INTRODUCTORY STATEMENT AND GENERAL INVESTIGATIONAL PLAN

Interleukin-12, a heterodimeric cytokine secreted by activated monocytes and macrophages, has been shown in murine tumor models to inhibit tumor growth and modulate immune responses by enhancing the cytolytic activity of natural killer (NK) and cytotoxic T-lymphocytes (CTL), and by inducing the production of gamma interferon (IFN-γ) by these cells. Clinical trials of systemically administered human recombinant interleukin-12 have shown however limited antitumor activity despite significant dose- and schedule-dependent toxicities.

To test the hypothesis that delivery of high concentrations of interleukin-12 by in vivo intratumoral gene therapy can be effective, we have constructed an E1 and E3 deleted replication defective adenoviral vector (Adv.RSV-mIL12) expressing the murine interleukin-12 cDNA under the control of the Rous sarcoma virus promoter, and have investigated its efficacy in an orthotopic murine model of established hepatic tumors. In this model, tumors from syngeneic murine cell lines (MCA26 colon carcinoma and JC mammary adenocarcinoma) were established in the liver by direct implantation. We have shown that intratumoral injection of Adv.RSV-mIL12 at doses of produced tumor regression, survival prolongation, and antitumor immunity mediated by natural killer and cytolytic T cells. At therapeutically effective doses (2.5 to 5.0 x 10^8 pfu), Adv.RSV-mIL12 was well tolerated without serious toxicities. At higher doses, toxicities in the liver, lung, white blood cell and platelet counts were seen, and were similar to toxicities seen in clinical trials of the human recombinant IL12 protein.

To test the translational safety and efficacy of these preclinical studies, we have constructed a replication defective E1/E3 deleted serotype 5 adenoviral vector expressing the human interleukin-12 cDNA (Adv.RSV-hIL12 or ADV-hIL12). We propose to study in a Phase I design the safety of Adv.RSV-hIL12 when administered by intratumoral injection in patients with malignant tumors in the liver. Two trials will be performed concurrently: one trial in aptients with metastatic breast cancer to the liver, and the other trial in patients with metastatic non-breast or primary malignant neoplasms in the liver. Data will also be collected on tumor regression and immune responses. The vector will be injected into one hepatic tumor by skinny needles placed percutaneously under ultrasound guidance. Dose escalation of the vector will be performed in half log increments from 10^8 to 10^{12} pfu in cohorts of three patients each. Dose limiting toxicity (DLT) is defined as any nonhematologic grade 3 irreversible toxicity or any grade 4 toxicity except for reversible grade 4 fever, leucopenia, neutropenia, lymphopenia, or thrombocytopenia. The maximal tolerated dose (MTD) for each trial is defined as the highest cohort level at which less than two instances of DLT are observed among six patients treated. 30-36 patients will be treated in each trial. The planned duration of the trials will be 16 months. Both trials will be conducted at the Mount Sinai School of Medicine in New York.

We have developed a percutaneous injection procedure for intratumoral delivery of adenoviral vectors in patients with malignant tumors in the liver, and have tested it in a Phase I trial of intratumoral adenoviral vector delivery of the Herpes Simplex Virus thymidine kinase gene (Adv.RSV-tk) and followed by intravenous ganciclovir in patients with primary or metastatic malignant tumors in the liver. The vector was injected via percutaneous placement of up to three skinny needles into a hepatic tumors under ultrasound guidance. 16 patients were treated on five cohort levels from 10^9 to 10^{12} pfu Adv.RSV-tk. The percutaneous vector injection procedure was tolerated; no serious toxicities were encountered. Hepatotoxicity included grade 1 transient elevations in serum aminotransferases in 3/16 patients (BBIND #6906, ORDA #9610-164).

The vector expressing human interleukin-12 (Adv.RSV-hIL12, also termed ADV-hIL12, or Ad.hIL-12), a BL2 agent, has been produced for the proposed trials under GMP conditions by the University of Pennsylvania Institute for Human Gene Therapy. The trial in metastatic breast cancer is sponsored by the U.S. Army Medical Research Acquisition Activity (Grant No. DAMD17-98-1-8322). The trial in patients with metastatic (nonbreast) or primary malignant tumors in the liver is sponsored by the Mount Sinai School of Medicine.