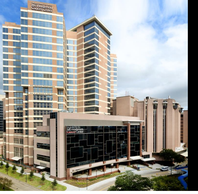


Combination therapy of oncolytic adenovirus with NK cells for the treatment of aggressive solid tumors

Ehulises Rodriguez, Xin Ru Jiang, Candelaria Gomez-Manzano MD, Juan Fueyo MD, Rafet Basar MD, Katy Rezvani MD

MD Anderson Cancer Center

THE UNIVERSITY OF TEXAS MD Anderson Cancer Center Making Cancer History®

Background

NK cells are a type of innate lymphocyte that specializes in killing of virally infected cells and tumor cells. Adoptive NK therapy has been successful for the treatment of many types of liquid tumors, including leukemia and lymphomas. However, due to the suppressive tumor microenvironment (TME), NK cells are not effective for targeting solid tumors. Oncolytic virus delta24RGD has been shown to induce inflammation in the TME following local injection. In addition, NK cells have been shown to be effective in eliminating adenovirus-infected cells. Therefore, this study will examine whether the combination of delta24RGD and NK cells can be effective for treating highly aggressive solid tumors and what the mechanisms behind the synergy is. This study will focus on investigating 1) the phenotypic changes in tumors following infection by the oncolytic virus, 2) the phenotypic and functional changes in NK cells following exposure to infected tumor cells, 3) the changes to the TME following viral infection of the tumor cells and how these changes can affect NK function and persistence. The results from this study can lay the foundation for potentially moving this treatment into the clinic. In addition, the mechanistic discoveries can be helpful for future genetic engineering of oncolytic viruses and NK cells be even more effective at targeting solid tumors.

Materials

PDAC and glioblastoma tumor cells:

For this aim, as a proof-of-principle experiment, we will be using glioblastoma (GB) and pancreatic ductal adenocarcinoma (PDAC) as tumor models for aggressive solid tumors because these two are one of the most difficult tumors to target with immunotherapy so far. The GB cell lines used are U87, which is an example of an adherent and differentiated GB cell line and GSC 8-11, which is an example of a glioma stem cell cell line. Both cell lines are used to determine whether the combination therapy can target both the main GB tumor and the GB stem cells. For PDACs, we will be using CAPAN1 as an example of a slow-growing PDAC model and BXPC3 as an example of a fast-growing PDAC model to cover a wider variety of PDAC types.

NK cells:

All the NK cells used in this aim are cord blood NK cells that are isolated from fresh cord blood, expanded with IL-2, and stimulated with UAPC every 7-8 days. All NKs are used between day 11-28 after isolation to ensure optimum and consistent cytotoxicity quality and reflect the clinical use of cord blood NKs.

Virus:

All delta24RGD and delta24RGDOX viruses are synthesized by the Gomez-Manzano lab and Fueyo lab

Objectives

1.1 Aim 1: investigate the phenotypic changes in tumor cells after infection by oncolytic viruses.

We will characterize the change in surface ligand expression in the infected tumor cells including upregulation of stress ligand expression and downregulation of MHC expression. In addition, we will knock out and/or block the ligands that have an expression level change to see if it can negate the synergistic effect of the combination therapy.

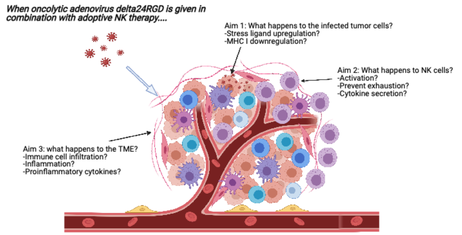
1.2 Aim 2: Determine the phenotypic and functional changes in NK cells when co-incubated with tumor cells infected by the oncolytic virus.

We will investigate the changes in the cytotoxic capabilities and activation/exhaustion status of NK cells induced by the oncolytic virus in vitro. We will then confirm the results in vivo.

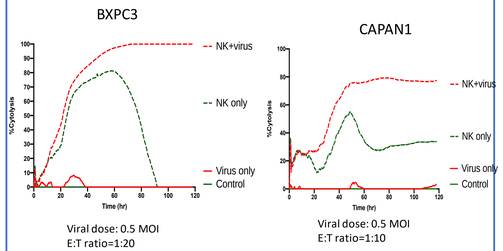
1.3 Aim 3: Determine the change in TME in vivo under the combination treatment and its long-term effect on the activity of adoptively transferred NK cells.

We will measure the changes in immune cell infiltration and cytokine secretion in the tumor microenvironment in the primary tumor as well as the non-injected secondary tumor. In addition, we will determine the ability of the adoptively transferred NK cells to infiltrate the tumor, the exhaustion profile of those NK cells and duration of persistence after initial injection.

The experiments described in this proposal will characterize the efficacy of combination therapy with oncolytic virus and NK cells and provide a mechanistic explanation of it. The findings of this study may serve as the preclinical data to justify the use of oncolytic viruses with NK cells in the clinic to treat aggressive solid tumors that were previously resistant to immunotherapy.



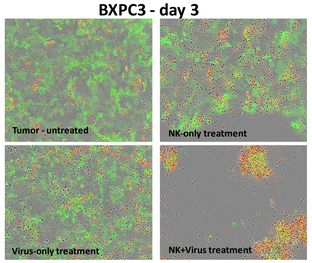
Delta24RGD and NK cells can synergistically kill tumor cells



Viral dose: 0.5 MOI
E:T ratio=1:20

Viral dose: 0.5 MOI
E:T ratio=1:10

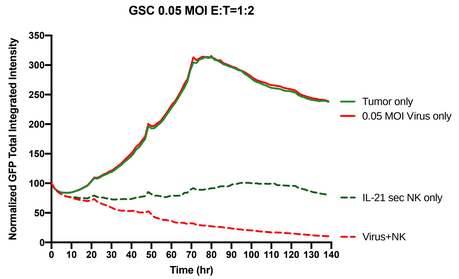
BXPC3 - day 3



Green = tumor cells
Red = dead-cell stain

The experiments described provided significant data towards the idea of combination therapy between oncolytic adenoviruses and natural killer cells. As expressed in the first graph that explores the cell line BXPC3, our low viral dose does insignificant killing against our tumor cells. In comparison, when we add our NK cells to our tumor cells, we are presented with significant killing but around the 60-hour mark, our NK cells slow down and eventually and stop killing. However, when we combine the NK cells with our virus, we are able to see a synergistic effect in which killing does not stop and rather continues on past 100 hours. As depicted on the images above, we are able see a grand difference between the tumor untreated in comparison to the other three treatments. It is important to note how our pictures support the information presented on the graph. After trying the same procedure with a different cell line (CAPAN1), we were able to see the same synergistic effect in which the combination between NK cells and oncolytic viruses does a greater killing of tumor cells.

Delta24RGD and IL21 secreting NK cells can synergistically kill glioma stem cells



In the graph above, we are presenting the results of a similar procedure as the one realized on the BXPC3 and CAPAN1 cell lines. In comparison to the graphs shown prior to this one, the Y axis represents the normalized GFP intensity which is a straight correlation to the number of GFP+ GSC tumor cells alive. As depicted in the graph, and in accordance with our previous graphs, the addition of our virus did no significant, with some of our data points overlapping with our tumor only treatment. It is also important to note how our virus only treatment follows a similar, if not, identical pattern as our control. However, with the addition of our IL-21 secreting NK cells we can see a constant killing of tumor cells that denotes a massive difference in comparison to the tumor only treatment. However, when we combine our Delta24RBG virus and IL21 secreting NK cells, we are able to recognize a synergistic effect in which the glioma stem cells are killed efficiently, eventually reaching an integrated intensity that approximates zero.

Conclusions

After analyzing the previous graphs that encompass three different cell lines and express similar results to the same procedure, we are able to conclude that the combination between oncolytic adenoviruses and natural killer cells is more efficient against treating highly aggressive solid tumors. Additionally, we are able to conclude that oncolytic viruses, by themselves are highly inefficient against tumor cells. Lastly, NK cells are better at eliminating tumor cells, but overtime lose their efficacy. The findings from this research allow for questions regarding the behavioral and phenotypic changes in NK cells after they have been co-incubated with tumor cells that have been exposed to the oncolytic virus. The findings from these experiments can potentially allow for further exploration which includes but is not limited to new therapy for solid tumors and testing on mice. Additionally, these findings could be used towards preclinical data to justify the use of combination therapy between oncolytic adenoviruses and NK cells in a clinical setting where it could be used to treat solid tumors previously resistant to immunotherapy.

References

Body systems and cancer. Cancer Research UK. (2020, May 13). https://www.cancerresearchuk.org/what-is-cancer/body-systems-and-cancer.

Can you really boost your immune system? Cedars. (n.d.). https://www.cedars-sinai.org/blog/boosting-your-immune-system.html#:~:text=Your%20immune%20system%20works%20to,a%20virus%20or%20an%20infection.

Home. Agilent. (n.d.). https://www.agilent.com/en/product/cell-analysis/real-time-cell-analysis/rtca-reagents-kits-accessories/xcelligence-immunotherapy-kits-741233.

May 27, 2021, May 20, 2021, & April 22, 2021. (n.d.). *Using oncolytic viruses to treat cancer*. National Cancer Institute. https://www.cancer.gov/news-events/cancer-currents-blog/2018/oncolytic-viruses-to-treat-cancer.

Racaniello, V. (2011, January 13). *Vincent Racaniello*. virology blog. https://www.virology.ws/2011/01/13/multiplicity-of-infection/#:~:text=Multiplicity%20of%20infection%20(MOI)%20is,and%20the%20MOI%20is%200.1.

U.S. National Library of Medicine. (2020, July 30). *The innate and adaptive immune systems*. InformedHealth.org [Internet]. https://www.ncbi.nlm.nih.gov/books/NBK279396/.

Xu, Y., Zhou, S., Lam, Y. W., & Pang, S. W. (1AD, January 1). *Dynamics of natural killer Cells Cytotoxicity IN Microwell arrays with Connecting Channels*. Frontiers. https://www.frontiersin.org/articles/10.3389/fimmu.2017.00998/full#:~:text=Effector%20(E)%20to%20target%20(,cytotoxicity%20in%20population%2Dbased%20study.

Acknowledgements

The research presented in this poster could not have been made possible without the help of Xin Ru Jiang, Dr. Rafet Basar, and Dr. Katy Rezvani. I want to thank Xin Ru for guiding me through my first time in a laboratory setting as well as helping me figure out what's the best route for me post-college. Additionally, I could not be more grateful for the knowledge and inspiration she has bestowed on me. I would like to Dr. Basar for his help and patience throughout these experiments. Lastly, I want to thank Dr. Rezvani for making this experience possible by allowing me to partake in her laboratory. Her charming and wholesome personality allowed for me to find comfort in a new setting, and I could not be more thankful.