Background Non-small cell lung cancer (NSCLC) is a leading cause of cancer deaths, with a 5-year survival rate of 19%. Improvements in treatment are crucial for reducing both morbidity and mortality. In this study, we examined the therapeutic benefit of combining oncolytic vesicular stomatitis virus (VSV) expressing reovirus-derived fusion-associated small transmembrane (FAST) proteins (p14 or p15), PD-1 checkpoint blockade, and natural killer T (NKT) cell immunotherapy in a genetic mouse model of lung adenocarcinoma.

Methods Mice containing a tamoxifen-inducible Cre recombinase gene driven by the (Club cell-secretory protein) CCSP promoter, were crossed with mice containing a Lox-Stop-Lox KRAS<sup>G12D</sup> mutation and a floxed <sup>p53</sup> allele. CCSP-KP mice were treated with VSV-GFP, VSV-p14, or VSV-p15 on days 40, 42, and 44, followed on day 45 by treatment with <sup>α</sup>-galactosylceramide-loaded dendritic cells to activate NKT cells. Anti-PD-1 (ip. 300 mg) was given once a week for a total of 4 doses (days 48, 55, 62, and 69). MTT assays were performed to examine the oncolytic potential of the VSV-FAST constructs (VSV-p14, -p15, -p14delta, -p14endop15, -p10 ARV, -p10 NBV) in comparison to UV-inactivated VSV and VSV-GFP controls in murine (LLC, CMT-167) and human lung cancer cell lines (A549).

Results MTT cell viability assays demonstrate that VSV-FAST constructs VSV-p14, -p15, and chimeric -p14endop15 have increased tumor cell killing ability when compared to VSV-GFP in both mouse and human lung cancer cells. CCSP-KP mice receiving combinatorial treatment of VSV-p14 or VSV-p15, NKT cell activation, and PD-1 blockade exhibited increased overall survival in comparison with untreated or VSV-GFP combination therapy treated mice CCSP-KP mice. Furthermore, signs of morbidity (heavy or labored respirations, hunched posture, weight loss, etc.) were considerably delayed in treated mice.

Conclusions Our study demonstrates that VSV-FAST constructs are effective at killing lung cancer cells and when combined with NKT cell immunotherapy and immune checkpoint blockade, can prolong survival in a mouse model of NSCLC.

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Ethics Approval This study was approved by the Dalhousie University Committee on Laboratory Animals; approval number 20-100.