder cancer (MIBC) is associated with high recurrence and life-threatening metastasis. Although the standard therapy for MIBC is a radical cystectomy to prevent metastasis, this approach has been associated with distant recurrence in 75 % of cases and local recurrence in 25 % of cases with a median time to recurrence of 12 months, leading to adverse impact on quality of life [1]. For the past two decades, combination therapy consists of the platinum-based drugs, MVAC (methotrexate, vinblastine, Adriamycin, and cisplatin), and gemcitabine and cisplatin (GC) have been the mainstay of treatment for locally advanced and/or metastatic urothelial cancer. The overall response rate associated with the GC regimen remains modest, ranging from 40 to 50%, and no other groundbreaking treatment emerged until the introduction of pembrolizumab (anti-PD-1 antibody) in 2017. Recently, the JAVELIN Bladder 100 study demonstrated that maintenance therapy (continuous treatment after complete or partial response following chemotherapy) targeting programmed death-ligand 1 (PD-L1) administered after platinum-based combination chemotherapy significantly improved overall and progression-free survival [3], which

subsequently led to the approval of avelumab (anti-PD-L1 antibody) in Japan in 2021. Pembrolizumab and avelumab are immune checkpoint inhibitors (ICIs) that are broadly used in the treatment of various cancers nowadays.

While the development of ICIs is a drastic paradigm shift in the treatment of urothelial cancer, some patients receiving ICIs experience not only immune-related adverse events but also accelerated tumor proliferation and rapid progression. These cases, termed hyper progressive disease (HPD), have been reported in various cancer types, with incidences ranging from 4 to 30% [4-6]. Several studies have sought to elucidate the mechanism underlying HPD. In particular, infiltrating regulatory T cell [7], exhausted TIM-3+ T cells [8], M2 like macrophages expressing FcγR-I [9], MDM2/4 amplification [10] and/or EGFR mutation [11] have been extensively studied, and these factors are considered to act either independently or synergistically.

Our recent report [12] detailed a case of rapid metastatic tumor growth following avelumab maintenance therapy despite an initial complete response to GC therapy. Our multiomics analysis revealed that TGF-β and IL-8 may

orchestrate the activity of M2-like macrophage, leading to HPD and rapid disease progression—manifesting merely six months after the first dose of avelumab, even in the context of a 24 months complete response to GC therapy with pelvic irradiation. These finding necessitate further discussion on several aspects that were not addressed in our article.

First, is there a possibility that radiation therapy might contribute to the rapid progression of disease in the context of immune checkpoint inhibitors (ICIs)? This issue remains controversial, and no definitive conclusion has been reached yet. On one hand, the abscopal effect suggests that radiation therapy can enhance the presentation of tumor-associated antigens and activate immune-mediated antitumor responses [13]. Furthermore, a secondary analysis of KEYNOTE-001 phase 1 trial revealed that patients who had previously received radiation therapy before receiving pembrolizumab exhibited significantly longer progression-free survival [14], supporting a synergistic effect between ICIs and radiation therapy. Conversely, radiation therapy is considered a double-edged sword in anti-tumor immunity, as it may impair immune cell function and alter the tumor microenvironment [15]. Indeed, several investigators have suggested that combining radiation therapy with ICIs is associated with locoregional recurrence in the irradiated field and with HPD [16-18]. The proposed underlying mechanism includes radiation-induced upregulation of VEGF, which promotes tumor angiogenesis [19], and enhances TGF- β that induces epithelial-mesenchymal transition and activates cancer-associated fibroblast [20]. Although the patient in our case received radiation therapy in combination with GC therapy, it is noteworthy that we have also administered radiation therapy (50 Gy) as part of bladder preservation therapy in 43.3% of other patients (201 out of 464 patients recorded in our biobank data), and none of these patients experienced HPD. This suggests that radiation therapy alone is unlikely to be the primary cause of HPD in our case.

Additionally, we characterized the genetic profile of our case, revealing intact *TP53* and *EGFR*, along with the presence of *FGFR3*-T757P (NM_000142), *BRCA2*-I3412V(NM_000059), and *ATM*-V2166A(NM_000051); *TP53* alterations are often observed in MIBC, while *FGFR3* alterations are in non-muscle invasive bladder cancer. Among these genes, *FGFR3* is recently focused and our previous study showed that *FGFR3* alteration could alter immune cells component in the tumor microenvironment of bladder cancer [21]. Several reports have identified *MDM2/4* amplification [22] and/or *EGFR* mutations [23] as predictive markers of HPD. Specifically, *MDM2/4* are known to block the p53 transactivation domain and promote proteasomal, ubiquitin-dependent degradation of p53 [24], while *EGFR* mutations are frequently associated with the upregulation of PD-1/PD-L1, thereby facilitating immune escape [11]. Indeed, among 155 patients analyzed for genomic mutations, *MDM2/MDM4* amplification (odds ratio = 10.2; $p = 0.002$) and *EGFR* alterations (odds ratio >11.9; $p=0.001$) correlated with time to

treatment failure within two months [6]. However, in our case, no *MDM2/4* amplification was observed, and both *EGFR* and *TP53* remained intact, suggesting that cancer-associated gene alterations did not play a significant role in the development of HPD in our patient.

To further explore the immunological mechanisms underlying HPD, we are currently undertaking a cellular neighborhood analysis of tumor tissue using spatial profiling with the PhenoCycler platform (Akoya bioscience, Marlborough, MA, USA), as a part of OMPU-NCC Cancer Consortium Project [21,25]. Thus far, our analysis has revealed that terminally exhausted T cells, such as CD8⁺PD-1⁺TIM-3⁺ T cells, are scarce, while CD8⁺IFN γ ⁺ T cells are observed in the metastatic region of HPD. Cellular clustering from neighborhood analysis showed that CD8⁺IFN γ ⁺ T cells predominantly localize to Region 3 and 6 (R3 and 6); where R3 is enriched with CD4⁺CD25⁺FOXP3⁺ cells, and R6 comprises tumor cell, thereby delineating distinct tumor microenvironment (**Figure 1A**). Comparative analysis between primary and metastatic tumor sites indicated that while CD163⁺ cells do not correlate with tumor cells in primary lesions, a positive correlation is observed in metastatic sites. Furthermore, CD8⁺PD-1⁺ T cells exhibited a positive correlation with tumor cells and PD-L1 expression in primary lesions, whereas CD8⁺IFN γ ⁺ T cells were correlated with those cells in metastatic site, suggesting a dynamic alteration of the tumor microenvironment (**Figure 1B**). Although IFN γ is widely recognized as a pro-inflammatory cytokine, it has been shown to upregulate IL-8 gene expression in monocytic lineage cells, as our sequence data supports [26]. Furthermore, emerging evidence highlights that increased IFN- γ in the tumor microenvironment can promote immune evasion by upregulating PD-L1 expression on tumor cells through the JAK-STAT pathway. This is supported by clinical studies showing that the clinical response to PD-1 blockade can be predicted by the IFN- γ mRNA profiling of immune cells [27,28]. However, as the precise contribution of IFN γ and PD-L1 to hyper progression remains unclear, additional investigations are necessitated to understand these immunological mechanisms.

In conclusion, significant uncertainties persist regarding HPD. First, a consensus definition of HPD has not been established. Although criteria such as RECIST (response evaluation criteria in solid tumor), TGR (tumor growth rate), TGK (tumor growth kinetics), and TTF (time to treatment failure) have been employed in several studies, it remains unclear which parameter most precisely characterizes HPD [29-31]. Given these challenges, the identification of reliable biomarkers plays a pivotal role. Specifically, two types of biomarkers are crucial: diagnostic biomarkers that can clearly define HPD when it occurs, and predictive biomarkers that can identify patients at risk of developing HPD before initiating treatment. The presence of intra-tumoral heterogeneity further complicates biomarker evaluation, as biomarker levels may vary among samples from different sites, even within the same patient

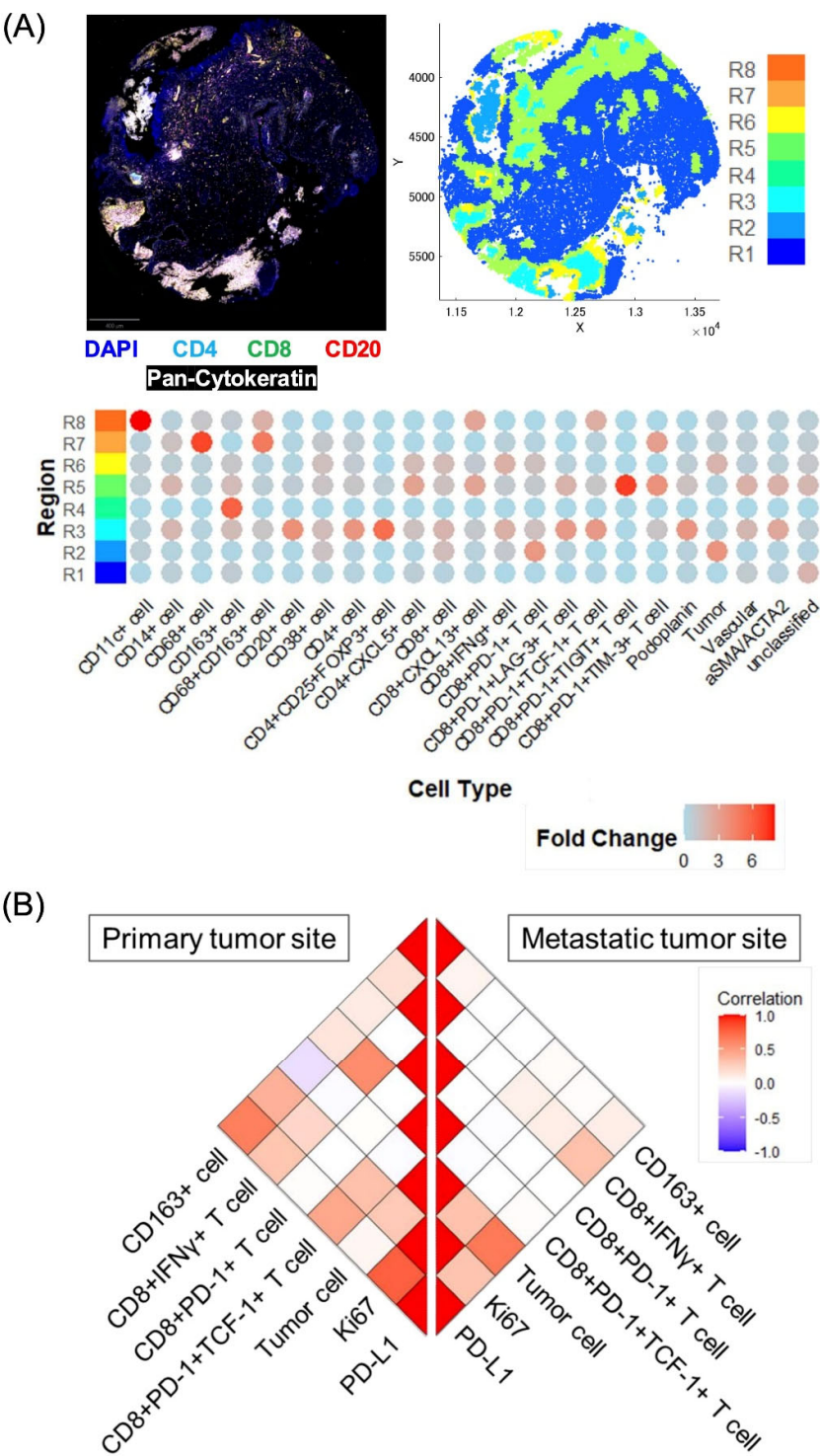


Figure 1. Cellular neighborhood analysis of hyperprogressive disease (HPD) tissues. **(A)** Tissue microarray (TMA) cores from patients receiving avelumab were stained with 41 immunofluorescent antibodies using the PhenoCycler system (top left). The resulting image data were processed using CytoMAP to perform cellular neighborhood analysis, which revealed distinct clustering patterns within the tumor microenvironment (top right: clustering map overlaid on TMA; bottom: heatmap of the identified cluster; “R” indicates regions defined by these clustering patterns). **(B)** Correlation heatmap comparing primary and metastatic tumor sites from the same patient.

[32]. Since HPD is now increasingly acknowledged as a distinct and clinically significant phenomenon that arises specifically in response to ICIs, rather than being a result of natural disease progression or treatment failure, further studies are essential to define robust diagnostic criteria, identify predictive parameters, and elucidate the underlying mechanism. Such efforts will improve patient selection, optimize treatment strategies, and ultimately reduce adverse outcomes associated with HPD.

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