Combination therapy with reovirus and PD-1 blockade effectively establishes tumor control via innate and adaptive immune responses

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Introduction

Previous work from our group and others has developed the use of reovirus, a naturally occurring oncolytic virus, as a systemically delivered anti-cancer agent in both pre-clinical models and early phase clinical trials. The agent has direct oncolytic activity against a wide range of human and murine tumor cells, in part due to dysfunction of the PKR-mediated anti-viral response in malignant cells. However, in addition, we have demonstrated reoviral-mediated immune activation in association with virus replication in tumor cells; these responses comprise both innate immune activation against virally infected tumor cells and generation of adaptive immunes responses against tumor-associated antigens following *in vivo* priming secondary to immunogenic cell death.

Given the multifaceted immune effects of reovirus therapy, we hypothesized that immune checkpoint inhibition would augment the efficacy of this agent. This hypothesis was tested using our immune-competent murine model in which reovirus is injected into B16 melanomas growing subcutaneously (SC) in C57BL/6 mice.

Key result: Combination therapy of reovirus with PD-1 blockade delivers significant survival benefit, by augmenting tumor-specific NK responses and specifically attenuating tumor-specific immunosuppression.

Figure 1: Reovirus plus PD-1 blockade prolongs survival of mice with melanomas

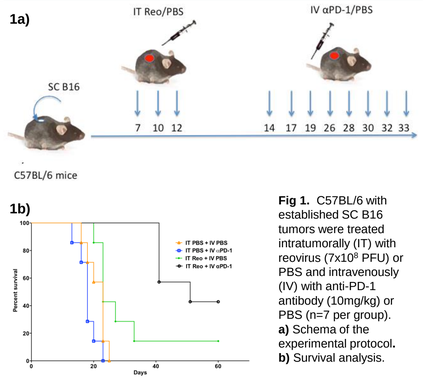


Fig 1. C57BL/6 with established SC B16 tumors were treated intratumorally (IT) with reovirus ($7x10^8$ PFU) or PBS and intravenously (IV) with anti-PD-1 antibody (10mg/kg) or PBS (n=7 per group).
a) Schema of the experimental protocol.
b) Survival analysis.

Figure 2: Reovirus plus PD-1 blockade induces robust IFNγ memory T cell responses

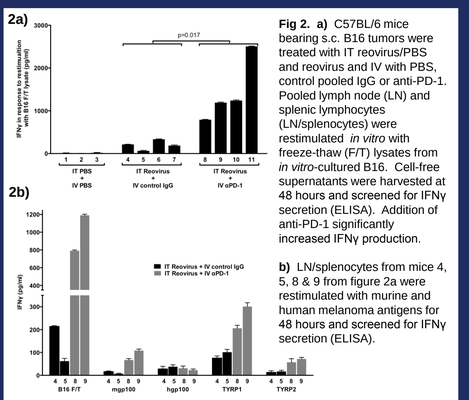


Fig 2. a) C57BL/6 mice bearing s.c. B16 tumors were treated with IT reovirus/PBS and reovirus and IV with PBS, control pooled IgG or anti-PD-1. Pooled lymph node (LN) and splenic lymphocytes (LN/splenocytes) were restimulated *in vitro* with freeze-thaw (F/T) lysates from *in vitro*-cultured B16. Cell-free supernatants were harvested at 48 hours and screened for IFNγ secretion (ELISA). Addition of anti-PD-1 significantly increased IFNγ production.

b) LN/splenocytes from mice 4, 5, 8 & 9 from figure 2a were restimulated with murine and human melanoma antigens for 48 hours and screened for IFNγ secretion (ELISA).

Figure 3: PD-1 blockade augments reovirus-induced NK cytokine production and killing

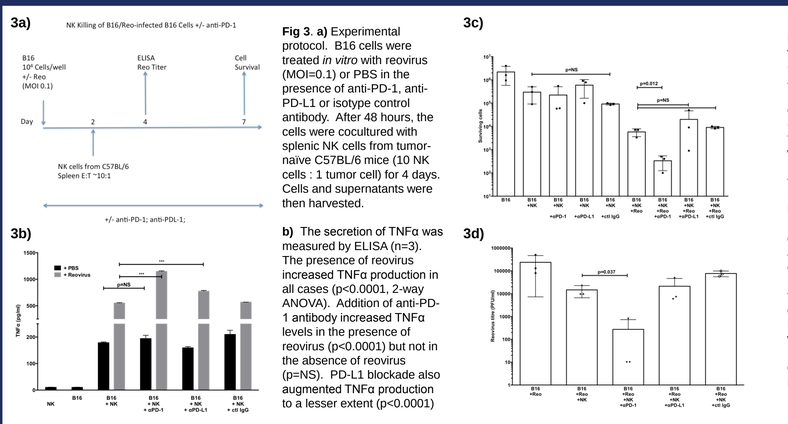


Fig 3. a) Experimental protocol. B16 cells were treated *in vitro* with reovirus (MOI=0.1) or PBS in the presence of anti-PD-1, anti-PD-L1 or isotype control antibody. After 48 hours, the cells were cocultured with splenic NK cells from tumor-naive C57BL/6 mice (10 NK cells : 1 tumor cell) for 4 days. Cells and supernatants were then harvested.

b) The secretion of TNFα was measured by ELISA (n=3). The presence of reovirus increased TNFα production in all cases (p<0.0001, 2-way ANOVA). Addition of anti-PD-1 antibody increased TNFα levels in the presence of reovirus (p<0.0001) but not in the absence of reovirus (p=NS). PD-L1 blockade also augmented TNFα production to a lesser extent (p<0.0001)

c) After coculture, viable cells were counted (n=3). Addition of anti-PD-1, anti-PD-L1 or control IgG to B16-NK coculture had no effect on tumor cell survival (p=NS). However, when tumor cells had been treated with reovirus, addition of anti-PD-1 markedly impaired tumor cell survival (p=0.012). Interestingly PD-L1 blockade had no effect (p=NS)

d) Reovirus titers were assayed in supernatants after coculture. Addition of anti-PD-1 to coculture of reovirus-treated B16 and NK cells decreased the recovered reovirus titer (p=0.037), whereas addition of anti-PD-L1 had no effect (p=NS) and control IgG minimally increased the titer (p=0.093)

Figure 4: PD-1 blockade ablates tumor--specific immune suppression by T_{reg}s.

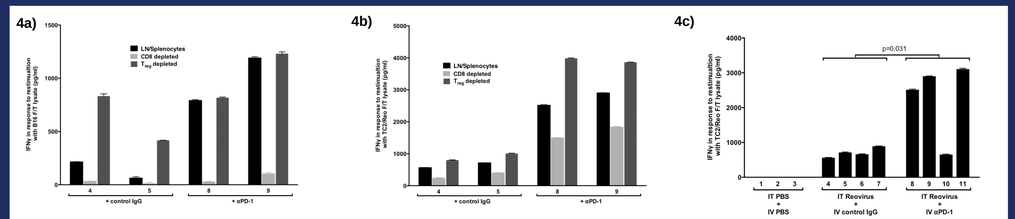


Fig 4. Lymph node and splenic lymphocytes were harvested from mice 4, 5, 8 & 9 (figure 2a); cells were kept whole or depleted of CD8 cells or regulatory T cells (T_{reg}) then restimulated *in vitro* with F/T lysates of B16 **(a)** or reovirus-infected TC2, a murine prostate cancer cell line **(b)**. After 48 hours, supernatants were screened for secreted IFNγ by ELISA (n=3).

c) Lymph node and splenic lymphocytes from mice in figure 2a were restimulated *in vitro* with F/T lysates of reovirus-infected TC2. Addition of anti-PD-1 significantly increased IFNγ production in response to restimulation with this cell line expressing reovirus antigens (p=0.031).

Figure 5: Both innate and adaptive immunity mediate *in vivo* efficacy of Reovirus + αPD-1

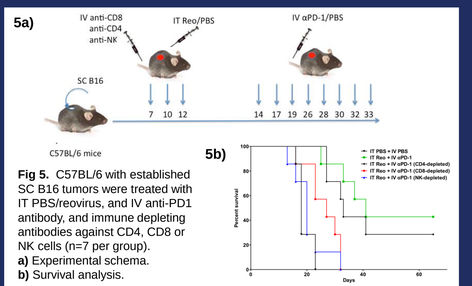
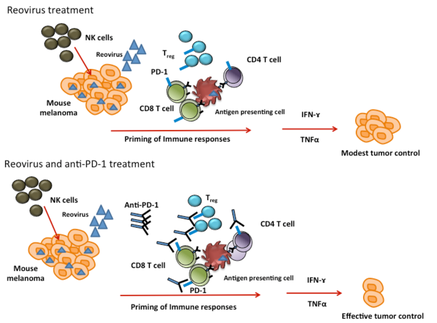


Fig 5. C57BL/6 with established SC B16 tumors were treated with IT PBS/reovirus, and IV anti-PD1 antibody, and immune depleting antibodies against CD4, CD8 or NK cells (n=7 per group).
a) Experimental schema.
b) Survival analysis.

Summary



- Combining reovirus with αPD-1 therapy induces durable anti-tumor responses of established tumors
- Combined therapy results in augmented T cell recall responses against both freeze-thaw tumor lysates and specific tumor-associated antigens.
- Addition of PD-1 blockade augments reovirus-induced TNFα secretion and tumor cell killing by NK cells; recovered viral titers were actually decreased by the combined treatment, presumably reflecting a reduced 'pool' of viable tumor cells to support replication.
- CD8-dependent IFNγ recall responses against freeze-thaw tumor lysates were suppressed *in vitro* by T_{regs} – this effect was nullified by PD-1 blockade. Interestingly, T_{reg} cells continued to suppress anti-viral adaptive immunity, even in the presence of PD-1 blockade
- *In vivo* depletion of CD8 or CD4 cells reduced the efficacy of the reovirus-αPD-1 combination, however, NK cell depletion had an even more marked effect, confirming the central role of NK cell-mediated cell death in tumor control.

Acknowledgements