[CANCER RESEARCH 61, 5453–5460, July 15, 2001]

CV706, a Prostate Cancer-specific Adenovirus Variant, in Combination with Radiotherapy Produces Synergistic Antitumor Efficacy without Increasing Toxicity[1]

Yu Chen, Theodore DeWeese, Jeanette Dilley, Yiwei Zhang, Yuanhao Li, Nagarajan Ramesh, Jake Lee, Rukmini Pennathur-Das, John Radzyminski, Joseph Wypych, Dominic Brignetti, Sara Scott, Jennifer Stephens, David B. Karpf, Daniel R. Henderson, and De-Chao Yu[2]

Calydon Incorporated, Sunnyvale, California 94089 [Y. C., J. D., Y. Z., Y. L., N. R., J. L., R. P-D., J. R., J. W., D. B., D. B. K., D. R. H., D-C. Y.], and Division of Radiation Oncology and the Department of Urology, The Johns Hopkins Hospital, The Johns Hopkins University School of Medicine, Baltimore, Maryland 21287 [T. D., S. S., J. S.]

ABSTRACT

Radiation is an effective means of treating localized prostate cancer. However, up to 40% of men with certain risk factors will develop biochemical failure 5 years after radiotherapy. CV706, a prostate cell-specific adenovirus variant, is currently in clinical trials for the treatment of recurrent organ-confined prostate cancer. We demonstrated previously that a single administration of CV706 at 5×10^8 particles/mm^3 of tumor eliminated established tumors within 6 weeks in nude mouse xenografts (Rodriguez *et al.*, *Cancer Res. 57:* 2559–2563, 1997). We now demonstrate that CV706-mediated cytotoxicity is synergistic with radiation. *In vitro*, addition of radiation to CV706 resulted in a synergistic increase of cytotoxicity toward the human prostate cancer cell line LNCaP and a significant increase of virus burst size, with no reduction in specificity of CV706-based cytopathogenicity for prostate cancer cells. *In vivo*, prostate-specific antigen (+) LNCaP xenografts of human prostate cancer were treated with CV706 (1×10^7 particles/mm^3 of tumor), 10 Gy of single fraction local tumor radiation, or both. Tumor volumes of the group treated with CV706 or radiation was 97% or 120% of baseline 6 weeks after treatment. However, when the same dose of CV706 was followed 24 h later with the same dose of radiation, the tumor volume dropped to 4% of baseline at this time point and produced antitumor activity that was 6.7-fold greater than a predicted additive effect of CV706 and radiation. Histological analyses of tumors revealed that, compared with CV706 or radiation alone, combination treatment with two agents increased necrosis by 180% and 690%, apoptosis by 330% and 880%, and decreased blood vessel number by 1290% and 600%, respectively. Importantly, no increase in toxicity was observed after combined treatment when compared with CV706 or radiation alone. These data demonstrate that CV706 enhances the *in vivo* radioresponse of prostate tumors and support the clinical development of CV706 as a neoadjuvant agent with radiation for localized prostate cancer.

INTRODUCTION

It has been known for many years that between 20 to 30% of localized prostate cancer patients continue to have positive biopsy specimens after radiotherapy (1). In the case of EBRT,[3] evidence supporting incomplete tumor ablation has come from several posttreatment biopsy studies (2) that find histopathology consistent with cancer even when digital rectal examination demonstrates a favorable tumor response. With the advent of PSA testing to evaluate patients after radiotherapy, it has been observed that approximately 80% of patients show progressive decreases in PSA in the first 12~18 months after radiotherapy, but by 7 years after therapy, as many as half of the patients will have a PSA that is progressively increasing, depending on pretreatment prognostic factors of the cancers (3–5). Even for men with T_1/T_2 disease, up to 40% experience biochemical failure 5 years after standard EBRT (6). Therefore, strategies to improve the outcome of local therapy with irradiation through radiosensitization are needed (7, 8).

Recent clinical and animal studies (9, 10) have described improved results when androgen ablation is combined with radiation. The clinical gains from such combination therapy have been encouraging in some groups of patients (9) but have unfortunately been associated with significant long-term side effects (11). We have developed previously (12–14) two selectively replication-competent adenovirus variants, CV706 and CV787, both of which replicate preferentially in and destroy prostate cells that produce PSA. A Phase I/II clinical trial of men with locally recurrent prostate cancer after radiotherapy has shown that half of the patients treated with a single intraprostatic administration of CV706 at 1×10^{13} particles demonstrated at least a 50% decrease in PSA, clearly demonstrating antitumor activity without serious side effects (15). Recently (16), synergistic antitumor efficacy was observed in human prostate cancer xenografts when the prostate cancer-specific adenovirus variant CV787 was combined with taxanes (paclitaxel and docetaxel). Because adenovirus E1A is known to be a potent inducer of chemosensitivity and radiosensitivity through p53-dependent and independent mechanisms (17), we have investigated the possible radiosensitizing effects of CV706 on prostate tumor cells. The present report provides evidence that CV706 has a synergistic antitumor effect both on irradiated human prostate cancer cells and tumor xenografts.

MATERIALS AND METHODS

Cell Culture and Virus. The human LNCaP (prostate carcinoma), OVCAR-3 (ovary carcinoma), and HBL-100 (breast epithelia) cell lines were obtained from the American Type Culture Collection (Rockville, MD). The human embryonic kidney cell line, 293, which expresses the adenoviral *E1A* and *E1B* gene products, was purchased from Microbix Biosystem, Inc. (Toronto, Ontario, Canada). Cells were maintained at 37°C with 5% CO_2 in RPMI 1640 supplemented with 10% fetal bovine serum (Hyclone, Utah), 100 units/ml penicillin, and 100 μg/ml of streptomycin (Life Technologies, Inc., Gaithersburg, MD).

CV706 is a prostate-specific replication competent adenovirus variant that was engineered at Calydon. One prostate-specific TRE, the human PSA promoter and prostate-specific enhancer, was inserted upstream of the E1A-encoding region in the viral genome (12). Similarly, CV787 is also a prostate-specific replication competent adenovirus variant, which contains two prostate-specific TREs, the probasin promoter and prostate-specific enhancer, inserted upstream of the E1A- and E1B-encoding regions in the viral genome, respectively (14). Both CV706 and CV787 are currently in clinical trials, the former for organ-confined prostate cancer and the latter for metastatic hormone refractory prostate cancer.

Cell Viability and Radiation. MTT assays were performed to measure cell viability as described previously (14). Briefly, HBL-100, OVCAR-3, and

Received 3/6/01; accepted 5/7/01.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked *advertisement* in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

[1] Supported in part by NIH/NCI CA58236 (to T. D., S. S., and J. S.).

[2] To whom requests for reprints should be addressed, at Calydon, Inc., 1324 Chesapeake Terrace, Sunnyvale, CA 94089. Phone: (408) 752-9877; Fax: (408) 734-2670; E-mail: dyu@calydon.com.

[3] The abbreviations used are: EBRT, external beam radiotherapy; CV, Calydon virus; FTV, fractional tumor volume; MTT, 3-(4,5-dimethylthiazol-2–4)-2,5-diphenyl-2H-tetrazolium bromide; MOI, multiplicity of infection; PSA, prostate-specific antigen; TRE, transcription response element; i.t., intratumoral; TUNEL, terminal deoxynucleotidyl transferase-mediated nick end labeling; PFU, plaque-forming unit.

LNCaP cells (2×10^4 cells/well; 96-well plate) were either infected with CV706 or CV787 at various MOIs (from 0.0001 to 1) and/or treated with radiation at the indicated dosages. Cells were incubated in growth medium for 24 h to allow for viral replication. After 24 h, cells were exposed to a single dose of γ-radiation (0~40 Gy; Mark 1 Research Irradiator Model #1608A; Cesium 137 source). Cell viability was measured at the times indicated by removing the media and replacing it with 50 μl of 1 mg/ml solution of MTT (Sigma Chemical Co., St. Louis, MO) and incubating for 3 h at 37°C (14).

Statistical Analysis. The dose-response interactions between CV706 and radiation at the point of IC_{50} were evaluated by the isobologram method of Steel and Peckham (18) as modified by Aoe *et al.* (19). The IC_{50} is defined as the concentration of drug that produces 50% cell growth inhibition; *i.e.*, a 50% reduction in absorbance. Isobolograms (three isoeffect curves; mode 1 and mode 2) were computed as described previously (16). FTV relative to untreated controls was determined based on the method described previously (16, 20).

One-step Growth Curve and Virus Yield. One-step growth curves of data obtained from CV706 grown in LNCaP cells in the presence and absence of radiation were charted and used to determine burst size. Monolayers of LNCaP cells were infected with CV706 at MOIs of 0.01, 0.1, and 1. After 24-h incubation at 37°C with 5% CO_2, cells were exposed to a single dose of γ-radiation at 10 Gy. At the indicated times thereafter, duplicate cell samples were harvested and lysed by three cycles of freeze thawing. Virus yield was determined by plaque assay as described (13).

***In Vivo* Antitumor Efficacy.** Six-to-eight-week-old athymic BALB/c *nu/nu* mice were obtained from Simonson Laboratories (Gilroy, CA) and acclimatized to laboratory conditions 1 week before tumor implantation. Xenografts were established either by injecting 1×10^6 LNCaP cells s.c. near the small of the back suspended in 100 μl of RPMI 1640 and 100 μl of Matrigel (back tumor) or by injecting cells into the right gastrocnemius muscle (i.m.; leg tumor). When tumors reached between 300 mm^3 and 500 mm^3, mice were randomized into groups of four. The first group received CV706 at day 0 via i.t. administration. CV706 was diluted in PBS containing 10% glycerol and injected into the tumor as 0.4 μl of diluted virus (1×10^7 particles/mm^3 of tumor) using a 28-gauge needle. The second group was given radiation only. For radiation, mice were immobilized in lucite chambers, and their whole body was shielded with lead except for the tumor-bearing sites on their back or tumor-bearing hind leg. This tumor-bearing site was irradiated with a Mark 1 Research Irradiator (Model #1608A; J. H. Shepherd Associates) at various doses (0, 5, 10, and 20 Gy) 1 day after CV706 injection or mock injection. The third group was given CV706 i.t. at day 0 and irradiated at the same doses at day 1. As a control, a fourth group was treated with virus dilution buffer (*i.e.*, control) i.t. at day 0. Tumors were measured weekly in two dimensions by external caliper, and the volume of back tumors was estimated by the formula {[*length* (mm) $\times$ *width* $(mm)^2$]/2; Ref. 14}. The volume of i.m. leg tumors was determined using the following formula: *volume* $(cm^3) = d'^3 - (0.6)^2 d'$, where d' is the average diameter of the tumor-bearing leg (cm), and the product $(0.6)^2 d'$ is the correction factor for normal leg volume (21). Animals were killed humanely when their tumor burden became excessive. The difference in relative tumor volumes between treatment groups was compared for statistical significance using the type 2 (two-sample equal variance), two-tailed *t* test. Blood samples were collected at various time points by retro-orbital bleed for determining PSA (14). Federal and institutional guidelines for animal care were followed.

Histochemistry Analysis. Four groups of mice ($n = 6$) were treated with vehicle, CV706 (1×10^7 particles/mm^3 of tumor), radiation (10 Gy), or a combination of CV706 and radiation. Half the animals were sacrificed on day 7, and the other half were sacrificed on day 14. The tumor samples were embedded in paraffin blocks, and 4-μm sections were cut and stained with H&E. Histology methods for detecting adenovirus antigens were as described (14). The necrotic cells were scored on coded slides by light microscopy at 400× magnification. The extent of necrosis was based on scoring 500 cells/section as either necrotic or nonnecrotic. The average necrosis score was calculated based on counting 10 fields distributed evenly across the area of the tumor section. The light-microscopic features used to identify necrosis included cell size, indistinct cell border, eosinophilic cytoplasm, loss or condensation of the nucleus, and associated inflammation (22). To assess the effect of CV706, radiation, or combination treatment on tumor vascularization, the number of blood vessels was counted at a magnification of 400×, and the average number of blood vessels was calculated from 10 fields distributed evenly across the area of whole tumor section (23). Apoptotic cells were detected using TUNEL assay (Roche Molecular Biochemicals, Indianapolis, IN) as suggested by the manufacturer. The morphological features used to identify apoptosis in the tumor sections have been described previously (22), associated with positive TUNEL staining. The apoptotic cells were scored on coded slides at 400× magnification, and the average score of apoptotic cells was calculated from 10 fields of nonnecrotic areas selected randomly across each tumor section. Anti-CD31 antibody was purchased from BD PharMingen (San Diego, CA), and the immunohistochemical staining procedure for detecting of CD31 antigen was performed as described by the manufacturer's protocol (24–26).

RESULTS

CV706 in Combination with Radiation Produces Synergistic Cytotoxicity in Prostate Carcinoma LNCaP Cells. To study the potential interaction between the prostate-specific adenovirus variant CV706 and radiation *in vitro*, the effectiveness of combined treatment of several combinations of CV706 and radiation at various doses was evaluated in the PSA-producing prostate carcinoma LNCaP cell line. LNCaP cells were either mock-infected or infected with CV706. One day later, the cells received a single dose of γ-radiation (0, 5 Gy, 10 Gy, and 20 Gy), and the cell viability was then determined at various time points by the MTT assay. Several viral MOIs and radiation doses were tested to determine the dose-response curves in LNCaP cells, such that the selected dose shows greater combined efficacy with radiation plus virus but minimal cell killing when treated with the same dose of virus or radiation alone. As shown in Fig. 1*A*, infecting LNCaP cells with CV706 at an MOI of 0.01 resulted in 80% cell survival 5 days after infection, whereas radiation at a dose of 10 Gy resulted in 78% survival 5 days after treatment. However, when CV706 and radiation were combined at these doses, cell survival dropped to 20% 5 days after treatment (Fig. 1*A*). Cell viability dropped further to 8% 9 days after combination treatment, whereas cells treated with virus alone at an MOI of 0.01 or radiation 10 Gy alone retained 70% or 60% cell viability, respectively. To determine whether the timing of administration for the tested agents affected the combined cytotoxic effect, LNCaP cells were treated with radiation 24 h before or after infection with CV706. Results showed that there

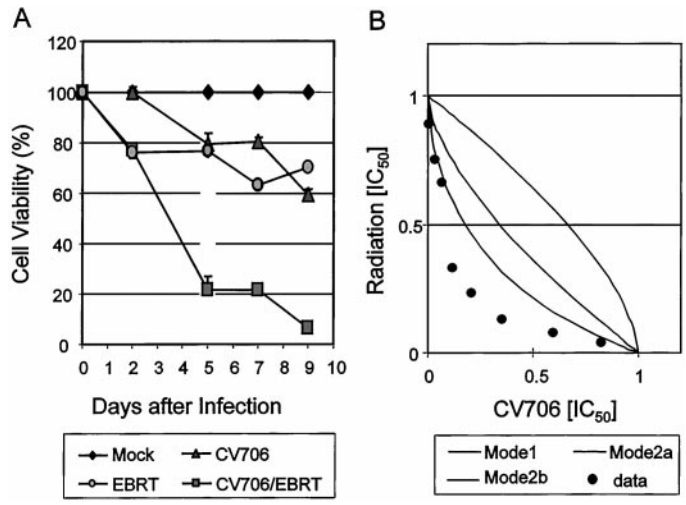

Fig. 1. Viability of prostate cancer LNCaP cells treated with vehicle, CV706, radiation, or CV706 plus radiation. *A*, LNCaP cells were treated with CV706 (MOI, 0.01) 24 h before exposure to radiation (10 Gy). *B*, isobologram analysis of the observed data from the combination of CV706 and radiation. The concentration that produced IC_{50} is expressed as 1.0 in the ordinate and the abscissa of the isobologram. *Y* axis, CV706 (IC_{50}); *X* axis, radiation (IC_{50}).

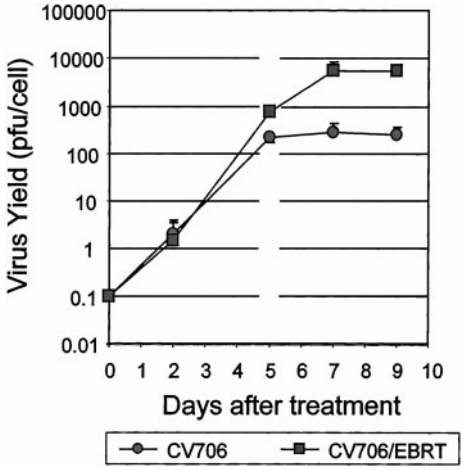

Fig. 2. One-step growth curve of CV706 (MOI, 0.1) in human prostate cancer LNCaP cells with (■) or without (●) radiation (10 Gy).

were no significant differences in cytotoxic activity between cells treated with radiation before infection or after infection with CV706 (data not shown).

Isobolograms were generated from the modes to determine the presence of synergy, additivity, or antagonism between CV706 and radiation. Dose-response curve analysis indicated that the IC_{50} at day 7 in LNCaP cells for CV706 and radiation was 0.028 MOI and 7.57 Gy, respectively. Fig. 1*B* shows isobologram representation of the statistical modeling used to analyze the interaction between CV706 and radiation. The combined data points fell to the left of the envelope of additivity, indicating sequential exposure to CV706 followed by radiation produced synergistic cytotoxicity. The enhanced cytotoxicity was also observed in LNCaP cells when CV787, a second prostate-specific adenovirus variant, was combined with radiation (data not shown). Taken together, our *in vitro* data demonstrate that prostate-specific adenovirus variants in combination with radiation produce synergistic cell cytotoxicity in prostate carcinoma LNCaP cells.

Radiation Increases CV706 Burst Size in LNCaP Cells. To examine the effect of radiation on virus replication, we performed a one-step growth curve experiment. LNCaP cells were infected with CV706 at an MOI of 0.1 for 24 h, followed by radiation at a dose of 10 Gy. Cells were harvested at various times after infection, and the number of infectious virus particles was determined on 293 cells by standard plaque assay (14). As shown in Fig. 2, cells treated with CV706 plus radiation produced a larger burst size, although the initial rate of increase of CV706 in cells treated with combined CV706 and radiation was similar to that of cells treated with CV706 alone; *e.g.*, cells treated with CV706 plus radiation produced 8000 PFU/cell 9 days after infection, whereas cells infected with CV706 alone generated about 500 PFU/cell at this time point (Fig. 2). Combination treatment with radiation and CV706 at MOIs of 0.01 or 1 also resulted in larger virus burst sizes. Cells treated with CV706 alone at MOIs of 0.01 and 1 produced 15 and 3500 PFU/cell, respectively, whereas cells treated with the same doses of CV706 combined with radiation produced 4750 and 8700 PFU/cell, respectively, 9 days after virus infection (data not shown). This observation was confirmed by quantitative PCR, which determines the number of copies of CV706 genome (data not shown). Thus, rather than inhibiting CV706 replication, the addition of radiation significantly increased virus propagation.

Cytotoxicity of CV706 in Combination with Radiation Does Not Alter Viral Specificity to Prostate Cancer Cells. To evaluate whether the addition of radiation could change the specificity of CV706-mediated cytotoxicity, we assessed the specificity of the combination treatment of CV706 and radiation by measuring the viability of various infected cell lines using the MTT assay. LNCaP, HBL-100, and OVCAR-3 cells were infected with CV706 at an MOI of 0.01 for 24 h, followed by a single dose of radiation at 10 Gy. The percentage of cell viability *versus* time after treatment was plotted in Fig. 3. The combination of CV706 and radiation was toxic to LNCaP cells but not to HBL-100 and OVCAR-3 cells. There were few surviving LNCaP cells 9 days after infection. In contrast, the viability of HBL-100 and OVCAR-3 cells treated with CV706 and radiation remained at >90% throughout the course of the experiment, similar to that of cells treated with radiation alone. These data demonstrate that combination with radiation does not alter the specificity of CV706 for PSA(+) cells.

Synergistic Efficacy of CV706 in Combination with Radiation *in Vivo*. The *in vivo* antitumor efficacy of CV706 in combination with radiation was assessed in the LNCaP mouse xenograft model. We have shown previously (12) that a single intratumoral administration of CV706 at 5×10^8 particles/mm^3 of tumor can eliminate s.c. xenograft tumors in 6 weeks. Established human prostate cancer xenografts (LNCaP cells) were treated with either vehicle, CV706 (1×10^7 particles/mm^3; a 50-fold lower dose), radiation (10 Gy), or both CV706 and radiation. For the combination treatment, animals were i.t. injected with CV706, and 24 h later, animals received a single dose of radiation. In this study, a single dose of 10 Gy was used because it caused a tumor growth delay in a previous pilot study. The dose of 1×10^7 CV706 particles/mm^3 of tumor was selected to produce a modest antitumor effect, based on our previous studies of antitumor efficacy (14).

The tumor volume data presented in Fig. 4*A* shows that there was a significant decrease in tumor volume between control and all of the treatment groups. In all of the cases, although a single dose of CV706 or radiation was effective in producing tumor growth inhibition, the combination of the two showed significantly greater tumor regression; *e.g.*, tumor volume of the group treated with radiation (10 Gy) was 120% of baseline 6 weeks after treatment, and the tumor volume of the group treated with CV706 was 97% of baseline at this time point. However, when the same dose of CV706 was followed 24 h later with the same dose of radiation, a statistically significant drop in the relative tumor volume to 4% of baseline was observed ($P < 0.01$; Fig. 4*A*). Twelve weeks after treatment, no visual tumors were observed in the group of animals that received a combination treatment of CV706 plus radiation, whereas tumor volume of the group treated with

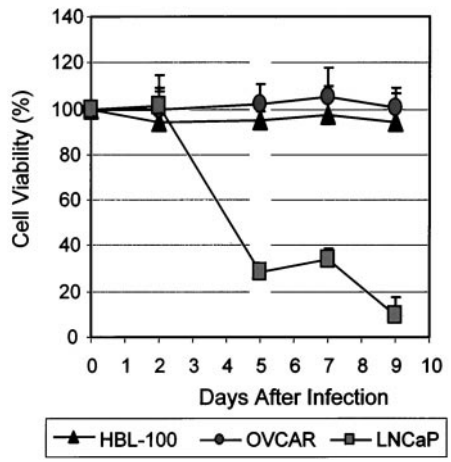

Fig. 3. Effect of radiation on the replication-mediated cytotoxicity of CV706. LNCaP, HBL-100, and OVCAR-3 cells were infected with CV706 (MOI, 0.01) 24 h before exposure to radiation (10 Gy). Cell viability was determined by MTT assay; *error bars* equal the SE.

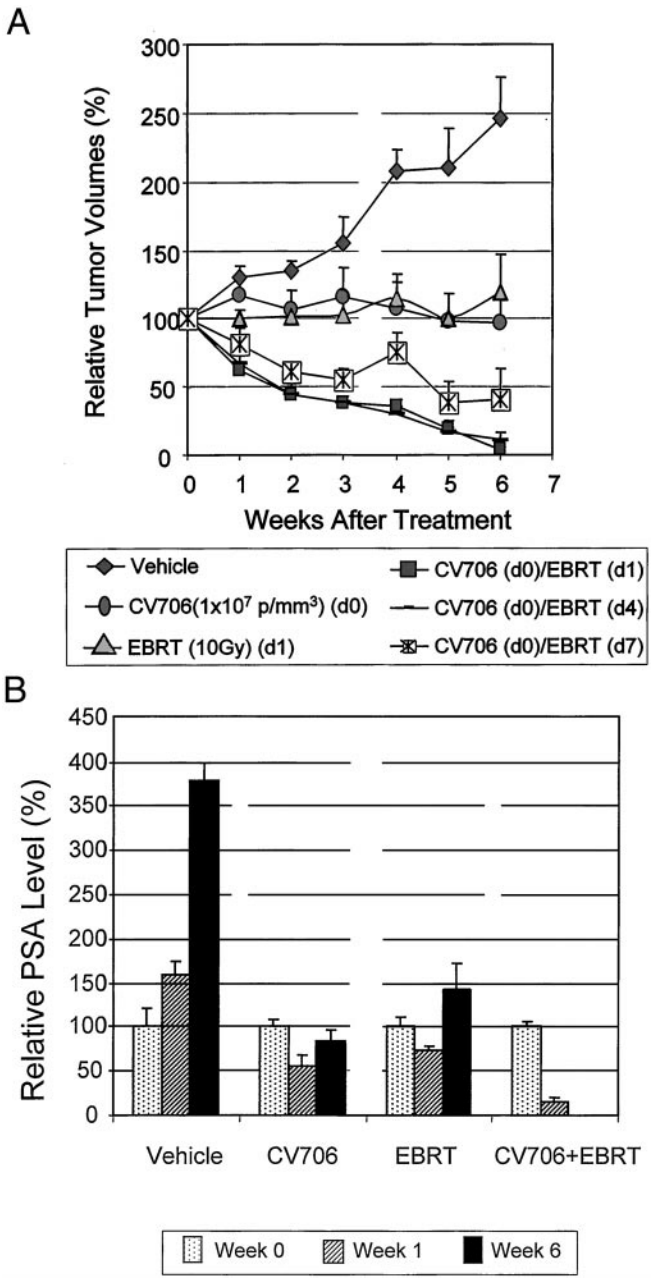

Fig. 4. *In vivo* efficacy of i.t. administered CV706 and radiation against nude mouse LNCaP xenografts. *A*, tumor volume of LNCaP xenografts treated with either vehicle (◆), CV706 alone (1×10^7 particles/mm^3 of tumor; ●), radiation alone (10 Gy; △), CV706 (1×10^7 particles/mm^3 of tumor) on day 0 plus radiation (10 Gy) on day 1 (■), CV706 on day 0 plus radiation on day 4 (*line*), or CV706 on day 0 plus radiation on day 7 (*cross line*). *B*, serum PSA values in the mice treated with either vehicle, CV706 (1×10^7 particles/mm^3 of tumor), radiation (10 Gy), or CV706 (1×10^7 particles/mm^3 of tumor) on day 0 plus radiation (10 Gy) on day 1. Groups consisted of eight mice each.

radiation was 510% of baseline and the tumor volume of the group treated with CV706 was 86% of baseline at this time point (data not shown). Serum PSA levels were also monitored in the mice to assess antitumor efficacy. The mean PSA level in mice receiving vehicle treatment increased to 370% of baseline after 6 weeks. In mice treated with radiation (10 Gy) alone, PSA increased only to 139% of baseline after 6 weeks, and PSA was reduced to 84% of baseline 6 weeks after treatment with CV706 (1×10^7 particles/mm^3) alone. In contrast, PSA levels in mice treated with CV706 plus radiation decreased substantially by 1 week after treatment and reached less than 1% of baseline within 6 weeks.

Table 1 summarizes the relative tumor volume of control and treated groups at six different time points. Combination treatment demonstrated greater than an additive effect on tumor growth inhibition at all of the time points studied after day 7. On day 21, there was a 2-fold improvement in antitumor activity in the combination group when compared with the expected additive effect. At this time point, CV706 and radiation (10 Gy) individually inhibited tumor growth by 26% and 34%, respectively (fractional tumor volumes of 0.7419 and 0.6645, respectively) when compared with the control group. A simple additive effect would predict a fractional tumor volume of 0.4930 from combination treatment (51% tumor inhibition), whereas treatment with CV706 plus radiation resulted in an average measured fractional volume of 0.2452, representing a 75% inhibition. This antitumor activity further improved with time. On day 42, the group treated with the combination of CV706 and radiation showed a 6.7-fold greater inhibition of tumor growth over that expected from an additive effect alone (Table 1). These observations further support the conclusion of synergy between CV706 and radiation in the eradication of LNCaP xenografts.

Enhanced antitumor efficacy was also observed in the animal model in which the prostate cancer tumors are implanted in hind limb of mice. In this study, tumors were produced by inoculation of 1×10^6 cells into limb muscle. When the tumors attained a volume of 200 mm^3 to 300 mm^3, the mice were randomized into four groups and treated as described above for back tumors. As before, the weekly tumor volume measurements showed that combination treatment with CV706 plus radiation led to significant antitumor activity in comparison with either CV706 or radiation alone. Tumor volume of the group treated only with radiation (20 Gy) was 70% of baseline 4 weeks after treatment, whereas the tumor volume of the group treated solely with CV706 (5×10^7 particle/mm^3 of tumor) was 75% of baseline at this time point. However, when CV706 was combined with radiation at these dose levels, the tumor volume dropped to 8% of baseline (data not shown).

A series of experiments was then designed to examine the effects of various factors, including the temporal sequence of the two agents, the timing of radiation after virus administration, and radiation fractionation. The effect of order of administration for the tested agents was examined in an *in vivo* study using the back tumor xenograft model. LNCaP xenografts were irradiated either 24 h before or after CV706 administration. Tumor volume (measured weekly) indicated that treatment with CV706 before radiation was superior to radiation followed by CV706 within the first 6 weeks; however, the difference became insignificant 8 weeks after treatment (data not shown).

The second study was designed to evaluate the timing of radiation after virus administration. Tumors were treated with CV706 at day 0 and followed by radiation at various time points. The results indicated similar antitumor efficacy when tumors treated with CV706 at day 0

Table 1 *Combination treatment with CV706 and radiation*

	FTV relative to untreated controls[a]				
			Combination treatment		
Day[b]	CV706	Irradiation	Expected[c]	Observed	Ratio of expected FTV/observed FTV[d]
7	0.9070	0.7752	0.7031	0.4806	1.46
14	0.7910	0.7537	0.5962	0.3280	1.82
21	0.7419	0.6645	0.4930	0.2452	2.01
28	0.5144	0.5481	0.2819	0.1683	1.66
35	0.4667	0.4762	0.2222	0.0905	2.46
42	0.3943	0.4837	0.1907	0.0285	6.69

[a] FTV (mean tumor volume experimental)/(mean tumor volume control).
[b] Day after tumors treated with agents.
[c] (mean FTV of CV706) × (mean FTV of radiation).
[d] Obtained by dividing the expected FTV by the observed FTV. A ratio of >1 indicates a synergistic effect, and a ratio of <1 indicates a less than additive effect.

were followed by radiation either 1 day or 4 days after virus administration; both treatments eliminated tumors within 6 weeks of treatment (Fig. 4*A*). However, the antitumor activity was decreased when the tumors were treated with radiation 7 days after CV706 administration (Fig. 4*A*).

The third study was designed to assess the effect of radiation fractionation on antitumor efficacy. Animals with human prostate cancer tumors on their backs were randomized into five groups. Two groups were treated with either CV706 on day 0, followed by a single dose of radiation at 10 Gy on day 1, or CV706 on day 0, followed by four fractional doses of radiation at 2.5 Gy on days 1, 3, 6, and 8, to provide a cumulative dose of 10 Gy. Tumor volume data, measured weekly, indicated that both treatments eliminated preexisting tumors 6 weeks after treatment and produced comparable synergistic antitumor activity when compared with either agent alone. No significant difference in antitumor efficacy was observed between these two virus/radiation combination groups as long as the total doses of radiation were comparable (data not shown).

Synergistic antitumor efficacy of CV706 in combination with radiation was further documented by histological analysis of the tumors. More necrotic cells were observed in the tumors treated with CV706 plus radiation than those treated with either agent alone (Table 2; and Fig. 5, *A* and *B*). The amount of necrosis in tumors treated with CV706 alone was higher than that seen in the control tumors or in the tumors treated with radiation alone. Evidence of necrosis and multifocal inflammation was observed in a small portion of tumors treated with radiation. In the tumors treated with both CV706 and radiation, only a few virus-infected cells were detected (Fig. 5*A*, *Panel R*). Most of the cells in the sections were empty and virtually devoid of cellular content. A significant increase in the extent of necrosis was the dominant histological feature, which involved about 95% of the tumor mass in this treatment group (Fig. 5*A*, *Panel R*). The average necrosis scores in a 400× magnification of the tumors treated with vehicle, radiation, CV706, or CV706 plus radiation were 5.4 ± 2.2, 67.0 ± 48.2, 258.2 ± 80.8, and 461.6 ± 37.9, respectively (Table 2). The presence of mass necrosis in the tumors treated with CV706 or CV706 plus radiation verifies the *in vivo* antitumor efficacy based on tumor volume analysis. Analysis of the necrosis data by a Student *t* test revealed that tumor cell necrosis induced by CV706 in combination with radiation was significantly greater than by CV706 ($P < 0.03$) or radiation alone ($P < 0.0001$). Likewise, a significantly greater number of apoptotic cells were observed in the treated tumors (Table 2). The number of apoptotic cells detected using TUNEL assay (22, 23) in the tumors treated with CV706 and radiation was 16.7-fold higher than with vehicle, 8.8-fold higher than with radiation alone, and 3.2-fold higher than with CV706 alone (Table 2).

Additionally, we observed a significant reduction in the number of blood vessels in the tumors treated with CV706 in combination with radiation, as shown in Fig. 5*B*. The average number of blood vessels observed at a magnification of 400× in tumors treated with vehicle, CV706, radiation, or the combination of CV706 and radiation was 87.5 ± 6.3, 27.5 ± 8.9, 58.5 ± 3.1, and 4.5 ± 1.9, respectively (Table 2). Significantly reduced numbers of blood vessels in tumors treated with combination in comparison with CV706 alone or radiation alone ($P < 0.01$) suggest that the reduction of tumor vascularization may contribute to enhanced tumor regression. It is unclear at this time as to the precise mechanism by which this reduction in blood vessel number is achieved. This can result either through direct damage of endothelial cells or through the destruction of tumor vasculature by extensive necrosis indirectly. CD31 is expressed constitutively on the surface of adult and embryonic endothelial cells and has been used as a marker to detect angiogenesis (24, 25). Immunohistochemical staining was performed to examine the effect of virus and radiation treatment on tumor angiogenesis by using a monoclonal antibody against CD31 (26). Tumors treated with CV706 followed by radiation showed a significantly lower level of CD31-positive cells when compared with tumors treated with either radiation ($P = 0.003$) or CV706 alone ($P = 0.03$; Table 2). When compared with untreated mice, CV706/radiation treated mice exhibited significantly lower (4.0-fold) CD31-positive blood cells ($P < 0.0001$), whereas radiation-treated or CV706-treated mice displayed 1.6-fold ($P = 0.03$) or 2.3-fold ($P = 0.004$) lower CD31-positive blood vessels. These observations suggest that CV706 in combination with radiation may be inhibiting tumor angiogenesis to a significant extent.

Finally, combination treatment of virus and radiation does not seem to impair the general health of the treated animals in comparison to the treatment with either virus or radiation alone. The quality of life of the animals seemed to be greatly improved, as evidenced by both general appearance and a significant gain in body weight. Indeed, animals treated with both CV706 and radiation gained 38% more weight than untreated control animals, 22% more than CV706-treated animals, and 25% more than radiation-treated animals (Table 3). The combination treatment was also found to protect the animals from the transient weight loss observed in the case of animals treated with radiation alone (Table 3).

Table 2 *Effect of CV706, radiation, or both on necrosis, apoptosis, and vascularization in LNCaP tumor xenografts*

LNCaP tumor-bearing animals were treated with CV706 (i.t. administration of 1×10^7 particles/mm^3 of tumor), radiation (local tumor radiation at a dose of 10 Gy), or both as described in "Materials and Methods." Tumor samples were harvested 14 days after treatment and analyzed by histological staining (H&E for necrosis and blood vessel and immunohistochemical staining with anti-CD31 antibody for angiogenesis) and TUNEL assay (for apoptotic cells). Groups consisted of six mice each.

Treatment	Necrosis	Blood vessel	CD31$^+$ cells	Apoptosis
Vehicle	5.4 ± 2.2	87.5 ± 6.3	33.7 ± 8.2	4.7 ± 1.2
CV706	258.2 ± 80.8	58.5 ± 3.1	14.8 ± 3.9	24.6 ± 7.2
Radiation	67.1 ± 48.2	27.5 ± 8.9	20.6 ± 7.3	8.9 ± 2.2
CV706 + Radiation	461.6 ± 37.9	4.5 ± 1.9	8.4 ± 2.7	78.3 ± 12.2

DISCUSSION

Although radiation is capable of permanently eradicating localized prostate tumors, as many as 40% of patients with clinically localized prostate cancer treated with potentially curative doses of standard, external beam radiation relapse at the site of the irradiated tumors within 5 years (3, 6, 8). Specifically, the eradication of locally advanced or high-risk prostate cancer with radiation has proven more difficult than believed previously (5). A modest increase in efficacy can be achieved by increasing the radiation dose, but this approach is limited by a concomitant increase in toxicity (5). Thus, a relatively nontoxic means of enhancing radiosensitivity could represent a useful clinical approach. The results presented above demonstrate that substantial synergy can be achieved when radiation treatment is complemented with a tumor-specific oncolytic virus.

Initial *in vitro* experiments in LNCaP prostate cancer cells demonstrated synergistic cytotoxicity when CV706 was combined with radiation. LNCaP cells treated with CV706 plus radiation had significantly decreased viability compared to cells treated with either agent alone. Surprisingly, LNCaP cells treated with CV706 plus radiation exhibited a greater burst size, suggesting that radiation treatment enhanced viral replication. Importantly, the addition of radiation to the cells treated with CV706 did not alter the specificity of replication-mediated cytotoxicity for PSA(+) cells. Thus, the CV706-radiation treatment retains the inherent high selectivity of the CV706 virus.

The *in vitro* demonstration of synergistic antitumor efficacy of combination therapy with CV706 and radiation was supported by

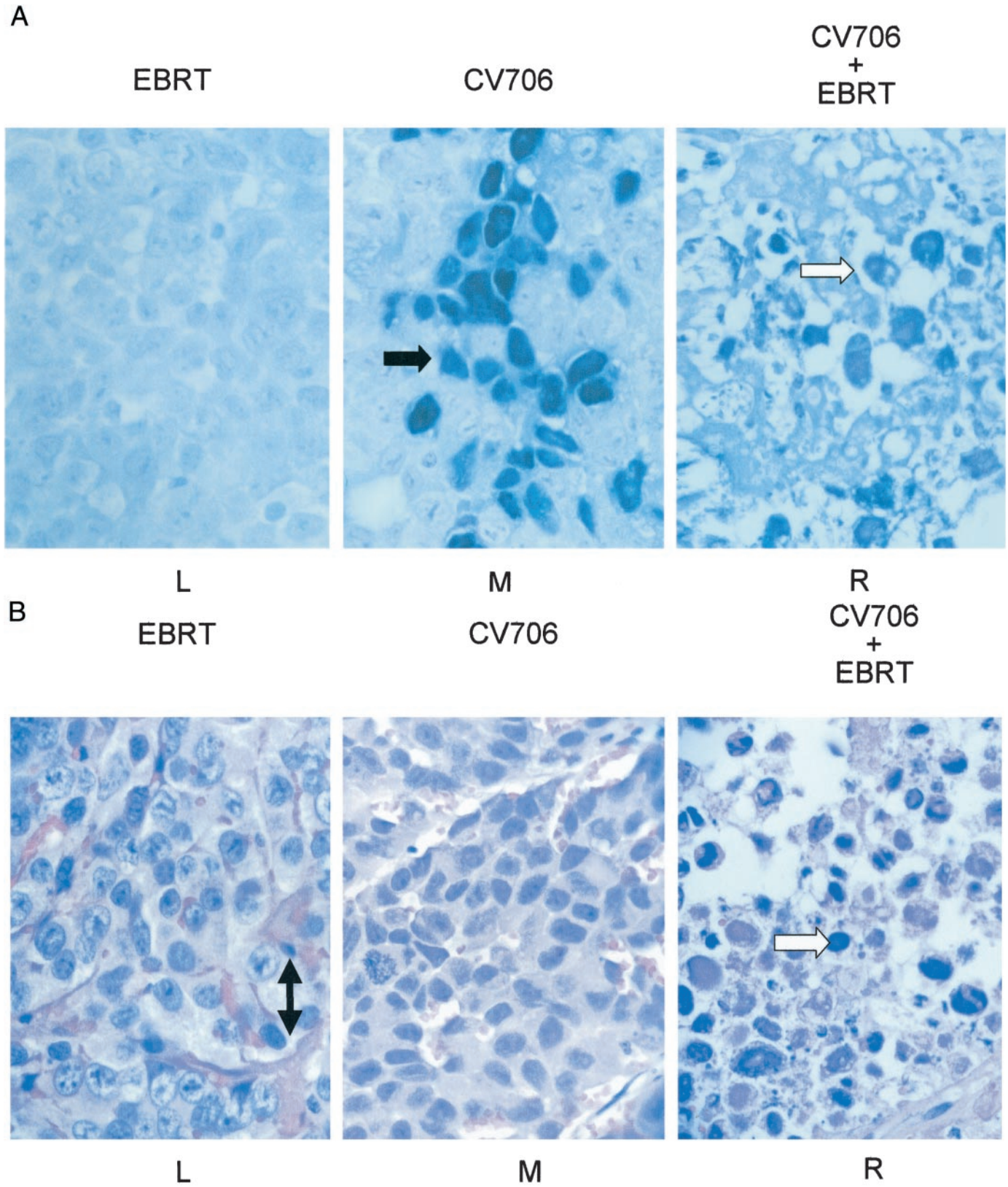

Fig. 5. Histological analysis of LNCaP tumors. LNCaP xenografts were treated with vehicle, CV706 alone (1×10^7 particles/mm^3 of tumor), radiation (10 Gy), or CV706 on day 0 plus radiation on day 1 and harvested on day 14 for histological analysis. *A*, immunohistochemical staining with anti-adenovirus polyclonal antibodies. *B*, H&E staining for blood vessels and necrotic cells. Representative histological features of LNCaP tumors at 400× magnification were documented. Adenovirus-infected cells stain dark blue as indicated by a *filled arrow*, blood vessels as indicated by a *double arrow*, and necrotic cells as indicated by the *open arrow*.

Table 3 *Relative body weight of animals bearing LNCaP tumor xenografts*

Groups consisted of eight animals treated with either vehicle, CV706 (1×10^7 particles/mm^3 of tumor), radiation (10 Gy), or CV706 plus radiation as described in the legend of Figure 4.

Treatment	Week 0	Week 1	Week 4	Week 6
Vehicle	100	97.2	92.4	96.3
CV706	100	101.3	106.5	112.9
Radiation	100	96.9	105.7	109.2
CV706 + Radiation	100	105.7	126.4	134.7

comparable synergy in the *in vivo* LNCaP xenograft model. Previous *in vivo* studies have demonstrated that large prostate tumors were completely eliminated within 6 weeks by a single i.t. administration of CV706 at a dose 5×10^8 particles/mm^3 of tumor (12, 14, 27). When combined with radiation (10 Gy), comparable eradication of tumors was observed with a 50-fold lower dose of virus (1×10^7 particles/mm^3). Under similar conditions, this dose of CV706 or radiation alone could only inhibit further tumor growth, but neither agent resulted in tumor eradication. Statistical analysis of the *in vivo* studies indicated that the CV706 and radiation combination resulted in significant antitumor synergy, with a 1.5–6.7-fold greater inhibition of tumor growth than expected from a simple additive effect during whole treatment period (Table 1). Subsequent studies have shown that similar synergistic antitumor efficacy can be achieved when 1×10^7 particles of CV706 are combined with only 5-Gy radiation (data not shown). Additional *in vivo* studies are in progress to determine the effective minimum dose of CV706 in combination with radiation required for complete regression of tumors in this xenotransplant model.

The timing of radiation after virus administration was found to be critical. The antitumor activity was decreased when the tumors were treated with radiation 7 days after CV706 administration, although no difference in antitumor effect was observed when the radiation was given 1 day or 4 days after CV706 injection. Conventional radiation therapy for localized prostate cancer in the clinic is delivered by a fractionated radiation schema. This is one fraction of radiation/day, 5 days/week. Our *in vivo* study indicated that no significant difference in antitumor efficacy was observed when tumors were treated with CV706 and a single dose of radiation or four doses of radiation as long as the total dose of radiation was the same. This is particularly interesting because the fractionated radiation protocol used in this study is actually less biologically effective in killing tumor clones than the single radiation dose fraction, yet it provided similar tumor control. In addition, fractionated radiation therapy is known to cause less damage to normal tissues when compared with radiation given in only one or several large fractions. The data from this study suggest that it may be possible to use less total radiation to control tumors if CV706 is given concomitantly, demising the risk of radiation-induced injury to normal tissues and thereby improving the therapeutic ratio. This hypothesis will require further study to verify.

A preliminary assessment of the synergistic activity of CV706 plus radiation treatment reveals several mechanistic possibilities. First, as mentioned above, radiation at the synergistic dose significantly increases virus replication. A one-step growth curve study showed that radiation significantly increased the burst size of CV706 in LNCaP cells without altering the kinetics of virus replication (Fig. 2). The burst size of CV706 in LNCaP cells treated with CV706 for 24 h followed by radiation was 500-fold higher than that in cells treated with CV706 (0.01 MOI) alone (Fig. 2; and data not shown). Radiation kills mammalian cells by inducing a reproductive (also known as mitotic) cell death, apoptotic death pathways, and by breaking DNA strands. Most radiation-induced DNA double-stranded breaks are rapidly repaired by constitutively expressed DNA repair mechanisms (28). DNA repair becomes more active in irradiated cells, potentially allowing for greater replication/multiplication of the episomal adenoviral DNA. Because of its small target size, the adenoviral genome (36 kb) is far less likely to sustain radiation-induced damage because it is 10^5-fold smaller than that of the DNA in human cells (3×10^6 kb). Therefore, the more active cellular DNA synthesis machinery in the irradiated cells may facilitate viral DNA synthesis and virus replication. Secondly, CV706 may be augmenting the antitumor activity of radiation. The adenovirus *E1A* gene is the only viral gene expressed during the first 2.5 h of infection and encodes a multifunctional transcriptional factor also known to induce apoptosis (29, 30). It is believed that the adenovirus E1A gene product represents a potent inducer of chemosensitivity and radiosensitivity through both p53-dependent and independent mechanisms. Malignant tumors, when expressing adenovirus E1A, are very sensitive to treatment with DNA-damaging agents *in vivo*, including radiation (17, 31–33). The histological features, which included intravascular thrombosis and massive necrosis positioned more centrally within tumors (data not shown), support the involvement of the vascular effect on tumor reduction. This seems to be in agreement with a recent finding (34) that inhibition of angiogenesis leads to increased tumor radioresponse. Indeed, CV706 in combination with radiation induced a significant reduction of CD31-positive cells in blood vessels, indicating a potential antiangiogenic effect of the combination treatment (24, 25).

Recently, a similar effect of radiation was reported when a colon cancer-specific replication competent adenovirus variant was combined with radiation in colon tumor cells (Dr. William Wold, CapCure Gene Therapy Summit 2001). Additionally, a replication-selective adenovirus, ONYX-015, in combination with radiation was also shown to have a greater effect than either individual modality (35). Thus, it is suggesting that the combination of adenovirus-based therapy with radiation for the cancer treatment appears to be promising.

Combination therapy using cytotoxic agents with differing mechanisms of action is a mainstay of oncology treatment, because it affords several important advantages not achievable with single-agent treatment, including: maximal cell kill within the range of toxicity tolerated by the patient for each agent (assuming dosing is not compromised); a broader range of interactions between the therapeutic modalities and tumor cells with different genetic abnormalities (in a heterogeneous tumor population); and the potential for preventing or slowing the development of treatment resistance (36). As demonstrated here, radiation can be successfully combined with cytolytic adenoviral therapy because it increases virus replication. In addition, the damaging effects are primarily limited to the irradiated host cell. In human prostate tumor xenografts, at least 50 times less CV706 is required to eliminate preexisting tumors in the combination treatment compared with single treatment. Moreover, combination treatment leads to significantly increased necrosis and a significant decrease in tumoral blood vessels and angiogenesis. Finally, no additional toxicities were noted from the combined treatment. In fact, the animals receiving combination treatment appeared healthier than those treated with radiation alone, as judged by a significant weight gain compared with the groups treated with either agent alone. If these results are replicated in human studies, it opens the potential of improved treatments for localized prostate cancer, offering enhanced efficacy while minimizing toxicity.

ACKNOWLEDGMENTS

We thank Natalie Nguyen, Heini Ilves, Eric Memarzaden, Joseph Oh, Pinky Amin, Mike McGlothlen, and Tina Le for technical help and Dr. Albert Owens, Dr. W. K. Joklik, and Gary Brouwer for thoughtful discussion.

REFERENCES

1. Kurth, K. H., Altwein, J. E., Skoluda, D., and Hohenfellner, R. Follow-up of irradiated prostatic carcinoma by aspiration biopsy. J. Urol., *117:* 615–617, 1977.
2. Pollack, A., and Zagars, G. K. External beam radiotherapy dose response of prostate cancer. Int. J. Radiat. Oncol. Biol. Phys., *39:* 1011–1018, 1997.
3. Hanks, G. E., Hanlon, A., Schulthess, T. E., Pinover, W. H., Movsas, B., Epstein, B. E., and Hunt, M. A. Dose escalation with 3D conformal treatment: five year outcomes, treatment optimization, and future directions. Int. J. Radiat. Oncol. Biol. Phys., *41:* 501–510, 1998.
4. Stamey, T. A., Kabalin, J. N., and Ferrari, M. Prostate specific antigen in the diagnosis and treatment of adenocarcinoma of the prostate. III. Radiation treated patients. J. Urol., *141:* 1084–1087, 1989.
5. Zelefsky, M. J., Leibel, S. A., Gaudin, P. B., Kutcher, G. J., Fleshner, N. E., Venkatramen, E. S., Reiter, V. E., Fair, W. R., Ling, C. C., and Fuks, V. Dose escalation with three-dimensional conformal radiation therapy affects the outcome in prostate cancer. Int. J. Radiat. Oncol. Biol. Phys., *41:* 491–500, 1998.
6. Horwitz, E. M., and Hanks, G. E. External beam radiation therapy for prostate cancer. CA Cancer J. Clin., *50:* 349–375, 2000.
7. Cowen, D., Salem, N., Ashoori, F., Meyn, R., Meistrich, M. L., Roth, J. A., and Pollack, A. Prostate cancer radiosensitization *in vivo* with adenovirus-mediated p53 gene therapy. Clin. Cancer Res., *6:* 4402–4408, 2000.
8. Zerati, M., and Pontes, J. E. Treatment of local failure of prostate cancer after radiotherapy and surgery. *In:* D. Raghavan, H. I. Scher, S. A. Leibel, and P. Lange (eds.), Principles and Practice of Genitourinary Oncology, pp. 567–571. Philadelphia: Lippincott-Raven Publishers, 1997.
9. Bolla, M., Gonzalez, D., Warde, P., Dubois, J. B., Mirimanoff, R. O., Storme, G., Bernier, J., Kuten, A., Steinberg, C., Gil, T., Collette, L., and Pierart, M. Improved survival in patients with locally advanced prostate cancer treated with radiotherapy and goserelin. N. Engl. J. Med., *337:* 295–300, 1997.
10. Joon, D. L., Hasegawa, M., Sikes, C., Khoo, U. S., Terry, N. H., Zagars, G. K., Meistrich, M. L., and Pollack, A. Supraadditive apoptotic response of R3327-G rat prostate tumors to androgen ablation and radiation. Int. J. Radiat. Oncol. Biol. Phys., *38:* 1071–1077, 1997.
11. Pilepich, M. V., Caplan, R., Byhardt, R. W., Lawton, C. A., Gallagher, M. J., Mepic, J. B., Hanus, G. E., Coughlin, C. T., Porter, A., Shipley, W. U., and Grignon, D. Phase III trial of androgen suppression using goserelin in unfavorable-prognosis carcinoma of the prostate treated with definitive radiotherapy: report of Radiation Therapy Oncology Group Protocol 85–31. J. Clin. Oncol., *15:* 1013–1021, 1997.
12. Rodriguez, R., Schuur, E. R., Lim, H. Y., Henderson, G. A., Simons, J. W., and Henderson, D. R. Prostate-attenuated replication competent adenovirus (ARCA) CN706: a selective cytotoxic for prostate-specific antigen positive prostate cancer cells. Cancer Res., *57:* 2559–2563, 1997.
13. Yu, D. C., Sakamoto, G. T., and Henderson, D. R. Identification of the transcriptional regulatory sequences of human kallikrein 2 and their use in the construction of Calydon virus 764, an attenuated replication competent adenovirus for prostate cancer therapy. Cancer Res., *59:* 1498–1504, 1999.
14. Yu, D-C., Chen, Y., Seng, M., Dilley, J., and Henderson, D. R. The addition of the Ad5 Region E3 enables Calydon virus 787 to eliminate distant prostate tumor xenografts. Cancer Res., *59:* 4200–4203, 1999.
15. DeWeese, T. L., Rioseco-Camacho, N., Ramakrishna, N. R., Henderson, D., Sawyers, C. L., and Simons, J. W. Results of a Phase I study of CN706, a replication-competent cytolytic adenovirus, for the treatment of adenocarcinoma of the prostate which is locally recurrent following radiation therapy. Int. J. Radiat. Oncol. Biol. Phys., *48:* 172, 2000.
16. Yu, D-C., Chen, Y., Dilley, J., Li, Y., Embry, M., Zhang, H., Nguyen, N., Amin, P., Oh, J., and Henderson, D. R. Antitumor synergy of CV787, a prostate cancer-specific adenovirus, and paclitaxel and docetaxel. Cancer Res., *61:* 517–525, 2001.
17. Martin-Duque, P., Sanchez-Prieto, R., Romero, J., Martinez-Lamparero, A., Cebrian-Sagarriga, S., Guinea-Viniegra, J., Dominguez, C., Lleonart, M., Cano, A., Quintanilla, M., Ramon, Y., and Cajal, S. *In vivo* radiosensitizing effect of the adenovirus *E1A* gene in murine and human malignant tumors. Int. J. Oncol., *15:* 1163–1168, 1999.
18. Steel, G. G., and Peckham, M. J. Exploitable mechanisms in combined radiotherapy-chemotherapy: the concept of additivity. Int. J. Radiat. Oncol. Biol. Phys., *5:* 85–91, 1993.
19. Aoe, K., Kiura, K., Ueoka, H., Tabata, M., Matsumura, T., Chikamor, I. M., Matsushita, A., Kohara, H., and Harada, M. Effect of docetaxel with cisplatin or vinorelbine on lung cancer cell lines. Anticancer Res., *19:* 291–299, 1999.
20. Yokoyama, Y., Dhanabal, M., Griffioen, A. W., Sukhatme, V. P., and Ramakrishnan, S. Synergy between angiostatin and endostatin: inhibition of ovarian cancer growth. Cancer Res., *60:* 2190–2196, 2000.
21. Alfieri, A. A., and Hahn, E. W. An *in situ* method for estimating cell survival in a solid tumor. Cancer Res., *38:* 3006–3011, 1978.
22. Milross, C. G., Tucker, S. L., Mason, K. A., Hunter, N. R., Peters, L. J., and Milas, L. The effect of tumor size on necrosis and polarographically measured pO2. Acta Oncol., *36:* 183–189, 1997.
23. Milas, L., Mason, K., Hunter, N., Petersen, S., Yamakawa, M., Ang, K., Mendelsohn, J., and Fan, Z. *In vivo* enhancement of tumor radioresponse by C225 antiepidermal growth factor receptor antibody. Clin. Cancer Res., *6:* 701–708, 2000.
24. Giatromanolaki, A., Sivridis, E., Koukourakis, M. I., Georgoulias, V., Gatter, K. C., and Harris, A. L. Intratumoral angiogenesis: a new prognostic indicator for stage I endometrial adenocarcinomas? Oncol. Res., *11:* 205–212, 1999.
25. Giatromanolaki, A., Koukourakis, M. I., Theodossiou, D., Barbatis, K., O'Byrne, K., Harris, A. L., and Gatter, K. C. Comparative evaluation of angiogenesis assessment with anti-factor-VIII and anti-CD31 immunostaining in non-small cell lung cancer. Clin. Cancer Res., *3:* 2485–2492, 1997.
26. Horak, E. R., Leek, R., Klenk, N., LeJeune, S., Smith, K., Stuart, N., Greenall, M., Stepniewska, K., and Harris, A. L. Angiogenesis, assessed by platelet/endothelial cell adhesion molecule antibodies, as indicator of node metastases and survival in breast cancer. Lancet, *340:* 1120–1124, 1992.
27. Chen, Y., Yu, D-C., Charlton, D., and Henderson, D. R. Pre-existent adenovirus antibody inhibits systemic toxicity and antitumor activity of CN706 in the nude mouse LNCaP xenograft model: implications and proposals for human therapy. Hum. Gene Ther., *11:* 1153–1167, 2000.
28. Garzotto, M., Haimovitz-Friedman, A., Liao, W-C., Whit-Jones, M., Huryk, R., Heston, W. D., Cardon-Cardo, C., Kolesnick, R., and Fuks, Z. Reversal of radiation resistance in LNCaP cells by targeting apoptosis through ceramide synthase. Cancer Res., *59:* 5194–5201, 1999.
29. Jones, N., and Shenk, T. An adenovirus type 5 early gene function regulates expression of other early viral genes. Proc. Natl. Acad. Sci. USA, *76:* 3665–3669, 1979.
30. Horwitz, M. S. Adenovirus and their replication. *In:* B. N. Fields and D. M. Knipe (eds.), Virology, Ed. 2, pp. 1679–1721. New York: Raven Press, Ltd., 1990.
31. Sanchez-Prieto, R., Lleonart, M., and Ramon y Cajal, S. Lack of correlation between p53 protein level and sensitivity of DNA-damaging agents in keratinocytes carrying adenovirus E1a mutants. Oncogene, *11:* 675–682, 1995.
32. Sanchez-Prieto, R., Lleonart, M., and Ramon y Cajal, S. Carcinoma cell lines become sensitive to DNA-damaging agents by the expression of the adenovirus *E1A* gene. Oncogene, *13:* 1083–1092, 1996.
33. Sanchez-Prieto, R., Quintanilla, M., Martin, P., Lleonart, M., Cano, A., Dotto, G. P., and Ramon y Cajal, S. *In vivo* antitumor effect of retrovirus-mediated gene transfer of the adenovirus *E1a* gene. Cancer Gene Ther., *5:* 215–224, 1998.
34. Gorski, D. H., Beckett, M. A., Jaskowiak, N. T., Calvin, D. P., Mauceri, H. J., Salloum, R. M., Seetharam, S., Koons, A., Hari, D. M., Kufe, D. W., and Weichselbaum, R. R. Blockage of the vascular endothelial growth factor stress response increases the antitumor effects of ionizing radiation. Cancer Res., *59:* 3374–3378, 1999.
35. Rogulski, K. R., Freytag, S. O., Zhang, K., Gilbert, J. D., Paielli, D. L., Kim, J. H., Heise, C. C., and Kirn, D. H. *In vivo* antitumor activity of ONYX-015 is influenced by p53 status and is augmented by radiotherapy. Cancer Res., *60:* 1193–1196, 2000.
36. Chu, E., and DeVita, V. T., Jr. Principles of cancer management: chemotherapy. *In:* J. Vincent, T. DeVita, S. Hellman, S. A. Rosenberg (ed.), Cancer: Principles and Practice of Oncology, pp. 289–306. Philadelphia: Lippincott Williams & Wilkins, 2001.

CV706, a Prostate Cancer-specific Adenovirus Variant, in Combination with Radiotherapy Produces Synergistic Antitumor Efficacy without Increasing Toxicity

Yu Chen, Theodore DeWeese, Jeanette Dilley, et al.

Cancer Res 2001;61:5453-5460.