Combination therapy of Reovirus and Checkpoint Inhibition. Shane Zaidi\textsuperscript{1,3}, Kevin Shim\textsuperscript{1}, Varishma Rajani\textsuperscript{1}, Rosa Diaz\textsuperscript{1}, Elizabeth Ilett\textsuperscript{2}, Timothy Kottke\textsuperscript{1}, Jill Thompson\textsuperscript{1}, Peter Selby\textsuperscript{2}, Kevin Harrington\textsuperscript{3}, Hardev Pandha\textsuperscript{4}; Alan Melcher\textsuperscript{2}, Matt Coffey\textsuperscript{5}, Richard Vile\textsuperscript{4,2,6}. \textsuperscript{1}Department of Molecular Medicine, Mayo Clinic, Rochester, MN 55905; \textsuperscript{2}Leeds Institute of Cancer and Pathology, St. James' University Hospital, Leeds, UK; \textsuperscript{3}The Institute of Cancer Research, 237 Fulham Road, London, SW3; \textsuperscript{4}University of Surrey, Guildford, UK; \textsuperscript{5}Oncolytics Biotech Incorporated, Calgary, Canada; \textsuperscript{6}Department of Immunology, Mayo Clinic, Rochester, MN 55905

We have developed the use of reovirus as a systemically delivered oncolytic agent in both pre-clinical models and in early Phase clinical trials. Reovirus has direct oncolytic activity against many human/murine tumor cells, partly because of disruption of the PKR-mediated anti-viral response in malignant cells. In addition, however, we have shown that anti-tumor therapy is directly associated with immune activation by virus replication in tumors. The immune mechanisms of therapy include both innate immune activation against virally infected tumor cells, as well as the generation of adaptive anti-tumor immune responses as a result of in vivo priming against tumor associated antigens released during that killing. Therefore, to exploit the immune components of reovirus anti-tumor therapy, we tested the combination of oncolytic therapy with systemic checkpoint inhibition. To do this, we used our murine, immune-competent, model and a protocol in which injection of reovirus into subcutaneous (s.c.) B16 melanomas generates moderate therapy following intra-tumoral injection of reovirus into 5d established subcutaneous B16 melanomas growing in C57Bl/6 mice. In this model, provision of systemic anti-FD-1 antibody along with i.t. reovirus, significantly enhanced survival compared to i.t. reovirus alone (p<0.01) and led to >40% of mice being cured long term. Immune analysis suggests that the enhanced therapeutic benefit of reovirus plus checkpoint inhibition is contributed by at least two factors. Thus, blockade of PD-1 significantly enhanced the ability of NK cells to recognize (TNF-α secretion), and kill, reovirus-infected target tumor cells. Second, antiPD-1 antibody led to a significant reduction in Treg activity in reovirus-treated mice, with the overall effect of increasing the adaptive CD8+ anti tumor T cell response. We also showed that timing of the checkpoint inhibitor antibody was significant in balancing toxicity with anti tumor therapy. These data suggest that combination of checkpoint inhibition therapy with reovirus oncolytic/immunotherapy represents a readily translatable method to enhance the therapeutic value of either alone.
Day 60 Survival:

Percent survival

Days

i.t. PBS/i.v. αPD1
i.t. REO/i.v. αPD1
i.t. PBS/i.v. PBS
i.t. REO/i.v. PBS
Combination therapy with reovirus and PD-1 blockade effectively induces 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 parovirus, as a systemically delivered and cancer agent that can both directly lyse cancer cells and stimulate a robust immune response. 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 reovirus-mediated immune activation in association with virus replication in tumor cells; these responses operate both to create immune activation against virtually any virus that can infect tumor cells and to specifically attenuate tumor-specific immunosuppression. This hypothesis was tested using our immune-competent murine model in which reovirus is injected into B16 melanomas growing subcutaneously (SC). Previous work from our group and others has developed the use of reovirus, a naturally occurring oncolytic virus, as a systemically delivered anti-cancer therapy (1). Treatment with reovirus prolongs survival of mice with melanomas and melanoma bearing s.c. B16 tumors were treated with reovirus and IV with PBS (n=7 per group).

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

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

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

Figure 4: Reovirus plus PD-1 blockade ablates tumor-specific immune suppression by Treg.s

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

Summary

- Combined therapy results in augmented T cell recall responses against both these tumor-species and specific tumor-associated antigens.
- Addition of PD-1 blockade augments reovirus-induced TNFα secretion and tumor cell killing by NK cells; recovered virus titers were actually decreased by the combined treatment, presumably reflecting a reduced ‘pool’ of viable tumor cells to support replication.
- Combination therapy results in augmented T cell recall responses against both reovirus-infected tumor-lung and specific tumor-associated antigens.
- Addition of PD-1 blockade augments reovirus-induced IFNγ memory T cell responses.
- PD-L1 blockade also increases reovirus-induced TNFα production in vivo with reovirus and IV with PBS, anti-PD-1, anti-PD-L1 or control IgG to B16-bearing C57BL/6 mice (n=3). Addition of anti-PD-L1 or control IgG minimally increased TNFα production in vitro with reovirus, whereas addition of anti-PD-1 markedly impaired reovirus-induced TNFα production (p<0.0001, 2-way ANOVA). Addition of anti-PD-L1 had no effect (p=NS) and reovirus titer in the absence of reovirus had no effect (p=NS). The presence of reovirus increased TNFα production (p=0.037), whereas addition of anti-PD-1 inactivated TNFα production (p<0.0001) or reovirus-infected TC2. Addition of anti-PD-1 to a lesser extent (p<0.0001) augmented TNFα production compared to reovirus plus PD-L1 antibody or isotype control. Interestingly PD-L1 blockade had no effect (p=NS) on reovirus or reovirus-infected tumor cell survival (p=0.037). Moreover, addition of anti-PD-L1 markedly impaired tumor cell survival (p<0.0001). In vivo, reovirus-infected TC2 tumor cell infusion resulted in a significant increase in tumor cell killing by NK cells (n=7 per group).

Acknowledgements

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In vivo, reovirus-induced TNFα secretion was suppressed by the combined treatment, presumably reflecting a reduced ‘pool’ of viable tumor cells to support replication.

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