Background Oncolytic virus (OV)-based therapies have demonstrated considerable promise for treating various solid tumor types. However, mounting evidence indicates OVs are negatively impacted by Type I interferon(s) (IFN) produced in solid tumors with functional cytosolic nucleic acid sensing mechanisms, including the cGAS/STING and/or RIG-I pathways. VAX014 is a novel, clinical stage oncolytic agent based on recombinant bacterial minicells (rBMCs) and is designed to target and deliver a pre-formed oncolytic protein toxin, perfringolysin O (PFO), directly to integrin-expressing tumor cells. Here we characterize the interaction and impact of tumor intrinsic STING and RIG-I on the antitumor activity of VAX014 in vitro and in vivo.

Methods Targeted deletion of STING or RIG-I in wild type MB49 murine urothelial carcinoma cells was performed via CRISPR-Cas9. Following extensive orthogonal in vitro functional characterization to confirm genotype/phenotype, MB49STING KO and MB49RIG-I KO tumor cell lines were utilized in in vitro preclinical pharmacology studies in wild type C57BL/6 mice to assess any influence of tumor intrinsic STING and/or RIG-I on the efficacy of VAX014 following intratumoral (i.t.) administration to intradermal (i.d.) tumors. Individual tumor growth rates and survival curves were plotted against respective saline and wild type controls.

Results In vitro analysis demonstrated wild type MB49 cells express STING and RIG-I (but not cGAS) and upregulate PD-L1 and MHC-I in a Type I IFN and Tank-binding kinase-1 (TBK-1) dependent manner following treatment with VAX014. Targeted genetic ablation of either STING or RIG-I (MB49STING KO and MB49RIG-I KO) reduced this response. In MB49STING KO cells, pharmacologic inhibition of TBK-1 led to complete elimination of Type I IFN production and subsequent PD-L1/MHC-I upregulation, as did pharmacologic inhibition of STING in MB49RIG-I KO cells. In vivo, both the MB49STING KO and MB49RIG-I KO tumor cell lines had similar i.d. tumor growth rates to wild type MB49. Consistent with previous work, weekly i.t. administration of VAX014 to wild type MB49 tumors led to a 100% durable complete response (CR) rate. In contrast, the loss of tumor intrinsic STING or RIG-I reduced CR rates and lengthened the time to respond in MB49STING KO or MB49RIG-I KO tumors.

Conclusions VAX014 activates both the murine STING and RIG-I pathways and the presence of tumor-intrinsic STING and/or RIG-I leads to optimal antitumor activity of VAX014 following i.t. administration. This unique mechanism pairs STING and RIG-I agonism and subsequent Type I IFN production with oncolysis-mediated availability of tumor antigens, which together, may lead to better antitumor T cell priming.

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Ethics Approval This study was approved by San Diego State University’s Institutional Animal Care and Use Committee under approved Animal Protocol number IACUC-22-064.

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