Editorial Commentary

Novel G9a/DNMT first-in-class dual reversible inhibitor has potent antitumor effect in bladder cancer

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Epigenetic changes, which are heritable changes that alter gene expression without changing the primary DNA sequence, are a hallmark of cancer. The epigenome is a therapeutic target in cancer. There are two main forms of epigenetic modifications: those that directly modify DNA (i.e., DNA methylation) and those that modify DNA-binding proteins (i.e., histone modifications).

G9a is overexpressed in various cancers. The methylation of its target, histone 3 lysine 9 (H3K9), is correlated with silence of transcription (1-3). Some researchers have reported that suppression of G9a expression leads to decreased tumor cell proliferation, delayed cancer progression, and blocked tumor metastasis (1,2,4,5). In addition, G9a interacts with DNA methyltransferase-1 (DNMT1) to regulate methylation of DNA and histone while cell division, which promotes transcriptional silence of the target gene (6,7). A decrease in methylation levels of DNA and H3K9 promote reactivation of tumor suppressor genes that suppress tumor cell growth (8,9). However, the basic mechanism is not fully understood.

CM-272 is a first-in-class potent, dual and reversible inhibitor of G9a (GLP) and DNMT with novelty. The article of Segovia *et al.* (10) presents a potential new strategy for the treatment of bladder cancer (BC) from the use of an epigenetic inhibitor which previously unknown in combination with immune checkpoint inhibitors (ICIs).

Role of G9a/DNA methyltransferase-1 in bladder cancer

G9a (EHMT2), a histone methyltransferase that catalyzes lysine 9 of histone 3 (H3K9), showed high levels of expression in several malignancies like breast cancer and head and neck squamous cell carcinoma. Previous studies reported that G9a was highly expressed in bladder transitional cell carcinoma (TCC), and that G9a inhibition notably diminished cell proliferation (11). These results have attracted attention towards the role of G9a in cellular metabolism and suggest that G9a may be a therapeutic target for bladder TCC. Although G9a overexpression in non-muscle-invasive bladder cancer (NMIBC) is associated with poor clinical outcomes, recurrence in NMIBC is influenced by many confounding factors. Hence, further analysis is needed to correlate G9a RNA levels with NMIBC prognosis. Studies are also needed on G9a RNA levels to determine its association with molecular subtypes and with their prognosis.

Use of specific G9a (A-366) and DNMT (decitabine) inhibitors in combination, or use of small interfering RNA (siRNA)has a synergistic effect.

Mode of action of CM-272

CM-272 is a novel reversible dual inhibitor against G9a and DNMTs. It lengthens survival in *in vivo* models of hematologic cancers by causing immunogenic cell death (12). While CM-272 does not directly affect EZH2 methyltransferase activity, it leads to a decrease in H3K27me3 levels exclusively as well as H3K9me2 levels in BC. Knockdown or overexpression of EZH2 confers elevated resistance or sensitivity, respectively, to CM-272 in

BC. Cumulatively, above results represent the presence of a regulatory loop between EZH2 and G9a in BC, without direct effect of CM-272 on methyltransferase activity of EZH2.

Segovia and colleagues report that CM-272 induced up-regulation of genes involved in the regulation of immune responses in BC cell lines, and that the combination of CM-272 and anti-PD-L1 was effective against primary and metastatic tumors in mice, producing a sustained reduction in, or elimination of, tumor burden (10).

Clinical implications of CM-272 in BC treatment

For decades, standard treatment for advanced urothelial carcinoma (UC) has been focused almost exclusively on cisplatin-based chemotherapy. ICIs have been demonstrated to have a durable long-term response and tolerable safety in recent clinical trials. However, in about 70–80% of patients with metastatic BC the tumor is unresponsive to ICIs. Therefore, further research on the inhibition of G9a/DNMT network in BC could present a potential new strategy for the treatment of BC from the use of a previously unknown epigenetic inhibitor in combination with ICIs.

Segovia *et al.* (10) demonstrated that treatment of CM-272 + cisplatin shows statistically significant regression in immunocompetent quadruple-knockout (PtenloxP/loxP; Trp53loxP/loxP; Rb1loxP/loxP; Rbl1$^{-/-}$) transgenic mouse models. However, the immune system of the mice in this study is different from that of humans. Therefore, this effect needs to be demonstrated again in the humanized mouse model.

The treatment of CM-272 + cisplatin reveals statistically significant regression of established cancers and metastases. Recent studies (12,13) have shown that the responses of type I interferon impact to the efficacy of chemotherapeutic agents. This could be one of the mechanisms of action of CM-272 + cisplatin treatment.

The anticancer effect is notably enhanced with the combination of CM-272 with anti-programmed cell death ligand 1 (PD-L1), even in the absence of cisplatin. These effects correlate with an endogenous anticancer immune response and immunogenic cell death, with the conversion of a cold tumor into a hot tumor. The PD-L1 expression change after CM-272 treatment *in vivo* needs to be studied further.

In the study of Segovia *et al.* (10), on day 28, 75% of the mice in the anti-PD-L1 treatment group had evidence of cancers and metastases, whereas only 17% of the mice in the CM-272 + anti-PD-L1 treatment group, with or without cisplatin (CDDP)-based chemotherapy, had evidence of a primary cancer or metastatic disease. To enhance the effects of ICIs, extensive studies are being conducted to investigate the therapeutic effects of combinations of anticancer drugs and ICIs. Therefore, Segovia *et al.* (10) need to explain whether the effect of treatment with CM-272 plus anti-PD-L1 plus CDDP is lower than that of CM-272 + anti-PD-L1 due to renal toxicity or other reasons.

The recent National Comprehensive Cancer Network (NCCN) Guideline recommends the use of ICIs as the primary treatment in CDDP-ineligible patients whose tumors express high levels of PD-L1 (14).

Carboplatin-based combination chemotherapy is recommended when PD-L1 expression is not increased, in which case an objective response rate of about 30–40% for 9 to 10 months has been reported (15). This study included a cohort of CDDP-ineligible patients (1 responder and 2 non-responders) with advanced or metastatic UC who were treated with first-line anti-PD-1 therapy. In order to interpret the results more accurately, additional information about PD-L1 expression may be required.

G9a expression was associated with resistance to programmed cell death protein 1 (PD-1) inhibition in patients with BC. Further research is needed to determine whether G9a expression can be used to predict the effects of ICIs.

Conclusions

Targeting or modulating G9a/DNMT methyltransferase activity might emerge as a new area in the treatment of BC. The results of the study of Segovia *et al.* (10) have furthered the implications of investigating G9a/DNMT. Validation of CM-272-induced up-regulation of genes involved in the regulation of immune responses in a larger cohort of patients with BC could lead to the discovery of a new combination therapy of CM-272 and ICIs and provide biomarkers for immunotherapy in patients with metastatic BC.

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Footnote

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