Exploring the Effects of Oncolytic Viruses on the NK Cell Killing of Solid Tumors

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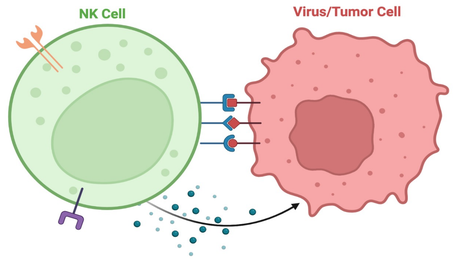
Introduction

Natural killer (NK) cells are integral to the innate immune system and function to immediately recognize and lyse virally infected cells and tumor cells[1,4]. NK cell therapy is largely and solely directed towards liquid tumors rather than solid tumors[1,4]. Solid tumors, including glioblastoma and pancreatic ductal adenocarcinoma, exhibit severe tumor hypoxia and maintain a suppressive tumor microenvironment through the secretion of NK sensitive-inhibitory cytokines[1,4].

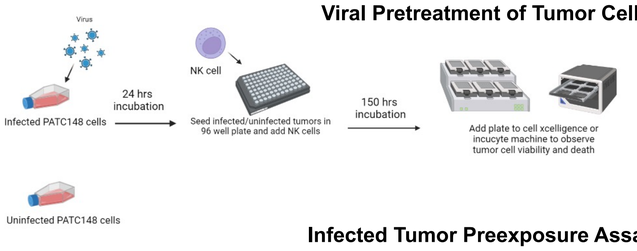


TGF-β is an immunosuppressive cytokine released by tumor cells into the microenvironment and generally functions as an inhibitory response to innate immune response of NK cells[5].

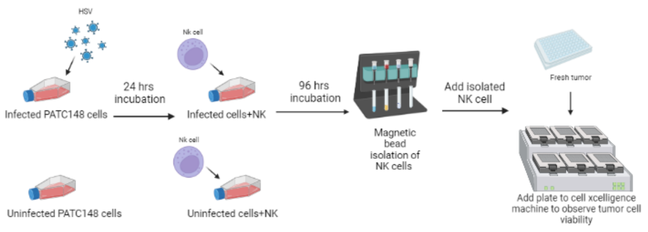
Oncolytic viruses are genetically-engineered viruses to specifically target and infect tumor cells and rather than normal cells. This includes 'Oncolytic-Herpes Simplex Virus (oHSV)' and 'Δ24RGD (Adenovirus)[2,3,6].'

Methods

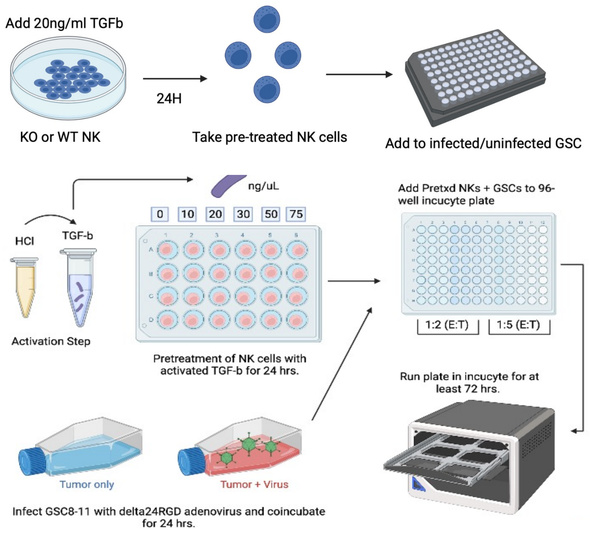
Viral Pretreatment of Tumor Cells



Infected Tumor Preexposure Assay



TGF-beta - NK Cell Pretreatment and Titer Assay



Experimental Questions and Results:

1. Can oncolytic viruses stimulate NK cell killing of solid tumors?
2. How do oncolytic viruses enhance NK cell killing?
3. Why does the TGF-beta knockout + virally pre-treated tumor cells exhibit the greatest NK cell killing response?

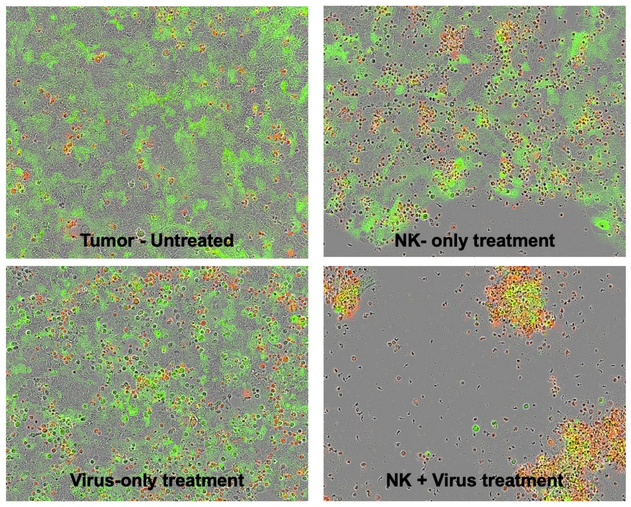


Figure 1: NK+Virus increases the cytotoxicity of NK cells against pancreatic tumor cells (PDAC).
Day 3 Incucyte fluorescence imaging of BXPC3. 1:10 effector to target ratio. Green fluorescence highlights live tumor cells and red, the dead cell stain. delta24-RGD, 0.5 MOI.

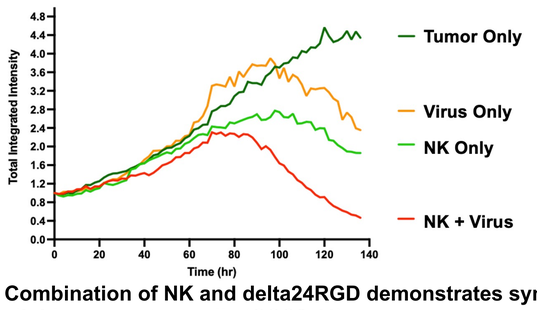


Figure 2: Combination of NK and delta24RGD demonstrates synergistic killing of glioblastoma stem cells (GSC8-11).
Incucyte, red Integrated Intensity of GSC8-11. Δ24RGD tumor pretreatment, 0.05 MOI. 1:2 effector to target ratio.

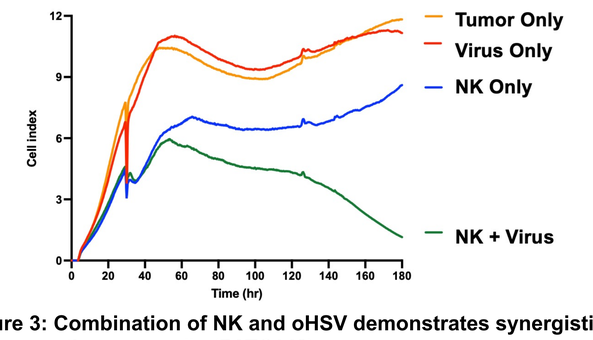


Figure 3: Combination of NK and oHSV demonstrates synergistic killing of pancreatic tumor cells (PATC148).
XCelligence of PATC148. oHSV tumor pretreatment, 0.01 MOI. 2:1 effector to target ratio.

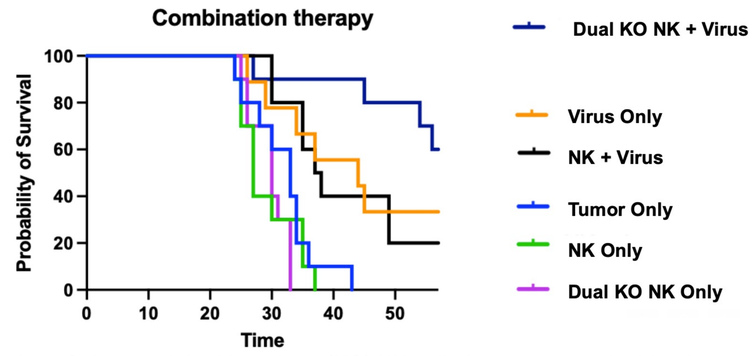


Figure 4: Combination of virus and dual knockout (KO) NK cells improves survival *in vivo*.
Survival analysis of mice injected with GSC8-11, Δ24RGD, and NK cells, respectively. Dual Knockout indicates lack of TGF-beta and NR3C1 Glucocorticoid receptor. n=10.

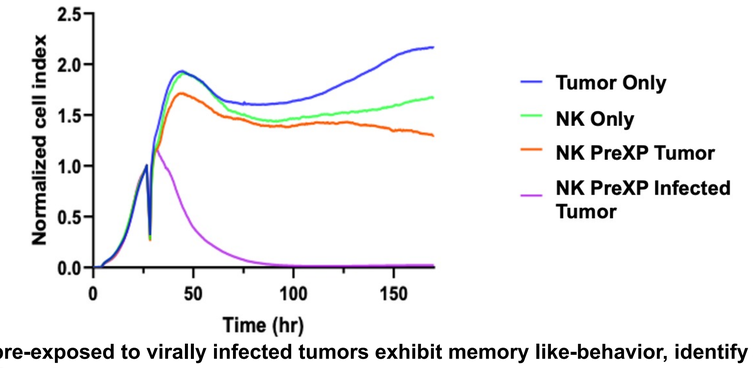


Figure 5: NK cells pre-exposed to virally infected tumors exhibit memory like-behavior, identify and effectively kill uninfected tumors.
XCelligence of PATC148. oHSV tumor pretreatment, 2 MOI. 4:1 effector to target ratio.

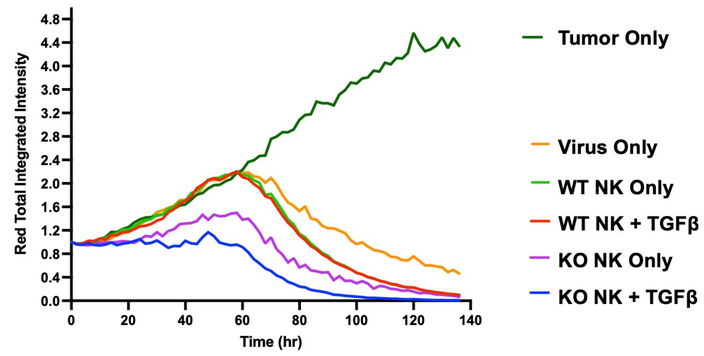


Figure 6: Wild-type (WT) and Knockout (KO) NK cell killing is unaffected by TGF-beta pretreatment
Incucyte: red Integrated Intensity of GSC8-11. Δ24RGD tumor pretreatment, 0.25 MOI.

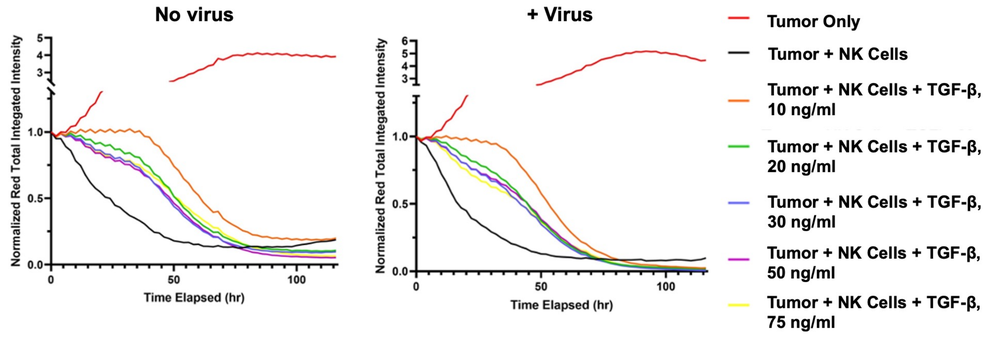


Figure 7: TGF-beta pretreatment reduces NK cell anti-tumor killing
Incucyte: red Integrated Intensity of GSC8-11. 1:2 effector to target ratio. Δ24RGD tumor pretreatment, 0.05 MOI.

Conclusions

- Oncolytic viruses enhance the NK cell killing response of solid tumors.
- Dual Knockout NK Cells + Virally pretreated tumors exhibit the greatest NK cell tumor lysis.
- NK cells exhibit memory-like behavior upon infected tumor preexposure.

Future Directions

- Optimization of the TGF-beta pretreatment assay to better understand the mechanism behind the dual knockout enhanced NK cell tumor lysis.
- ATAQ Seq and RNA Seq to understand changes in gene expression at different timepoints within our preexposure assay.

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