

Two is better than one

Complementing oncolytic virotherapy with gemcitabine to potentiate antitumor immune responses

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Abbreviations: APC, antigen presenting cell; COX2, cyclooxygenase 2; CTLA4, cytotoxic T-lymphocyte associated protein 4; DC, dendritic cell; IDO1, indoleamine 2,3-dioxygenase 1; IL, interleukin; MDSC, myeloid-derived suppressor cell; OV, oncolytic virus; TAA, tumor-associated antigen, TGF β 1, transforming growth factor β 1; Treg, regulatory T cell

Oncolytic viruses (OVs) preferentially infect and kill cancer cells. Additionally, OV-induced immune responses subvert cancer-associated immunosuppression and promote antitumor immunity. We have recently demonstrated that the complementation of oncolytic virotherapy with gemcitabine accentuates its immunostimulatory effects, hence exerting superior antineoplastic activity.

Exhaustive evidence indicates that the presence of strong, multivalent and polyclonal antitumor T-cell responses correlate with positive disease outcomes and improved survival in cancer patients. To initiate such a multi-dimensional adaptive immune response, T cells require 2 signals from antigen-presenting cells (APCs): tumor-associated antigens (TAAs) presented in the context of MHC molecules and co-stimulatory cues. However, since tumors usually develop from altered “self” tissues, they fail to undergo proper antigen processing by APCs. Moreover, tumors often generate an immunosuppressive milieu that hampers the expression of co-stimulatory molecules on APCs. Indeed, immunosuppressive cytokines such as interleukin-10 (IL-10) and transforming growth factor β 1 (TGF β 1), inhibitory surface receptors such as programmed cell death 1 (PDCD1, best known as PD-1) and cytotoxic T lymphocyte-associated protein 4 (CTLA-4), as well as immunosuppressive cells including regulatory T cells (Tregs) and myeloid-derived suppressor

cells (MDSCs) are often abundant within the cancer microenvironment. In the context of such an immunosuppressive microenvironment, APCs fail to deliver one or both activatory signals to tumor-specific T cells, which hence remain inactive.

Despite the obstacles mentioned above, mounting evidence shows that efficient antitumor immune responses can be promoted, especially when the appropriate therapeutic interventions are employed to mitigate tumor-dependent immunosuppression.¹ The most surprising revelation in this setting is that many among the conventional anticancer therapeutics that are employed nowadays in the clinic inadvertently prime anticancer immune responses and reap the additional therapeutic benefits provided by the host immune system (reviewed in Zitvogel et al.).²

Reovirus, which in its natural, unmodified form is a benign human pathogen, has been shown to kill malignant cells of multiple origin, including breast, brain, colon, lymphoid tissue, ovaries, spinal

cord, and bladder, and is currently being tested as an anticancer intervention in several multicenter Phase I-III clinical trials. The primary mode of action for reovirus-based virotherapy is oncolysis, i.e., the direct destruction of cancer cells.³ However, recent studies have shown that OVs also elicit an immune response that attack cancer cells (reviewed in Melcher et al.).⁴ Using murine models of melanoma as well as lung, ovarian, and prostate cancer, we have shown that the immunostimulatory effects of reovirus override various mechanisms set in place by malignant cells to avoid immune responses, hence promoting the establishment of a therapeutically meaningful, protective antitumor immunity.⁵⁻⁷ Therefore, if properly managed, reovirus-based oncolytic virotherapy can simultaneously target tumors through 2 distinct mechanisms: (1) upon direct oncolysis, and (2) by stimulating anticancer immune responses. Such a double attack can destroy existing cancer cells and establish an immunosurveillance system that prevents relapse.

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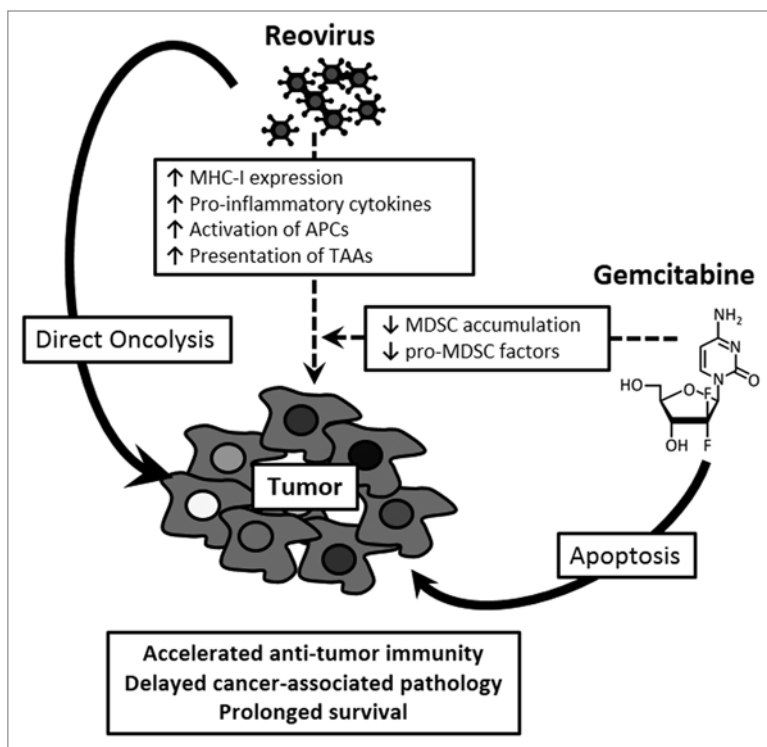


Figure 1. Gemcitabine potentiates oncolytic virotherapy-induced antitumor immune responses. Reovirus-based virotherapy and gemcitabine primarily target cancer cells by mediating direct oncolytic and pro-apoptotic effects, respectively. In addition, both these interventions promote immunological events that support the development of beneficial antitumor immunity. Interestingly, the addition of gemcitabine to reovirus-based oncolytic virotherapy further potentiates reovirus-induced antitumor immune responses. Thus, the combination of reovirus and gemcitabine produces better cancer outcomes as compared with the either therapeutic intervention alone.

In the context of oncolytic virotherapy, immune responses can have positive as well as negative implications, since they come in 2 different flavors: antiviral and antitumor. On one hand, antitumor immunity is a highly desirable outcome, as it targets cancer cells. On the other hand, antiviral immunity is often unwanted, as it inhibits viral replication and thus hampers direct oncolysis. It has now become clear that oncolytic virotherapy can exert optimal effects only when the accompanying immunological events are carefully managed.

We have previously observed that the administration of reoviral particles in tumor-bearing animals initiates a robust accumulation of immunosuppressive cell populations, including $\text{Gr1}^+\text{CD11b}^+$ MDSCs and $\text{CD4}^+\text{CD25}^+\text{FOXP3}^+$ Tregs, within tumor microenvironment.^{5,8} These findings contrasted with the ability of reovirus to induce functional antiviral and antitumor T-cell responses. It can be hypothesized that the immunosuppressive

cells recruited immediately upon the administration of OV serve to protect the host against the cytotoxic activity of immune effector cells and limit undesirable collateral damage. Alternatively, reovirus might recruit immunosuppressive cells as a mechanism to evade antiviral immunity during the early stages of infection, hence establishing a productive infection that would otherwise be curtailed by the attack of immune effector cells. In either scenario, the presence of immunosuppressive cells, especially MDSCs, in the tumor microenvironment limits the immunological benefits of oncolytic virotherapy. We hypothesized that inhibiting the accumulation of MDSCs during reovirus-based oncolytic virotherapy would greatly improve its antitumor efficacy. This was the rationale behind our recent study, in which we combined the administration of reovirus with gemcitabine.⁹

Gemcitabine is a deoxycytidine analog with a well-established antineoplastic profile. While gemcitabine triphosphate is

incorporated into DNA, causing chain termination and cell death, the diphosphate form also inhibits ribonucleotide reductase, limiting the pool of deoxynucleotide available for DNA synthesis and promoting apoptosis. Importantly, gemcitabine is also known for its MDSC-depleting activity.¹⁰ As summarized in Figure 1, we hoped that combining reovirus-based oncolytic virotherapy with gemcitabine would result in superior antineoplastic effects owing to: (1) the direct oncolytic activity mediated by reovirus; (2) the direct pro-apoptotic effects mediated by gemcitabine, and (3) improved antitumor immune responses elicited by a decrease in tumor-infiltrating MDSCs during the early phase of therapy. Our results demonstrate indeed that the combination of gemcitabine and reovirus-based oncolytic virotherapy retards tumor progression and improves the survival of tumor-bearing hosts as compared with either therapeutic intervention alone. Our findings also demonstrate that gemcitabine limits the reovirus-induced accumulation of MDSCs in the tumor microenvironment. Such a decrease is accompanied by the downregulation of MDSC-supporting factors including cyclooxygenase 2 (COX2), indoleamine 2,3-dioxygenase 1 (IDO1), $\text{IL-1}\beta$, and $\text{TGF}\beta 1$. Most importantly, gemcitabine turned out to accelerate virotherapy-driven antitumor immune responses. Taken together, our results indicate that gemcitabine enhances the efficacy of reovirus-based oncolytic virotherapy through immunological mechanisms. These findings lend further support to the emerging notion that many conventional chemotherapeutics promote beneficial antitumor immune responses.

Oncolytic virotherapy represents a promising anticancer strategy. The efficacy of this approach, however, ultimately depends on a delicate balance between antiviral and antitumor immune responses. The strategic management of virotherapy-induced immune responses through synergistic and complementary immunomodulators holds the key to achieving superior therapeutic effects in cancer patients.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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