Enhancing response to immunotherapy in urothelial carcinoma by targeted inhibition of the histone methyltransferase G9a pathway

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Provenance: This is an invited article commissioned by the Section Editor Xiao Li (Department of Urology, Jiangsu Cancer Hospital & Jiangsu Institute of Cancer Research & Nanjing Medical University Affiliated Cancer Hospital, Nanjing, China).
Comment on: Segovia C, San José-Enériz E, Munera-Maravilla E, *et al*. Inhibition of a G9a/DNMT network triggers immune-mediated bladder cancer regression. Nat Med 2019;25:1073-81.

Bladder cancer (BCa) is the fourth most common malignancy among men, and is the eighth most common cause of cancer death in the US (1). Once muscle-invasive, the treatment options for BCa are limited, and include radical cystectomy with or without neoadjuvant platinum-based chemotherapy or organ sparing chemoradiotherapy (2). In 2016, the treatment of metastatic BCa (mBCa) was revolutionized by results of a phase II trial of immune checkpoint programmed death-ligand 1 (PD-L1) inhibitor atezolizumab in 311 patients with mBCa who progressed after chemotherapy (3,4). The trial found a response rate of 15% in patients receiving 1,200 mg atezolizumab for 3 weeks, which was a significant improvement over systemic chemotherapy historical controls, which had a response rate of 10% (2). Several additional trials of other inhibitors of the PD-1/PD-L1 pathway in mBCa, such as pembrolizumab (5), nivolumab (6), durvalumab (7), and avelumab (8), also found sustained response in mBCa patients.

Despite the success of PD-1/PD-L1 inhibitors over conventional chemotherapy, the majority of mBCa patients fail to respond to PD-1/PD-L1 inhibitors. There have been attempts to use PD-L1 expression as a biomarker for potential response to PD-1/PD-L1 inhibitors (9). PD-L1 expression does appear to correlate with higher likelihood of responding to PD-1/PD-L1 inhibition, but response can still be achieved in low PD-L1 expressing patients (2). Therefore, there is a current need to determine biomarkers that would improve selection of therapy for mBCa patients, and to discover drugs that would improve the efficacy of PD-1/PD-L1 inhibitors.

G9a (*EHMT2*) is a histone methyltransferase which methylates histone 3 at lysine residue 9 twice (H3K9), forming dimethylated H3K9 (me1, me2). This recruits HP1, which inhibits transcription, and DNA methyltransferase DNMT1, which methylates nearby DNA and further reinforces transcription inhibition (10). Physiologically, G9a is involved in embryological development (10) and in the inhibition of hypoxia responsive genes during hypoxia (11). Pathologically, G9a is overexpressed in bladder, breast, ovarian, and many other cancers (12), and is associated with poor prognosis, due to its ability to suppress tumor suppressor genes such as *Trp53* (13).

Segovia *et al*. (14) report on the effects of CM-272, a dual G9a and DNMT1 inhibitor. They demonstrate that CM-272 with chemotherapy is superior in terms of reducing tumor and metastatic burden in a quadruple-knockout (QKO) mouse model of mBCa (inactivated *Pten, Trp53, Rb1, Rbl1*) when compared to CM-272 alone or chemotherapy alone. The investigators performed transcriptome analysis of tumor cells from the QKO mice treated with CM-272 with chemotherapy, and determined that the mechanism of CM-272 was the induction of immunogenic cell death, due to the upregulation of cytokines (interferon-α, interferon-γ, tumor necrosis factor-α) and increased expression of major histocompatibility genes such as β2 microglobulin. They also found that the addition of CM-272 caused extensive infiltration of CD8+ T cells and natural killer cells into the tumor, suggesting that CM-272 was able to induce inflammation within the tumor, and thus increase the tumor's sensitivity to an immune checkpoint inhibitor.

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The investigators then treated QKO mice with a PD-L1 inhibitor in combination with CM-272, and found that only 17% of mice with PD-L1 + CM-272 had tumor at the end of treatment, compared to 75% of mice treated with PD-L1 alone. The mice treated with PD-L1 + CM-272 had infiltrations of CD3+, CD8+, and NK cells, suggesting that the CM-272 was capable of reactivating the immune system against tumors.

The investigators then assessed whether G9a was overexpressed in mBCa patients who failed therapy with PD-L1 inhibition. They measured G9a levels in 3 mBCa patients who failed PD-L1 therapy, and found that G9a expression was roughly doubled in the PD-L1 resistant patients compared to patients who responded to PD-L1 blockade. This indicates that G9a is a potential biomarker that could be used to predict patient response to PD-L1 therapy (15).

Prior studies show that G9a-deficient helper T cells are impaired in their production of Th2 lineage-specific cytokines (IL-4, IL-5, IL-13) (16). This cytokine expression profile creates an environment that favors M1 macrophage differentiation and Th1/CD8+ T cell proliferation. Increasing the population of CD8+ T cells results in enhanced anti-tumor effect of PD-L1 inhibitors due to a greater number of effector cells recruited to the area of the tumor. Patients initially resistant to PD-L1-inhibitor treatment due to low CD8+ T cell concentration in the tumor microenvironment should experience improvement in their response to PD-L1 inhibitors if G9a inhibition stimulates the immune system to increase proliferation of CD8+ T cells.

Other studies have also demonstrated that G9a inhibition leads to increased expression of inflammatory mediators such as TNF-alpha and IFN-alpha (17,18). Increased IFN-alpha expression results in an antiviral response that is more robust after G9a inhibition. Fibroblasts with G9a inhibition are converted into cells that produce large amounts of IFN-alpha, resulting in greater resistance to viral infection (18).

This study by Segovia *et al.* (14) did not show a change in the proliferation of FOXP3+ or CD4+ T cells after treatment with CM-272. However, another study found that deleting DNMT1 decreased the number and function of peripheral regulatory T cells (Tregs) and could impair conversion of T cells into FOXP3+ T regs (19). The resulting loss of immunosuppressive function increased the risk of lethal autoimmunity in mice, suggesting that reduction of DNMT1 activity below the threshold necessary for maintenance of a classical Treg lineage may

lead to serious adverse outcomes in the treatment of cancer patients. Other studies have supported the development of colitis in mice treated with G9a inhibitors, associated with pathologic intestinal inflammation due to the decreased differentiation of naïve T cells into Th17 and Treg cells (20). The study by Segovia *et al.* (14) did not report on adverse effects of treatment with CM-272 besides the association of combined treatment of CM-272 and anti-PD-L1 with the development of renal toxicity. Therefore, when evaluating the effects of CM-272 in large human populations, special caution must be used in establishing safe dosage and monitoring for harmful autoimmune effects.

We could hypothesize that treatment with CM-272 could activate the immune system in a way that would be permissive for greater BCa treatment response with other treatment options that are currently used. For instance, intravesical Bacillus Calmette-Guerin (BCG) can be administered with IFN-alpha as a salvage treatment for patients who experience non-muscle-invasive bladder cancer (NMIBC) recurrence after initial trial of BCG alone (21). Since CM-272 treatment increases production of IFN-alpha as part of its immune system activating effects, CM-272 treatment might be a more effective therapy than IFN-alpha if used in combination with BCG for high-risk NMIBC.

In summary, the findings from Segovia *et al.* (14) are consistent with findings from previous studies elucidating the relationship between G9a/DNMT1 inhibition and its activating effects on the immune system. Although there is concern for widespread immune system dysregulation, use of CM-272 in combination with PD-1/PD-L1 inhibitors opens an exciting avenue for treatment of advanced BCa. Future studies of the use of CM-272 in combination with other treatment options for BCa could yield similarly promising results.

Acknowledgments

Footnote

to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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