

## SCIENTIFIC ABSTRACT

### Adoptive Cellular Therapy of Cancer Combining Direct HLA-B7/ $\beta$ 2-microglobulin Gene Transfer with Autologous Tumor Vaccination for the Generation of Vaccine-Primed Anti-CD3 Activated Lymphocytes

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Lipid complex-mediated gene transfer with plasmid vectors provides an apparently safe approach to modify tumor cells such that they are more readily recognized by the immune system. Since 1991, we have investigated the effect of lipofecting allogeneic class I molecules into poorly immunogenic murine tumors. These experiments have contributed to two direct intratumoral allogeneic HLA class I proposals (Rac approval Feb. 1992 and June 1993 - P.I. Dr. Gary J. Nabel). Our laboratory efforts have primarily focused on two subclones of B16BL6 (A9 and D5) which are defined as poorly immunogenic, since immunity cannot be induced against them by standard immunization-challenge experiments (other investigators might define these as non immunogenic). Using these models we have demonstrated that direct intratumoral allogeneic class I gene transfer, which does not effect the progressive growth of these tumors, does augment the sensitization of pre-effector T cells in the tumor-draining lymph nodes (TDLN). *In vitro* activation of these TDLN from allogeneic class I-modified tumors generates T cell populations which are significantly more therapeutic than normal TDLN (Wahl 1992, Wahl 1995). Recently, we have demonstrated that *in vitro* lipofection of the tumor vaccine combined with subsequent *in vitro* anti-CD3-stimulated activation of the tumor vaccine-draining lymph node cells (TVDLN) is significantly more therapeutic than combining an optimal dose of BCG with the tumor vaccine (3 of 3 determinations, Table 1 - appendices). This observation suggests that allogeneic HLA class I gene transfer into an autologous tumor vaccine may be more effective than our current approach of adding BCG to the autologous tumor vaccine. Therefore we propose the following modifications to our currently active tumor vaccination/adoptive cellular immunotherapy protocol.

Patients with histologically proven metastatic renal cell carcinoma or melanoma which is judged to be incurable by standard modalities will undergo surgical retrieval of tumor for use as a tumor vaccine. Patients will be vaccinated with irradiated autologous tumor and BCG in one limb, and irradiated autologous tumor transfected with a plasmid vector encoding both HLA-B7 and 2-microglobulin (HLA-B7/2m - VCL-1005) and combined with nothing, an escalating dose of HLA-B7/2m DNA/lipid-complex, or BCG in the contralateral limb. Regional draining lymph nodes will be removed 7-14 days after immunization to obtain lymphocytes for activation with an anti-CD3 monoclonal antibody (OKT3) and subsequent expansion in IL-2 *in vitro*. After *in vitro* activation these lymphocytes will be adoptively transferred to the patient along with IL-2, which will be administered systemically for 5 days. Three patients will be treated in each of 6 groups for a total of 18 patients. The toxicity and immunologic effects of treatment will be monitored.