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Review Article

Replication-competent viruses for cancer therapy

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Abstract

Most cases of cancer, when detected at an advanced stage, cannot be cured with conventional therapeutic modalities. Therefore, novel approaches such as gene therapy are needed. Nevertheless, while the safety record of gene therapy for cancer has been excellent, clinical efficacy has been limited. Moreover, it has become evident that clinical efficacy is directly determined by gene delivery efficacy. For this reason, the use of replication-competent oncolytic viruses has been proposed. Oncolytic viruses are potentially attractive therapeutics for cancer because they selectively amplify the input treatment dose in target cells. Both naturally occurring and genetically engineered oncolytic viruses have been described. The most advanced clinical results are reported for conditionally replicating adenoviruses (CRAds). In this review, we will describe the use of oncolytic viruses as single-agent and combination treatments for cancer.

Introduction

Although chemotherapeutics and radiation therapy target various different cellular structures and pathways, most of them kill cancer cells by inducing apoptosis. As malignant cells are characterized by an impressive ability to adapt to the environment, apoptosis-resistant clones frequently develop during treatment. Each cancer cell has a statistical possibility for gaining resistance and therefore the number of malignant cells directly determines the overall likelihood of relapse. Furthermore, during subsequent treatment regimens, resistance usually occurs more rapidly, and there is a tendency for cross-resistance between agents. Therefore, new approaches are needed. Importantly, new agents should have novel mechanisms of action, thereby lacking cross-resistance with currently available treatments. Tumor-targeted oncolytic viruses might prove useful in this regard. These viruses have a cytolytic nature, *i.e.*, the replicative life cycle of the virus results in host cell destruction. Infection of tumor cells results in replication, oncolysis and subsequent release of the virus progeny. Normal tissue is spared due to lack of replication.

The ideal replication-competent virus should infect, replicate in and destroy cancer cells including nondividing cells. From a safety standpoint, the respective wild-type virus should cause only mild, well-characterized human disease. Further, a nonintegrating virus would decrease the risk of mutagenesis. Also, stability *in vivo* and established production of high titers of current good manufacturing practices (cGMP) quality are desirable features. For interpretation of these attributes, a reasonable understanding of virus biology is needed.

Oncolytic virus therapy

To achieve tumor-selective replication and subsequent oncolysis, some viruses can be genetically attenuated to preferentially replicate in malignant cells. Specifically, adenovirus, herpes simplex and vaccinia

viruses have been genetically engineered to confine replication to tumor cells. In contrast, it has been suggested that some viruses, such as reovirus, vesicular stomatitis and Newcastle disease virus, might have intrinsic selectivity for replication in tumors.

Viruses with inherent tumor selectivity

Reovirus, vesicular stomatitis, measles and Newcastle disease viruses are RNA viruses (1). During their replication cycle, double-stranded RNA, a stimulator of protein kinase R (PKR), is formed. Protein kinase R inhibits protein synthesis and promotes apoptosis, thereby controlling the spread of the virus infection. Double-stranded RNA can also stimulate release of interferons (IFNs), which activate PKR in adjacent, uninfected cells. Importantly, tumors are frequently defective in the PKR signaling pathway, allowing the replication of these viruses in malignant cells.

Reoviruses are nonenveloped double-stranded RNA viruses commonly isolated from the human respiratory and gastrointestinal tracts, although they seem to be nonpathogenic (1). Recently, it was shown that reoviruses replicate in cancer cells with the activated Ras signaling pathway. *Ras* is found mutated in 30% of all tumors, but some tumor types display high *ras* mutation incidences, *e.g.*, pancreatic cancers with a 90% incidence. Furthermore, the Ras pathway can be activated by upstream elements in the absence of mutation of *ras* itself. Therefore, up to 80% of tumors might be susceptible to reovirus replication.

Vesicular stomatitis is an enveloped, negative-strand RNA virus which causes a self-limiting disease in livestock, but is essentially nonpathogenic in humans (1). It is extremely sensitive to the antiviral actions of IFNs. Therefore, it replicates inefficiently in normal cells with a functional IFN/PKR system, while the opposite is true for malignant cells with defects in the PKR pathway.

Wild-type measles is a negative-strand RNA virus causing rash, fever, cough and conjunctivitis (1). Although the disease is usually mild, and infected persons recover completely, it causes almost a million deaths worldwide each year. The virus strain used for cancer gene therapy is derived from an attenuated vaccination strain (MV-Edm). Tumor cell killing is a consequence of cell-to-cell fusion and subsequent syncytial formation. However, the basis for the tumor cell-specific replication is unclear. An extensive vaccination program in Western countries has ensured that about 80% of the population has preexisting anti-measles antibodies, which might hinder the infectivity and spread of the virus.

Newcastle disease virus is a negative-stranded RNA virus which causes respiratory and central nervous system infections in fowl (1). However, in humans it is a mild pathogen causing conjunctivitis. Tissue culture-adapted strains of the virus show potent oncolytic activity in human cancer cells, possibly due to a defect in the IFN signaling pathway.

Herpes simplex virus type-1 (HSV-1)

HSV-1 is a double-stranded DNA virus and is a natural human pathogen that can cause recurrent oropharyngeal or genital sores. Pathogenicity is reduced by mutating one or more of the crucial virulence genes (*e.g., ICP6, γ34.5, UL24, UL56*) resulting in replication competence only in cycling cells (1, 2). Due to their neurotropism, oncolytic HSV-1 viruses were initially constructed for brain tumor therapy. Nevertheless, preclinical studies have shown efficacy against various solid tumors *in vitro* and *in vivo*.

Vaccinia

Vaccinia virus is a member of the poxvirus family. It is related to smallpox and it has therefore been used as a smallpox vaccine. However, vaccinia is a mild pathogen and it may cause rash, fever and body aches. It has a double-stranded DNA genome of about 200 kb. Most genetically modified vaccinia viruses have the thymidine kinase (TK) gene deleted, which might help to give selectivity for dividing cells (1, 2). This deletion makes the virus dependent on host cell nucleotides, which are more available in dividing cells. Also, other viral genes have been mutated to achieve tumor selectivity.

Adenovirus

The human adenovirus is a nonenveloped icosahedral particle that encapsulates up to a 36-kb double-stranded DNA genome. Hexon is the most abundant structural protein. Penton base units are located at each of the twelve vertices of the capsid, and the twelve fiber homotrimers protrude from the penton bases. Hexon appears to play only a structural role as a coating protein, while the penton base and fiber are responsible for cellular attachment and internalization. As shown in Figure 1, initial high-affinity binding of the most common adenoviral gene therapy vector, serotype 5 (Ad5), occurs via direct binding of the fiber knob domain to the primary receptor, the coxsackie-adenovirus receptor (CAR) (3). Other receptors have been described, *i.e.*, the major histocompatibility complex I and heparan sulfate glycosaminoglycans, but their role is currently unclear. Cell surface binding is followed by the internalization of the virus, mediated by the interaction of a penton base Arg-Gly-Asp (RGD) motif and cellular $\alpha_v\beta$ integrins, which triggers endocytosis of the virion via clathrin-coated pits. In the endosome, the virus is disassembled followed by endosomal lysis. Thereafter, viral DNA is transported to the nucleus through a microtubule-mediated process and viral genes are expressed. The adenoviral genome can be divided into immediately early (*E1A*), early (*E1B, E2, E3, E4*), intermediate (*IX, IVa2*) and late genes. The latter encode structural proteins. The early genes encode mainly regulatory proteins that prepare the host cell for virus

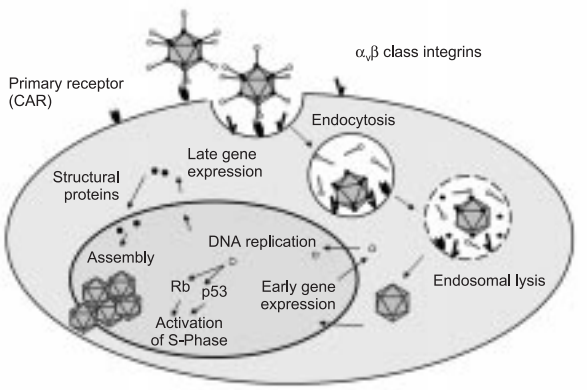

Fig. 1. The adenovirus infection pathway. Cell entry is initiated by high-affinity binding of the fiber knob domain to its primary receptor, CAR. Endocytosis is mediated by interaction of penton base RGD motifs with cellular $\alpha_v\beta$ integrins. After endosomal lysis, viral DNA is transported to the nucleus through a microtubule-mediated process and viral genes or transgenes are expressed.

DNA replication and block antiviral mechanisms. The E1A protein and its binding to the retinoblastoma (Rb) protein leads to the release of E2F family transcription factors, which force the host cell into S phase. Furthermore, to prevent apoptosis the adenovirus utilizes E1B proteins. Proteins encoded from the *E3* region regulate host immune responses and enhance cell lysis and release of virus progeny.

Conditionally replicating adenoviruses (CRAds)

The first clinical cancer trial with replicating adenovirus was done in 1956 with various wild-type strains. More recently, the increased understanding of adenovirus replication and its interactions with cellular proteins has inspired the construction of CRAds. Infection of tumor cells results in replication, oncolysis and subsequent release of the virus progeny (Fig. 2). Importantly, this replication cycle allows dramatic local amplification of the input dose and, in theory, a CRAd would replicate until all cancer cells are lysed. Conceivably, CRAd released from the tumor tissue might disseminate and infect distant metastases. Furthermore, eradication of systemic disease might be enhanced by the immune response directed against infected tumor cells (4).

Deletion-mutant CRAds

Type I CRAds feature loss-of-function mutations in the virus genome, which are compensated by cellular factors present in cancer but not normal cells. For example, this can be achieved by incorporating deletions in the immediate early (*E1A)* or early *(E1B)* adenoviral genes resulting in mutant E1 proteins unable to bind the cellular proteins necessary for viral replication in normal cells, but not in cancer cells.

The first published CRAd was ONYX-015 (a virus was initially reported as dl1520), which has two mutations in the gene coding for the E1B-55 kD protein (5). The purpose of this protein is binding and inactivation of p53 in infected cells, for induction of S phase, which is required for effective virus replication. Thus, this virus should only replicate in cells with an aberrant p53-p14^{ARF} pathway, a common feature in human tumors. While this is still subject to debate, initial studies suggested that this agent replicates more effectively in tumor than in normal cells. Unfortunately, the function of E1B-55 kD is not limited to p53 binding, which results in inefficient replication of ONYX-015 in comparison to wild-type adenovirus.

Ad5-Δ24 contains a 24-bp deletion in the constant region 2 (CR2) of *E1A*, and the modified protein is unable to bind the cellular Rb protein for induction of S phase (6). Therefore, viruses with this type of deletion have a reduced ability to overcome the G_1-S checkpoint and replicate efficiently only in cells where this interaction is not necessary, *e.g.*, tumor cells defective in the Rb-p16 pathway. It has been suggested that all human cancers may be deficient in this crucial pathway.

In normal cells, virus-associated (VA) RNAs I and II inactivate the RNA-dependent PKR, which otherwise would block protein translation in response to infection. However, an activated Ras/MAPK pathway can also inactivate PKR. Cascallo *et al.* studied a deletion-mutant CRAd, dl331, which has a deletion in the VA region and therefore is unable to replicate efficiently in normal cells, but retains Ras/MAPK-dependent replication (7).

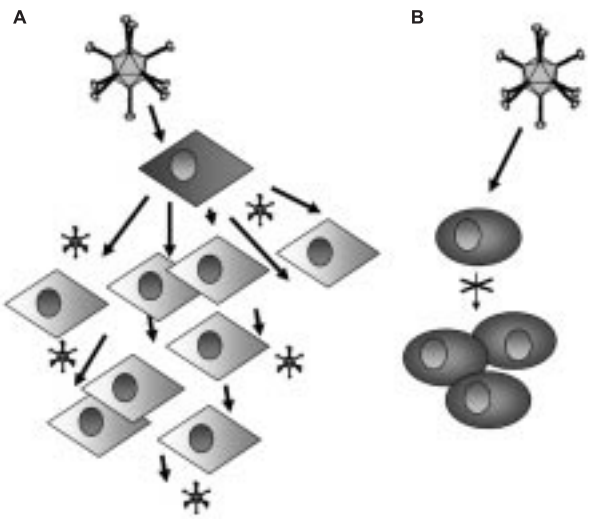

Fig. 2. Conditionally replicating adenoviruses. (a) Infection of tumor cells results in replication, oncolysis and subsequent release of virus progeny. Importantly, replication allows local amplification of the input dose. (b) Benign cells are spared due to lack of replication.

Promoter-inducible CRAds

In type II CRAds, tumor- or tissue-specific promoters (TSPs) replace endogenous viral promoters. This restricts viral replication to target tissues actively expressing the transcription factors that stimulate the TSP. Usually, a TSP is placed to control *E1A*, but alternatively or in addition, other early genes can also be regulated. Various promoters have been used to control viral replication. AvE1a04i, containing the *E1A* gene under the control of the α-fetoprotein (AFP) promoter, selectively replicates in hepatocellular carcinomas that express AFP (8). The same approach was used for breast cancer with the DF3/MUC1 gene promoter (9), a truncated L-plastin promoter (10) and estrogen-responsive elements from the pS2 gene promoter (11). The midkine differentiation factor promoter was used for advanced neuroblastoma and Ewing's sarcoma (12).

Telomerase activity is present in almost all human tumors. Therefore, tumor-specific hTERT-regulated CRAds were shown to have oncolytic activity in various types of tumors (13). OV798 has the CEA promoter driving *E1A* expression. It displayed oncolysis of CEA-expressing colorectal cancer cells *in vitro* and *in vivo* (14). Human prostate-specific antigen (PSA) promoter and rat probasin promoters have been utilized for prostate cancer-specific replication. CV706 has the PSA promoter and enhancer controlling *E1A* expression (15), while CV787 has the rat probasin promoter controlling *E1A* and the PSA promoter and enhancer driving *E1B* (16). Furthermore, Ad-OC-E1a has been used for treatment of both androgen-dependent and androgen-independent prostate cancer bone metastasis (17). The cyclooxygenase type-2 (COX-2) promoter has been explored in the context of pancreatic carcinoma and ovarian cancer (18, 19). Ahmed *et al.* described a novel CRAd whose tumor selectivity was based on control of gene expression at the level of mRNA stability. They ligated the COX-2 3'-untranslated region (UTR) downstream of the *E1A* gene. This results in mRNA stabilization regulated by an activated Ras/MAPK pathway (20).

Finally, a novel CRAd combining both type I and type II approaches featured a melanoma-specific tyrosinase promoter driving a CR2 deletion-mutated *E1A* (21). Also, this deletion has been combined with E2F1 promoter-controlled *E1A* and *E4* expression (22).

Multimodal therapy

Oncolytic tumor killing differs from conventional anticancer therapies, providing a possibility for additive or synergistic interactions in a multimodal antitumor approach. Moreover, the toxicity profiles may be different, which could result in enhanced efficacy without increased adverse effects.

There are several studies suggesting enhanced cell killing activity when CRAds and chemotherapeutics were combined. ONYX-015 has been combined *in vitro* and *in vivo* with 5-fluorouracil (5-FU) for the treatment of squamous cell carcinoma of the head and neck (SCCHN) and colon carcinoma (23). Also, ONYX-015 was combined with 5-FU + cisplatin (SCCHN, ovarian cancer) (24), cisplatin (SCCHN) (23) and cisplatin + paclitaxel (non-small cell lung cancer) (25). Interestingly, Heise *et al.* suggested that the efficacy is highly dependent on the sequencing of the agents, *i.e.*, simultaneous treatment or administration of the virus before 5-FU + cisplatin was superior to chemotherapy followed by virus (24). Also, the combination of prostate cancer-specific CV787 and paclitaxel or docetaxel displayed synergistic efficacy both *in vitro* and *in vivo* (26).

Combined ONYX-015 and radiotherapy exhibited additive antitumor cell-killing effects in glioma (27), colon cancer (28) and cervical cancer (29) xenograft models. Importantly, viral replication *in vitro* after radiation was not significantly inhibited. A Δ24-based virus, Ad5-Δ24RGD, displayed enhanced oncolysis for glioma in combination with radiation *in vitro* and *in vivo* (30). Furthermore, type II CRAd CV706 exhibited synergistic antitumor efficacy in combination with radiotherapy (31).

Armed and infectivity-enhanced CRAds

To further increase the oncolytic effect, transgenes for cytokines or prodrug-activating enzymes have been included in CRAds (32, 33). Such "armed CRAds" couple the lytic capability of the virus with the capacity to deliver therapeutic factors into tumor cells. In the prodrug-based strategy, genes encoding prodrug-activating enzymes are utilized. Common approaches include HSV-TK and *Escherichia coli* cytosine deaminase (CD), which convert systemically administrable and relatively nontoxic prodrugs (ganciclovir [GCV] and 5-fluorocytosine, respectively) into toxic products. The activated drugs can spread into surrounding cells (local bystander effect). The HSV-TK strategy may also allow noninvasive imaging (34) and abrogation of virus replication in case of toxicity. This approach has led to an enhanced antitumor effect compared to virus alone (29, 33, 35). However, other studies have suggested that addition of GCV did not increase oncolysis, possibly due to the inhibition of viral replication by GCV (36). Freytag *et al.* introduced a TK/CD fusion gene in the deleted *E1B-55 kD* region of dl1520. Furthermore they combined this double suicide gene therapy to radiotherapy with encouraging results (33).

Most published CRAds rely on CAR for entry into cells. Recently, it was demonstrated that the oncolytic potency of replicating agents is directly determined by their capability for infecting target cells (37, 38). Thus, variable CAR expression on cancer cells could hinder CRAd-mediated oncolysis. Therefore, methods to circumvent CAR deficiency and improve cell killing have been evaluated in the context of CRAds.

Ad5-Δ24RGD features the 24-bp *E1A* CR2 deletion and an RGD-4C modification of the fiber (39). The combination results in similar or enhanced oncolytic

potency in comparison to wild-type virus in various cancer cells. This virus was able to replicate in ovarian cancer primary cell spheroids and resulted in significantly prolonged survival in an aggressive orthotopic ovarian cancer model (40). The Ad5/3-Δ24 fiber features the knob from Ad3, which retargets the cell binding to the Ad3 receptor, which is currently unknown but is probably distinct from CAR (41). This chimerism resulted in dramatically enhanced infectivity of cancer but not normal cells, which translated into increased oncolysis of target cells. Furthermore, the antitumor efficacy in orthotopic animal models of ovarian cancer was impressive. Also, an *E1B-55 kD*-deleted CRAd has been modified with the heparan sulfate-binding polylysine residue at the C-terminus of the fiber (42). The first TSP-controlled infectivity-enhanced CRAd has recently been constructed and tested on ovarian and pancreatic cancer substrates (18, 19). Replicative specificity was achieved with a COX-2 promoter controlling expression of *E1A*, while the fiber was modified with RGD-4C.

Targeting with adapter molecules has been tested in the context of CRAds. Coinfection of a replication-deficient virus coding sCAR-EGF and Ad5-Δ24 resulted in enhanced oncolysis (38). However, this approach did not increase infectivity as a single-component oncolytic virus, D24sCAR-EGF, which incorporates sCAR-EGF fusion protein in the deleted *E3* region (43). This suggests that the expression of biologically active adapter proteins can interfere with virus production and oncolysis. In contrast, van Beusechem *et al.* introduced the bispecific single-chain (sFv) antibody 425-S11 (recognizes EGFR and the fiber knob) into the *E3* region of Ad5-Δ24. This secretory retargeting moiety increased the killing of CAR-deficient glioma cells *in vitro* and *in vivo* (44).

Monitoring CRAd efficacy

Imaging techniques can provide fundamental safety and efficacy information on experimental therapeutic approaches (45). Specifically, utilizing an orthotopic animal model for monitoring CRAd efficacy is advantageous, as it may resemble the clinical scenario more closely than subcutaneous tumors, but this approach is also problematic as tumors are not accessible to diameter measurements. Another important feature of noninvasive imaging is the possibility of performing repeated measurements. Therefore, various imaging systems have been evaluated (45). For example, expression of somatostatin receptor subtype 2, coded by an adenovirus vector, can be imaged with a radioisotope gamma camera after administration of the somatostatin analogue ^{99m}Tc-P2045 (34). Also, optical charge-coupled device (CCD) imaging has been used to detect bioluminescence emitted from D-luciferin reacting with firefly luciferase, coded by an adenovirus vector. Other approaches include magnetic resonance and positron emission tomography imaging of positron-emitting ligands (45). Cancer cells expressing reporter genes such as firefly luciferase and green fluorescence protein are useful means of following tumor growth. We used an orthotopic ovarian cancer model with SK-OV-3-luc cells, which emit light after intraperitoneal administration of D-luciferin (41). Using *in vivo* bioluminescence imaging, we were able to detect and measure intraperitoneal tumor cell killing by the virus. Oncolytic killing of tumor cells corresponded with reduction of signal in comparison to control animals.

As both safety and efficacy relate to persistence and propagation of the treatment agent, a secretory marker protein whose expression correlates with replication might allow noninvasive detection of these variables. Consequently, we constructed a retargeted CRAd featuring a secreted marker protein, soluble human carcinoembryogenic antigen (hCEA), which can be measured in growth medium or plasma (46). We found that virus replication closely correlated with hCEA secretion. In addition, antitumor efficacy and the persistence of the virus could be deduced from plasma hCEA levels. When plasma hCEA measurements were combined with noninvasive light-based imaging, it was possible to correlate virus replication to antitumor efficacy.

Clinical trials with oncolytic viruses

ONYX-015 is the most comprehensively clinically evaluated CRAd (Table I). Safety data has been excellent, but demonstration of efficacy has been limited. ONYX-015 has been well tolerated at doses from 2×10^{12} to 2×10^{13} vp by intratumoral, intraperitoneal, intravenous and intra-arterial routes. In a phase II study of intratumorally administered ONYX-015 in 40 patients with head and neck cancer, 3 complete and 2 partial responses were reported (47). In contrast, when the same virus was given in combination with 5-FU and cisplatin, 27% and 36% of patients had complete and partial responses, respectively (48). This suggests that initial clinical applications may feature combination treatments. However, most completed trials have employed CRAds such as ONYX-015, with low replicativity and therefore low oncolytic potency. Thus, single agent efficacy may be more impressive with more potent viruses. This was demonstrated by the high rate of PSA responses in a preliminary report of a trial featuring systemic treatment of disseminated prostate cancer with CG7870 (formerly CV787) (49).

All completed trials have evaluated CAR-binding CRAds. As CAR deficiency may be a universal phenomenon associated with carcinogenesis, this may have decreased the efficacy of approaches utilized thus far. The first trial featuring a transductionally targeted CRAd (Ad5-Δ24RGD) (39) has received National Cancer Institute funding and may soon start enrolling glioma and ovarian cancer patients (Hemminki, personal communication). Clinical CRAd trials are discussed in more detail elsewhere (50).

Several cancer trials have been performed recently with viruses other than adenovirus (2, 51). All of

Table I: Clinical trials with CRAds.[a]

Virus	Genetic alterations/ Concurrent treatment	Phase	Pts.	Route[b]	Tumor tagets[c]	Results[d]	Refs.
ONYX-015	*E1B-55 kD* deletion	I	22	i.t.	SCCHN	1 x 10^{11} pfu, 2 PR	55
ONYX-015	*E1B-55 kD* deletion	I	23	i.t.	Pancreatic ca.	1 x 10^{11} pfu, no responses	56
ONYX-015	*E1B-55 kD* deletion	I	10	i.v.	Metastatic solid tumor	2 x 10^{13} vp, no responses	57
ONYX-015	*E1B-55 kD* deletion	I	16	i.p.	Ovarian ca.	1 x 10^{11} pfu for 5 days, no responses	58
ONYX-015	*E1B-55 kD* deletion ± 5-fluorouracil and leucovorin	I	11	i.ha.	Colorectal ca. metastatic to liver	2 x 10^{12} vp, 1 PR with combination therapy	59
ONYX-015	*E1B-55 kD* deletion	I	22	m.w.	Premalignant oral dysplasia	1 x 10^{11} pfu for 5 days, followed by 1 dose/week for 5 weeks, 2 CR, 1 PR	60
ONYX-015	*E1B-55 kD* deletion + irinotecan and 5-fluorouracil	I	5	i.v.	Colon ca.	2 x 10^{12} vp, no responses	61
ONYX-015	*E1B-55 kD* deletion + interleukin 2	I	5	i.v.	Solid tumor	2 x 10^{11} vp, no responses	61
CV706	PSA promoter-enhancer controlling *E1A*	I	20	i.t.	Prostate ca.	1 x 10^{13} vp, ≥50% PSA decrease in 5/20 pts.	62
Ad5-CD/TK*rep*	*E1B-55 kD* deletion, insertion of TK/CD + GCV/5-fluorocytosine	I	16	i.t.	Recurrent prostate ca.	1 x 10^{12} vp, ≥50% PSA decrease in 3/16 pts.	63
Ad5-CD/TK*rep*	*E1B-55 kD* deletion, insertion of TK/CD + GCV/5-fluorocytosine and radiation	I	15	i.t.	Newly diagnosed prostate ca.	1 x 10^{12} vp, significant decline in PSA level in all pts.	64
ONYX-015	*E1B-55 kD* deletion + 5-fluorouracil (in phase II)	I-II	16	i.t., i.ha., i.v.	HCC and colorectal ca. metastatic to liver	3 x 10^{11} pfu, no responses, in phase II 50% CEA decrease in 3/7 pts.	65
ONYX-015	*E1B-55 kD* deletion + 5-fluorouracil and leucovorin	II	27	i.ha.	Gastrointestinal ca. metastatic to liver	2 x 10^{12} vp, 3 PR	66
ONYX-015	*E1B-55 kD* deletion	II	40	i.t.	SCCHN	2 x 10^{11} vp for 10 days, 2 PR, 3 CR	47
ONYX-015	*E1B-55 kD* deletion + cisplatin and 5-fluorouracil	II	37	i.t.	SCCHN	1 x 10^{10} pfu for 5 days, 11 PR, 8 CR	48
ONYX-015	*E1B-55 kD* deletion ± gemcitabine	I-II	21	i.t.	Pancreatic ca.	2 x 10^{11} vp, 8 injections over 8 weeks, 2 PR	67
ONYX-015	*E1B-55 kD* deletion	II	18	i.v.	Metastatic colorectal ca.	2 x 10^{12} vp every 2 weeks, no responses	68
CG7870	Rat probasin promoter controlling *E1A* and PSA promoter and enhancer driving *E1B*	I-II	20	i.t.	Locally recurrent prostate ca.	1 x 10^{13} vp, 25-50% PSA decrease in 8/12 evaluable pts.	49

[a]Includes clinical cancer gene therapy trials that have completed patient enrollment. [b]i.t. = intratumoral, i.v. = intravenous, i.p. = intraperitoneal, i.ha = intrahepatic artery, m.w. = mouthwash. [c]SCCHN = squamous cell carcinoma of the head and neck, HCC = hepatocellular carcinoma. [d]PR = partial response, CR = complete response.

these early-phase trials reported good safety data, while efficacy seemed modest at best. Thus, CRAds are currently the most promising oncolytic agents. Nevertheless, armed variants of the other oncolytic viruses could improve their efficacy.

Conclusions

Although the safety data with CRAds in clinical trials has been excellent, there are some hurdles to overcome in order to achieve efficient oncolysis in metastatic disease. Most obvious is the rapid clearance of adenovirus from blood by tissue macrophages, such as Kupffer cells of the liver (52). This is a non-CAR-mediated process and results in rapid blood clearance and virus degradation. After intravenous injection, the majority of virus accumulates in the liver and is rapidly cleared. However, Kupffer cells can be saturated with approximately 1-2 x 10^{10} viral particles in mice, and thereafter virus bioavailability and subsequent delivery to other tissues are increased (53). Alternatively, Kupffer cells can be depleted prior to virus

administration (52). It is currently unknown if these aspects are similar in humans.

In conclusion, the use of selectively replicating adenoviruses holds significant promise as a future cancer therapy approach. The combination of replication-competent viruses with standard chemo- and radiotherapies has great potential for increasing tumor cell destruction without concurrent increases in side effects. Finally, the immunogenicity of adenovirus-mediated tumor cell killing could be useful for eradication of distant metastases (54) and long-term antitumor immune surveillance, but needs to be studied further.

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References

1. Russell, S.J. *RNA viruses as virotherapy agents.* Cancer Gene Ther 2002, 9: 961-6.

2. Nemunaitis, J. *Selective replicating viral vectors: Potential for use in cancer gene therapy.* Biodrugs 2003, 17: 251-62.

3. Russell, W.C. *Update on adenovirus and its vectors.* J Gen Virol 2000, 81: 2573-604.

4. Bauerschmitz, G.J., Barker, S.D., Hemminki, A. *Adenoviral gene therapy for cancer: From vectors to targeted and replication competent agents.* Int J Oncol 2002, 21: 1161-74.

5. Bischoff, J.R., Kirn, D.H., Williams, A. et al. *An adenovirus mutant that replicates selectively in p53-deficient human tumor cells.* Science 1996, 274: 373-6.

6. Fueyo, J., Gomez-Manzano, C., Alemany, R. et al. *A mutant oncolytic adenovirus targeting the Rb pathway produces anti-glioma effect in vivo.* Oncogene 2000, 19: 2-12.

7. Cascallo, M., Capella, G., Mazo, A., Alemany, R. *Ras-dependent oncolysis with an adenovirus VAI mutant.* Cancer Res 2003, 63: 5544-50.

8. Hallenbeck, P.L., Chang, Y.N., Hay, C. et al. *A novel tumor-specific replication-restricted adenoviral vector for gene therapy of hepatocellular carcinoma.* Hum Gene Ther 1999, 10: 1721-33.

9. Kurihara, T., Brough, D.E., Kovesdi, I., Kufe, D.W. *Selectivity of a replication-competent adenovirus for human breast carcinoma cells expressing the MUC1 antigen.* J Clin Invest 2000, 106: 763-71.

10. Zhang, L., Akbulut, H., Tang, Y. et al. *Adenoviral vectors with E1A regulated by tumor-specific promoters are selectively cytolytic for breast cancer and melanoma.* Mol Ther 2002, 6: 386-93.

11. Hernandez-Alcoceba, R., Pihalja, M., Wicha, M.S., Clarke, M.F. *A novel, conditionally replicative adenovirus for the treatment of breast cancer that allows controlled replication of E1a-deleted adenoviral vectors.* Hum Gene Ther 2000, 11: 2009-24.

12. Adachi, Y., Reynolds, P.N., Yamamoto, M. et al. *A midkine promoter-based conditionally replicative adenovirus for treatment of pediatric solid tumors and bone marrow tumor purging.* Cancer Res 2001, 61: 7882-8.

13. Wirth, T., Zender, L., Schulte, B. et al. *A telomerase-dependent conditionally replicating adenovirus for selective treatment of cancer.* Cancer Res 2003, 63: 3181-8.

14. Li, Y., Chen, Y., Dilley, J. et al. *Carcinoembryonic antigen-producing cell-specific oncolytic adenovirus, OV798, for colorectal cancer therapy.* Mol Cancer Ther 2003, 2: 1003-9.

15. Rodriguez, R., Schuur, E., Lim, H., Henderson, G., Simons, J., Henderson, D. *Prostate attenuated replication competent adenovirus (ARCA) CN706: A selective cytotoxic for prostate-specific antigen-positive prostate cancer cells.* Cancer Res 1997, 57: 2559-63.

16. Yu, D.C., Chen, Y., Seng, M., Dilley, J., Henderson, D.R. *The addition of adenovirus type 5 region E3 enables calydon virus 787 to eliminate distant prostate tumor xenografts.* Cancer Res 1999, 59: 4200-3.

17. Matsubara, S., Wada, Y., Gardner, T.A. et al. *A conditional replication-competent adenoviral vector, Ad-OC-E1a, to cotarget prostate cancer and bone stroma in an experimental model of androgen-independent prostate cancer bone metastasis.* Cancer Res 2001, 61: 6012-9.

18. Yamamoto, M., Davydova, J., Wang, M. et al. *Infectivity enhanced, cyclooxygenase-2 promoter-based conditionally replicative adenovirus for pancreatic cancer.* Gastroenterology 2003, 125: 1203-18.

19. Kanerva, A., Bauerschmitz, G.J., Yamamoto, M. et al. *A cyclooxygenase-2 promoter based conditionally replicating adenovirus with enhanced infectivity for treatment of ovarian adenocarcinoma.* Gene Ther 2004, 11: 552-9.

20. Ahmed, A., Thompson, J., Emiliusen, L. et al. *A conditionally replicating adenovirus targeted to tumor cells through activated RAS/P-MAPK-selective mRNA stabilization.* Nat Biotechnol 2003, 21: 771-7.

21. Nettelbeck, D.M., Rivera, A.A., Balague, C., Alemany, R., Curiel, D.T. *Novel oncolytic adenoviruses targeted to melanoma: Specific viral replication and cytolysis by expression of E1A mutants from the tyrosinase enhancer/promoter.* Cancer Res 2002, 62: 4663-70.

22. Johnson, L., Shen, A., Boyle, L. et al. *Selectively replicating adenoviruses targeting deregulated E2F activity are potent, systemic antitumor agents.* Cancer Cell 2002, 1: 325-37.

23. Heise, C., Sampson-Johannes, A., Williams, A., McCormick, F., Von Hoff, D.D., Kirn, D.H. *ONYX-015, an E1B gene-attenuated adenovirus, causes tumor-specific cytolysis and antitumoral efficacy that can be augmented by standard chemotherapeutic agents.* Nat Med 1997, 3: 639-45.

24. Heise, C., Lemmon, M., Kirn, D. *Efficacy with a replication-selective adenovirus plus cisplatin-based chemotherapy: Dependence on sequencing but not p53 functional status or route of administration.* Clin Cancer Res 2000, 6: 4908-14.

25. You, L., Yang, C.T., Jablons, D.M. *ONYX-015 works synergistically with chemotherapy in lung cancer cell lines and primary cultures freshly made from lung cancer patients.* Cancer Res 2000, 60: 1009-13.

26. Yu, D.C., Chen, Y., Dilley, J. et al. *Antitumor synergy of CV787, a prostate cancer-specific adenovirus, and paclitaxel and docetaxel.* Cancer Res 2001, 61: 517-25.

27. Geoerger, B., Grill, J., Opolon, P. et al. *Potentiation of radiation therapy by the oncolytic adenovirus dl1520 (ONYX-015) in human malignant glioma xenografts.* Br J Cancer 2003, 89: 577-84.

28. Rogulski, K.R., Freytag, S.O., Zhang, K. et al. *In vivo antitumor activity of ONYX-015 is influenced by p53 status and is augmented by radiotherapy.* Cancer Res 2000, 60: 1193-6.

29. Rogulski, K.R., Wing, M.S., Paielli, D.L., Gilbert, J.D., Kim, J.H., Freytag, S.O. *Double suicide gene therapy augments the antitumor activity of a replication-competent lytic adenovirus through enhanced cytotoxicity and radiosensitization.* Hum Gene Ther 2000, 11: 67-76.

30. Lamfers, M.L., Grill, J., Dirven, C.M. et al. *Potential of the conditionally replicative adenovirus Ad5-Δ24RGD in the treatment of malignant gliomas and its enhanced effect with radiotherapy.* Cancer Res 2002, 62: 5736-42.

31. Chen, Y., DeWeese, T., Dilley, J. et al. *CV706, a prostate cancer-specific adenovirus variant, in combination with radiotherapy produces synergistic antitumor efficacy without increasing toxicity.* Cancer Res 2001, 61: 5453-60.

32. Bauzon, M., Castro, D., Karr, M., Hawkins, L.K., Hermiston, T.W. *Multigene expression from a replicating adenovirus using native viral promoters.* Mol Ther 2003, 7: 526-34.

33. Freytag, S.O., Rogulski, K.R., Paielli, D.L., Gilbert, J.D., Kim, J.H. *A novel three-pronged approach to kill cancer cells selectively: Concomitant viral, double suicide gene, and radiotherapy.* Hum Gene Ther 1998, 9: 1323-33.

34. Hemminki, A., Zinn, K.R., Liu, B. et al. *In vivo molecular chemotherapy and noninvasive imaging with an infectivity-enhanced adenovirus.* J Natl Cancer Inst 2002, 94: 741-9.

35. Wildner, O., Blaese, R.M., Morris, J.C. *Therapy of colon cancer with oncolytic adenovirus is enhanced by the addition of herpes simplex virus-thymidine kinase.* Cancer Res 1999, 59: 410-3.

36. Lambright, E.S., Amin, K., Wiewrodt, R. et al. *Inclusion of the herpes simplex thymidine kinase gene in a replicating adenovirus does not augment antitumor efficacy.* Gene Ther 2001, 8: 946-53.

37. Douglas, J.T., Kim, M., Sumerel, L.A., Carey, D.E., Curiel, D.T. *Efficient oncolysis by a replicating adenovirus (Ad) in vivo is critically dependent on tumor expression of primary Ad receptors.* Cancer Res 2001, 61: 813-7.

38. Hemminki, A., Dmitriev, I., Liu, B., Desmond, R.A., Alemany, R., Curiel, D.T. *Targeting oncolytic adenoviral agents to the epidermal growth factor pathway with a secretory fusion molecule.* Cancer Res 2001, 61: 6377-81.

39. Suzuki, K., Fueyo, J., Krasnykh, V., Reynolds, P.N., Curiel, D.T., Alemany, R. *A conditionally replicative adenovirus with enhanced infectivity shows improved oncolytic potency.* Clin Cancer Res 2001, 7: 120-6.

40. Bauerschmitz, G.J., Lam, J.T., Kanerva, A. et al. *Treatment of ovarian cancer with a tropism modified oncolytic adenovirus.* Cancer Res 2002, 62: 1266-70.

41. Kanerva, A., Zinn, K.R., Chaudhuri, T.R. et al. *Enhanced therapeutic efficacy for ovarian cancer with a serotype 3 receptor-targeted oncolytic adenovirus.* Mol Ther 2003, 8: 449-58.

42. Shinoura, N., Yoshida, Y., Tsunoda, R. et al. *Highly augmented cytopathic effect of a fiber-mutant E1B-defective adenovirus for gene therapy of gliomas.* Cancer Res 1999, 59: 3411-6.

43. Hemminki, A., Wang, M., Hakkarainen, T., Desmond, R.A., Wahlfors, J., Curiel, D.T. *Production of an EGFR targeting molecule from a conditionally replicating adenovirus impairs its oncolytic potential.* Cancer Gene Ther 2003, 10: 583-8.

44. van Beusechem, V.W., Mastenbroek, D.C., van den Doel, P.B. et al. *Conditionally replicative adenovirus expressing a targeting adapter molecule exhibits enhanced oncolytic potency on CAR-deficient tumors.* Gene Ther 2003, 10: 1982-91.

45. Gambhir, S.S., Barrio, J.R., Herschman, H.R., Phelps, M.E. *Assays for noninvasive imaging of reporter gene expression.* Nucl Med Biol 1999, 26: 481-90.

46. Kanerva, A., Zinn, K.R., Peng, K. et al. *Non-invasive dual modality in vivo monitoring of the persistence and potency of a tumor targeted conditionally replicating adenovirus.* Submitted.

47. Nemunaitis, J., Khuri, F., Ganly, I. et al. *Phase II trial of intratumoral administration of ONYX-015, a replication-selective adenovirus, in patients with refractory head and neck cancer.* J Clin Oncol 2001, 19: 289-98.

48. Khuri, F.R., Nemunaitis, J., Ganly, I. et al. *A controlled trial of intratumoral ONYX-015, a selectively-replicating adenovirus, in combination with cisplatin and 5-fluorouracil in patients with recurrent head and neck cancer.* Nat Med 2000, 6: 879-85.

49. DeWeese, T., Arterbery, E., Michalski, J. et al. *A phase I/II dose escalation trial of the intra prostatic injection of CG7870, a prostate specific antigen-dependent oncolytic adenovirus in patients with locally recurrent prostate cancer following definitive radiotherapy.* Mol Ther 2003, 7: S446.

50. Hemminki, A., Alvarez, R.D. *Adenoviruses in oncology: A viable option?* Biodrugs 2002, 16: 77-87.

51. Hermiston, T.W., Kuhn, I. *Armed therapeutic viruses: Strategies and challenges to arming oncolytic viruses with therapeutic genes.* Cancer Gene Ther 2002, 9: 1022-35.

52. Alemany, R., Suzuki, K., Curiel, D.T. *Blood clearance rates of adenovirus type 5 in mice.* J Gen Virol 2000, 81: 2605-9.

53. Tao, N., Gao, G.P., Parr, M. et al. *Sequestration of adenoviral vector by Kupffer cells leads to a nonlinear dose response of transduction in liver.* Mol Ther 2001, 3: 28-35.

54. Todo, T., Rabkin, S.D., Sundaresan, P. et al. *Systemic antitumor immunity in experimental brain tumor therapy using a multimutated, replication-competent herpes simplex virus.* Hum Gene Ther 1999, 10: 2741-55.

55. Ganly, I., Kirn, D., Eckhardt, G. et al. *A phase I study of Onyx-015, an E1B attenuated adenovirus, administered intratumorally to patients with recurrent head and neck cancer.* Clin Cancer Res 2000, 6: 798-806.

56. Mulvihill, S., Warren, R., Venook, A. et al. *Safety and feasibility of injection with an E1B-55 kDa gene-deleted, replication-selective adenovirus (ONYX-015) into primary carcinomas of the pancreas: A phase I trial.* Gene Ther 2001, 8: 308-15.

57. Nemunaitis, J., Cunningham, C., Buchanan, A. et al. *Intravenous infusion of a replication-selective adenovirus (ONYX-015) in cancer patients: Safety, feasibility and biological activity.* Gene Ther 2001, 8: 746-59.

58. Vasey, P.A., Shulman, L.N., Campos, S. et al. *Phase I trial of intraperitoneal injection of the E1B-55-kd-gene-deleted adenovirus ONYX-015 (dl1520) given on days 1 through 5 every 3 weeks in patients with recurrent/refractory epithelial ovarian cancer.* J Clin Oncol 2002, 20: 1562-9.

59. Reid, T., Galanis, E., Abbruzzese, J. et al. *Intra-arterial administration of a replication-selective adenovirus (dl1520) in patients with colorectal carcinoma metastatic to the liver: A phase I trial.* Gene Ther 2001, 8: 1618-26.

60. Rudin, C.M., Cohen, E.E., Papadimitrakopoulou, V.A. et al. *An attenuated adenovirus, ONYX-015, as mouthwash therapy for premalignant oral dysplasia.* J Clin Oncol 2003, 21: 4546-52.

61. Nemunaitis, J., Cunningham, C., Tong, A.W. et al. *Pilot trial of intravenous infusion of a replication-selective adenovirus (ONYX-015) in combination with chemotherapy or IL-2 treatment in refractory cancer patients.* Cancer Gene Ther 2003, 10: 341-52.

62. DeWeese, T.L., van der Poel, H., Li, S. et al. *A phase I trial of CV706, a replication-competent, PSA selective oncolytic adenovirus, for the treatment of locally recurrent prostate cancer following radiation therapy.* Cancer Res 2001, 61: 7464-72.

63. Freytag, S.O., Khil, M., Stricker, H. et al. *Phase I study of replication-competent adenovirus-mediated double suicide gene therapy for the treatment of locally recurrent prostate cancer.* Cancer Res 2002, 62: 4968-76.

64. Freytag, S.O., Stricker, H., Pegg, J. et al. *Phase I study of replication-competent adenovirus-mediated double-suicide gene therapy in combination with conventional-dose three-dimensional conformal radiation therapy for the treatment of newly diagnosed, intermediate- to high-risk prostate cancer.* Cancer Res 2003, 63: 7497-506.

65. Habib, N.A., Sarraf, C.E., Mitry, R.R. et al. *E1B-deleted adenovirus (dl1520) gene therapy for patients with primary and secondary liver tumors.* Hum Gene Ther 2001, 12: 219-26.

66. Reid, T., Galanis, E., Abbruzzese, J. et al. *Hepatic arterial infusion of a replication-selective oncolytic adenovirus (dl1520): Phase II viral, immunologic, and clinical endpoints.* Cancer Res 2002, 62: 6070-9.

67. Hecht, J.R., Bedford, R., Abbruzzese, J.L. et al. *A phase I/II trial of intratumoral endoscopic ultrasound injection of ONYX-015 with intravenous gemcitabine in unresectable pancreatic carcinoma.* Clin Cancer Res 2003, 9: 555-61.

68. Hamid, O., Varterasian, M.L., Wadler, S. et al. *Phase II trial of intravenous CI-1042 in patients with metastatic colorectal cancer.* J Clin Oncol 2003, 21: 1498-504.