Newcastle disease virus mediated CD47/SIRPα immune checkpoint blockade potentiates anti-tumor immunity

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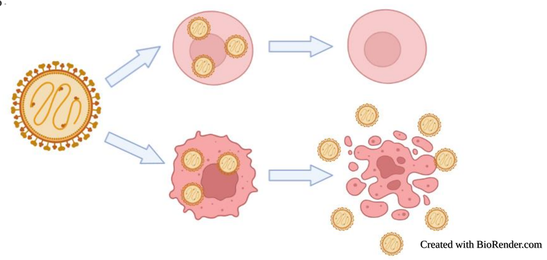
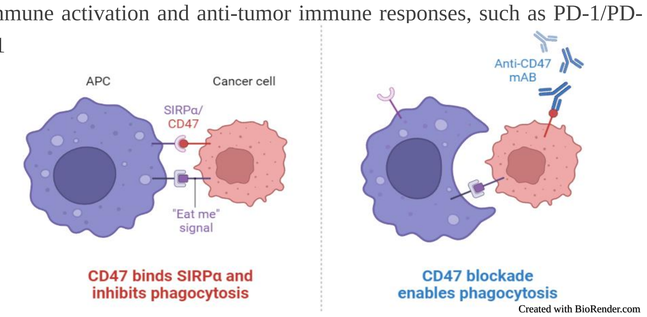
BACKGROUND

- Oncolytic viruses (OVs) are viruses which selectively replicate in tumor cells(1-4). This promotes tumor cell lysis and anti-tumor immune responses.



- Checkpoint blockade (ICB) is a cancer immunotherapy where inhibitory signalling pathways are interfered with(1-4). This acts to help prolong immune activation and anti-tumor immune responses, such as PD-1/PD-L1

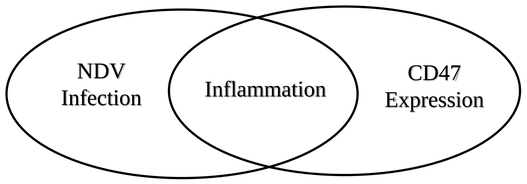


- Systemic administration of ICB, like anti-CD47, is associated with several adverse effects due to its ubiquitous expression(1-4). This can be circumvented by using an oncolytic viral vector such as Newcastle disease virus (NDV) to deliver anti-CD47 ICB directly to the tumor.

PURPOSE

When oncolytic viruses such as NDV are administered as a cancer immunotherapy, what role does the CD47/SIRPα signaling pathway play?

- Is this role beneficial or detrimental to the oncolytic ability of NDV?
- By antagonizing the CD47/SIRPα signaling pathway can the oncolytic ability of NDV be improved?



Hypothesis:

Given the overlap of CD47 expression and NDV infection with pro-inflammatory mediators, engineering oncolytic NDV to mediate immune checkpoint blockade of CD47/SIRPα through production of anti-CD47 antibody will improve the oncolytic ability of NDV.

RESULTS

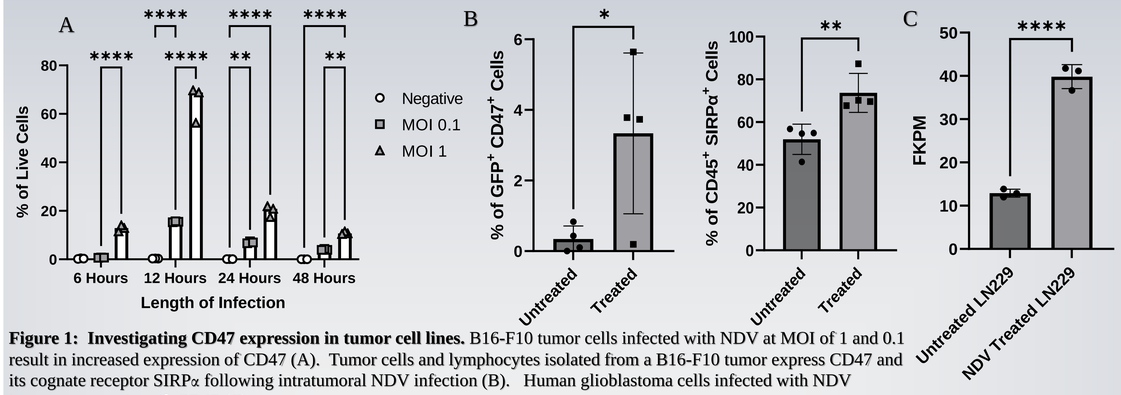


Figure 1: Investigating CD47 expression in tumor cell lines. B16-F10 tumor cells infected with NDV at MOI of 1 and 0.1 result in increased expression of CD47 (A). Tumor cells and lymphocytes isolated from a B16-F10 tumor express CD47 and its cognate receptor SIRPα following intratumoral NDV infection (B). Human glioblastoma cells infected with NDV increase expression of CD47 (C).

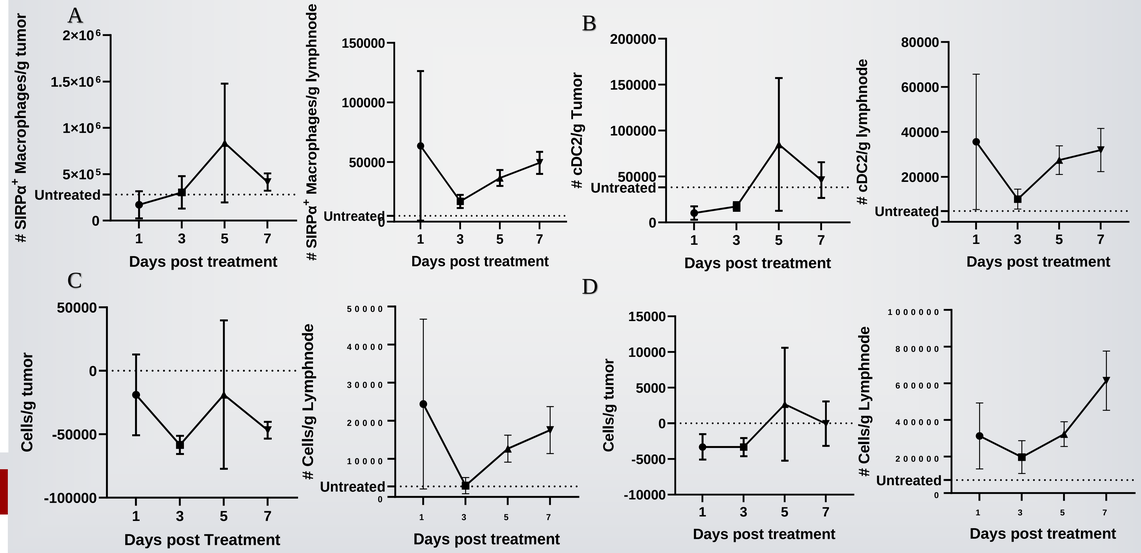


Figure 2: Immune cell responses following intratumoral administration of $1x10^8$ PFU of NDV. The infiltration of cells typically involved in phagocytosis like Macrophages (A), type II classical dendritic cells (B), type I classical dendritic cells (C) and plasmacytoid dendritic cells (D) were characterized in the tumor and tumor draining lymphnode following a period of 7 days post NDV treatment.

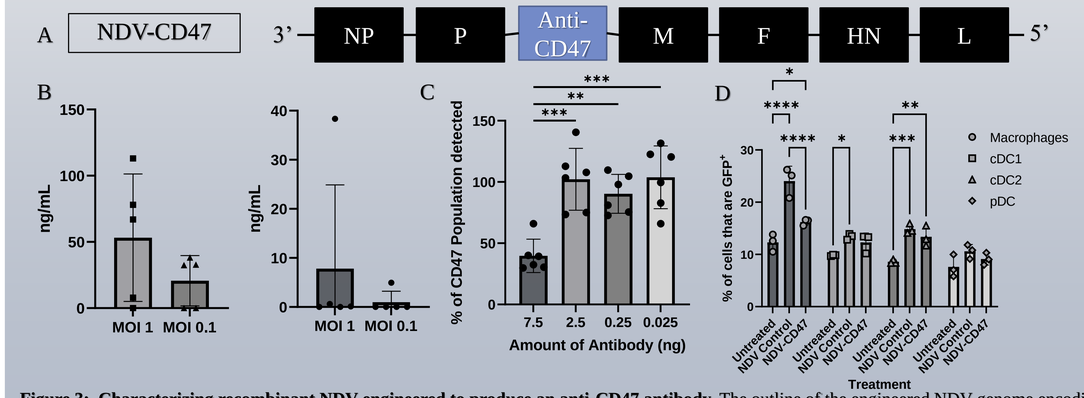


Figure 3: Characterizing recombinant NDV engineered to produce an anti-CD47 antibody. The outline of the engineered NDV genome encoding the anti-CD47 antibody containing a human IgG Fc domain (A). Production of anti-CD47 antibody in cell supernatants (left) and in cell lysates (right) (B). A competitive binding assay showing anti-CD47 antibody prevents binding of fluorescently conjugated anti-CD47 antibody (C). B16-F10 tumor cells uninfected or infected with NDV or NDV-CD47 impact tumor cell phagocytosis by various antigen presenting cells (D).

Summary of Results

- Treatment of tumor cells both *in vitro* and *in vivo* increases CD47 expression and results in the recruitment of immune cells to the tumor and tumor draining lymphnode which express the cognate receptor, SIRPα.
- NDV can be engineered to produce an anti-CD47 monoclonal antibody capable of binding CD47 on the surface of tumor cells. Infection of tumor cells with NDV and NDV-CD47 improves tumor cell phagocytosis by macrophages and type II dendritic cells.
- The treatment of B16-F10 tumor bearing mice with NDV or NDV-CD47 improves survival relative to untreated controls.

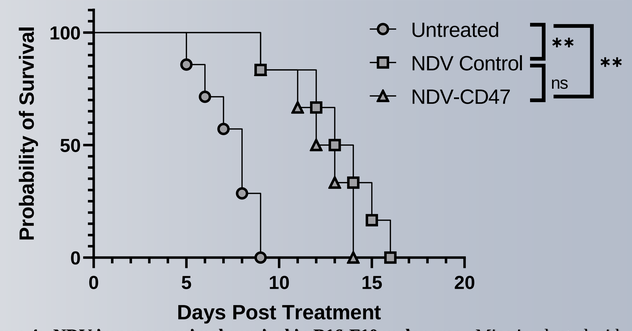


Figure 4: NDV improves animal survival in B16-F10 melanoma. Mice implanted with $2x10^5$ tumor cells were administered three consecutive doses of $1x10^8$ PFU once tumors reached 5x5mm.

CONCLUSIONS

- NDV is an immunostimulatory and advantageous as a cancer immunotherapy.
- NDV encoding an anti-CD47 antibody does not significantly improve its efficacy. However, the combination therapy of NDV and CD47 immune checkpoint blockade should be evaluated separately
- The production of anti-CD47 antibody may be a limitation impacting the efficacy of NDV mediated anti-CD47 immune checkpoint blockade. NDV encoding a SIRPα-Fc immunoadhesin may lead to increased production and improved anti-CD47 checkpoint blockade

REFERENCES

1. Logtenberg, M. E. W., Scheeren, F. A. & Schumacher, T. N. The CD47-SIRPα Immune Checkpoint. *Immunity* **52**, 742–752 (2020).

2. Liu, X. *et al.* CD47 blockade triggers T cell–mediated destruction of immunogenic tumors. *Nature Medicine 2015 21:10* **21**, 1209–1215 (2015).

3. Billerhart, M. *et al.* CD47-targeted cancer immunogene therapy: Secreted SIRPα-Fc fusion protein eradicates tumors by macrophage and NK cell activation. *Mol Ther Oncolytics* **23**, 192 (2021).

4. Son, J. *et al.* Inhibition of the CD47-SIRPα axis for cancer therapy: A systematic review and meta-analysis of emerging clinical data. *Front Immunol* **13**, (2022).

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