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. 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 10^6 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 10^6 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(hi)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.

Acknowledgements National Institute of Health, Maryland Tedco, Willowcroft Foundation, and Cigarette Restitution Fund

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.