# B and T lymphocyte attenuator (BTLA) and PD-L1 significantly upregulated in reovirus treated TRAMP-C2 tumours.

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**Introduction: Whilst cancer immunotherapy with monoclonal antibodies against immune checkpoints has revolutionized the treatment of patients affected by certain cancer types, prostate cancers are generally considered to be a 'cold' tumour with minimal T cell infiltrates, lack of a type I IFN signature and chemokines and containing immunosuppressive cells such as myeloid derived suppressor cells. This non-inflamed phenotype is thought to be largely responsible for the disappointing lack of sensitivity of prostate cancer patients to immune checkpoint blockade (ICB) therapy. However, the use of oncolytic viruses can overcome pre-existing mechanisms of resistance to immunotherapy in prostate cancers by transforming these cold tumours into 'hot', immune cell infiltrated, tumours.**

**Aims: In the current study, we investigated whether the effectiveness of oncolytic viral therapy for prostate cancer could be improved with targeted blockade of PD-1 and/or CD73.**

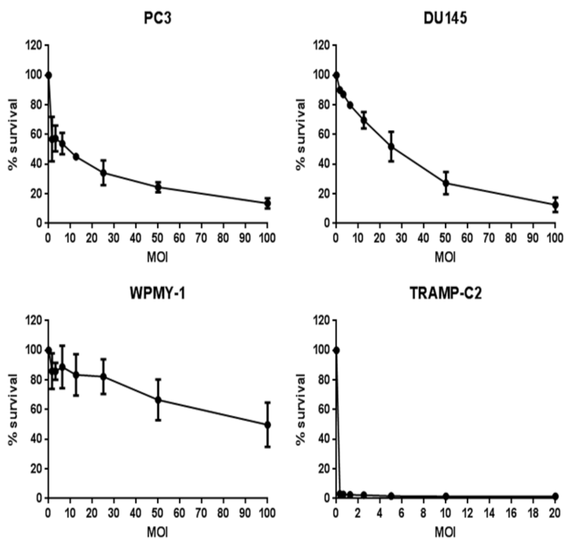



**Figure1: In vitro susceptibility to Reovirus infection of a panel of prostate cancer cell lines.** Cell monolayers of human prostate cancer cell lines (PC3 and DU145), a human prostatic stromal myofibroblast cell line WPMY-1 and the transgenic adenocarcinoma mouse prostate cell line TRAMP-C2 were infected with doubling dilutions of a stock preparation of Reovirus ($3x10^9$pfu/ml). Following incubation at 37°C for 72h, cell survival was determined by MTS assay. Data is presented as the average ± SD (n=2).

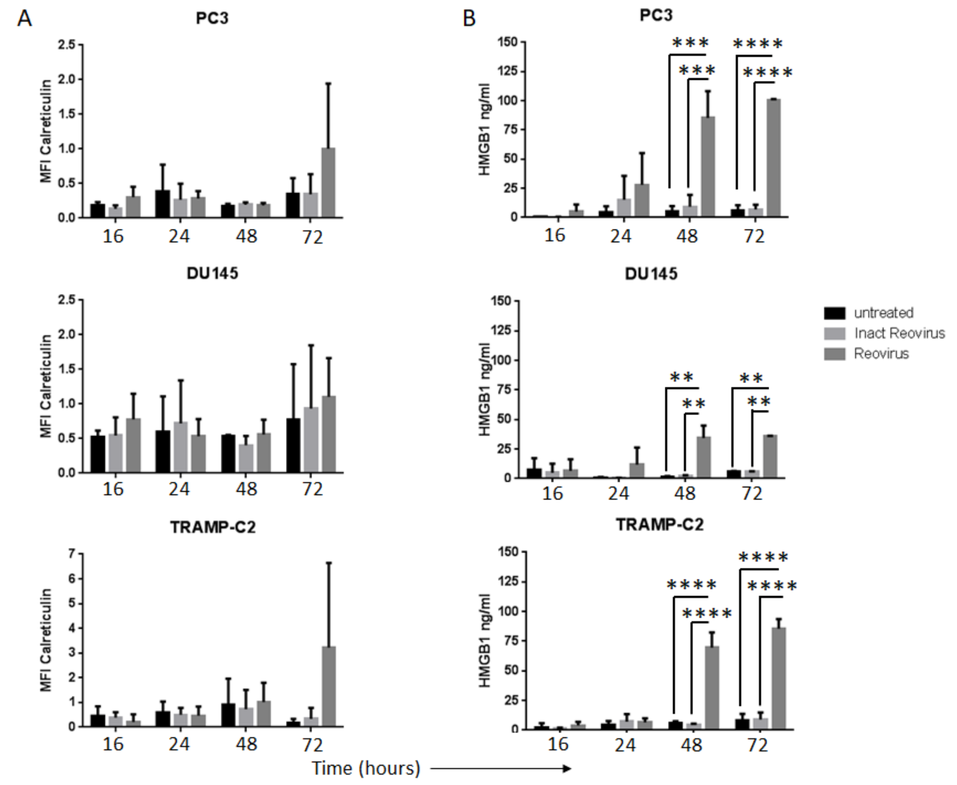



**Figure 2: Induction of ICD determinants in response to reovirus infection in prostate cancer cell lines.** The cell lines PC3 and DU145 and the mouse cell line TRAMP-C2 were treated with reovirus at an MOI of 3 for PC3, 40 for DU145 and 0.06 for TRAMP-C2. (A) Cells were harvested at 16, 24, 48 and 72hour time-points and flow cytometry was performed. The MFI of calreticulin positive cells was gated on viable cells (ViViD negative cells) thus detecting surface exposed calreticulin rather than total calreticulin. B) Supernatants were harvested at 16, 24, 48 and 72hour time-points. Reovirus triggered extracellular HMGB1 accumulation was determined by ELISA analysis of supernatants (significant differences between untreated or inactivated virus and reovirus-infected cultures as determined by two-way ANOVA; ** p<0.01,*** p<0.001, ****p<0.0001).

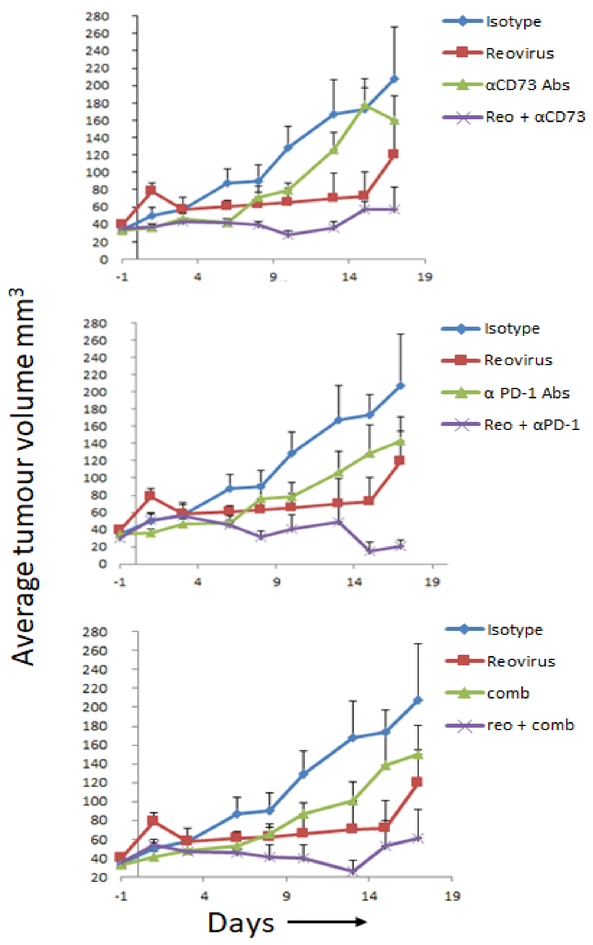
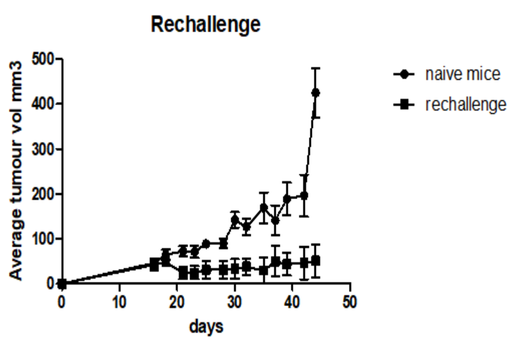



**Figure 4. Reovirus infection of tumours is needed before a therapeutic effect of anti-immune inhibitory/suppressive antibodies is seen.** Anti-CD73 and anti-PD-1 alone or in combination was tested as a monotherapy as well as in combination with reovirus infection. Mice were first intratumorally administered with reovIrus at $0.75x10^8$ pfu on days 0, 2 and 5 before the addition of anti-CD73, anti-PD-1 or a combination of the antibodies twice weekly IP.




**Reovirus-initiated antitumor immunity protects against subsequent tumour challenge.** C57BL/6 mice who had demonstrated complete remission of their tumours following reovirus plus antibody therapy were subsequently further challenged with $5x10^6$ TRAMP-C2 cells.

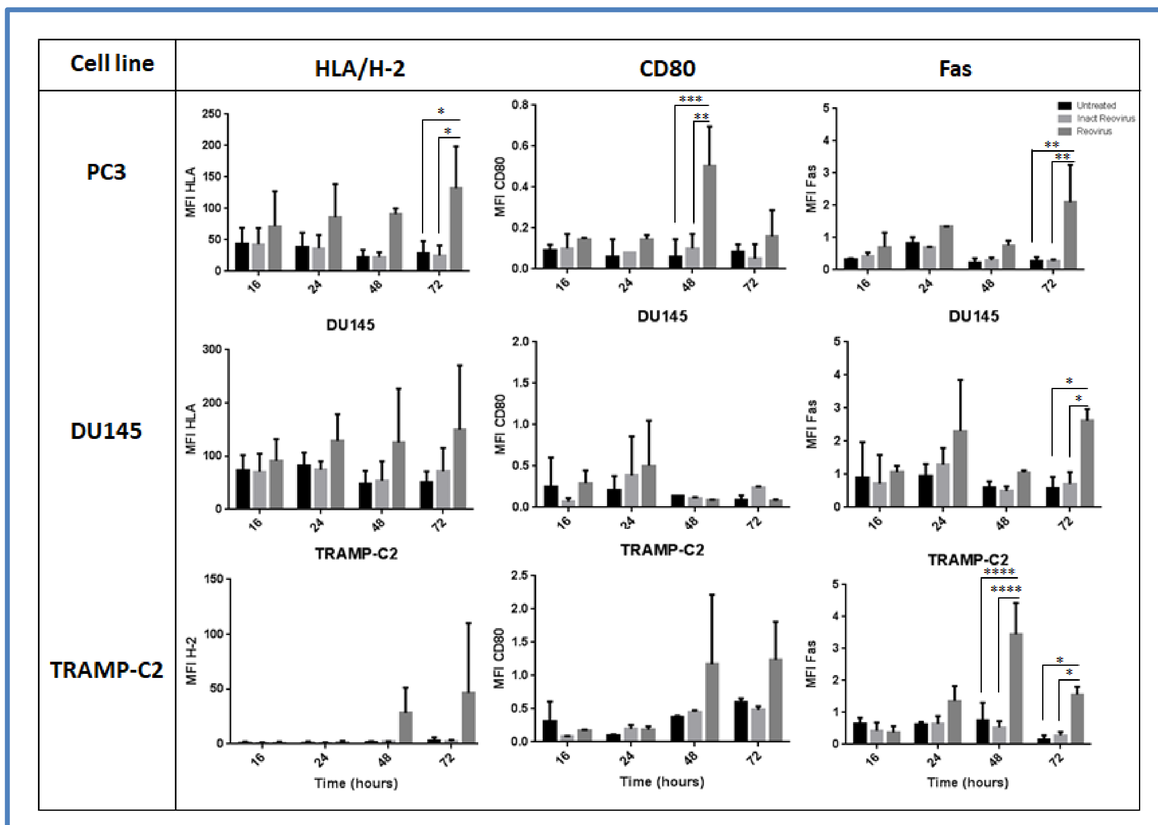



**Figure 3: Reovirus infection of prostate cancer cell lines up-regulates cell surface molecules associated with susceptibility to immune attack.** The human prostate cancer cell lines PC3 and DU145 and the mouse transgenic adenocarcinoma prostate cell line TRAMP-C2 were treated with reovirus at an MOI of 3 for PC3, 40 for DU145 and 0.06 for TRAMP-C2. Cells were harvested at 16, 24, 48 and 72hour time-points and HLA/H-2, CD80 and Fas expression were assessed by flow cytometry. Results are from two independent experiments (mean ± SD).

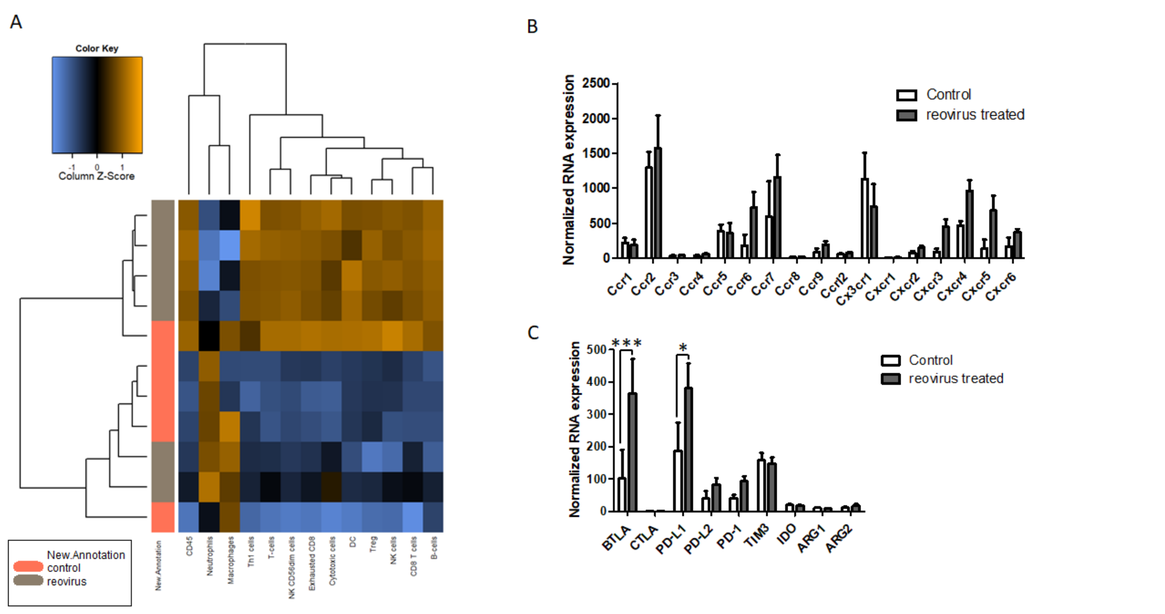



**Figure 5: Nanostring's PanCancer Immune Profiling demonstrated the ability of reovirus infection to cause an increase in innate (NK cells and DCs) and adaptive immune cell types (T cells and B cells) within the virus-treated TRAMP-C2 tumours as compared to untreated tumours (a).** An increased chemokine receptor expression (CCR6, CCR7 and CXCR3 involved in recruitment/activation of T cells, NK cells and DCs, and CXCR5 involved in B cell migration) was observed within the reovirus-treated tumours **(b)**. Of the panel of negative regulators studied only B and T lymphocyte attenuator (BTLA) and PD-L1 were significantly upregulated in the reovirus treated TRAMP-C2 tumours compared to untreated tumours **(c).**

## CONCLUSIONS:

- **Reovirus infection of prostate cancer cell lines up-regulates cell surface molecules associated with susceptibility to immune attack and immunogenic cell death determinants.**
- **We show that treatment of subcutaneous TRAMP-C2 prostate tumours with a combination of intratumoral reovirus and anti-PD-1 or anti-CD73 antibody significantly enhanced survival of mice compared to reovirus or antibody therapy alone.**
- **Only the combination therapy led to rejection of pre-established tumours and protection from tumour rechallenge.**
- **Nanostring immune profiling of reovirus-treated and untreated tumours confirmed the ability of reovirus to increase tumour immune cell infiltration.**
- **Of the panel of negative regulators studied only B and T lymphocyte attenuator (BTLA) and PD-L1 were significantly upregulated in the reovirus treated TRAMP-C2 tumours compared to untreated tumours.**
- **Studies are underway to determine whether blockade of BTLA can synergise with anti-PD-1 to further enhance the therapeutic outcome.**