SCIENTIFIC ABSTRACT

Patients bearing tumors of different histologic origins have elevated levels of Transforming Growth Factors-βs (TGF-βs), which are associated with immunosuppression. TGF-βs suppresses T-cell activation in part by inhibiting the gamma chain of high affinity cytokine receptor.

Genetic modification of tumor cells to block TGF-β secretion has made the tumor cells more immunogenic and suitable for active immunotherapy. We have shown the efficacy of this approach in four different tumor models, a murine squamous NSCLC (KLN-205), two rat glial tumors, and one murine ovarian teratoma (MOT). Others have also shown the efficacy of TGF-β gene therapy in inducing anti tumor effects in cultured cells and animal tumor models.

PRECLINICAL: In the murine lung carcinoma two injections with 5 x 10^5 TGF-β2 antisense gene modified cells protected the animals against a subsequent intra peritoneal tumor challenge with 10^6 cells. In the rat 9L glial tumor model inoculation of tumor bearing rats with TGF-β antisense gene modified 9L cells resulted in eradication of intracranial tumors in all animals. In the second glial tumor, the low TGF-β producing C-6 glioma, TGF-β antisense gene therapy eradicated intracranial tumors in 60 percent of animals. In the murine ovarian teratoma (MOT) tumor model, however, only the group inoculated with TGF-β antisense and IL-2 gene modified cells resulted in protection of approximately 70% of animals from a subsequent tumor challenge. This protection level was significantly higher than protection in the control groups treated with all other combinations. *In vitro* cytotoxicity assays in animal tumor models using lymph node effector cells taken from immunized animals and activated *in vitro* in the presence of irradiated tumor cells and 50 BRMP units of IL-2 revealed a 4-to-8 fold increase in lytic activity for the animals immunized with TGF-β2 antisense gene modified tumor cells compare to control groups.

PHASE I: This approach has been tested in a phase I clinical trial in patients with WHO grade IV astrocytoma. In this study it was shown that injections of either 5 x 10^6, 1 x 10^7, or 2 x 10^7 TGF-β antisense gene modified autologous tumor cells was safe and did not cause toxicity. Immune histology of injection site biopsies and secondary tumor resections demonstrated significantly higher number of immune infiltrates in comparison to biopsies taken prior to initiation of gene therapy.

PHASE II: A phase II randomized, dose-variable clinical trial was conducted in 75 patients diagnosed with stage II through stage IV non-small cell lung cancer. The vaccine cocktail, Lucanix™ (belagenpumatucel-L) consisted of an equal number of each of the four irradiated TGF-β antisense gene modified NSCLC cell lines (NCI-H-460, NCI-H-520, SK-Lu1, and Rh-2). The number of injected cells in the three cohorts was 1.25 x 10^7, 2.5 x 10^7, and 5 x 10^7 cells, respectively with injections administered on a monthly or bi-monthly schedule for a maximum of 16 injections.

Patient's response to treatment with Lucanix™ were evaluated according to standard evaluation criteria of no response, stable disease, partial response and complete response. A 15% partial response rate was achieved in the 16 late stage (stages IIIB/IV) patients. A dose-related survival rate was seen in patients who received $\geq 2.5 \times 10^7$ cells/injection. No significant toxicities were seen in each of the dose regimens. One- and two-year combined survival rates in stages IIIB and IV NSCLC was 61% and 41%, respectively. The one- and two-year survival rates for the low-dose group was 39% and 20%, respectively. At week 12, immune function in the 61 late stage patients showed increased cytokine production in clinical responders as compared to patients with progressive disease. Clinical responders also showed an elevated anti-body-mediated response to vaccine HLAs. In addition, a correlation trend was seen in clinical responders who achieved stable disease or better by a positive enzyme-linked immunospot reaction to Lucanix™.

Based on these results, and how well tolerated Lucanix™ was in this patient population, a phase III clinical trial in patients with stages IIIA, IIIB, and IV non-small cell lung cancer is planned to investigate survival in patients randomized into one of two arms: the control arm using an approved drug, pemetrexed (Alimta®), and the investigational arm using Lucanix™ (belagenpumatucel-L). Patients enrolled in either treatment group will be evaluated monthly during treatment. Patients assigned to the vaccine treatment group will be injected monthly for a total of 18 months, and then once each quarter for two quarters. Thus patients may receive up to a total of 20 vaccine injections. Additionally, clinical response, time to tumor progression, and survival will be evaluated.

The results of this study may be used as the basis of an application to the US Food and Drug Administration seeking marketing authorization for the sale of the vaccine in the United States.