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CG7870

Treatment of Prostate Cancer

CN787
CV787

Attenuated replication-competent adenovirus containing the rat probasin promoter (–426 to +28) driving the Ad5 E1A gene and the human prostate-specific enhancer/promoter (–5322 to –3875 and –230 to +7) driving the Ad5 E1B gene plus the entire Ad5 E3 region

EN: 274504

Abstract

Human prostate-specific antigen (PSA) is a widely used tumor marker for prostate cancer diagnosis, prognosis and management. A prostate-specific oncolytic adenovirus, CG7060 (formerly CN706 and CV706), was made by applying transcriptional regulatory elements (TREs) from the human PSA gene to control the expression of the adenoviral E1A gene. Transcriptional control restricts viral E1A gene expression in a tissue-specific manner and results in preferential viral replication in PSA-producing cells, thereby causing tumor-specific oncolysis. In order to increase antitumor specificity and potency, CG7870 (formerly CV787), an improved prostate-specific oncolytic virus, was generated using dual prostate-specific TREs for greater selectivity while retaining an intact E3 gene for higher antitumor potency. Preclinical *in vitro* and *in vivo* studies have shown that CG7870 replication is attenuated approximately 10,000-fold in PSA-negative cells compared to the wild-type adenovirus and is approximately 100 times more specific for PSA-producing cells than CG7060. A single dose of CG7870, delivered intratumorally or intravenously, can eliminate established tumors in LNCaP xenograft models. CG7870 is 10-fold more potent than CG7060 in killing PSA-producing cells or xenografts, probably due to the intact E3 region. Combination of CG7870 with chemotherapy or radiation therapy results in synergistic antitumor efficacy in tumor models. In clinical studies, CG7870 was well tolerated after intraprostatic or intravenous administration, and exhibited evidence of antitumor activity in patients with locally recurrent or hormone-refractory metastatic prostate cancer.

Introduction

Prostate cancer is the most common non-skin cancer in the United States among adult men. About 1 in every 9 American men over age 65 will be diagnosed with prostate cancer (1). In the prostate gland, the predominant cell type is the luminal cell, a differentiated androgen-dependent cell that produces prostatic secretory proteins, including prostate-specific antigen (PSA), which can be detected in the blood. It has been suggested that prostate cancer cells arise from the transiently proliferating progenitor compartment and thus may partly represent a luminal cell phenotype characterized by PSA secretion (2). Therefore, elevated PSA is common in prostate cancer patients, and it is used as an important marker for the diagnosis, prognosis and management of prostate cancer.

The expression of PSA is regulated by androgens (*e.g.*, testosterone). Binding of androgen to the androgen receptor (AR) will activate the androgen response element (ARE) in the transcriptional regulatory region of the PSA gene. This interaction regulates PSA gene expression and is required for the development of both the normal prostate gland and prostate cancer (3, 4). In the early stages of prostate cancer, most cancer cells are androgen-dependent and express both AR and PSA (5). These cancer cells respond well to androgen ablation therapy, and therefore hormone treatment can be an effective systemic therapy at early stages of prostate cancer. However, the androgen dependence changes to an androgen-independent status within approximately 2 years in virtually all patients (6). Thus, prostate cancer will eventually progress to a hormone-refractory stage for which new therapies are needed.

In hormone-refractory prostate cancer, the AR in cancer cells can undergo changes by a poorly understood mechanism (7-10). Some of the cancer cells may become androgen-independent after altered AR expression or AR mutagenesis (11, 12). However, there is evidence to suggest that AR is still involved in the growth of androgen-independent prostate cancer (12). The cancer cell population may become mixed, with many cancer cells still AR-positive and thus responsive to androgen induction.

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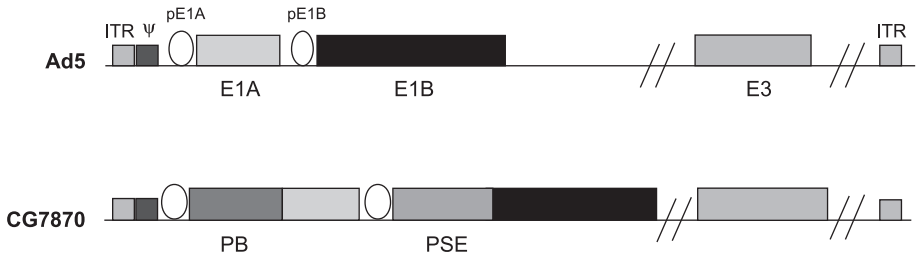

Fig. 1. Schematic structure of CG7870. E1A, E1B and E3 are respective adenoviral early genes. In wild-type adenovirus serotype 5 (Ad5), E1A and E1B genes are controlled by their endogenous promoters pE1A and pE1B. In CG7870, the rat probasin (PB) promoter and human prostate-specific antigen promoter and enhancer (PSE) are inserted into the viral genome to control the expression of the Ad E1A and E1B genes, respectively. ITR: adenoviral inverted terminal repeat. ψ: packaging signal.

Meanwhile, mutation of tumor suppressor genes or oncogenes in cancer cells may contribute to the failure of hormone therapy. For example, overexpression of the bcl-2 oncogene leads to inhibition of apoptosis in prostate cancer cells, thereby mitigating the effects of hormone treatment (13, 14). Mutations of p53 or Rb are also common and may also contribute to the failure of hormone therapy (15, 16). Due to these changes in advanced prostate cancer, new therapies targeting the AR should follow different paths than classical androgen ablation therapy.

The presence of the AR on normal and malignant prostate cells is key to the expression of several prostate-specific genes, including PSA (17), human glandular kallikrein (18, 19), probasin (PB) (20), prostatic steroid-binding protein (21) and prostatic acid phosphatase genes (22). All these genes are androgen-inducible, mediated by interaction of the androgen/AR complex with the functional ARE in their promoter region (23). Based on the specific expression of these genes in the prostate, unique tissue-specific promoters have been used for transcriptional targeting of prostate cells (17, 24, 25). The transcriptional targeting of transgene expression has been widely used in cancer therapy (26-28). In this strategy, the transgene is specifically transcribed in target cells in which the promoter is active. In normal or nontargeted cells, the promoter will be silent and the transgene will not be expressed.

In order to apply transcriptional targeting to a potent anticancer therapy, we have chosen recombinant adenoviruses as a platform to develop oncolytic viruses for prostate cancer. The adenovirus subtype Ad5 is common in the human population and does not cause serious health problems. In 1956, 11 different serotypes of wild-type adenoviruses, including type 5, were used to treat 30 patients with cervical cancer via intratumoral, intra-arterial or intravenous administration. More than half of the patients treated with live virus exhibited tumor regression without evidence of toxicity (29). In the last two decades, recombinant replication-defective adenoviruses have been extensively used for gene therapy in both the preclinical and clinical settings (30). The use of replication-competent oncolytic adenoviruses for the treatment of a variety of cancers is also being aggressively pursued (31). CG7870 is a selective, replication-competent adenovirus that is being actively developed for the treatment of prostate cancer.

Virus construction

Two different prostate-specific transcriptional regulatory elements (TREs) were utilized in the CG7870 viral genome. The rat probasin (PB) promoter and a minimal enhancer/promoter fragment (termed the prostate-specific enhancer, or PSE) derived from the human PSA gene were used to control the adenovirus serotype 5 (Ad5) E1A and E1B genes, respectively (32). These exogenous TREs were engineered into a recombinant adenoviral genome by molecular cloning. The 0.45-Kb PB promoter was inserted at nucleotide 551 of the Ad5 genome to control the expression of the E1A gene. Additionally, the 1.7-Kb PSE fragment was inserted at nucleotide 1682 of the Ad5 genome to control the E1B gene. The resulting recombinant virus, CG7870, is otherwise unchanged relative to wild-type Ad5 (Fig. 1).

Pharmacological Actions

During adenoviral infection, viral gene expression is a cascade process initiated by the expression of the E1 genes. Expression of the E1 genes is essential to activate other viral genes and, by extension, viral replication. Therefore, transcriptional control of the E1 genes will subsequently control the entire viral replication process, as demonstrated in several transcriptionally controlled recombinant adenoviral variants (19, 24, 32-34). We have shown that transcriptional control of E1 restricts expression of the mRNA and protein products of E1 to those permissive cells where the TRE is active. The selective expression of E1 restricts viral replication to permissive cells. In nonpermissive cells, E1 expression and viral replication will not occur due to the silence of the TRE.

Following specific E1 expression, the virus utilizes the host cell for productive viral replication. Eventually, virus-induced host cell shut-off mechanisms will lead to cell death. In addition, many different viral products are

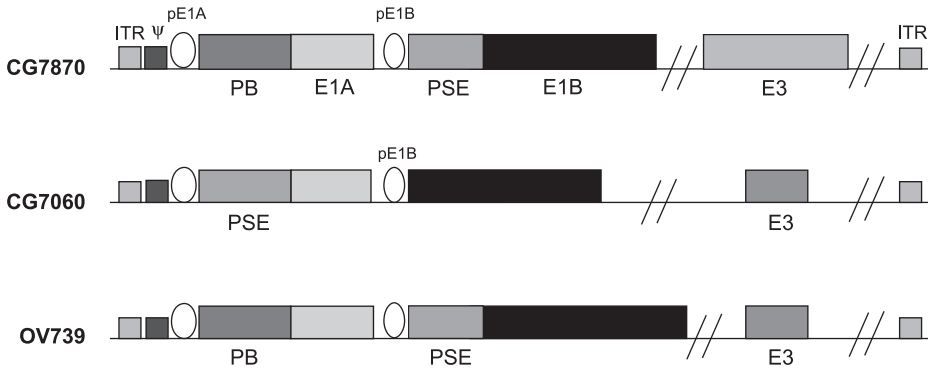

Fig. 2. Structure comparison of different prostate-specific adenoviral variants. E3: intact wild-type E3 region. ΔE3: deleted E3 region.

produced that are toxic to the cells. For example, viral fiber proteins have been found to inhibit macromolecular synthesis in cells permissive to adenovirus (35). Viral E1A products induce p53-dependent and -independent apoptosis (36). The E1a protein can also sensitize the cell to tumor necrosis factor (TNF)- (37, 38), natural killer (NK) cell- and cytotoxic T-lymphocyte (CTL)-mediated killing (39, 40). The adenovirus death protein (ADP, E3-11.6-kDa protein) is involved in cell lysis for viral release (41). The E4orf4 protein can coordinate with E1a to cause apoptosis independent of p53 and caspases (42). In summary, recombinant adenovirus-induced oncolysis is the result of tissue-specific transcriptional control of the E1 gene family leading to replication, cytotoxic protein elaboration and cell death.

CG7870 has improved specificity and efficacy compared to the previous-generation virus CG7060 (Fig. 2), a PSA-producing cell-specific oncolytic adenovirus that has been tested in patients (43). In CG7870, the prostate-specific rat PB promoter and the PSE were used to drive E1A and E1B expression, respectively. The PB promoter is selectively regulated by androgens through androgen receptor binding sites (ARBS-1 and ARBS-2) (20). Furthermore, it can target transgene expression specifically to the epithelial cells of transgenic mouse or human prostate cells (44-46). The PSE is derived from the PSA gene minimal enhancer and promoter with the ARE structure and has been used previously in CG7060 for prostate cancer-specific gene expression (17, 24). Therefore, both PB and PSE are androgen-responsive and active in cells containing the AR, such as LNCaP cells. A specific interaction between the androgen-AR complex and the ARE regions in the PB or PSE promoters turns on these promoters for E1A and E1B expression.

With these two prostate-specific TREs in CG7870, expression of the E1 genes is more tightly controlled and viral replication is therefore further restricted compared to CG7060. *In vitro* characterization showed that CG7870 was specific to PSA-producing cells and attenuated at least 10,000-fold in PSA-negative cells as compared to the wild-type virus. When compared to the single TRE-controlled CG7060, CG7870 demonstrated a higher specificity for PSA-positive cells in a variety of *in vitro* tests. For example, CG7870 had about a 100-1,000-fold lower burst size than CG7060 in PSA-negative HBL-100 and PA-1 cells, respectively, demonstrating its greater selectivity. Alternatively, similar replication to wild-type adenovirus was demonstrated for CG7870 in PSA-positive cells (32). The cytotoxicity of CG7870 in PSA-negative cells was also lower than CG7060, as measured by the methylthiazoletetrazolium (MTT) assay for cellular mitochondrial activity and cytopathic effect, again indicating superior selectivity. The improved tissue specificity of CG7870 compared to CG7060 is likely due to the placement of E1A and E1B under the control of dual prostate-specific TREs. This improved specificity confers an improved therapeutic index, an advantage that strongly supports the use of CG7870 as an oncolytic agent.

Another improvement of CG7870 over CG7060 is the intact E3 region. The adenovirus E3 region contains several genes, including ADP, which is required for efficient cell lysis (47). Direct comparison to CG7060 showed that CG7870 had an increased burst size compared to CG7060 in the permissive LNCaP cell line, suggesting enhanced viral replication and potency (32). Further examination of viral plaque morphology showed that CG7870 had a much larger plaque than the E3-deleted virus OV739, an otherwise identical counterpart (Fig. 2). The plaque-forming kinetics of these viruses were also different, CG7870 plaques appearing a few days earlier than those of OV739 and CG7060, suggesting that CG7870 is more cytotoxic to permissive cells. This enhanced cytotoxicity was confirmed in cell viability assays in which CG7870 killed LNCaP cells faster than the E3-deleted virus. Thus, the presence of E3 in CG7870 increases viral cytotoxicity, facilitates virus spread from cell to cell and restores the viral potency to a level similar to that of wild-type virus.

The enhanced anticancer potency of CG7870 is also evident in animals bearing LNCaP xenografts (32). *In vivo*, CG7870 retains the expected specificity profile, preferentially eliminating xenografts derived from PSA-positive tumor cells but not PSA-negative cells (33). With

intratumoral viral treatment, about 100-fold less CG7870 than CG7060 was needed to achieve a similar tumor reduction in LNCaP xenograft models. Single intratumoral administration of CG7870 at 1 x 10^8 particles/mm^3 tumor completely eliminated LNCaP tumors within 6 weeks and a single i.v. administration of CG7870 at a dose of 1 x 10^{11} viral particles eliminated established, distantly located LNCaP tumors. In this study, 8 of 14 treated mice were tumor-free and the rest were immunohistologically devoid of PSA. Considering all the preclinical characterization results, CG7870 is about 10-100 times more potent and 100-1,000 times more specific to PSA-positive cells than CG7060.

With *in vivo* treatment of CG7870, virus replication within LNCaP xenografts could be shown by Ad5 immunostaining at different time points after injection (32). The *in vivo* replication and spread in LNCaP xenografts were sequentially examined at days 3, 7, 21 and 28 in nude mice (32). Early after viral injection, a large cluster of cells in limited tumor sections stained positive with anti-Ad5 polyclonal antibodies and tumor necrotic areas were seen around the infected cells. By day 21 or 28, the percentage of infected area in the tumor increased, with more than 90% of the microscopic fields in most of the tumor sections positively stained. These results indicate that CG7870 predominantly replicated and spread in the tumor by way of infectious progeny viruses. Immunohistochemical analysis of virus-treated xenografts showed that the apoptotic staining zone and virus staining area were closely associated, indicating virus-induced cell killing. The viral infection of adjacent cells within the tumor was also associated with progressive necrosis, indicating the therapeutic effect of CG7870. Immunohistochemical staining of CD31, an angiogenesis marker, suggested that oncolytic viral treatment significantly lowered the angiogenic activity in tumors. Although the *in vivo* studies were carried out in immunodeficient nude mice, which cannot provide a complete picture of the oncolysis caused by CG7870, especially with respect to the adaptive immunity from the host, the combined effects of viral replication and the host immune response should work together for greater antitumor efficacy.

Preclinically, the combination of CG7870 with conventional radiation and chemotherapy was evaluated for synergistic anticancer effect (48, 49). A synergistic effect was demonstrated in the treatment of LNCaP xenografts with CG7870 and taxanes (paclitaxel or docetaxel). The combination increased CG7870 burst size and lowered the intravenous dose of CG7870 required for tumor elimination by about 1,000-fold (48). The combination also significantly elevated p53 expression and thereby increased the cell sensitivity to chemotherapy-induced apoptosis (48). Additionally, treatment with taxanes has been shown to increase the expression of adenovirus receptors, which may in turn sensitize the cells to CG7870 infection (50). Synergy was also demonstrated in combination with radiation therapy (49; Yu *et al.*, unreported results). In animal models, the combination of oncolytic virus with radiation for local prostate cancer treatment decreased the curative single dose of CG7870 at least 50-fold. Radiation kills proliferating cells by breaking DNA strands and inducing DNA repair mechanisms. Thus, irradiated cells provide active cellular machinery for viral DNA synthesis and replication (51). *In vitro*, viral burst size increased 500-fold when cells were irradiated. An *in vivo* study also demonstrated that radiation significantly reduced CD31-positive blood vessels, suggesting an enhanced antiangiogenic effect of the combination versus either treatment alone. In a recent study, Toth *et al.* (52) also demonstrated that radiation increased viral spread and cell killing. In combination with conventional therapy, the potency of CG7870 is significantly increased and may provide relevant clinical benefit.

Clinical Studies

CG7870 has been tested in patients with prostate cancer in two phase I/II studies (53, 54). A phase I/II trial of CG7870 in patients with locally recurrent prostate cancer with rising PSA levels following definitive external beam irradiation was initiated in 1999 at 5 medical centers including the Brady Urological Institute of the Johns Hopkins Oncology Center. The virus was administered under spinal anesthesia using a brachytherapy template and ultrasound 3D imaging with the MMS Terapac Plus 6.6 B3DTUI (Charlottesville, VA) treatment planning software for implantation of radioactive seeds. Virus was initially administered in 0.1-ml aliquots from up to 40 brachytherapy needles. Twenty patients were enrolled in this trial. Ten patients were treated at a dose level of $1x10^{12}$ viral particles (vp), 5 patients at $3x10^{12}$ vp and 5 patients at $1x10^{13}$ vp. Treatment was well tolerated overall with no serious adverse events. The most frequently reported adverse events were local injection-site reactions, flu-like symptoms and urinary tract symptoms consistent with the underlying disease process. One NCI-CTC treatment-related grade 3 adverse event occurred in a patient treated at the $1x10^{13}$ vp dose level. The patient had a grade 3 elevated D-dimer associated with grade 2 mild liver function test elevations, which resolved in 29 days. Patients were evaluable for PSA response if the baseline PSA value was 5 ng/ml or above. The PSA value decreased 25-49% in 8 of 12 evaluable patients (67%), and a decrease was observed in all patients in the highest dose cohort. With a median follow-up of approximately 6 months, 75% of evaluable patients remained progression-free; the median PSA progression-free survival has not been reached. No patient demonstrated a complete or partial response based on the Prostate Cancer Working Group criteria. These data indicate that intraprostatic administration of CG7870 is feasible and associated with an acceptable toxicity profile. The decrease in PSA levels observed in most evaluable patients after a single dose of CG7870 suggests biological antitumor activity for CG7870 (53).

A phase I/II trial of intravenous CG7870 was conducted in patients with hormone-refractory metastatic prostate cancer (54). Twenty-three patients were treated, each with a single i.v. bolus of CG7870 at doses of $1x10^{10}$-$6x10^{12}$ vp (3 patients per dose level except 2 at $6x10^{12}$ vp). Overall, CG7870 was well tolerated at all dose levels. The major toxicity experienced, besides flu-like symptoms (fever, fatigue, rigors, nausea, and vomiting) associated with intravenous infusion, was a transient, mild to moderate elevation of liver function tests, and a transient, nonsignificant coagulopathy in a minority of patients. Pharmacokinetic investigations revealed peak virus levels within the first hour after injection with a rapid clearance from the blood; a secondary peak indicative of *in vivo* replication is seen in most patients beginning about 3 days after administration. Stabilization of PSA was seen in 6/23 (26%) patients (median duration of 4 months) and < 50% declines of PSA were observed in 3/23 (13%) patients. These preliminary data indicate that a single i.v. dose of CG7870 was safe and associated with anti-PSA activity at the doses administered.

Based on the encouraging preclinical results with CG7870 in combination with radiation therapy, a new clinical protocol is actively enrolling patients with newly diagnosed, intermediate-risk prostate cancer. In this protocol, patients will receive intraprostatic injections of CG7870 concurrent with a standard course of 3D-CRT (conformal radiotherapy) or IMRT (intensity-modulated radiotherapy).

Conclusions

Preclinical studies have demonstrated CG7870 to be highly selective and potent as a prostate cancer-directed cytotoxic. CG7870 is attenuated 10,000:1 compared to the wild-type virus in PSA-negative cells, yet is capable of eliminating mouse xenograft tumors with a single i.v. injection. In addition, synergy between CG7870 and conventional chemotherapy and radiation therapy has been proven. Recently completed clinical studies of CG7870 as a single agent in prostate cancer have established that the agent is well tolerated and has antitumor activity as measured by PSA.

It is likely that doses of $1x10^{13}$ vp or greater will have limiting toxicities (55). Furthermore, given that 40-60% of the adult population have pre-existing antibodies to adenovirus and an increase in antibody titer to adenovirus has been observed in all patients thus far tested, concomitant treatment with immunosuppressive chemotherapy will be necessary in order to achieve adequate systemic delivery of adenoviral vectors (56). Conversely, clinical investigations of local intratumoral delivery of adenoviral vectors have indicated that repeated dosing is more feasible and continued efficacy possible, with the anti-adenovirus immune response apparently less critical to this application. Ongoing clinical trials of attenuated replication-competent viruses, including CG7870, will further evaluate virotherapy for the treatment of cancer.

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Source

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