

**TITLE: A Phase I Study of Sequential Vaccinations with Fowlpox-CEA(6D)-TRICOM (B7.1/ICAM/LFA3) and Vaccinia-CEA(6D)-TRICOM, in Combination with GM-CSF and Interferon-Alfa-2B in Patients with CEA Expressing Carcinomas.**

The present trial will employ two novel anti-CEA vaccines; recombinant (r) Vaccinia-CEA(6D)-TRICOM and recombinant Fowlpox-CEA(6D)-TRICOM. These viruses are capable of infecting professional antigen presenting cells which in turn present part of the CEA gene (a modified 9-MER CEA peptide) in the context of HLA class II to CD4+ T cells. These viral vectors also direct the expression of three co-stimulatory molecules (B7-1, ICAM-1, and LFA-3) each of which is capable of providing a critical second activating signal to antigen-specific T cells.

In the present trial we will be evaluating the safety and efficacy of the vaccine regimen when combined with interferon-alpha-2b (IFN- $\alpha$ -2b). It has been previously demonstrated that IFN- $\alpha$ -2b upregulates CEA expression on established tumor cell lines and tumor cells isolated from malignant ascites. A phase I study by Roselli *et al.* revealed that administration of IFN- $\alpha$  at a dose of  $3 \times 10^6$  U/d thrice weekly (TIW) to patients with colorectal cancer led to significant increases in tumor CEA levels. Numerous studies have also shown that IFNs upregulate the expression of HLA-class I molecules. It has also been shown that treatment of human tumor cells with IFN renders them more susceptible to lysis by a CEA-specific CD8<sup>+</sup> T-cell line (Zaremba *et al.*, reference No. 52). In addition, it has been shown that the combination of IFN- $\alpha$  and GM-CSF induces a surprisingly rapid maturation of monocytes into dendritic cells that are functionally superior to those induced by treatment with IL-4 plus GM-CSF.

The vaccine containing the viral vector will be administered on day 1 followed by GM-CSF on days 1-4. In Cycle 1 patients will receive thrice weekly injections of IFN- $\alpha$ -2b via the subcutaneous (s.c.) route the week after the rVaccinia-CEA(6D)-TRICOM vaccine is administered. 7 days will have passed since vaccination so that the IFN- $\alpha$ -2b will not inhibit replication of the vaccinia virus. In Cycles 2-4 patients will receive three s.c. injections of IFN- $\alpha$ -2b at the same time that the rFowlpox-CEA(6D)-TRICOM vaccine and GM-CSF are being administered, since IFN- $\alpha$ -2b can augment dendritic cell maturation and will not inhibit the activity of the Fowlpox vector. We hypothesize that this treatment will increase levels of CEA and HLA Class I expression on patient tumor cells and lead to an improved anti-CEA immune response. Clinical correlates will be performed to determine the effect of IFN- $\alpha$ -2b administration on tumor expression of CEA. Dose escalation of the IFN- $\alpha$ -2b component of therapy will take place in cohorts of three patients. There will be no intra-patient dose escalation of IFN- $\alpha$ -2b. IFN- $\alpha$ -2b will be administered s.c. at rotating sites located away from the vaccination site during Cycle 1. In Cycles 2-4 the IFN- $\alpha$ -2b will be given along with the GM-CSF at the vaccination site.

There will be three patients in each of the four cohorts. HLA-A2 status will be determined for these patients, but HLA-A2 positivity will not be a requirement for entry into the study. In the process of determining the MTD, one cohort will be expanded with an additional 3 patients. These additional 3 patients must be HLA-A2 positive.

Patients who do not progress and do not have unacceptable toxicity after completing the initial phase of vaccinations will be offered additional vaccinations of rFowlpox-CEA(6D)-TRICOM (in combination with GM-CSF and IFN- $\alpha$ -2b) as long as adequate drug supply is available. Patients

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will receive the same dose of vaccine and IFN- $\alpha$ -2b as per the treatment arm on which they were enrolled every 28 days up through vaccination #6 and then every 3 months thereafter for up to two years. Off-study criteria and clinical/immunologic monitoring will continue unchanged for these patients. Patients will be re-staged every two months, or more frequently if clinically indicated.