Transgenic Oncolytic Viruses and Ruxolitinib as Neoadjuvant Combination Therapy for Pancreatic Ductal Adenocarcinoma

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Introduction

Pancreatic Ductal Adenocarcinoma (PDAC)

- PDAC is currently the 4th leading and projected to become the 2nd leading cause of cancer related deaths in the US by 2030.
- It is a highly invasive malignancy with poor prognosis and represents about 85 - 90% of all pancreatic cancers.
- PDAC is characterized by a dense stroma coupled with immunosuppression in a desmoplastic tumor microenvironment (TME) which makes it resistant to radiation, chemotherapy and immunotherapy.

Oncolytic Viruses (OVs)

- OVs such as vesicular stomatitis virus (VSV) are biological anticancer agents that replicate selectively within tumor cells.
- OVs are being explored in PDAC treatment because of their cell specific cytotoxicity and ability to improve immune activation within TME.
- VSV has been shown to be effective against most PDAC cell lines, but some are highly resistant to VSV.
- The treatment of resistant PDAC cell lines with Janus kinase 1/2 inhibitors such as ruxolitinib has been shown to improve the sensitivity of some resistant cells to VSV infection.

This research evaluated some transgenic VSV and ruxolitinib as potential neoadjuvant therapy for PDAC.

Methods

- This research is based on the *in vitro* ell culture model and all experiments and assays were done in triplicate.
- For the viral cytotoxicity and VSV-ruxolitinib treatments experiments, cells were seeded in a 96-well plate at a density of 5,000 cells per well.
- After 24 hours and at 80-90% confluency, the cells were infected with different transgenic VSV at a multiplicity of infection (MOI) of 1 or 10 based on BHK-21 cells.
- Cell viability as a percentage of no viral treatment was analyzed by MTS assay using CellTiter-Glo® at 3 days post infection (p.i.).

Cytotoxicity of Viral Treatments

The cytotoxicity of transgenic VSV strains namely, VSV-GFP, VSV-Decorin, VSV-Relaxin, and VSV-PH20 against H6C7, Panc1, MiaPaCa2, BX-PC3, Capan1 and HPAF-II PDAC cell lines was investigated.

VSV-Ruxolitinib Treatments

The effect of different transgenic VSV strains and ruxolitinib treatment on BX-PC3, HS766T, KPC, and HPAF-II PDAC cell lines was studied.

Cell Invasion Assay

The invasiveness of MiaPaCa2, AsPC-1 and HS766T PDAC cell lines treated with VSV-Decorin at MOI of 1 or 10 and 5 µM ruxolitinib was assessed using the MILLIPORE QCM™ 96-well cell invasion assay (Fig. 1). Invaded cells on the low side of the membrane were stained with 0.1% Crystal violet solution.

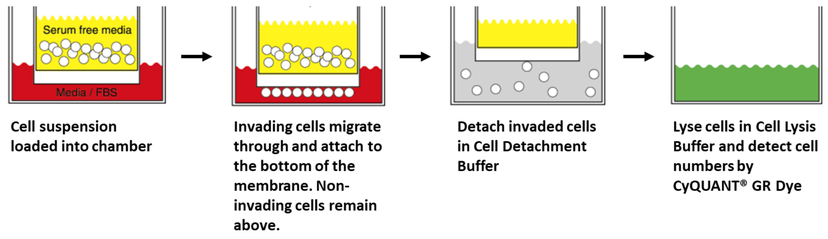

Figure 1: Overview of MILLIPORE QCM™ 96-well Cell Invasion Assay.

Results

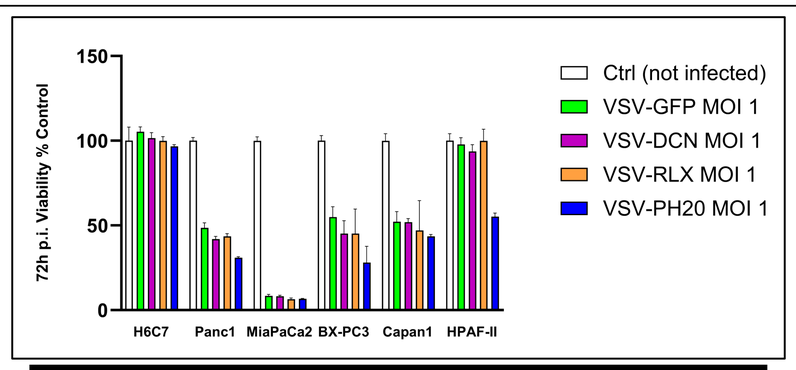

Figure 2: Cytotoxic Effects of Viral Treatments on PDAC Cell Lines.

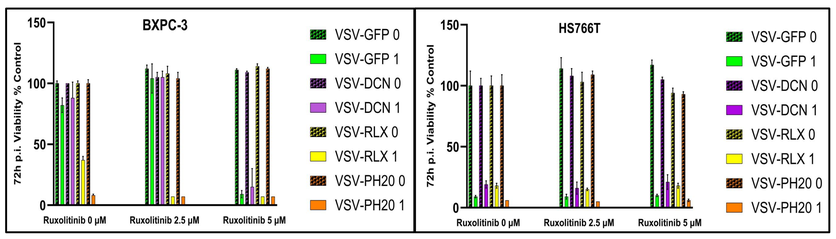

Figure 3: Effect of VSV-Ruxolitinib Treatment on BXPC-3 and HS766T.

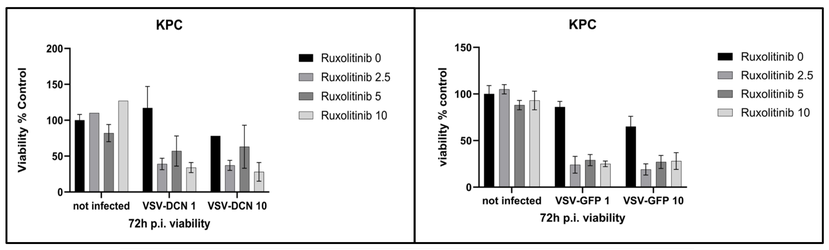

Figure 4: Effect of VSV-Ruxolitinib Treatment on KPC.

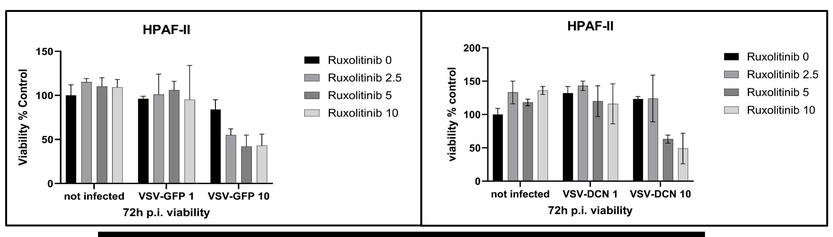

Figure 5: Effect of VSV-Ruxolitinib Treatment on HPAF-II.

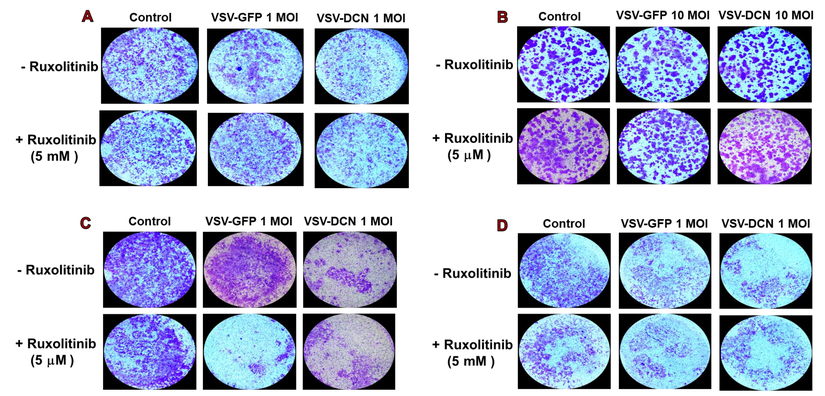

Figure 6: Photomicrogrpahs of Crystal Violet Stained Invasive PDAC Cells.
A - AsPC-1; B - HPAF-II; C - HS766T; D - MiaPaCa-2

Summary of Results

- The results of viral cytotoxicity showed MiaPaCa2 as the most sensitive. Panc1, BX-PC3 and Capan1 were partially sensitive, and HPAF-II was resistant to VSV infection (Fig. 2).
- In the VSV-ruxolitinib treatments, 5 µM ruxolitinib was the most effective for all VSV strains used on BX-PC3 (Fig. 3). Ruxolitinib did not significantly improve the infection of HS766T by transgenic VSV (Fig. 3). In the KPC, VSV-Decorin at MOI of 10 and 10 µM ruxolitinib was the most effective (Fig. 4). For the HPAF-II, the results of infection with VSV-Decorin at MOI of 10 and 5 µM/10 µM ruxolitinib were comparable in outcome (Fig 5).
- The cell invasion assay revealed an overall reduction of invasiveness in MiaPaCa2, AsPC-1 and HS766T with VSV-Decorin at MOI of 1 and in HPAFII with VSV-Decorin at MOI of 10 (Figs. 6 a – d).

Outlook

- Organoids based experiments and *in vivo* studies with animal models.
- Fluorimetry for quantification of invasive cells in the MILLIPORE QCM™ 96-well cell invasion assay.

Conclusion

This study showed that a combination of transgenic VSV strains coupled with ruxolitinib treatment was very effective in the infection of both sensitive and resistant PDAC cell lines. This shows great potential for oncolytic virotherapy in the neoadjuvant treatment of PDAC.

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