Background: Malignant peripheral nerve sheath tumor (MPNST) is a rare, aggressive soft-tissue sarcoma with a poor prognosis and is insensitive to immune checkpoint blockade (ICB) therapy. Loss-of-function of the histone-modifying polycomb repressive complex 2 (PRC2) components, EED or SUZ12, is one of the main mechanisms of malignant transformation. Our ongoing collaborative work revealed that in a murine model of MPNST, PRC2-loss tumors have an 'immune desert' phenotype and intratumoral (IT) delivery immunogenic modified vaccinia virus Ankara (MVA) sensitized the PRC2-loss tumors to ICB. IT MQ833, a second-generation recombinant modified vaccinia virus Ankara virus, results in neutrophil recruitment and activation and neutrophil-dependent tumor killing. MQ833 was engineered by deleting three viral immune evasion genes, E5R, E3L, and WR199, and expressing three transgenes, including the two membrane-bound Flt3L and OX40L, and IL-12 with an extracellular matrix anchoring signal. In this study, we explored strategies to enhance anti-tumor effects of MQ833 by co-administration of granulocyte colony-stimulating factor (G-CSF) and elucidated the mechanisms of action.

Methods: We first assessed the innate immune effects and transgene expression of MQ833 infection in murine and human MPNST cell lines. We then evaluated antitumor effects of MQ833 in immune-competent mice. Wild-type C57BL/6J mice were implanted subcutaneously with PRC2-loss SKP605 cells (Nf1-/- Cdkn2a-/- Cdkn2b-/- Eed-/-). IT MQ833 was performed twice weekly. Tumor sizes were measured and survival of mice was monitored. For antibody depletion experiment, mice were treated with anti-CD4, CD8, NK1.1, CSF1, or Ly6G antibodies to deplete CD4, CD8, NK, macrophages, and neutrophils. To assess whether G-CSF co-administration improves MQ833 therapeutic efficacy, G-CSF was co-delivered with MQ833 twice weekly into the tumors. In addition, to evaluate how co-administration of G-CSF with MQ833 alters the immunosuppressive tumor microenvironment, we analyzed the tumor-infiltrating myeloid cells and T cells by flow cytometry.

Results: MQ833 infection potently induced type I IFN production in human and murine MPNST cells. IT MQ833 induced CD4 and CD8 T cell activation and neutrophil recruitment into PRC2-loss tumors. Antibody depletion experiment revealed the critical role of neutrophils, macrophages and CD4+ T cells in MQ833-induced antitumor immunity. In addition, co-administration of MQ833 and human G-CSF generated more potent antitumor efficacy in PRC2-wt tumors than either agent alone. Finally, our results show that MQ833 and hG-CSF combination treatment increased activated neutrophils in vivo.

Conclusions: In conclusion, MQ833 represents a promising therapeutic approach for ICB-resistant tumors by mobilizing and activating neutrophils and macrophages, addressing a common mechanism of tumor resistance to ICB.