

## 1.1 Scientific Abstract

Prostate cancer currently is the most common (noncutaneous) malignant neoplasm and the second leading cause of cancer-specific death among males in the US. In 1998, 184,500 new patients were estimated to have been diagnosed with prostate cancer, and 39,200 deaths were attributed to the disease, and similar numbers are predicted for 1999 (8,9). The incidence of this disease has increased dramatically over the past 25 years, in part because of an increase in the treatment of benign prostatic hypertrophy (BPH) with transurethral resection of the prostate, and more recently, because of an increase in screening for elevated PSA (10, 11). Thus, despite the fact that prostate cancer may grow somewhat more slowly and be diagnosed later in life than several other neoplasms, it represents the most common of all the serious cancers, and is responsible for more cancer deaths among men than all but lung cancer. In fact, the death rates for prostate cancer among men and breast cancer among women are quite comparable (12, 13), highlighting the substantial contribution of prostate cancer to overall cancer incidence, prevalence, and mortality in the US.

Of men diagnosed very late in life with very favorable tumors (Gleason score 2-4, and PSA < 10), only a minority will die of their disease, as they face only a 4-7% chance of dying from prostate cancer within 15 years of diagnosis (14). However, the majority of patients present with less favorable tumors (either GS  $\geq$  5 or PSA > 10), which carry substantially worse prognoses. For example, the risk of dying of prostate cancer within 15 years is 18% to 30% for GS = 6, 42% to 60% for GS = 7, and up to 87% for GS = 8-10 tumors (14). Thus, while it true that many men will die with (but not necessarily of) prostate cancer, a quite substantial percentage of men diagnosed with prostate cancer will die of metastatic disease, often with unremitting pain from multiple skeletal metastases (8).

Treatment for early stage (T1, T2) prostate carcinoma in which disease is clinically confined to the prostate has generally consisted of either radical prostatectomy or radiation therapy (external beam or implant) (15-17). These therapies appear to offer an excellent chance of long term disease-free survival to men with very good prognosis (low risk) tumors, but they all show substantially less efficacy in men with higher-risk disease; in fact, the risk of subsequent biochemical failure and disease progression approaches 50% after 5 years for patients with intermediate-risk tumors (18). Thus, new therapeutic modalities are needed for this group of patients.

One way to attempt to increase the long term tumor control is to combine therapies. However, all of the current treatments share substantial chronic toxicities, most notably the risks of urinary incontinence, impotence, and (for the two radiotherapy techniques) significant rectal complications, so combining such therapies is problematic. Adding EBRT and brachytherapy exposes the urethra, penile bulb, and rectum to substantially greater radiation than either therapy alone, which increases the risk of these side-effects that so adversely affect the quality of men's lives. Furthermore, none of these treatments can be repeated in the event of local recurrence, which can occur in between 56% to 80% of patients treated with EBRT alone (19, 20). Improving the long term tumor control for these patients without exposing them to an unacceptable risk of chronic sexual, GU and rectal complications represents a current unmet medical need. Accomplishing this goal with a

treatment that could potentially be repeated in the event of local recurrence would be particularly useful.

Human adenoviruses were first cultured from the tonsils and adenoids of children in 1953 (21). Adenovirus type 5 (Ad5) is associated with a self-limiting febrile respiratory illness and ocular disease in humans. In immunosuppressed individuals it has also been associated with renal impairment and hepatic necrosis, and with gastric erosions (22, 23). Ad5 has been reported to have little or no oncogenic potential in mammals (24). A recent serologic survey has revealed that 57% of the adult population in the U.S. has neutralizing antibodies to Ad5, indicating that virtually all of the adult population has been exposed (25). Several attempts have previously been made to exploit the cell killing properties of replication-competent adenoviruses in the treatment of cancer (26-30).

A study of the local injection of ONYX-015, a genetically modified replication-competent adenovirus, was performed in patients with refractory cancer of the head and neck and reported at the American Society of Clinical Oncology (ASCO) (27). This virus is an E1B- and E3-deleted group C adenovirus that selectively replicates in and lyses p53-deficient tumor cells, albeit with substantially less efficiency than wild-type. When the virus was combined with the chemotherapeutics cisplatin and 5-fluorouracil, nine out of ten patients experienced a greater than 50% reduction in tumor size, with two complete remissions. Adverse events were reported to be mild to moderate in severity.

In several clinical trials, adenovirus was injected directly into the prostate. These studies utilized a replication-defective adenovirus as a vector for a target transgene (p53 or the Herpes simplex tk). In 31 patients with locally recurrent prostate cancer, a single-dose, dose-escalation study ( $1 \times 10^8$  -  $1 \times 10^{11}$  infectious units) was conducted using an adenovirus vector with the HSV thymidine kinase gene (Adv/HSV-*tk*) injected transrectally into one lobe of the prostate followed by ganciclovir administration (29). There was no local toxicity; the most common adverse events reported were liver transaminase elevations (three Grade 1 and one Grade 4) fever, thrombocytopenia, and leukopenia. In another multiple-dose, dose-escalation study, RPR/INGN201, a replication-defective adenovirus with a wild type p53 was injected intraprostatically in 17 patients with locally advanced disease every two weeks for a total of up to three treatments prior to radical prostatectomy at dosages ranging from  $3 \times 10^{10}$  to  $3 \times 10^{12}$  virus particles (31). Three patients received two courses of therapy (a total of six intraprostatic treatments and 18 mL of injectate). Adverse events reported were fever, chills, pain (at the injection and/or biopsy site and in the scrotum), hemorrhage (scrotal), and hematuria. Most events were considered mild in toxicity.

Calydon's products for prostate cancer use the regulatory genes responsible for the production of PSA, a biochemical marker commonly used to screen for prostate cancer and to track the progression or regression of the disease. PSA is normally produced by 5 percent of normal prostate cells. However, nearly all prostate cancer cells produce high levels of PSA. As a result, PSA has become the most widely used marker for any type of cancer. Calydon discovered a 'switch' (termed the prostate specific enhancer or PSE) that controls where PSA is produced in the body. By genetic engineering, this gene can be used to control the growth of adenoviruses and restrict the replication of these viruses to the same

cells that express PSA, almost always prostate cancer cells. The result of productively infecting PSA-producing cells with our attenuated adenovirus is cell death.

CV706 (CV706 was formerly known as CN706)- Inserting this prostate specific gene into an adenovirus Calydon created a virus that *in vitro* replicates at least 100 times better in PSA<sup>+</sup> cells (e.g. prostate cancer) than in PSA<sup>-</sup> cells. In laboratory animal models of prostate cancer we were able to eliminate the tumor after a single injection of CV706 directly into the tumor at doses of  $5 \times 10^8$  particles/mm<sup>3</sup>. Recently, a series of preclinical studies (*in vitro* and *in vivo*) have demonstrated substantial and significant synergy between CV706 and radiation therapy. When CV706 treatment of human prostate cancer tumors in nude mice were followed by radiation, tumor eradication was seen with doses as low as  $1 \times 10^7$  particles/mm<sup>3</sup>; e.g., complete tumor responses were seen with the combination of CV706 and radiation at doses where little effect was seen when each agent was used individually.

A clinical trial of Calydon's virus CV706 (*A Phase I Dose Escalation Trial of the Intraprostatic Injection of CN706, a Prostate-Specific Antigen Gene-Regulated Cytolytic Adenovirus, in Patients with Locally Recurrent Prostate Cancer Following Definitive Radiotherapy-Protocol Number CN706-001*) has been completed. Twenty patients have been treated, at doses of up to  $1 \times 10^{13}$  viral particles injected, under stereotactic ultrasound guidance, directly into the prostate. In general, treatments have been well tolerated. Adverse events consistently seen within the 30 days post-treatment have included transient and self-limiting mild to moderate fever and/or flu-like symptoms, generally occurring the afternoon or evening of treatment, which were easily treated with antipyretics. Most patients' complaints have been related to the physical procedure: perineal pain, edema and bruising post-operatively, and urinary problems (urethral pain, dysuria, hematuria, etc.) due to the extended prophylactic indwelling Foley catheterization required by that protocol.

A transient, small and clinically non-significant decrease in platelets was seen consistently two days following treatment, with levels returning to baseline by one week after treatment. Similar transient and even smaller changes in RBC were observed in most patients, but this has not correlated with fibrin split product or D-dimer levels, and has been reversible without medical intervention. A transient, clinically non-significant decrease in absolute lymphocyte levels has been observed one or two days post treatment, with lymphocyte levels generally back in the clinically normal range by the next blood draw (4 days post-treatment). These transient and self-limited hematology findings are entirely consistent with a typical acute phase response, are inconsistent with marrow suppression, and are neither clinically alarming nor sufficient to be dose-limiting.

Post-treatment liver enzymes have been generally normal. Minor transient elevations in AST to just above the ULN were seen in 3 patients in Cohort #4 (patients 13, 14 and 15) one week following treatment, which in all cases returned to normal at the next timepoint one week later. Two of these patients (14 and 15) showed comparable minor elevations of ALT at the same timepoint, which also resolved spontaneously within one week. No increases in transaminases were seen in any patients in the highest dose group, Cohort #5. Thus far there have been no NCI grade 2 toxicities in any patient for liver transaminases. Based on the interim data analysis of Protocol CN706-001, we can state that doses of up to  $1 \times 10^{13}$  virus particles have safely been administered to patients with locally recurrent prostate cancer.

Dose-limiting toxicities were not observed in any patient, therefore the MTD has not been defined.

Release of virus into the circulation was observed. An estimated 0.1 to 1% of the total administered dose ended up in circulation during or immediately after treatment, with as many as  $6 \times 10^9$  CN706 particles found in circulation 30 minutes post-treatment. A significant secondary peak of circulating CN706 was observed in most of the patients within 3 days after treatment, confirming CN706 virus replication in these patients. In every patient, the secondary peak dropped off or was eliminated by day 8, suggesting a limited time of virus propagation. Testing of patient's urine samples showed that virus was shed in urine of most patients' on Day 2, in only 2 patients at Day 8, and was not detected thereafter. This also supports a limited time of viral propagation. Importantly, it was proven that the viable virus particles were not wild type-like virions.

The proposed study, titled: "*A Phase 2 Randomized Comparison Study of an Intraprostatic Injection of CV706 Followed By External Beam Radiotherapy versus External Beam Radiotherapy Alone in Patients with Intermediate-risk, Clinically Localized Prostate Cancer*" will assess the safety and efficacy of CV706 initially at 18 months and then again at 5 years. This study is designed to take advantage of the striking synergy seen in the preclinical studies between CV706 and radiation therapy. Patients are followed for 60 days then every 3 months for the first 18 months, then every 6 months until the 5-year time point, and then annually by postcard (to capture late clinical disease progression and deaths). Unless contraindicated, all patients will undergo an 18-month biopsy. Additionally, a prostate biopsy will be performed after the 18-month visit in all patients with biochemical or clinical failure.

Consistent with standard of care, a bone scan will be performed in patients with new bone pain that cannot be attributed to intercurrent illness (e.g., trauma), and diagnostic imaging studies (e.g., standard radiography, tomography, or MRI) will be performed as appropriate to confirm the diagnosis of metastatic disease.

The 18 month analysis will compare the efficacy of Treatment Arm I versus Arm II and be based on the PSA response (nadir and timecourse), supported by prostate biopsies and assessment of tumor response by endorectal 3D-spectroscopic MRI (MRI/3D-MRSI, an exploratory endpoint in a subset of patients). At the 5-year analysis, the efficacy comparison will be based on the proportion of patients free from disease progression (biochemical failure, local recurrence and development of metastases).