Editorial Commentary

Imposter among us: field cancerization in the bladder

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Comment on: Strandgaard T, Nordentoft I, Birkenkamp-Demtröder K, *et al.* Field Cancerization Is Associated with Tumor Development, T-cell Exhaustion, and Clinical Outcomes in Bladder Cancer. Eur Urol 2024;85:82-92.

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Every year, 80,000 individuals are diagnosed with bladder cancer in the United States, of which the majority are histologically classified as urothelial carcinoma arising from cells in the transitional epithelium. Non-muscle-invasive bladder cancer (NMIBC) comprises approximately 75% of new bladder cancer diagnoses in the United States (1). Most patients diagnosed with NMIBC can look forward to a favorable prognosis, with cancer-specific survival (CSS) approaching 70–85% 10 years after diagnosis (2). However, NMIBC remains difficult to treat for the portion of patients that experience disease recurrence and progression after intravesical bacillus Calmette-Guérin (BCG) therapy. For those who experience metastasis, 5-year survival plummets to 5% (3). There is therefore a critical unmet need to identify factors associated with recurrence in patients with NMIBC.

In 1953, Slaughter *et al.* proposed the concept of field cancerization upon observing that oral squamous carcinomas tend to spread laterally across oral epithelial tissue instead of vertically downward or inward (4). Moreover, in resected oral tumors, the benign epithelium contained signs of hyperplasia and hyperkeratinization accompanied by fibrosis and atrophy of the surrounding tissues. Beyond directly adjacent tissue of the original tumor, separate foci of oral squamous cell carcinoma were discovered in normal-appearing tissue, supporting the idea of multicentric tumor origin. This discovery, along with lateral tumor spread and histologically normal tissue displaying abnormal cell growth, led to their proposal of the hypothesis of field cancerization: mutations in histologically normal tissue predispose a patient to develop synchronous or metachronous tumors due to long-term carcinogen exposure (4).

Subsequent advances in molecular biology have enabled genetic analyses to confirm the hypothesis that a field of normal tissue harboring genetic alterations frequently exists prior to initial cancer development (5). Multiple authors (6-8) have used next generation DNA sequencing to observe abnormal changes in DNA methylation and the presence of mutations and copy number variations in histologically normal bladder samples. Despite the confirmation of Slaughter's original hypothesis of field cancerization through advances in high throughput DNA sequencing technologies, little work has been done to evaluate the clinical impact of field cancerization. In their recent study, Strandgaard *et al.* investigated the association of field cancerization with recurrence, disease progression, response to treatment, and the tumor microenvironment (TME) in patients with high-risk NMIBC (9).

In this retrospective study, 751 selected site biopsies (SSBs) were collected from dysplastic/cancerous lesions (n=79), histologically normal urothelium (n=662), and sites

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with other abnormal characteristics (n=10) from 70 patients with NMIBC who received at least five BCG instillations (9). Using deep-targeted sequencing, the mutational profiles of tumors and normal SSBs were compared. Unsurprisingly, tumor samples displayed higher levels of non-synonymous mutations compared to normal SSBs. However, normal SSBs did carry a high proportion of bladder cancer driver gene mutations. Specifically, normal SSBs already harbored 33% of high-impact cancer driver gene mutations originally observed in tumors, including mutations in *TP53*, *STAG2*, *ARID1A*, *KMT2D*, and *PIK3CA*. Most of these genes are central to the development of bladder cancer, and many are involved in chromatin remodeling. These findings are consistent with other recent reports of cancer driver gene mutations in normal-appearing urothelium, of which the most frequently mutated included chromatin remodeling genes (e.g., *KMT2D*, *KDM6A*, *ARID1A*) (7,10).

The authors then classified patients based on the highest observed level of field cancerization, defined as the maximum number of mutations detected in any normal-appearing urothelium sample from that patient. Patient smoking history was not found to be associated with level of field cancerization, despite prior hypotheses suggesting that exposure to cigarette smoke and other carcinogens may contribute. Interestingly, patients with high field cancerization levels experienced significantly worse high-grade recurrence-free survival (HG-RFS) within the first 9 months of follow-up, suggesting that field cancerization is associated with worse short-term patient outcomes. However, it is important to highlight that this association with HG-RFS was not statistically significant with longer follow-up beyond the initial 9 months, and there was no significant association with overall RFS or progression-free survival. The lack of a long-term association decreases the clinical impact of the study. Nevertheless, high-grade recurrences that occur within just a few months after completion of BCG therapy can still be clinically meaningful and impactful to a patient who must suffer the treatment-related physical discomfort and psychological consequences of such a rapid recurrence, including fear, anxiety, worry, and decreased health-related quality of life (11,12). Thus, field cancerization levels could potentially serve as a useful biomarker to identify higher risk patients who require closer follow-up during the first 9 months after BCG therapy.

Strandgaard and colleagues next sought to explain the link between elevated levels of field cancerization and higher rates of high-grade recurrence after treatment with BCG (9). Prior studies have demonstrated that persistent viral infections can induce $CD8^+$ T-cell exhaustion, resulting in loss of effector functions through loss of antigen binding effectiveness and expression of inhibitory receptors (13-15). Cancer is associated with a similar *modus operandi*, whereby continuous presentation of tumor antigens causes T-cells to undergo transcriptional and epigenetic changes that render them in an exhausted state (16). In the biopsy samples analyzed by Strandgaard *et al*., high levels of tumor mutational burden (TMB) and neoantigen load were associated with prominent levels of field cancerization prior to treatment, leading to the hypothesis that field cancerization may be a potential contributor to T-cell exhaustion due to chronic exposure to neoantigens (9).

To further evaluate the potential association of field cancerization with T-cell exhaustion, samples from pre- and post-BCG treated tumors were profiled for expression of immunoinhibitory genes to determine whether CD8 T-cell exhaustion correlated with a high field cancerization level. In pre-BCG samples, heightened T-cell exhaustion was indeed associated with increased pre-treatment field cancerization. Moreover, a previously developed predictor of post-BCG T-cell exhaustion (ExhP) based on pre-BCG tumor gene expression levels (17) was also associated with increased pre-treatment field cancerization, although this was not statistically significant. Together these results suggest that field cancerization may contribute to CD8 T-cell exhaustion though continuous presentation of neoantigens, resulting in constrained future anti-cancer immune responses. In this regard, patients with extensive field cancerization pre-treatment could warrant closer monitoring due to field cancerization precipitating post-treatment T-cell exhaustion and potential treatment failure.

The association of field cancerization with short-term high-grade disease recurrence suggests that further study is warranted on whether field cancerization levels should be considered as part of risk stratification of NMIBC patients. NMIBC is categorized into high, intermediate, or low-grade risk groups based on clinicopathologic features including histology, size, and multifocality (18). Assessing field cancerization may further risk stratify patients at the time of diagnosis. However, the potential incorporation of field cancerization into routine clinical practice faces several challenges with respect to standardizing the collection and analysis of healthy tissues. Random mapping biopsies of normal-appearing urothelium is often not recommended in

clinical guidelines due to the low sensitivity for carcinoma *in situ* and potential for damage to the bladder without clear benefit (18,19), although others advocate their use in high-risk clinical scenarios (2,20,21). Efforts are underway to standardize the collection of tissues in clinical trials (22), but such practices are not easily implemented in real-world clinical settings. Another impediment to assessing field cancerization is the high cost of next generation sequencing, although these costs have continued to decrease dramatically, outpacing Moore's Law (23). The use of targeted gene panels to assess field cancerization may be more cost-effective, widely available, and faster than whole exome sequencing, but it will be essential for the urologic and genitourinary pathology communities to come to a consensus regarding standardization of the optimal analytical approach.

A potential non-invasive alternative to normal bladder biopsies for assessment of field cancerization is a liquid biopsy biomarker such as urine tumor DNA (utDNA), which was demonstrated by Strandgaard *et al.* to reflect the total mutational burden from both tumors and field cancerization in the bladder (9). Additionally, utDNA can help monitor disease recurrence and progression after therapy and may even outperform traditional diagnostic surveillance methods. For example, the authors found in several cases that utDNA was positive for the presence of tumors despite cytology returning negative results. The authors also noted that patients who experienced recurrence and/or disease progression from NMIBC to muscle-invasive bladder cancer (MIBC) had higher levels of utDNA after BCG treatment, suggesting its potential as a monitoring tool and active biomarker of disease state. Other biomarkers such as circulating tumor DNA (ctDNA) and circulating tumor cells (CTCs) have also shown diagnostic and predictive value in monitoring disease course (24). Incorporation of such non-invasive biomarkers into clinical practice to monitor patients could help improve their short-term and long-term outcomes.

The possible connection between high field cancerization levels, poor short-term HG-RFS, and T-cell exhaustion also raises the question of whether programmed cell death protein 1 (PD-1) inhibitors, which can reverse T-cell exhaustion, could potentially be pushed earlier in the NMIBC treatment paradigm. Current guidelines stipulate that patients with BCG unresponsive NMIBC may be treated with the immune checkpoint inhibitor pembrolizumab, based on results from the KEYNOTE-057 study (18,25). However, this recent data from Strandgaard *et al.* suggests that patients with high-grade NMIBC and high field cancerization levels may experience early high-grade recurrence after BCG potentially due to T-cell exhaustion (9). For these patients, earlier administration of PD-1 inhibitors may have the potential to improve the effectiveness of BCG treatment and improve short-term HG-RFS. Although this hypothesis may be worth testing in future clinical trials, caution is warranted given the high financial costs and toxicities of immunotherapy (26). In addition, the interplay of high field cancerization with other treatment modalities such as chemotherapy, radiation therapy, and targeted therapies warrants further study.

In summary, this interesting paper by Strandgaard *et al.* highlights the potential association of field cancerization with worse short-term treatment outcomes for NMIBC patients treated with BCG (*Figure 1*) (9). Further study is needed regarding whether high field cancerization levels may also increase the risk of recurrence in patients with NMIBC that otherwise do not have high-risk features. Moreover, the potential connection between field cancerization and T-cell exhaustion raises questions regarding whether earlier administration of immune checkpoint inhibitors may further improve outcomes of high-risk NMIBC patients with high field cancerization levels, or whether it may be necessary to look further afield to other treatment options for these patients.

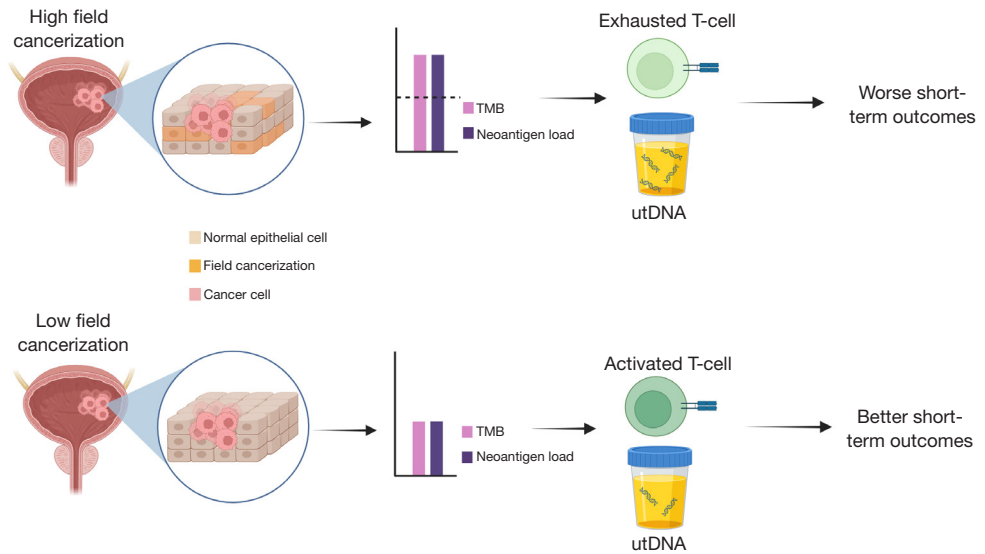

Figure 1 High field cancerization in bladder cancer leads to lower short-term HG-RFS after bacillus Calmette-Guérin therapy in part due to elevated TMB and neoantigen load and exhausted T-cells. Total mutational burden from both the tumor and the field cancerization is reflected in higher levels of utDNA. Created with BioRender.com. HG-RFS, high-grade recurrence-free survival; TMB, tumor mutational burden; utDNA, urine tumor DNA.

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