

## Minireview

# The Development of Conditionally Replicative Adenoviruses for Cancer Therapy<sup>1</sup>

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### Abstract

Replicative viral agents represent a novel approach for treating neoplastic disease. Tumor cell killing by the viral agent is achieved by direct consequence of the viral replication. Relative sparing of nontumor is, however, required to provide a therapeutic index of utility for cancer treatment. To this end, an ideal viral agent would, thus, possess several logical attributes, including stability and efficiency for infection and lateral spread *in vivo*, a preference for replication in tumor *versus* nontumor cells, and the capability of avoiding early detection—and eradication—by the immune system. To date, none of the agents has exhibited optimal characteristics with regard to the aforementioned attributes. Adenovirus, however, has lent itself to a process of extensive engineering that is dealing with each and every one of the major requirements and that is realizing its clinical potential. An advanced understanding of the cancer phenotype, as well as achievements in functionally exploiting viral plasticity, predicate the design and realization of conditionally replicative adenoviral agents with improved characteristics for cancer therapy.

### Introduction

The use of replicative viral agents represents a novel approach to neoplastic disease. In this strategy, target tumor cell killing by the viral agent is achieved by direct consequence of the viral replication (1). Furthermore, relative sparing of nontumor cells provides a therapeutic index of potential utility for cancer treatment. On this basis, it is apparent that the specificity of the viral agent for achieving tumor cell killing via replication (“oncolysis”) is the functional key to successful exploitation of

these agents for therapy. To this end, an ideal viral agent would, thus, possess several logical attributes: (a) such viruses must have the capacity to infect target cells *in situ*, that is, within the stringency imposed by direct *in vivo* delivery. Thus, a level of stability in the *in vivo* context is mandated to achieve an effective initial inoculum. Furthermore, such stability in the *in vivo* context would be critical for allowing replicated viruses to infect laterally, a key process to realizing effective amplification; and (b) the viral agent should possess a relative preference for replication in tumor *versus* nontumor cells. Thus, a useful viral agent would be well characterized in terms of entry biology and replicative physiology, such that these steps might be modified to achieve the desired tumor cell specificity, if thus required. Specifically, modulation of viral tropism, either by alteration of the initial attachment/entry steps or by modification of the functional aspects of viral genome replication and progeny-virus packaging, offers a means to achieve such specificity. Another potentially useful property for replicative viruses would be the capability of avoiding early detection and eradication by the immune system. Although a variety of viral agents have been used as replicative agents—including Bunyamwara, Coxsackievirus, dengue, mumps, Newcastle disease virus, vaccinia, West Nile virus, and adenovirus—none of the agents has exhibited optimal characteristics *vis-à-vis* the aforementioned desired attributes (2–4).

### Attributes of Adenovirus Recommend Its Use

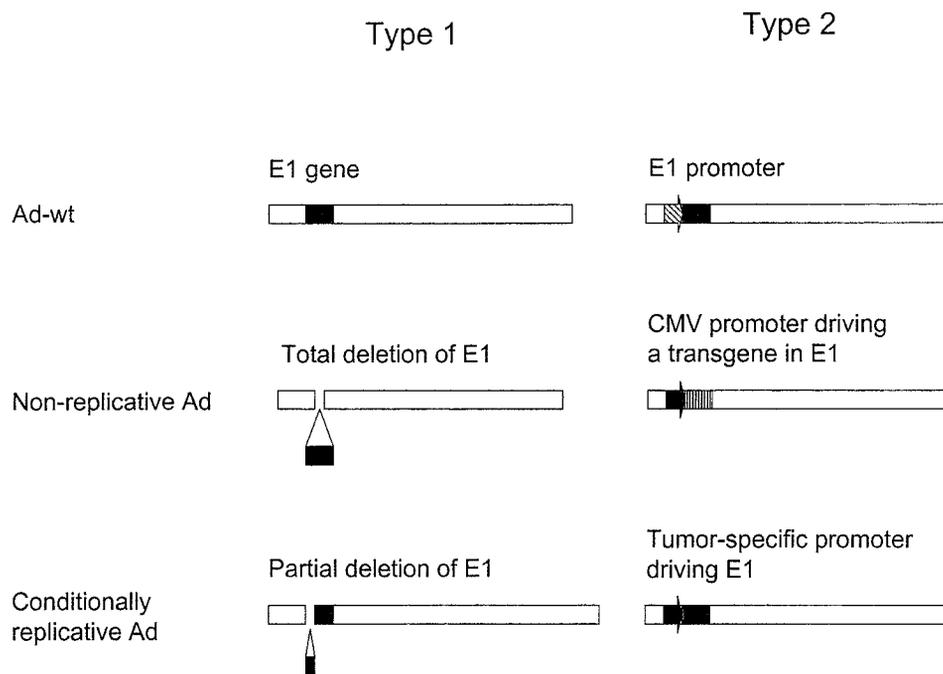
With respect to candidate replicative viral agents, adenoviruses possess many relevant attributes that recommend their use in this context (5). In this regard, adenoviral vectors have been used extensively for a variety of gene therapy applications (6, 7). In these various gene therapy schemas, adenovirus has exhibited an unparalleled efficiency allowing effective infection of target cells in the context of *in vivo* gene delivery. This attribute would logically predicate the ability of replicative adenoviruses to achieve a high initial inoculum to target tumor cells when used as a replicative agent. Of note, the entry pathway of the virus has been extensively characterized (8). On this basis, tropism modifications of the adenovirus have allowed rerouting of the virus through heterologous cellular pathways to allow achievement of cell specific gene delivery (9). Such biological plasticity would thus, in theory, allow infectious specificity to be achieved via restriction of binding exclusively to tumor cells. In addition, the replication cycle of the adenovirus has been the subject of investigation for several decades (10). Consequently, there exists a large database of information with respect to the viral regulatory mechanisms involved in the replicative cycle (11, 12). Thus, from the standpoint of inoculum efficiency and replicative specificity, adenovirus vectors offer potential utility as a conditionally replicative viral agent by providing the basis by which to modify the parent virus toward the requirements of a true CRAD reagent.

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**Fig. 1** Schematic representation of types of CRAD agents. Depicted are genomes of CRAD agents with illustration of the basis of conditional replication. For Type I CRADs, the design strategy of the transcomplementing genome is shown. Wild-type adenovirus (*Ad-wt*) has an intact *E1* gene that triggers early gene expression and adenovirus replication. A nonreplicative adenovirus (Ad) has a complete deletion of *E1A* and thus cannot propagate except in the context of *E1A*-expressing packaging cells. A conditionally replicative Ad may be derived by partial deletions of *E1* in which tumor cells provide the missing functions to allow replication. Ideally, the transcomplementing functions will be present in tumor cells but not in nontumor cells. For Type II CRADs, conditional expression of the *E1A* gene is achieved via a tumor-specific promoter. To achieve this end, replacement of the native *E1* promoter with the *tsp* would ideally allow *E1* expression only in promoter-inductive tumor cells. This *E1* expression could then trigger a replicative cycle for the adenovirus. Again, tumor-selective induction of the promoter is the basis of specificity. CMV, cytomegalovirus.

## Engineering Conditionality of Replication

### Specificity of Replication Based on Tumor Biology.

Initial attempts to derive CRADs<sup>3</sup> focused on the achievement of tumor selective replication (6). In this regard, by using the knowledge that components of the adenovirus replication cycle intrinsically interact with specific functional cellular proteins, one strategy has been the generation of CRAD vectors targeted to biological factors modified in cancer cells (Fig. 1). One such attenuated virus, containing mutations within an adenoviral early-transcribed gene, was developed to replicate only in cells lacking the cell cycle control protein p53 (6). Of note, cell cycle regulatory proteins, such as p53, are mutated in nearly all actively growing tumors (13); thus, the dependence of viral replication on the presence or absence of these proteins represents an ideal regulatory mechanism that potentially provides tumor-specific replication. On this basis, a mutated adenovirus, termed dl1520, was derived that contains two deletions within the *E1B-55* gene. Initial studies carried out with this agent demonstrated therapeutic potential, with the achievement of tumor regression and even complete elimination of tumors in

some murine xenograft models (6, 14). These findings resulted in the rapid translation of the virus into human Phase I, and then Phase II, clinical trials for carcinoma of the ovary and of head and neck cancer treatment (15). Of note, however, studies by Turnell *et al.* (16), and Goodrum *et al.* (17) determined that actual specificity of viral replication of dl1520 is not attributable to the absence or presence of p53 but is based on the timing of viral replication in tumor cells or other undefined (18, 19) factors. Replication of dl1520 is, therefore, not strictly linked to the presence of p53. In addition, replication in normal human primary cells has been noted (20). Thus, though the initial concept of targeting replication to the presence of a functional *p53* gene was not realized with this virus, empiric efficacy in tumor treatment has been suggested.

### Specificity of Replication Based on Transcriptional Control.

Given the inability to achieve absolute specificity with engineered replicative viruses via the aforementioned approach, investigators have used other methods (Table 1). In this regard, an alternate means for obtaining tumor specific adenoviral replication has been developed based on exploiting heterologous transcriptional control regions, or promoters, to restrict replication of the adenovirus to tumor. This has been accomplished by placing an essential adenoviral gene under the control of a heterologous genetic regulatory element the expression of which is limited to specific tissues or tumors. Two groups have

<sup>3</sup> The abbreviations used are: CRAD, conditionally replicative adenovirus/adenoviral; PSA, prostate-specific antigen; HCC, hepatocellular carcinoma; CAR, Coxsackie and adenovirus receptor.

Table 1 CRAD agents

Agent name <sup>a</sup>	Regulation method	Adenoviral region	Cancer type	Anti-tumor action	Research (Ref.)	Studies
CN706	TRAG <sup>b</sup>	E1A	Prostate	Oncolysis	Rodriguez <i>et al.</i> (7)	Human trials
787	TRAG	E1A and E1B	Prostate	Oncolysis	Yu <i>et al.</i> (24)	Animal models
AvE1a04I	TRAG	E1A	Hepatocellular carcinoma	Oncolysis	Hallenbeck <i>et al.</i> (21)	Animal models
Adv-E1AdB-F/K20	AGDCC	E1A	Glioblastoma	Oncolysis	Shinoura <i>et al.</i> (44)	Cell culture
Ad.TK <sup>RC</sup>	AGDCC	E1B	Colon	Oncolysis and toxin (thymidine kinase)	Wildner <i>et al.</i> (28)	Animal models
DI1520	AGDCC	E1B	Head and neck Ovary	Oncolysis	Bischoff <i>et al.</i> (6)	Human trials
Onyx-15, CD/HSV-1, TK	AGDCC	E1B	Cancer cell lines	Oncolysis and toxin (thymidine kinase and cytosine deaminase)	Freytag <i>et al.</i> (27)	Cell culture
Ad5dI309	AGDCC	E1 and E2	Cancer cell lines	Oncolysis	Medina <i>et al.</i> (45)	Cell culture

<sup>a</sup> Conditionally replicative adenoviruses engineered to date.

<sup>b</sup> TRAG, transcriptional regulation of adenovirus genome; AGDCC, adenoviral genome deleted to complement cellular genotype.

demonstrated the validity of this model by using such tumor-specific transcriptional regulatory elements, which control the essential early adenoviral genes (Table 1; Refs. 7, 21). In these instances, practical considerations dictated the strategy of heterologous control of the *E1A* gene. In addition, direct antitumor effects of *E1A*, based on apoptosis induction may be exploited in this manner (22). In this regard, the existence of *E1A*-transcomplementing cell lines, plus available plasmid packaging systems (23), allows for facile construction and rescue of such recombinant adenoviruses.

A variety of CRAD strategies have exploited this design strategy. In this regard, recognizing that levels of PSA are elevated in the prostate of individuals with prostate cancer, the transcriptional promoter sequences of the PSA gene have been configured into adenoviral vectors to regulate *E1* transcription (7). In mouse xenograft models, this replicative adenovirus eradicated large PSA-expressing tumors and abolished PSA production with a single intratumoral injection. Yu *et al.* (24) have presented studies using a CRAD vector containing dual promoter regulation within the *E1* region with promoters separately controlling expression of *E1A* and *E1B*. This replicative adenovirus was demonstrated to lyse PSA expressing cells with a selectivity of 10,000-fold over that of non-PSA-expressing cells. An alternative approach uses sequences that drive the expression of the HCC marker  $\alpha$ -fetoprotein, a gene that is singularly expressed in dividing hepatocytes and HCC (21). In addition, binary systems have also been developed as a means to achieve delivery that transcomplements *E1A* (25, 26).

### Multimodality Treatments

In addition to use as single agents, replication-competent adenoviruses have also been exploited in the context of combination treatment with conventional anticancer approaches. In this regard, several groups have examined the efficacy of this approach by configuring a toxin gene, such as *cytosine deaminase* or herpes *thymidine kinase*, into the context of replicative adenoviruses. In addition, Freytag *et al.* (27) have developed a replicative adenovirus that is configured with a *thymidine kinase/cytosine deaminase* fusion gene. The resultant toxin product kills cells with the administration of the prodrug, besides increasing the sensitivity of the tumor to radiation. Wildner *et*

*al.* (28, 29) and Heise *et al.* (30) have demonstrated that both of the therapy schemes bring additive effects to replicative viral cancer therapy. Furthermore, the resultant bystander effect seen from toxin-expressing cells is such that nontransduced tumor cells may likewise be eradicated, thereby accomplishing an additional mechanism for the achievement of an amplified antitumor effect. It has been proposed that utilization of this method may add a measure of safety to the use of oncolytic viruses in that one can effectively control the spread of virus via the addition of the prodrug analogue, which would selectively ablate virus-infected cells.

### Obstacles for Clinical Application of CRADs

Despite the various theoretical advantages of replicative adenoviral agents, the various strategies for use of CRADs will only allow true utility if they account for all of the relevant aspects of tumor biology.

#### Scarcity of Adenoviral Receptors in Human Tumors.

From the standpoint of inoculum efficiency, it has been noted that primary tumor is relatively refractory to adenoviral infection compared with cell line counterparts. This phenomenon is shown to occur on the basis of a relative deficiency of the primary adenovirus receptor CAR (31, 32). Clearly, the resistance of tumor targets to adenoviral infection will restrict not only the efficiency of the initial inoculum but also the ability of the virus to infect laterally postreplication. On this basis, in the absence of CRAD vectors that will infect with true tumor cell specificity, replicative adenoviral agents will at least need to possess the ability to achieve CAR-independent gene transfer (31). Indeed, such fundamental limits as tumor refractoriness to adenoviral infection may represent the major barrier to realizing the full benefit of CRAD agents translated into the clinical context at this point.

**True Tumor Specificity.** From the standpoint of replicative specificity, a number of design aspects used to date potentially undermine the goal of true tumor specificity. In the first regard, although transcomplementation of *E1A* offers practical advantages, a number of limits must be taken into account. In this regard, a number of tumors exhibit *E1A*-like activity and are, thus, capable of transcomplementing *E1A(-)* viruses (33, 34). Indeed, this capacity has actually been exploited in the

design of a class of CRAD agents that exploit interleukin 6-inducible E1A-like activity (33). The presence of intrinsic E1A-like activity would clearly operate to undermine the design of CRAD agents with E1A under control of tumor-specific promoters. In addition, promoter function in the adenoviral genome context is idiosyncratic, as has been noted in the context of a variety of adenoviral vectors designed to achieve transcriptional targeting of transgenes to tumor cells. Furthermore, this dysregulation of promoters is likely to be of even greater consequence in the context of cellular physiology induced by the replicative cycle of adenovirus. To address this, specific endeavors to understand heterologous promoter function in a CRAD context must be undertaken. Additional steps to maintain the fidelity of such promoters will require development and validation. Although some initiatives in this direction have been applied for adenovirus vectors, their relevance for CRAD vectors remains to be determined.

**Adenoviral Interaction with the Immune System.** Another key factor relevant to realizing the full therapeutic potential of CRAD agents is the interaction of the adenovirus with the immune system. In this regard, therapeutic efficacy of replicative adenovirus is predicated on the idea that replication and lateralization within tumors could occur without impairment via host eradication of the virus by immune mechanisms. Of note, Bramson *et al.* (35) have suggested that the intratumoral environment is a relatively privileged site in regard to adenoviral interaction with the immune system. Thus, appropriate physiology may exist within the tumor to allow further gain in viral amplification. On the other hand, Ikeda *et al.* (36) have shown that immunosuppression limits the utility of replicative herpes virus for antitumor therapy. On this basis, it may be argued that steps to attenuate the host immune response to adenovirus are rational. Although a variety of immunological approaches have been used to try to limit the host immune response to adenoviral vectors (37, 38), their use in the context of replicative adenoviruses raises particular safety concerns. Furthermore, at this time, mouse and rat tumors do not support efficient replication of human adenoviruses, so that syngeneic immunocompetent rodent tumor models are not available to evaluate the interaction between CRAD and the human immune system. Clearly, future studies are necessary to address the issue of immunomodulation of CRADs.

### The Clinical Indications for Using CRADs

Clinical translation of CRAD agents has progressed rapidly through Phase I and Phase II trials. These efforts have largely been carried out in the context of local or locoregional disease. This fact reflects the verity that the current generation of CRAD agents generally exhibits the promiscuous tropism of parent adenoviruses. On this basis, tumor-specific delivery is restricted to anatomical locations whereby the virus may be delivered and contained locally. This aspect of CRADs has limited the use of these agents for disseminated diseases, in which systemic delivery would be mandated. Thus, the ability to achieve cell-specific gene delivery via tropism modification of the parent virus would be required to allow the application of CRAD agents in the important context of disseminated disease (39). One key aspect of such a scenario is that the amplifying prin-

ciple nature of CRADs may allow the use of a much lower dose of administered adenovirus. On this basis, it may, in fact, be more feasible to use CRADs in a systemic manner for disseminated disease than to use adenoviral vector counterparts. This is especially relevant in the context of severe host reaction to i.v. injected adenovirus limiting the therapeutic efficacy of treatment (40).

### Conclusion

Despite these caveats, CRADs clearly represent antitumor agents of exciting promise. A greater understanding of precise patterns of tumor-specific gene expression will clearly offer additional venues for the derivation of viral tumor-specific replication. These endeavors will likewise be fostered by technologies to improve promoter specificity—via direct engineering of the adenoviral genome (41, 42) as well as via shuffling—and promoter evolution methods (43). In addition, dramatic strides have been made in adapting adenoviral vectors for cell-specific gene delivery. Clearly, these technologies will complement recent National Cancer Institute-directed efforts to a full characterization of unique surface molecules that distinguish tumor cells. Thus, on this basis, an advanced understanding of the cancer phenotype, as well as achievements in functionally exploiting viral plasticity, predicates the design and realization of CRAD agents with more improved characteristics for cancer therapy.

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