NATURAL KILLER T CELL IMMUNOTHERAPY COMBINED WITH FUSOGENIC ONCOLYTIC VIRUSES AND PD-1 BLOCKADE ENHANCES SURVIVAL IN A MOUSE MODEL OF SPONTANEOUS LUNG ADENOCARCINOMA.

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Aims: Lung cancer remains among Canada's leading cause of cancer deaths. Moreover, with a 5-year survival of 19%, improvements in treatment are crucial for reducing both morbidity and mortality. In this study, we examined the therapeutic benefit of combining NKT cell activation immunotherapy with PD-1 checkpoint blockade, and oncolytic vesicular stomatitis virus (VSV) expressing fusion-associated small transmembrane (FAST) proteins p14 or p15 on survival in a genetic mouse model of lung cancer. **Methodology:** We generated mice that contain a tamoxifen-inducible Cre recombinase gene driven by the CCSP promoter, with *KP* mice (LSL-KRAS^{G12D}; *p53$^{fl/fl}$)* to enable the time-dependent activation of oncogenic KRAS^{G12D} and ablation of one *p53* allele to drive lung cancer development (CCSP-KP mice). To first evaluate the importance of NKT cell subsets in lung cancer development, type I NKT (iNKT) cell deficient *TRAJ18$^{-/-}$ (Jα18)$^{-/-}$* or type I and II NKT cell deficient *CD1d$^{-/-}$* mice were bred with CCSP-KP mice (CCSP-KP/Jα18$^{-/-}$, CCSP-KP/CD1d$^{-/-}$) and monitored for survival against CCSP-KP mice. To evaluate therapeutic intervention outcomes in CCSP-KP mice, groups of CCSP-KP mice were treated with VSV-GFP, VSV-p14, or VSV-p15 (days 40, 42, 44) followed by an iv. treatment of α-galactosylceramide-loaded dendritic cells to activate iNKT cells (day 45). An ip. injection anti-PD-1 (300 μg) was given once a week for a total of 4 doses (days 48, 55, 62, 69). **Results:** CCSP-KP/Jα18$^{-/-}$ (n = 17) and CCSP-KP/CD1d$^{-/-}$ (n = 20) mice exhibited significantly reduced overall survival in comparison to CCSP-KP (n = 20) mice, which was more pronounced in females than males, demonstrating a protective role for iNKT cells. CCSP-KP mice receiving combinatorial treatment of iNKT cell activation with PD-1 blockade and VSV-p14endp15 or VSV-p15 (n = 9-10) demonstrated increased overall survival in comparison with untreated CCSP-KP mice (n = 10) and signs of morbidity (heavy or labored respirations, hunched posture, weight loss, etc.) were considerably delayed in treated mice. Two mice in the VSV-p15 triple combination group (2/10) survived until the experimental endpoint and displayed a *complete* reduction in respiratory distress. **Conclusion:** Our study demonstrates that iNKT cells have a protective role against lung cancer development and combining multiple immunotherapies with iNKT cell activation not only enhances and prolongs anti-tumor immune responses, ultimately increasing survival, but also minimizes treatment resistance due to the complex and distinct nature of each individual treatment.

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