

**Scientific Abstract**

Prostate cancer is the most common type of cancer and the second leading cause of cancer-related death in North American men. Although prostatic carcinoma is diagnosed earlier because of the widespread use of serum prostate-specific antigen (PSA), the mortality rate from this tumor remains relatively constant at approximately 40,000 deaths per year, comparable to the annual death rate from breast cancer in women.

Virtually all deaths are a consequence of metastatic disease most often involving skeletal sites. Metastatic disease is initially treated with androgen deprivation, which achieves stabilization or regression of disease in more than 80% of patients. However, despite androgen deprivation and secondary hormonal manipulations, all patients ultimately develop hormone-refractory prostate cancer (HRPC). Although symptomatic improvement and control of metastatic disease is obtained in most patients after androgen ablation, disease progression occurs at a median of 12 to 18 months, and once this occurs the median survival for these patients is approximately 1 year.

Metastatic, hormone refractory prostate cancer may be associated with over-expression of the anti-apoptotic protein bcl-2 which is known to be expressed in 65% of HRPC. *In vitro*, phosphorylation of bcl2 (bcl2-P) leads to inactivation, inducing apoptosis; and the FDA approved anti-cancer drug, docetaxel (Taxotere™), is the most potent inducer of bcl-2P *in vitro*. Because of several recent prostate cancer trial results utilizing chemotherapeutic agent(s), the perception that prostate cancer is chemoresistant has been changing. Early results of docetaxel used in the treatment of prostate cancer indicate a high degree of activity in HRPC, and it appears to be the single most active agent at this time.

Weekly infusions of taxanes (docetaxel, paclitaxel) have been of interest in the past few years and a weekly regimen of docetaxel is now increasingly being used in patients with metastatic hormone refractory prostate cancer. The administration of such a weekly low-dose of docetaxel markedly reduces the severity of myelosuppression compared with an once-every-3-week schedule and allows the dose intensity (mg/m<sup>2</sup>/wk) of treatment to be increased. In several clinical studies, this regimen has shown to be active in men with HRPC and cancer-related pain. The observed PSA response rates (PSA decrease by >50%) in these two studies were 34-43% and a palliative response rate of 44%.

However, chemotherapy has not extended the median survival of approximately 10-12 months for patients with hormone-refractory metastatic prostate cancer. Consequently, identification of new active agents and/or new combinations with existing agents will be necessary for progress against hormone-refractory prostate cancer.

**Preclinical Data - Summary**

In our laboratory, CV787 has been shown to be effective against human prostate tumors. However, total eradication of human LNCaP xenografts in our animal model required as high as one third of the lethal dose of virus alone, resulting in a narrow therapeutic index to obtain a complete response. Therefore, strategies to improve the efficacy (and thus, increase the curative therapeutic index) of this form of treatment are desirable. In efforts to augment the cytotoxic activity of CV787, we have demonstrated that the taxanes (paclitaxel and docetaxel) are equally effective neoadjuvants to CV787, both *in vitro* and *in vivo*.

A synergistic decrease of cell viability of LNCaP prostate cancer cells was observed when CV787 was combined with taxanes. LNCaP cells cultured with paclitaxel or docetaxel for 24 hrs before or after infection with CV787 had significantly less viability than cells treated with either agent alone. LNCaP cells treated with taxanes exhibited a greater burst size of CV787, whereas no significant effect on viral growth kinetics was seen. Varying the time of taxane administration with respect to CV787 treatment did not significantly alter the synergistic effectiveness of the combined therapy

In addition, combination treatment of CV787 with taxane did not alter the specificity of replication-mediated cytotoxicity. Cell viability assays indicated that CV787 in combination with taxane remains fully selective for PSA(+) cells. This result was confirmed using the one-step growth curve. CV787 has been shown previously to replicate preferentially in PSA-producing human prostate cancer cells 10,000 times more efficiently than in non-PSA-producing cells. In the presence of paclitaxel or docetaxel in the culture medium, CV787 replicated with the same degree of specificity.

Synergistic anti-tumor efficacy of combination therapy with CV787 and taxane was also observed in LNCaP prostate cancer xenografts. Previous studies have demonstrated that tumors were eliminated within 6 weeks by a single intravenous administration of CV787 at a dose of  $1 \times 10^{11}$  particles per animal. In combination with a 12.5 mg/kg dose of docetaxel, CV787 doses as low as  $1 \times 10^8$  particles per animal produced complete elimination of tumors within 4 weeks. Thus, the addition of a single dose of taxane reduced the dose of CV787 required for complete remission 1000-fold, from  $1 \times 10^{11}$  to  $1 \times 10^8$  particles per animal, while maintaining an acceptable safety profile. As a result, the curative therapeutic index was increased 1000-fold. Statistical analysis of the *in vivo* studies indicated that the CV787 plus taxane combination group demonstrated significant synergy, with a 6.4- to 9.2-fold higher inhibition of tumor growth than expected from a simple additive effect.

The mechanisms of action of oncolytic therapy (CV787) and chemotherapy (docetaxel) are very different, and a formal toxicology study by Calydon with CV787 and docetaxel in Balb/C mice has failed to show any significant synergistic or additive toxicity from the combined use of both agents.

#### **Oncolytic Adenoviruses and Chemotherapy- Summary of Clinical Data**

Weekly infusions of taxanes have been of increasing interest to clinicians in the past few years. The administration of a weekly low-dose of docetaxel markedly reduces the severity of myelosuppression compared with a once-every-3-week schedule and allows the dose intensity (mg/m<sup>2</sup>/wk) of treatment to be increased. Several phase 1 and 2 studies have shown weekly docetaxel (36-43 mg/m<sup>2</sup> for 6 weeks followed by a 2 week rest period) to be an active and reasonably well tolerated regimen in men with HRPC and cancer-related pain. The observed PSA response rates (PSA decrease by >50%) in these two studies were 34 -43% with a palliative response rate of 44%, while the incidence of treatment related grade 3 or 4 toxicity was 11% for diarrhea, 9% for leukopenia, neutropenia and asthenia, and 4% for stomatitis, anemia, ALT elevation, nausea, and vomiting.

Recently, clinical study results were reported an intravenous delivery of a replication-selective adenovirus (ONYX-015) in combination with carboplatin and paclitaxel (plus dexamethasone pretreatment), in patients with end-stage refractory carcinoma metastatic to the lung. A total of 10 patients with advanced carcinoma metastatic to the lung received study treatment; 6 of these patients received weekly Paclitaxel (80mg/m<sup>2</sup> IV) concurrently with systemic virus administration. Paclitaxel pre-medications were given per standard of practice for the clinic and were: dexamethasone 20mg/12 hours then 2 hours prior to dosing; diphenhydramine 25mg and ranitidine 50mg IV; then ondansetron 1mg and dexamethasone 10mg IV just prior to dosing.

It was concluded that the intravenous infusion of ONYX-015 (with or without chemotherapy plus dexamethasone pretreatment) was well tolerated at doses up to  $2 \times 10^{13}$  particles via weekly infusion within 21-day cycles in 10 patients with advanced carcinoma metastatic to the lung. The authors stated that the intravenous administration of genetically altered, replicating competent adenovirus is a feasible approach.

An intravenous administration of CV787 is currently being studied in a Phase 1-2 clinical study, titled "A Phase 1/2 Dose Finding Trial of the Intravenous Injection of Calydon CV787, a Prostate-Specific Antigen Cytolytic Adenovirus, in Patients with Hormone Refractory Metastatic Prostate Cancer (Calydon Protocol CV787-9902)". To date, a total of ten patients in four cohorts have received one intravenous injection of CV787 at escalating doses from  $1 \times 10^{10}$  to  $3 \times 10^{11}$  virus particles. No significant adverse events and/or dose-limiting toxicities have been observed in these patients. Therefore the maximum tolerated and clinically safe dose (MTD) has not yet been defined.

**CV787-2001 – Study Outline**

The proposed clinical study CV787-2001 is a “Randomized, Placebo Controlled Phase 2 Study of an Intravenous Injection of CV787, a Prostate-Specific Antigen Oncolytic Adenovirus, Plus Weekly Docetaxel in Patients with Metastatic Hormone Refractory Prostate Cancer” and will use a clinically safe and tolerable CV787 dose that is being established in the ongoing CV787-9902 phase 1-2 study.

The objectives of the clinical study are to:

1. Compare the antitumor activity of CV787 plus weekly docetaxel versus placebo (saline) plus weekly docetaxel, as measured by PSA response in patients with Metastatic Hormone Refractory Prostate Cancer.
2. Compare the pain response and palliation of metastatic bone pain (McGill scale and analgesics usage) in these two treatment arms in this patient population.
3. Compare the antitumor activity of this regimen as measured by objective response (CT/pelvic scan) and evaluable disease (bonescan) in these two treatment arms in this patient population.
4. Evaluate the quantitative and qualitative toxicities in these two treatment arms in this patient population.

This is a randomized, double-blind, placebo-controlled multi-center study and approximately 60 patients with Metastatic Hormone Refractory Prostate Cancer will be enrolled. The assignment ratio will be 2:1 (40 patients CV787 plus Docetaxel : 20 patients Placebo plus Docetaxel). Patients are randomized into one of the following two treatment arms:

Arm I: Patients receive CV787 administered as a single intravenous infusion over 10 minutes plus 36 mg/m<sup>2</sup> docetaxel IV over 60 minutes on day 1, followed by weekly docetaxel (36 mg/m<sup>2</sup>) for a total of 6 consecutive weeks, followed by 2 weeks without treatment (8 week cycle). The maximum tolerated and clinically safe CV787 dose to be used in this trial is currently being determined in the ongoing study CV787-9902.

Arm II: Patients receive placebo (saline) administered as a single intravenous infusion over 10 minutes plus 36 mg/m<sup>2</sup> docetaxel IV over 60 minutes on day 1, followed by weekly docetaxel (36 mg/m<sup>2</sup>) for a total of 6 consecutive weeks, followed by 2 weeks without treatment (8 week cycle).

Patients in both Arm I and II receive treatment with dexamethasone as docetaxel pretreatment; a single dose of dexamethasone (not to exceed 10mg/week) will be administered immediately before treatment. The dexamethasone dose will be standardized for the study based on a consensus of the clinical investigators.

CV787 (or placebo) treatment is only administered once (day 1); treatment with docetaxel continues in both arms for at least 3 cycles (24 weeks) in the absence of disease progression or unacceptable toxicity. Patients are followed up to 18 months post-treatment.

Once the first ten patients in the study have received their first month of study treatment, the adverse events observed in those patients will be analyzed by a safety monitoring board. If unacceptable toxicity attributed to either CV787 or the combination of CV787 plus docetaxel has been observed in this group, recruitment will be halted and the protocol may either be modified (e.g., CV787 dose reduced) or discontinued.

The endpoints for each patient are PSA reduction, pain control, safety and survival. An endpoint for patients with measurable disease will be objective response. This study is not designed to have the power to detect significant differences between the treatment groups. The control arm, placebo plus docetaxel, is present to provide a reference enhancing the reliability of the data collected on the experimental arm, IV injection of CV787 plus docetaxel. The primary analysis will include all randomized and treated patients (an intent to treat analysis).