Recent developments in oncolytic adenovirus-based immunotherapeutic agents for use against metastatic cancers

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INTRODUCTION
Malignant tumors rank among the highest worldwide causes of disease-related mortality and morbidity. Despite successful therapies for a variety of localized cancers, recurrent or metastatic cancers remain poorly responsive to conventional treatments, including surgery, radiation therapy and chemotherapy. Therefore, recurrent or metastatic diseases are associated with an extremely poor clinical outcome.1,2 Once malignant tumors have metastasized to distant organs, there are no conventional or alternative therapeutic strategies available for effective treatment. Thus, the highly disseminated characteristics of malignant tumors represent the major limitation for regression of the disease, and new effective therapies for disseminated metastatic disease are urgently required.

Oncolytic adenoviruses (Ads) have attracted considerable attention as anticancer agents because they can be readily engineered and exhibit multiple tumor-targeting mechanisms for enhanced antitumor properties.3 Genetically manipulated oncolytic Ad can specifically target and replicate in neoplastic cells but not in normal cells (Figure 1). Viral replication can result in tumor cell destruction. In a single replication cycle, a 10 000-fold amplification of progeny virus particles, which then may spread to neighboring tumor cells, can be achieved.4 In engineered oncolytic Ads containing therapeutic genes, the transgenes amplify along with their viral hosts, leading to augmented therapeutic efficacy.5 Importantly, viral infection and replication may be maintained until the whole tumor mass is eradicated. Based on these characteristics, oncolytic Ads are considered effective and used in clinical management of various malignancies.6–9 Recent results from clinical trials of oncolytic Ads have demonstrated safety of the virotherapy; however, the antitumor effects were disappointingly low. One problem with current Ad-based virotherapy is that applications are strictly limited to local treatment against primary tumors, due to obstacles of pre-existing immunity to Ad in the clinic and poor cancer-cell targeting. Such restrictions translate to limited success in the treatment of disseminated metastatic cancer, as well as eradication of primary tumor mass, with oncolytic Ad as a single agent. For more successful Ad-based virotherapies against both primary and metastatic cancers, new therapeutic strategies are needed to meet two challenges: improve the antitumor efficacy, and overcome the limitations imposed by in situ clinical treatment.

One approach to both these challenges is activation of a cancer patient’s antitumor immune response using a viral-mediated therapeutic gene delivery system. The mechanism here is direct delivery by oncolytic Ad vectors of immunostimulatory genes for expression in infected cancer cells.10,11 This strategy has a great potential for targeting and destroying cancer cells by activating tumor-specific immune responses in cancer-bearing hosts. More importantly, despite limitations of localized tumor application, the ideal viral agent can potently generate host antitumor systemic immune responses, such as tumor-specific immunological memory as well as immediate tumor-specific immune responses, thereby eliciting complete clearance of both primary and metastatic cancers and long-term suppression of recurrence. The rationale underlying this strategy is that robust infection and replication of the engineered viruses in cancer cells should elicit oncolysis and release of tumor-associated antigens (TAAs) in the...
specific tumor microenvironment (Figure 2). Subsequent capture of released TAAs by immunostimulatory transgene-activated antigen-presenting cells would then stimulate tumor-specific T cells. The overall result should be generation of a persistent, systemic antitumor immune response that would act to destroy both primary and disseminated metastatic cancers. In addition, in situ transfer of immunostimulatory genes by oncolytic Ads is predicted to lead to enhanced local production of immunostimulatory genes in the tumor milieu, leading to effective tumor-specific immunity without significant systemic toxicity. In reviewing this area, the choice of immunostimulatory transgenes capable of achieving more efficient systemic antitumor immunity is an important topic, as well as recent advances in Ad-based gene therapy in combination with either dendritic cell (DC) vaccination or radiation therapy.

AD AS ONCOLYTIC AGENT AND ITS CLINICAL LIMITATIONS

Virotherapy with oncolytic virus has attracted considerable attention and provided a new cancer therapy platform that selectively targets and destroys neoplastic cells while sparing normal cells, and Ad-based vectors are the most commonly used viral oncolytic agents in the clinic for several reasons. First, Ad can infect a wide variety of cell types and facilitate an S-phase-like state irrespective of the cell cycle stage of the infected cells (that is, Ad has the ability to infect both dividing and non-dividing cells). Viral replication induces selective killing of cancer cells, and a single cycle of virus replication results in a 10 000-fold increase in the number of viral copies and amplification of encoded therapeutic transgenes. In principle, Ad infection and replication persists until complete tumor eradication has been achieved. Second, the Ad genome remains in an unintegrated episomal condition, so that the risk of carcinogenesis by insertional mutagenesis is low. Third, the viral genome can be readily manipulated and will efficiently accommodate insertion of large and/or multiple therapeutic genes for augmented antitumor effects. Fourth, clinical-grade recombinant oncolytic Ad stocks can be manufactured at high yields and titers (10^{10} plaque-forming units per ml).

Many clinical trials based on forms of oncolytic Ad-based therapies have been reported. The ONYX-015 viral agent (d1520 and CI-1042; Pfizer Corp., Groton, CT, USA) is the first replication-selective oncolytic Ad used in clinical trials for cancer, and it contains a deletion of the gene encoding the p53-inactivating protein E1B 55 kDa. This alteration enables the strain to specifically replicate and kill tumor cells lacking a functional p53 without affecting normal cells, which are subject to p53-mediated cell cycle arrest. ONYX-015 has been extensively tested and optimized in clinical trials for a variety of cancers, including head and neck, hepatocellular, ovarian, prostate, colorectal, lung, pancreatic carcinomas and malignant gliomas, and to date, more than 18 clinical trials have been conducted with this viral agent. In phase I and II clinical trials of ONYX-015 in recurrent head and neck cancer, intratumoral administration was well tolerated, and promising localized therapeutic efficacy was observed. However, in the majority of the clinical trials, the antitumor efficacy of ONYX-015 as a single agent was low despite its marked safety margin. Therefore, alternative approaches are needed to enhance the antitumor efficacy of oncolytic Ad monotherapy.

There are additional obstacles that limit clinical use of Ad-based therapies. Following systemic administration, Ads are inactivated and cleared by interactions with immune cells. There are also unwanted toxic interactions with platelets and erythrocytes, and systemic treatment results in high nonspecific liver accumulation via virus capture by Kupffer cells or infection of hepatocytes, causing major Ad-related side effects. Moreover, because most adults have neutralizing antibodies directed against Ads, attempts to elevate exposure levels by repeated administration and high-titer stocks can elicit severe liver toxicity and an exaggerated immune response. Thus, current Ad-based clinical applications are strictly limited to in situ administration against primary tumors.

ONCOLYTIC AD-BASED IMMUNOTHERAPEUTICS FOR MAXIMIZING SYSTEMIC ANTI TUMOR IMMUNITY

Immunogene therapy against cancer involves the delivery of genes encoding immune stimulatory factors to boost the antitumor-adaptive immunity in the tumor milieu, with the result of heightened activation of tumor-specific immune responses in cancer-bearing hosts. Ad-based viral agents are particularly suited as vectors for this approach for reasons discussed above. In the following sections, we discuss advances in therapeutic strategies with Ad-based vectors containing cytokines and/or costimulatory molecules, chemokines or heat-shock proteins (HSPs).

Cytokine- and/or costimulatory molecule-expressing oncolytic Ad Cytokines have a pivotal role in regulating intercellular signaling involved in inflammation, immune responses and pathogenesis. Several immunostimulatory cytokines, such as interleukin (IL)-2, IL-12, IL-15, IL-18, interferon (IFN)-α, IFN-γ and granulocyte-macrophage colony-stimulating factor (GM-CSF), confer therapeut ic benefit and improved antitumor immune responses when delivered exogenously. Immunostimulatory effects and antitumor activities of more recently discovered cytokines, for example, IL-21, IL-23 and IL-27, have also been observed in a variety of preclinical cancer models, suggesting that they may offer promise in immunotherapy. In general, however, systemic administration of recombinant cytokines at therapeutic doses can induce serious dose-dependent systemic toxicity in cancer patients and animal models. Accordingly, customized expression tools are needed to control sustained production of therapeutic levels of cytokines for antitumor immune responses within the tumor milieu, while simultaneously minimizing systemic toxicity.

In situ delivery of antitumor cytokines by oncolytic Ad vectors has been proposed as an ideal method to attenuate systemic toxicity.
encodes GM-CSF, has been found to express selectively the effects.53 These results have been confirmed in a Syrian hamster were thought to result from a combination of IL-12 and Ad vector effects.50–52 Intratumoral expression of IL-12 mediated by the cytokines. It promotes T helper 1 (Th1) cell differentiation, oncolytic Ad constantly replicates in the tumor cells. over a period of weeks in the tumor microenvironment, as the colony-stimulating factor (GM-CSF)). This process, in turn, stimulates antigen-presenting cells that have been recruited and activated by immunostimulatory genes (for example, granulocyte–macrophage colony-stimulating factor (GM-CSF)). This process, in turn, stimulates tumor-specific T cells and ultimately leads to generation of a strong antitumor immune response. Moreover, expression of an immunostimulatory gene such as interleukin (IL)-12 facilitates proliferation as well as cytolytic effects of cytotoxic T lymphocytes, resulting in further augmentation of the antitumor immunity.

toxicity of cytokine therapy and promote antitumor immunity. The reason for this is the direct transfer and expression of therapeutic genes in cancer cells, mediated by Ad. Indeed, numerous preclinical reports have confirmed a potent antitumor immune response without accompanying toxicity through the intratumoral delivery and production of antitumor cytokines by oncolytic Ads.66–69 Expression levels of the cytokine transgene can persist over a period of weeks in the tumor microenvironment, as the oncolytic Ad constantly replicates in the tumor cells.

IL-12 is one of the most effective and promising antitumor cytokines. It promotes T helper 1 (Th1) cell differentiation, augments the cytolytic effect of natural killer (NK) cells and cytotoxic T lymphocytes (CTLs), and induces angiogenic effects.50–52 Intratumoral expression of IL-12 mediated by the oncolytic Ad YKL-IL-12 was shown to result in significant antitumor activity and lengthened survival. Both CD4+ and CD8+ T-cell infiltration and potent CTL-mediated cytotoxicity were thought to result from a combination of IL-12 and Ad vector effects.53 These results have been confirmed in a Syrian hamster cancer model, which is both immunocompetent and permissive to adenoviral replication in normal and neoplastic cells.54

As a vaccine adjuvant, GM-CSF improves antitumor cell-mediated immune responses by enhancing recruitment, maturation and migration of DCs as well as stimulating the cross-presentation of DCs, which is essential for recognizing cancer cells.55–57 The oncolytic Ad-based CG0070 vector, which encodes GM-CSF, has been found to express selectively the cytokine in retinoblastoma pathway-deficient cancer cells and to elicit selective oncolytic effects and immunostimulatory actions associated with local production of GM-CSF.58 The construct KH901, a modified congener similar to CG0070, has been found to be well tolerated in a phase I clinical study of in situ treatment in recurrent head and neck cancer patients, to result in local GM-CSF expression and inflammation at the site of administration, and to stimulate a systemic antitumor immune response.59 A variety of antitumor cytokines with different modes of action through a range of effector pathways, including IL-4, IL-18, IL-24, IFN-γ and IFN-β, have been proposed for delivery in oncolytic Ad-based therapeutics.36,37,48,60,61 Choi et al.62 have recently studied antitumor properties of the RdB/IL-12/IL-18 Ad-based vector coexpressing IL-12 and IL-18. The combined cytokine expression results in an enhanced and prolonged antitumor activity and greater survival.62 The antitumor immunity is based on an increased Th1/Th2 cytokine ratio and levels of IL-12, IL-18, IFN-γ and GM-CSF in RdB/IL-12/IL-18-treated tumors, suggesting that IL-12/IL-18 coexpression creates a tumor microenvironment more favorable to activation of tumor-specific immune cells, resulting in enhanced generation of fully differentiated CD4+ and CD8+ T cells expressing IL-12Rβ2 or IL-18Rα. In a study of two other coexpressed cytokines, IL-12 and GM-CSF (in the oncolytic Ad vector Ad-AB7/IL-12/GM-CSF), it was found that coexpression results in significantly higher local antitumor responses and systemic antitumor immune memory than expression of either IL-12 or GM-CSF alone.63 In this case, the synergistic therapeutic effects were mediated by the upregulation of Th1 cytokines in the tumor milieu, reversal of tumor-induced thymic atrophy and augmentation of systemic antitumor immune memory.

Professional antigen-presenting cell-expressed costimulatory molecules, such as B7-1 (CD80) and B7-2 (CD86), are important for optimal T-cell activation, even in the presence of specific T-cell receptor activated by major histocompatibility complex proteins bound to TAA molecules.64,65 However, the majority of cancer cells are defective in surface expression of costimulatory molecules, thus leading to T-cell anergy and escape of immune surveillance.66,67 In theory, tumor cell expression of a combination of costimulatory molecules and immunostimulatory cytokines by oncolytic Ad delivery could optimally induce antitumor immunity through full T-cell activation. This has been confirmed in studies of in situ delivery of oncolytic Ad engineered to express both GM-CSF and B7-1 (vector YKL-GB), where enhanced antitumor effects and longer-lasting systemic antitumor immune responses against tumor challenge were found versus control Ad (YKL-1).5 Importantly, the replicating YKL-GB vector was significantly more effective than replication-deficient Ad-coexpressing GM-CSF and B7-1, suggesting that vector replication greatly amplifies the GM-CSF and B7-1 gene expression. Coexpression of IL-12 and B7-1 (oncolytic Ad vector YKL-IL-12/B7) also results in stronger antitumor effects and a higher incidence of tumor regression than IL-12 expression alone.5 A recent study has described that coexpression of IL-12 and 4-1BB ligand (4-1BBL) in the oncolytic Ad against Ad-AB7/IL-12/4-1BBL greatly improves tumor reduction compared with expression of either molecule alone.68 This therapeutic benefit is associated with optimal generation of a Th1 immune response via enhanced tumor infiltration by DCs, antigen presentation and activation of tumor-specific T cells. Furthermore, intratumoral administration of Ad-AB7/IL-12/4-1BBL at the primary tumor site was found to inhibit significantly the formation of disseminated metastatic lesions at distal sites through potent systemic antitumor immunity.

These studies indicate the effectiveness of augmenting the systemic antitumor immune response by cytokine- and/or costimulatory molecule-mediated immunostimulation in combination with oncolytic Ad virotherapy. They provide further support for employment of oncolytic Ads in cancer immunogene
therapeutics, particularly for tumor-specific local delivery of synergistic immunostimulatory genes in a single replicating vector, and such approaches warrant further study in clinical trials.

Chemokine-expressing oncolytic Ad

Chemokines have important roles in eliciting innate and adaptive immunity by boosting the recruitment of immune effector cells to the tumor milieu, and they have been successfully employed in approaches to induce tumor-specific immunity.60–72 Accordingly, local delivery of chemokines by oncolytic Ads may be a promising therapeutic strategy. Intratumoral administration of oncolytic Ad-expressing RANTES (the chemokine ‘regulated upon activation, normal T-cell expressed and secreted’), also known as CCL5, results in diminished tumor metastasis formation as well as improved primary tumor regression through potent systemic antitumor immunity. RANTES enhances tumor infiltration of various immune cells, such as DCs, macrophages, NK cells and CTLs, and the generation of tumor-specific CTL and NK cell responses.73 In a similar immunotherapeutic approach, administration of replication-competent oncolytic Ad-expressing macrophage inflammatory protein 1α and Fms-like tyrosine kinase-3 ligand enhances DC and T-cell recruitment to the tumor site, and it suppresses not only local tumor growth but also growth of uninfected lung metastasis.74 Treatment with macrophage inflammatory protein 1α- and Ad-Fms-like tyrosine kinase-3 ligand-expressing oncolytic Ad, compared with macrophage inflammatory protein 1α- and Ad-Fms-like tyrosine kinase-3 ligand-expressing non-replicating Ad delivery, leads to improved maturation of infiltrating DCs and immune response against tumor-specific antigens, illustrating the essential contribution of the oncolytic Ads for antitumor immunity.

HSP-expressing oncolytic Ad

Attempts have been made to augment the immunotherapeutic potential of oncolytic Ad by incorporating HSPs as transgenes. As HSPs act as chaperone proteins that facilitate peptide folding and translocation, their overexpression in cancer cells could augment adaptive antitumor T-cell immunity by stimulating the processing and presentation of TAA to T cells via antigen-presenting cells.75,76 Moreover, HSPs can promote innate immunity through induction of cytokine release from DCs and activation of NK cells.77,78 Direct intratumoral delivery of HSV70-expressing oncolytic Ad in an immunocompetent host was found to inhibit markedly the growth of both primary tumors and distal untreated tumors, but not in an immunodeficient host.79 The oncolytic Ad also was found to suppress tumor growth following challenge with a second inoculation of tumor cells into the host, thereby indicating a vaccine effect by the induction of potent recall tumor-specific immunity. In addition, results of a phase I clinical trial of intratumoral HSV70-expressing oncolytic Ad (H103) in patients with advanced solid tumors has shown safety of the treatments and promising clinical antitumor activity.80 A study of oncolytic Ad armed with secreted or membrane-associated forms of HSP gp96 indicate a stimulation of protective and therapeutic antitumor immune response via oncolysis and tumor cell epitope presentation to antigen-presenting cells.81

ONCOLYTIC AD-BASED IMMUNOTHERAPEUTICS COMBINED WITH DC THERAPY

The innate immune response is thought to provide an important link to the generation of adaptive immune responses, mediated through the activity of DC. These cells process and present TAA to naïve T cells, thereby initiating an adaptive antitumor immune response involving both Th1 cells and CTLs. Hence, DC-based vaccines have emerged as a promising therapeutic option for the management of cancer.82,83 Preclinical results demonstrate that DCs loaded in various ways with tumor antigens, such as with whole-cell lysates, peptides, recombinant proteins, RNA or DNA, elicit protective and therapeutic tumor antigen-specific immune responses in murine tumor models.84,85 Moreover, clinical studies testing DC-based vaccines in cancer patients have been performed.86,87 Unfortunately, the clinical benefit was lower than expected, despite safety of the treatments. Emerging evidence suggests that the failure of DC vaccination might be based on functional impairment of DCs in the tumor milieu that results in suboptimal DC-induced adaptive antitumor immunity.88,89 Accordingly, various attempts have been made to overcome tumor-induced immunosuppression of DCs. To this end, Huang et al.90 investigated the potential therapeutic benefit of combining DCs with cytokine-expressing oncolytic Ad in the syngeneic B16 melanoma model. They found that coexpression of IL-12 and 4-1BBL in Ad synergistically promote efficacy of DC vaccination and lead to potent antitumor and antitumorigenic effects, as well as increased survival. The rationale underlying this approach is that Ad-mediated oncolysis generates TAA for presentation to administered DCs, and the capability of 4-1BBL to migrate DCs, together with local high expression of antitumor immune cytokines, such as IL-12, should generate an ideal tumor microenvironment for tumor-specific immune response. Recently, Zhang et al.90 demonstrated that oncolytic Ad-coexpressing IL-12 and GM-CSF in combination with DC vaccination result in strong and synergistic antitumor effects compared with individual treatments alone.90 The therapeutic mechanism of this combination therapy involves the removal of immunosuppression in the tumor microenvironment and stimulation of DC migration to draining lymph nodes. It is important to note that the order of administration of DC and virus in the combination therapies has an influence on antitumor efficacy; initial treatment with virus before DC vaccination reduces immunosuppression and facilitates favorable conditions within the tumor milieu for an optimal immune response.

The results of these studies highlight the potential therapeutic benefit of combined DC and cytokine-expressing oncolytic Ad treatments. Importantly, cytokine-expressing oncolytic Ad can function as a potent immune adjuvant for promoting Th1 antitumor immune response and overcoming the obstacles of DC vaccination. Therefore, these studies provide a proof of principle for feasibility of clinical studies of oncolytic Ad-based cytokine gene therapy in concert with DC-based vaccination in cancer.

ONCOLYTIC AD-BASED IMMUNOTHERAPEUTICS COMBINED WITH RADIATION THERAPY

Radiotherapy is one of the major therapeutic treatment modalities for solid tumors and an established local therapy for cancer.91,92 The key mechanism is considered to be damage of tumor DNA by ionizing irradiation, thereby eliciting apoptosis and necrosis of tumor cells. Importantly, radiation-induced cell death results in immunostimulatory effects,13–16 and this suggests that additional mechanisms of action are involved. For example, local irradiation treatment could have a pivotal role in slowing tumor growth, which would then give more time for targeting by the immune system. This concept is of relevance, as it is generally understood that therapeutic responses to cancer immunotherapeutics are not immediate, but rather need time to fully induce antitumor immunity in the cancer patient.

Radiation therapy-induced tumor apoptosis/necrosis may contribute to the efficacy of vaccination by creating a source of TAA. Recent studies have demonstrated that local tumor radiation leads to growth delay of murine hepatic cancer via stimulation of radiation-induced tumor-specific T cells, and T-cell-mediated antitumor immunity is essential for therapeutic response of
radiotherapy. The latter was shown by comparing tumor growth in immunodeficient and immunocompetent mice. Moreover, preclinical studies of DC vaccination in combination with local tumor radiation therapy have shown activated tumor-specific CD8+ T cells and potent antitumor effects when compared with radiation treatment alone.99,100 Other preclinical studies have demonstrated increased antitumor immune responses and longer animal survival in response to combined in vivo tumor irradiation and immunostimulatory agents, such as IL-12.101 Thus, the combination of local radiation therapy and oncolytic Ad-based immunotherapeutics appears to be a rational strategy to augment the systemic antitumor immune response, with efficacy for primary cancers as well as disseminated metastatic cancers and long-term inhibition of recurrence.

Recently, the first attempt to combine oncolytic Ad with in vivo tumor irradiation was carried out in an immunocompetent murine hepatocarcinoma model.102 Results indicate that oncolytic Ad-coexpressing IL-12 and GM-CSF, administered in concert with local radiation therapy, suppresses growth of the murine hepatocarcinoma HCa-1, which is a highly radiation-resistant solid tumor. The combination therapy also results in significant suppression of pulmonary metastasis. Elevation of tumor cell apoptosis as well as decrease in the number of CD31+ cells (a marker of angiogenesis) was observed in the combination regimen, suggesting a therapeutic mechanism for improvement of antitumor efficacy in the necrotic region of greatest tumor burden. Immunohistochemical analysis also exhibited an increased infiltration of CD4+ and CD8+ T cells and CD11c+ cells (a marker of DC) into the tissues surrounding the necrotic region of the tumor following in situ virus delivery and radiation treatment, when compared with individual treatments alone.

Even though oncolytic Ad-based immunotherapeutics in conjunction with local radiation therapy has opened new avenues for cancer therapeutics, additional studies are needed for clinical development. The timing and order of virus release versus radiotherapy in combined treatment may be important. For example, if viral oncolysis is potentiated by irradiation-mediated changes in the cancer cells, it could then be advantageous to first administer local radiotherapy. Conversely, if radiotherapy acts directly on the virus and/or has the potential for immunosuppressive effects and leukocyte killing, the combined therapy may be most effective when the virus is first administered. There is some evidence suggesting enhanced viral replication and oncolytic effects both in vitro and in vivo with initial radiation treatment.103–105 However, there are conflicting results from a study, which demonstrates no significant differences in long-term (8-week) tumor growth in animals treated with radiation either 24 h before or after viral inoculation.106 Therefore, larger studies will be required to assess whether such differences in regimen are general or limited to specific experimental situations, cancer cell lines or viral immunotherapeutics. Establishing the timing and order of the treatment regimen, and careful consideration of dose fractionation and scheduling, are necessary before combined oncolytic Ad-based immunotherapeutics and radiation therapy is taken into clinical trials.

CONCLUSIONS
Cancer virotherapy using oncolytic Ads has several advantages as an anticancer agent and has been safely used in clinical trials. However, there is limited efficacy in regression of primary tumors, and current clinical applications with oncolytic Ads are strictly limited to in situ administration, which points to the need for viable alternative therapeutic strategies in these approaches to successfully eradicate both primary and disseminated metastatic cancers. To this end, rational design of oncolytic Ads has incorporated immunostimulatory genes (cytokines, costimulatory molecules, chemokines and HSPs) in the vectors. The transgenes exert effects on systemic immune responses against the cancer cells and are active against both primary and metastatic cancers, even when only locally delivered. The direction of development of oncolytic Ad-based immunontherapeutics has been in direction of achieving a more efficient systemic antitumor immune response. The viral immunontherapeutics, in combination with either DC vaccination or radiation therapy, have led to a further augmentation of systemic tumor-specific immunity, in principle enabling complete suppression of both local and distant metastatic tumor growth. Preclinical studies of oncolytic Ad-based immunotherapeutics will provide the necessary support for moving optimal therapies forward into clinical trials. The optimal combination therapy to improve systemic antitumor immune responses will require careful consideration of dose prescription and treatment planning.

Clinical trials using oncolytic Ads have mainly been performed in advanced cancer patients for whom no alternative curative therapeutic options are available, and the objective antitumor responses have been disappointing. One reason for the limited therapeutic efficacy of oncolytic Ad-based immunontherapeutics may simply be the nature of treating advanced refractory cancer in late-stage patients, where it is known that the efficacy of cancer immunotherapeutic gradually decreases with increasing tumor mass.107,108 Accordingly, oncolytic Ad-based immunontherapeutics might elicit better clinical responses in earlier stage cancer patients with less refractory disease. In addition, the utility of oncolytic Ad-based immunontherapeutics as an adjuvant treatment before complete surgical resection of the tumor seems an attractive and promising strategy. The local delivery of oncolytic Ad-based immunontherapeutics in the tumor microenvironment can boost the generation of a systemic antitumor immune response, and this antitumor immunity can develop despite the resection of the primary tumor, thereby eliciting markedly decreased local recurrence, suppressed distant metastases and improved disease-free survival. Consequently, oncolytic Ad-based immunontherapeutics represent a potentially exciting new treatment paradigm for human cancers, and theoretical considerations as well as preclinical results strongly support a rationally directed clinical application of these agents in conjunction with established therapies. Thus, the oncolytic Ad-based immunontherapeutics should be tested in a variety of clinical settings as a means to maximize cancer treatment.

CONFLICT OF INTEREST
The authors declare no conflict of interest.

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