Radiation therapy is frequently used in the definitive management of patients with clinically-localized adenocarcinoma of the prostate (PCa). For men with low-risk, clinically-localized PCa, excellent 5 and 10 year disease-free survival can be expected. Men with intermediate or high-risk clinically-localized PCa have a lower disease-free survival regardless of the treatment modality. For patients treated with radiation therapy, about 10-50% of men who recur do so with a local recurrence as the first site of treatment failure depending on pre-treatment risk factors. Not surprisingly, men who do not suffer local recurrence are less likely to develop subsequent distant metastatic disease. Given these facts, the development of novel therapies that can minimize the risk of tumor recurrence and extend disease-free survival is logical and appropriate. Ideally, these novel therapies should have the potential for disease eradication and not be associated with a significant risk of toxicity and long-term complications.

One new antineoplastic approach involves exploitation of the cytolytic capacity of the adenovirus. It is well known that adenoviruses can induce cell death by cytolysis as part of their normal life cycle. Importantly, adenoviruses possess several important characteristics that make them attractive agents for prostate cancer gene therapy, including relatively high transduction efficiency and the ability to transduce and lyse non-replicating cells. The latter point is particularly important, as PCa typically possess a very low S-phase fraction of about 5% or less. Recently, molecular biologic techniques have allowed for genetic manipulation of the adenovirus providing the ability to restrict its replication to unique genetic profiles of the tumor type to be treated. These techniques include the production of replication-restricted adenoviruses that use heterologous tissue-specific promoters to control viral genes critical to replication.

We have recently reported on the development and clinical translation of a replication-competent, E3-deleted, cytolytic Ad5 adenovirus, with replication that is restricted to PSA-producing cells. This restricted replication is achieved by the insertion of a minimal promoter-enhancer construct of the human PSA gene (PSE) 5' of E1A, 3' of the E1A promoter, resulting in PSA-regulated expression of E1A. This E1A regulation, in turn, results in restriction of viral replication primarily to cells expressing PSA. Pre-clinical results as well as final results of our Phase I/II study will be presented. One of the concepts to be presented will include the "viral dosimetry" model we developed which allowed for practical and effective translation of the adenoviral therapy to the clinic. The model's underpinnings are based on pre-clinical data in animals and are undergoing extensive analysis and refinement at present.

Substantial improvements in tumor control are frequently achieved when rational combinations of cytotoxic therapies are employed. We have recently completed extensive preclinical evaluation of combinations of our PSA-selective, cytolytic adenovirus with radiation in a human PCa model. Both *in vitro* and *in vivo* analyses reveal that significant mathematical synergy exists between radiation and our PSA-selective adenovirus. This synergy can, in part, be explained by radiation-induced enhancement of viral replication as well as by the induction of widespread intratumoral necrosis and apoptosis. Results of these studies will be presented. Given these results, as well as the safety and activity data achieved in our earlier Phase I study, we have

designed and initiated a Phase I/II trial of conformal radiation therapy plus our PSA-selective adenovirus for the treatment of patients with newly diagnosed, intermediate-risk PCa.

It is clear that one of the primary areas in gene therapy that requires more detailed and focused attention is the refinement of intratumoral "viral dosimetry" which must logically accompany any intratumoral gene therapy program. The adaptation of radiation physics and dosimetric constructs to this problem is quite appropriate and logical. Concepts that need directed attention include intratumoral vector delivery, intratumoral vector dispersion, intratumoral pressure and flow dynamics and mathematical models that predict each of these. Once accomplished, "viral dosimetry", with the level of quality assurance we have come to expect from radiation therapy treatment planning, will allow a more logical and scientific advancement in the field. To achieve these goals, the medical physicist will be required to play an increasing role in the scientific and clinical development of viral-based gene therapy.