DIVERGENT THERAPEUTIC OUTCOMES OF STING AGONIST ADU-S100 IN INTRATUMORAL AND INTRAVESICAL TREATMENT REGIMENS IN SYNGENEIC MURINE MB49 AND IN THE N-METHYL-N-NITROSOUREA (NAT) RAT MODEL OF UROTHELIAL CARCINOMA

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Background STING agonist ADU-S100 as an intratumoral (IT) therapeutic regimen shows excellent CD8+ T cells-mediated antitumor immunity. Rapid absorption from IT sites, short terminal half-life and treatment related adverse outcomes caused withdrawal of ADU-S100 from clinical trials. We developed BCG-STING, a preclinical candidate for non-muscle invasive bladder cancer (NMIBC) that overexpresses a STING agonist (c-di-AMP) and confers superior antitumor immunity over BCG-WT by increasing tumor infiltrating CD4+ and CD8+ T cells and inflammatory macrophages.1 Due to similarities in the mechanism of action, comparative antitumor efficacies of BCG-STING and ADU-S100 were examined in an IT and intravesical (IV) dosing regimen.

Methods Syngeneic MB49 flank tumors in C57BL/6 female mice were given IT treatment of BCG-WT, BCG-STING (5 x 106 CFU) or ADU-S100 (100, 50 or 25 mg). End-point measurements included tumor volume and flow-cytometry based tumor immune infiltrate analyses (at 100 mg). MNU carcinogen rat model of NMIBC and the standard IV administration regimen was used for BCG-WT or BCG-STING (5 x 10⁶ CFU, 6x weekly) or ADU-S100 (25 mg, 6x weekly). Histopathological analyses of MNU rat bladders were performed for tumor involvement index (TII) and pathological tumor staging.

Results IT administration of ADU-S100 in MB49 tumor showed greatest tumor regression over BCG-WT or BCG-STING even at lowest dose (25 mg). ADU-S100 caused strongest infiltration of TNF-a+ MHCII+ F4/80+ CD11b+ macrophages and IFN-g+ CD8+ T cells as compared to BCG-STING or BCG-WT. We did observe a significant increase in immunosuppressive IL-10+ and ARG-1+ Ly6C(high)Ly6G(−) monocytic myeloid-derived suppressor cells (M-MDSCs) in MB49 tumors treated with ADU-S100 and BCG-WT, but not BCG-STING. In contrast to MB49 model, IV induction course of BCG-STING in MNU rat model showed the greatest antitumor effects with only 5% residual TII compared to 30% in ADU-S100 or 42% in BCG-WT. Tumor staging revealed residual T1 (50%), CIS (25%) and Ta (25%) tumors in ADU-S100 group, BCG-WT treated group showed a lower degree of invasion to the lamina propria with CIS (50%), T1 (25%) and Ta (25%) residual tumors. BCG-STING IV therapy resulted in 60% of rat bladders showing complete tumor regression while 40% had minimal residual non-invasive tumors.

Conclusions Divergent therapeutic outcomes of IT vs IV treatment regimens of ADU-S100 over BCG-STING or BCG-WT in different urothelial carcinoma models indicate the critical role of tumor microenvironment and dosing regimens on relative efficacy. Induction of immunosuppressive M-MDSCs by ADU-S100 or BCG-WT suggests unique advantages of BCG-STING. The therapeutic targeting of M-MDSCs as combination may improve clinical efficacies of BCGs.

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REFERENCES

Ethics Approval All protocols involving animals strictly adhered to US NIH guidelines and were approved by the Johns Hopkins Medical Institutions Animal Care and Use Committee under the protocols: MO18M58, MO20M20, and RA17M332.