SECTION 1 SCIENTIFIC ABSTRACT

The primary objective of this Phase I study is to test the hypothesis that the human sodium iodide symporter (hNIS) can be used as a reporter to monitor adenoviral gene therapy vectors non-invasively in humans. We have developed a replication-competent adenovirus (Ad5-yCD/*mut*TK_{SR39}*rep*-hNIS) expressing a yeast cytosine deaminase (yCD)/mutant herpes simplex virus thymidine kinase (HSV-1 TK_{SR39}) fusion gene in the E1 region and the hNIS gene in the E3 region. Using the dog as a model, we demonstrated previously that hNIS could be used to monitor the persistence and biodistribution of Ad5-yCD/*mut*TK_{SR39}*rep*-hNIS following intraprostatic administration of adenovirus and intravenous administration of sodium pertechnetate ($Na^{99m}TcO_4$). This Phase I study is a logical extension of our preclinical work and will determine whether hNIS can be used to monitor the persistence and biodistribution of adenoviral gene therapy vectors in humans.

The Phase I study described here has two parts. Progression to Part 2 will require that the safety and feasibility of the approach be established in Part 1.

Part 1 (Cohorts 1 and 2) is a safety and feasibility study to determine the maximum tolerated dose (MTD) and feasibility of using hNIS as a reporter gene to monitor adenoviral gene therapy vectors non-invasively in humans. Six (n = 6) subjects (two cohorts of 3 patients each) with clinically localized prostate cancer will receive a single intraprostatic injection (Cohort 1- 10^{11} vp; Cohort 2- 10^{12} vp) of Ad5-yCD/*mut*TK_{SR39}*rep*-hNIS on Day 1. Beginning three days later (Day 4), subjects will receive three weeks of 5-FC and vGCV prodrug therapy and a standard course (74 Gy) of intensity modulated radiation therapy (IMRT). Within 30 days prior to the adenovirus injection (Day 1), subjects will be administered 16 mCi $Na^{99m}TcO_4$ and will undergo whole body and pelvic planar scans using gamma camera scintigraphy to establish a baseline. On Day 4, subjects will be administered 16 mCi $Na^{99m}TcO_4$ and will undergo whole body and pelvic planar scans to determine whether Ad5-yCD/*mut*TK_{SR39}*rep*-hNIS-mediated $^{99m}TcO_4^-$ uptake can be detected in the prostate. If specific $^{99m}TcO_4^-$ uptake is detected, Single Photon Emission Computed Tomography (SPECT) of the pelvic region will be performed to obtain a three dimensional (3D) tomographical image of the prostate to estimate the volume of $^{99m}TcO_4^-$ uptake. The primary endpoint is toxicity through Day 90. The secondary endpoint is feasibility of using hNIS as a reporter gene to monitor the activity of adenoviral gene therapy vectors non-invasively *in vivo*.

Part 2 (Cohort 3) will determine the persistence and biodistribution of Ad5-yCD/*mut*TK_{SR39}*rep*-hNIS following intraprostatic administration. Six (n = 6) subjects will receive a single intraprostatic injection of Ad5-yCD/*mut*TK_{SR39}*rep*-hNIS at the MTD on Day 1 followed by 3 weeks of 5-FC and vGCV prodrug therapy and a standard course (74 Gy) of IMRT. Within 30 days prior to the adenovirus injection (Day 1), subjects will be administered 16 mCi $Na^{99m}TcO_4$ and will undergo whole body and pelvic planar scans using gamma camera scintigraphy to establish a baseline. On Days 4, 8, 15, and 22, subjects will be administered 16 mCi $Na^{99m}TcO_4$ and will undergo whole body and pelvic planar scans to determine the persistence and whole body distribution of the Ad5-

yCD/*mut*TK$_{SR39}$*rep*-hNIS adenovirus. If specific $^{99m}TcO_4^-$ uptake is detected in the prostate, SPECT of the pelvic region will be performed to obtain a 3D tomographical image to estimate the volume of $^{99m}TcO_4^-$ uptake. The primary endpoint is toxicity through Day 90. Secondary endpoints are persistence and biodistribution of the replication-competent Ad5-yCD/*mut*TK$_{SR39}$*rep*-hNIS adenovirus, and volume of $^{99m}TcO_4^-$ uptake in the prostate.

The three major questions we hope to answer are:

1) Can hNIS be used as a reporter gene to monitor adenoviral gene therapy vectors non-invasively in humans?
2) What is the persistence and biodistribution of replication-competent adenoviral gene therapy vectors following intraprostatic administration?
3) Can SPECT be used to estimate the volume of vector spread in the prostate?

We believe this Phase I study is important for several reasons. Having the capability to monitor cancer gene therapy vectors non-invasively *in vivo* will make it possible to investigate both in preclinical models and in the clinic a series of important questions regarding the efficacy and safety of cancer gene therapy. Studies designed to determine the optimal spatial distribution of viral deposits within the prostate gland are possible. Indeed, the same treatment planning algorithms used in clinical brachytherapy can be implemented. This is an important consideration for multi-focal diseases such as prostate cancer where sterilization of the whole organ is often necessary to eradicate tumor. Having the ability to monitor vector persistence in patients will help define the optimal duration of prodrug administration for vectors containing therapeutic suicide genes, such as Ad5-yCD/*mut*TK$_{SR39}$*rep*-hNIS. Having the ability to monitor vector biodistribution throughout the body will allow us to assess the risks of different routes of administration and injections procedures. This is important for suicide gene therapy, for if a significant amount of vector disseminates to a vital organ, such as liver, it could result in death if prodrugs are administered. We believe such novel imaging technologies have the potential to improve not only the efficacy, but also the safety, of human cancer gene therapy.