

## A one page scientific abstract of the protocol

The incidence of breast cancer is steadily increasing. Approximately 1 in 8 American women will develop breast cancer. At the present time, the metastatic form of breast cancer that has failed all conventional types of therapy has a poor prognosis. The ineffectiveness of hormonal therapy and chemotherapy has led investigators to evaluate a variety of immunotherapy regimens. The ultimate goal has been to consistently augment the immunological reactivity of breast cancer patients to their own tumor. In other human tumor systems, active specific immunotherapy (treatment with autologous tumor cells) has been shown to be effective in some patients with metastatic disease.

Berd et. al. immunized patients with irradiated autologous tumor cells and noted clinical responses in 25% of the patients treated. Other investigators have reported similar response rates in patients with renal cell carcinoma and colon carcinoma. Mitchell et. al. has reported clinical responses and stimulation of specific cytotoxic T-lymphocytes (CTL) in patients immunized with autologous melanoma cells. Seigler et. al. have serially injected high risk Stage I and Stage II melanoma patients using either x-irradiated melanoma cells or a vaccinia viral oncolysate of the tumor cells and have demonstrated improved survival in patients receiving adjuvant active treatment.

We have demonstrated that tumor specific CTLs can be generated *in vitro* by exposing patient lymphocytes to autologous tumor cells in the presence of a low dose interleukin-2 (unpublished data). The specific CTLs have effected cell mediated killing both *in vitro* and *in vivo* (unpublished data). Specific CTLs can also be generated against breast cancer specific antigens such as oncoproteins and peptides. In addition, CTLs were shown to recognize endogenously synthesized proteins and presented on the surface together with major histocompatibility (MHC) class I molecules (Maryanski JL, Pala P, et. al. 1986; Townsend ARM, Rothbard J, et. al. 1986; Moore MW, Carbone FR, et. al. 1988). Consequently, antigens include any primary amino acid sequence in any cellular proteins, either membrane bound or intracellular.

A variety of lymphokine genes have been introduced into tumor cells using retroviral vectors as well as non-viral delivery methods. The transduced cells express the inserted gene and secrete the specific lymphokine. We have used cationic liposomes to facilitate adeno-associated virus (AAV) plasmid transfections of primary and cultured cell types. AAV plasmid DNA complexed with liposomes (Avectin<sup>TM</sup>) showed levels of expression several fold higher than those of complexes with standard plasmids. In addition, long-term expression (>30 days) of the gene, unlike the transient expression demonstrated by typical liposome-mediated transfection with standard plasmids, was observed. Primary breast, ovarian, and lung cultured tumor cells were transfectable with the AAV plasmid DNA-liposome complexes. Transfected primary and cultured tumor cells were able to express transgene product even after lethal irradiation. Transfection efficiency ranged from 10 to 50% as assessed by intracellular IL-2 levels in IL-2 transfected cells.

The primary objective of this protocol will be to evaluate safety, biological response, and survival in patients serially treated with autologous breast cancer cells transduced with the gene for human Interleukin-2 (IL-2) via the Avectin<sup>TM</sup> method. We will monitor the safety and toxicity of the treatment and will attempt to determine its immunological effects on the patient. We will also evaluate the clinical responses and duration of any such response to this treatment.