SCIENTIFIC ABSTRACT

Patients bearing tumors of different histologic origin have elevated levels of Transforming Growth Factors-βs (TGF-βs), which are associated with immunosuppression. TGF-β2 inhibits T cell activation in response to antigen stimulation and is antagonistic to natural killer (NK) cell and lymphokine-activated killer (LAK) cell response.

Genetic modification of tumor cells to block their TGF-β secretion increases tumor cell immunogenicity and makes them 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).

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 106 cells. In the rat 9L glial tumor model (a high TGF-β2 expressive line) 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 (a low TGF-β2 producing C-6 glioma) TGF-β2 antisense gene therapy eradicated intracranial tumors in 60 percent of animals. In the KLN-205 lung tumor model survival benefit was demonstrated in mice vaccinated with TGF-β2 antisense modified lung cancer cells compared to unmodified cells (80% vs. 20% survival at 13 weeks respectively).

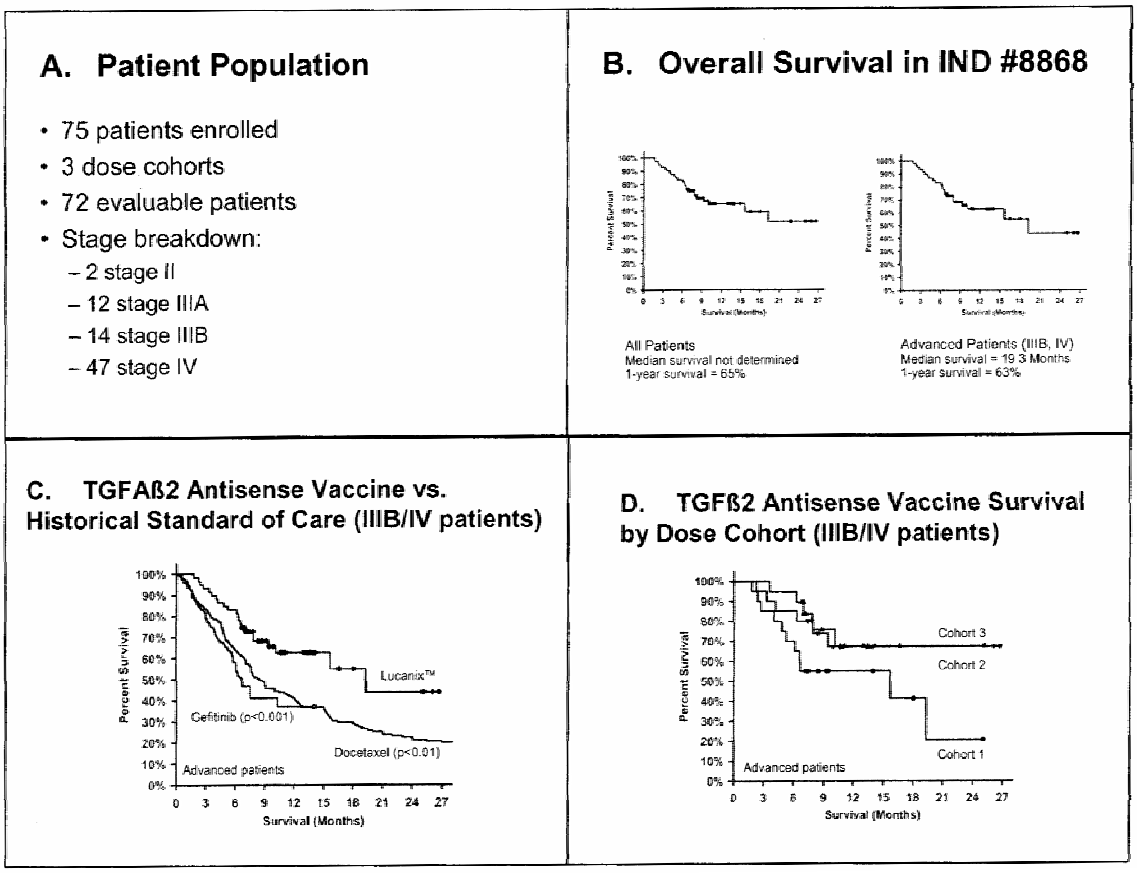
We have tested this approach in a phase I clinical trial in patients with glioblastoma. In this study we have thus far shown that injections of either 5 x 10^6, 1 x 10^7, or 2 x 10^7 TGF-β2 antisense gene modified autologous tumor cells is safe and does not cause toxicity. Immune histology of injection site biopsies and secondary tumor resections demonstrated significantly higher numbers of immune cells infiltrating the cancer mass in comparison to biopsies taken prior to initiation of gene therapy (manuscript in preparation).

A subsequent phase I/II trial in NSCLC patients was recently completed (IND # 8868). Briefly 75 patients (Figure 1A) with NSCLC were entered into trial and were treated with monthly injections of vaccine for 16 months (if no progressive disease). Sixty one patients had advanced disease (IIIB/IV) and 14 with early stage (IIIA, II) were entered. Preliminary survival results are shown in Figure 1B. Follow up of the last patient entered into trial is currently greater than 6 months. Survival of > 18 months was observed for the IIIB/IV NSCLC patients in comparison to the FDA approved trial results of taxotere and Iressa (6-7 months) (Figure 10). Furthermore, a dose related survival response between cohorts 2 and 3 was seen in comparison to cohort 1 (Figure 1D), thereby justifying a dose 2.5 x 10^7 cells.

Twenty-seven patients have deceased at last data analysis in IND #8868. In 25 cause of death was related to progressive disease and in 2 cause of death was due to other medical disease not related to vaccine (annual reports). Median survival of <u>only</u> the patients who died was 6.2 months. Median survival of the living patients has not been reached and is greater than 24 months at this time. Grade III/IV toxicity related to study vaccine was not observed. Common grade I/II toxicity related to vaccine included injection site erythema, rare grade I/11 toxicity included nausea, fatigue and bruising (at injection site).

In this pilot clinical trial we will use the same TGF-β2 antisense plasmid, same 4 NSCLC cell lines, same schedule of administration and will use the optimal dose as determined in IND # 8868, 2.5 x 10^7 cells per injection. The number of circulating cytokeratin positive tumor cells harvested by a 7.5 ml venous blood draw will be quantitatively measured by immunohistochemical analysis and correlated with patient disease outcome to vaccination.

Figure 1: Efficacy Results of IND #8868



Patients will be monitored and evaluated according to standard evaluation criteria of no response, stable disease, partial response and complete response. The results of this study will be used to evaluate the feasibility of monitoring circulating cytokeratin positive malignant cells in subsequent phase III clinical trial.