

## 1. SCIENTIFIC ABSTRACT

The identification of unique tumor-associated antigens capable of eliciting cytotoxic T-lymphocyte (CTL) responses has led to new methods of immunotherapy for cancer. One method is the insertion of a tumor antigen cDNA clone into a live virus that can be used in active immunization protocols. This approach was used to generate a vaccinia virus that expressed the human carcinoembryonic antigen (CEA) gene. Vaccinia was chosen as a vector because of its ability to accept a large amount of foreign DNA, its replicative stability, its ability to generate strong CD8<sup>+</sup> CTL responses, and its success as an immunizing agent in the smallpox eradication program. The recombinant vaccinia-CEA virus (rV-CEA) was shown to have therapeutic effectiveness in the prophylaxis and treatment of an established CEA-expressing murine adenocarcinoma tumor. Furthermore the construct elicited both anti-CEA antibodies and CTL directed against CEA in mice. The vaccine induced similar immune responses in a non-human primate model and was shown to be safe with few side effects.

The FDA and RAC granted permission to perform a clinical trial in human patients with this vaccine in 1992.

The National Cancer Institute (NCI) has conducted four studies using either ALVAC-CEA or ALVAC-CEA/B7.1:

1. A Phase I study in cancer patients of a replication-defective avipox recombinant vaccine that expresses human carcinoembryonic antigen; conducted by John Marshall et al.<sup>1</sup>
2. A pilot study of sequential vaccinations with ALVAC-CEA and vaccinia-CEA with the addition of IL-2 and GM-CSF in patients with CEA-expressing tumors; conducted by John Marshall, Jeffrey Schlom and Alfred Tsang<sup>2</sup>.
3. A Phase I pilot study of ALVAC-CEA/B7.1 immunization in patients with advanced adenocarcinomas expressing CEA; conducted by Margaret von Mehren et al<sup>3</sup>.
4. A Phase 1 clinical trial of a recombinant ALVAC-CEA/B7.1 vaccine in the treatment of advanced CEA-expressing adenocarcinomas; conducted by H. Horig et al<sup>4</sup>.

Some of these studies are still ongoing, and therefore not all results are available. In general, the treatments were well tolerated, with no significant product-related toxicities. The results demonstrated the ability of both ALVAC-CEA and ALVAC-CEA/B7.1 to induce CEA-specific CTL responses in patients with CEA-expressing carcinomas. The results to date from these studies can be summarized as follows:

Vaccination with ALVAC-CEA or ALVAC-CEA/B7.1 of patients with CEA-expressing tumors is safe and does not induce autoimmunity or serious adverse events, except for mild local reactions and some grade 4 toxicities such as fatigue, fever, and myalgia.

<sup>1</sup> Marshall JL, Hawkins MJ, et al. Phase I study in cancer patients of a replication-defective avipox recombinant vaccine that expresses human carcinoembryonic antigen. *J. Clin. Oncol.* 17(1): 332-337, 1999.

<sup>2</sup> Marshall, J, Hoyer R, et al. Granulocyte-macrophage colony-stimulating factor (GM-CSF) produced by recombinant avian poxviruses enriched the regional lymph nodes with antigen-presenting cells and acts as a biological vaccine adjuvant. Submitted for publication.

<sup>3</sup> Von Mehren M, Arlen P, et al. Pilot study of a dual gene recombinant avipox vaccine containing both carcinoembryonic antigen (CEA) and B7.1 transgenes in patients with recurrent CEA-expressing adenocarcinomas. *Clin. Cancer Res.* 6(6): 2219-28, 2000.

<sup>4</sup> Horig H, Lee DS, et al. Phase I clinical trial of a recombinant canarypoxvirus (ALVAC) vaccine expressing human carcinoembryonic antigen and the B7.1 co-stimulatory molecule. *Cancer Immunol. Immunother* 49(9): 504-514, 2000

Increases in T-cell precursor frequency, as demonstrated by limiting dilution assays, have been documented after vaccination. Frequency of T-cell responders and magnitude of the response may be increased by: adding GM-CSF as an immunoadjuvant, priming with vaccinia-CEA and then boosting with ALVAC-CEA, incorporating the B7.1 co-stimulatory molecule into the ALVAC-CEA construct.

The current study will investigate the administration of ALVAC-CEA/B7.1 concomitantly with chemotherapy.