

Releasing the Brake on Oncolytic Viral Therapy

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Oncolytic virus that selectively targets and eradicates tumor cells and immune checkpoint blockade that unleashes host antitumor immune responses show synergistic effects against cancer. This

combination holds great promise for future treatment of a broad range of cancers in patients. *Clin Cancer Res*; 21(24):5417–9. ©2015 AACR. See related article by Rojas et al., p. 5543

In this issue of *Clinical Cancer Research*, Rojas and colleagues report that combining an oncolytic virus and the immune checkpoint blockade inhibitor anti-CTLA-4 resulted in significantly better antitumor responses than either modality alone in two different mouse tumor models (1). These two therapies work in different ways. The oncolytic virus is tumor-selective due to specific deletion of genes that are crucial for replication of the virus in normal cells but dispensable in cancer cells (2). The immune checkpoint inhibitor, an antibody against cytotoxic T-lymphocyte-associated antigen 4 (CTLA-4), was the first drug of this kind approved by the FDA for treatment of cancer patients. CTLA-4 is exclusively expressed on T cells and regulates the amplitude of early T-cell activation (3). In this study, the authors revealed that administration of oncolytic virus and anti-CTLA-4 antibody resulted in activation of both innate and adaptive immune responses against the tumor and blocked immunosuppressive mechanisms within the tumor microenvironment.

The ability of viruses to lyse cancer cells has been well recognized. Genetically modified oncolytic viruses, such as herpes, vaccinia, and reovirus, have demonstrated encouraging results against cancers in both mouse models and human trials (2). Administration of talimogene laherparepvec (T-VEC) in human melanoma trials in particular has demonstrated encouraging responses at both the injected and distant tumor sites, including visceral lesions. T-VEC is a genetically modified herpes type 1 oncolytic virus that contains an insertion of the granulocyte-macrophage colony-stimulating factor (GM-CSF) gene that promotes local antigen presentation and systemic antitumor immunity (4). A similar approach using the vaccinia virus, JX-594, containing GM-CSF and deletion of the thymidine kinase gene has also generated therapeutic responses in patients with

advanced hepatocellular carcinoma, and these responses were also observed at distant untreated tumor sites (5).

However, despite these reports, the overall clinical benefit of oncolytic viruses as a monotherapy has been rather modest, and only a proportion of patients have benefited from this therapy. This may be due to the hostile tumor microenvironment that blocks viral induced antitumor immunity. Therefore, combining oncolytic viral therapy with approaches such as immune checkpoint blockade therapy that may release the brake on immune responses within the tumor microenvironment is a rational strategy. The application of oncolytic virus can induce strong infiltration of innate immune cells to the tumor microenvironment and promote the release of tumor antigens resulting in priming of an adaptive immune response against the tumor (Fig. 1). However, the immunoregulatory tumor microenvironment can effectively dampen effector T-cell responses induced by oncolytic viruses against the tumor resulting in immune escape. This may be overcome by administration of immune checkpoint inhibitors.

Immune checkpoint blockade has shown great promise for boosting immune responses against cancer by blocking checkpoint molecules on T cells and other effector immune cells. Two of the most studied immune checkpoint receptors are CTLA-4 and programmed cell death protein 1 (PD-1). These two receptors are both inhibitory but regulate immune responses by different mechanisms. CTLA-4 is exclusively expressed on T cells where it regulates the amplitude of early T-cell activation following binding to its ligands CD80 or CD86. The administration of anti-CTLA-4 antibody results in activation of effector T cells and depletion of regulatory T cells in the tumor that express high levels of CTLA-4 (6). In contrast, PD-1 expression is induced only when T cells become activated and its engagement with its ligands PD-L1 or PD-L2 inhibits kinases that are involved in T-cell activation. The use of either of these antibodies in the clinic has generated significant outcomes in a proportion of cancer patients, and recently the combination of these two drugs demonstrated significant advantage over either drug alone in patients with advanced melanoma (7). In a recent study, Victor and colleagues demonstrated that PD-1 and CTLA-4 blockade worked in a nonredundant way and combining the dual blockade with localized radiation generated synergistic antitumor effects (8). This study also highlighted the fact that the success of checkpoint blockade therapy is dependent in part on an ongoing immune response present within the tumor microenvironment. Thus, the combination of checkpoint inhibitors with approaches that can induce antitumor immune

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doi: 10.1158/1078-0432.CCR-15-1769

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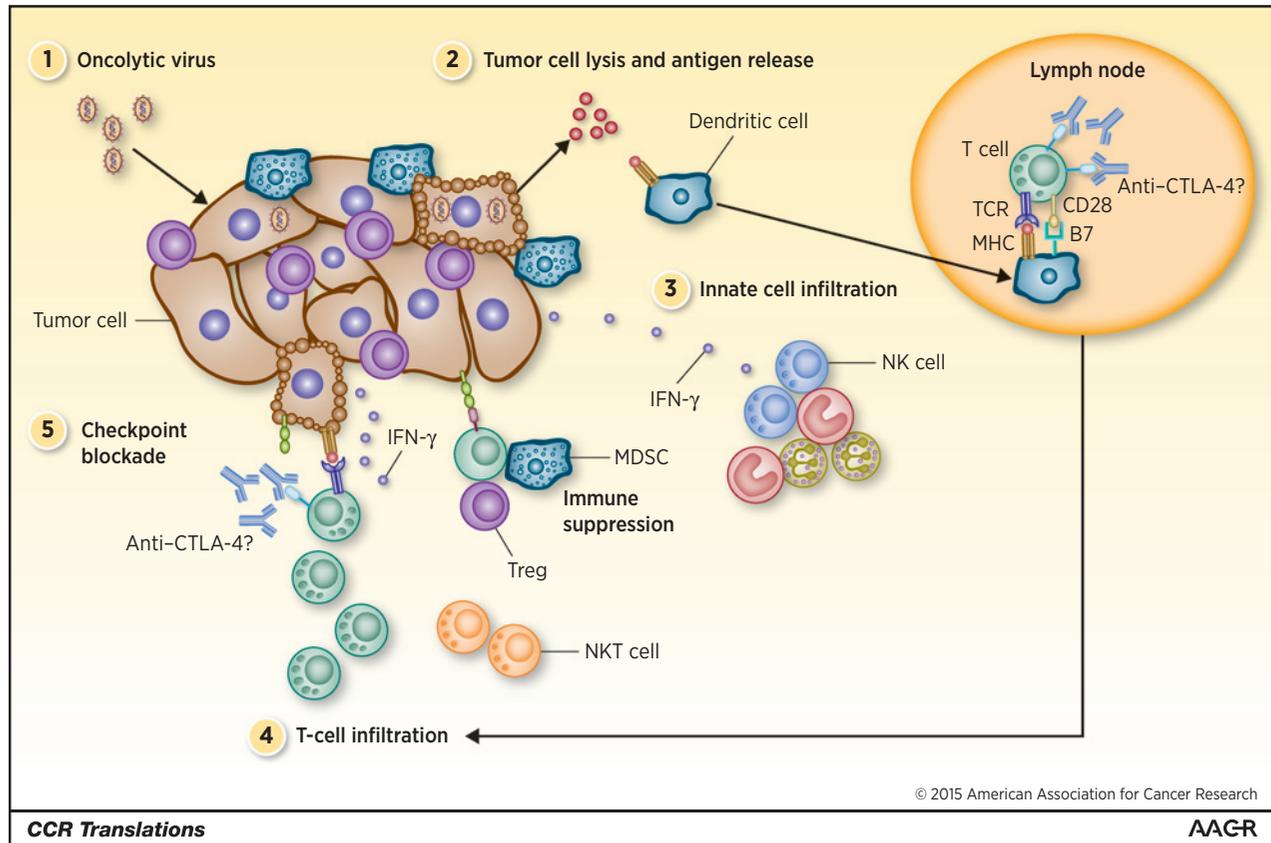


Figure 1.

Combined oncolytic virus and checkpoint blockade therapy against cancer. 1, the delivery of oncolytic virus leads to specific infection and replication within the tumor. 2, some of the infected tumor cells undergo cell lysis and release antigen. Antigen-presenting cells, such as dendritic cells, are activated and migrate to lymph nodes to present antigen to T cells, where the administration of anti-CTLA-4 mAb may increase the amplitude of T-cell activation. 3, concurrently, innate cells, including natural killer (NK) and natural killer T (NKT) cells, are recruited to the inflammatory tumor bed that may further contribute to antitumor immune responses, through secretion of cytokines such as IFN γ . 4, the activated T cells traffic to the tumor site and react against cancer cells through recognition of MHC/peptide complexes. 5, administration of CTLA-4 antibody may further contribute to increasing T-cell activity at the tumor site. MDSC, myeloid-derived suppressor cell; TCR, T-cell receptor.

responses *de novo*, such as oncolytic viruses, is anticipated to further increase treatment efficacy (1, 9).

The work undertaken by Rojas and colleagues utilized the oncolytic B18R-vaccinia virus that contains the deletion of thymidine kinase, an enzyme necessary for nucleic acid metabolism, and B18R, a gene encoding for the decoy receptor for type I interferons. The deletion of these two genes restricts the ability of the virus to replicate and spread within tumors, in preference to normal tissues. When B18R-vaccinia virus was given to mice followed by the administration of anti-CTLA-4 4 days later, antitumor effects were greatly improved compared with administration of virus alone. The enhanced therapeutic activity was accompanied by enhanced infiltration of CD8⁺ and CD4⁺ T cells, natural killer T cells, and natural killer cells into the tumor site. Interestingly, when the two components were given simultaneously, no therapeutic benefit was observed due to the premature clearance of the virus (1). This finding has important therapeutic implications. Increased persistence of the virus in the tumor is required to induce oncolysis and priming of the immune response. However, prolonged persistence of the virus may reduce overall antitumor immunity as the virus has evolved to suppress immunity in order to survive and replicate (10). It will be

important in ongoing studies to gain further insight into the complex interactions between the virus and host immunity, and the tumor and its microenvironment, which may lead to better design of utilizing oncolytic virus and immunomodulatory therapies such as anti-CTLA-4. In addition, the role of other innate cell types such as dendritic cells that present tumor antigen, and the role of specific chemokines and cytokines have yet to be addressed using this combined therapy. In future studies, it will be of interest to determine the effect of this combination approach on tumor vasculature and compare immune responses at various sites, including the draining lymph nodes and tumor.

In the clinic, the combination of Ipilimumab (monoclonal antibody to CTLA-4) and T-VEC is currently being tested in a phase III trial to treat advanced melanoma patients. The preliminary data are promising, demonstrating a significant synergistic effect (11). A further trial involving the checkpoint blocking antibody anti-PD-1 with oncolytic virus is planned for the treatment of melanoma patients (11). Whether this combined therapy leads to better control of distal tumor metastases remains to be seen. In summary, the combination of oncolytic virus and anti-CTLA-4 therapy appears to hold great promise for controlling cancer progression in patients and also opens up new options for

combining oncolytic virus with other immunomodulatory therapies. Additional approaches that amplify and prolong antitumor immune responses such as the agonist antibody anti-CD137 have been shown to effectively combine with oncolytic virus to significantly reduce tumor growth and metastases in mouse models (12). The insertion of genes into the oncolytic virus such as chemokines and cytokines for enhancing adaptive immune responses, and genes for targeting vasculature, angiogenesis, VEGF, and tumor stroma, have all been tested to enhance the oncolytic virus efficacy against cancer (2). Clearly, increased understanding of the interactions between oncolytic virus and host antitumor immunity will help in the design of more effective combination strategies for treatment of cancer in patients.

References

1. Rojas JJ, Sampath P, Hou W, Thorne SH. Defining effective combinations of immune checkpoint blockade and oncolytic virotherapy. *Clin Cancer Res* 2015;21:5543–51.
2. Chiocca EA, Rabkin SD. Oncolytic viruses and their application to cancer immunotherapy. *Cancer Immunol Res* 2014;2:295–300.
3. Pardoll DM. The blockade of immune checkpoints in cancer immunotherapy. *Nat Rev Cancer* 2012;12:252–64.
4. Andtbacka RH, Kaufman HL, Collichio F, Amatruda T, Senzer N, Chesney J, et al. Talimogene laherparepvec improves durable response rate in patients with advanced melanoma. *J Clin Oncol* 2015;33:2780–8.
5. Heo J, Reid T, Ruo L, Breitbart CJ, Rose S, Bloomston M, et al. Randomized dose-finding clinical trial of oncolytic immunotherapeutic vaccinia JX-594 in liver cancer. *Nat Med* 2013;19:329–36.
6. Simpson TR, Li F, Montalvo-Ortiz W, Sepulveda MA, Bergerhoff K, Arce F, et al. Fc-dependent depletion of tumor-infiltrating regulatory T cells co-defines the efficacy of anti-CTLA-4 therapy against melanoma. *J Exp Med* 2013;210:1695–710.
7. Larkin J, Chiarion-Sileni V, Gonzalez R, Grob JJ, Cowey CL, Lao CD, et al. Combined nivolumab and ipilimumab or monotherapy in untreated melanoma. *N Engl J Med* 2015;373:23–34.
8. Twyman-Saint Victor C, Rech AJ, Maity A, Rengan R, Pauken KE, Stelekati E, et al. Radiation and dual checkpoint blockade activate non-redundant immune mechanisms in cancer. *Nature* 2015;520:373–7.
9. Gao Y, Whitaker-Dowling P, Griffin JA, Barmada MA, Bergman I. Recombinant vesicular stomatitis virus targeted to Her2/neu combined with anti-CTLA4 antibody eliminates implanted mammary tumors. *Cancer Gene Ther* 2009;16:44–52.
10. Schneider-Schaulies S, Dittmer U. Silencing T cells or T-cell silencing: concepts in virus-induced immunosuppression. *J Gen Virol* 2006;87:1423–38.
11. Dolgin E. Oncolytic viruses get a boost with first FDA-approval recommendation. *Nat Rev Drug Discov* 2015;14:369–71.
12. John LB, Howland LJ, Flynn JK, West AC, Devaud C, Duong CP, et al. Oncolytic virus and anti-4-1BB combination therapy elicits strong antitumor immunity against established cancer. *Cancer Res* 2012;72:1651–60.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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Grant Support

C.Y. Slaney was supported by a National Breast Cancer Fellowship (PF-12-14). P.K. Darcy was supported by a National Health and Medical Research Council (NHMRC) Senior Research Fellowship (1041828).

Received August 17, 2015; accepted August 19, 2015; published OnlineFirst September 16, 2015.