

## 1. SCIENTIFIC ABSTRACT

Colorectal cancer is the third most common malignancy and the second most common cause of cancer death in this country. According to the American Cancer Society, approximately 150,000 new diagnoses of, and about 60,000 deaths from colorectal cancer occurred in the United States in 2003. Current therapeutic strategies for colorectal cancer have had limited success in the metastatic setting. The application of immunotherapy to the treatment of metastatic colorectal cancer has been proposed based on the identification of several well characterized tumor-associated antigens and the advent of increasingly sophisticated vectors for presenting antigen to the immune system. Almost all colorectal tumor cells express high levels of carcinoembryonic antigen (CEA), which can often be measured in peripheral blood. Several recent studies have identified HLA-restricted CEA epitopes that can be targeted by T-cells derived from patients vaccinated with CEA-specific vaccines. Based on these studies a single HLA-A2-restricted epitope was identified and a single amino acid modification of that epitope resulted in improved recognition by the CEA-specific T-cell receptor, and this modification has been incorporated into the CEA coding sequence used in viral construction. The mucin, MUC-1, is also highly expressed by nearly all colorectal tumors and can be targeted by T-cells in an HLA-independent manner. While numerous vaccine approaches have been described, the use of poxvirus vectors expressing tumor antigens, such as CEA and MUC-1, have been evaluated in previous clinical trials demonstrating safety, evidence for the induction of T-cell responses in a proportion of vaccinated subjects, and early suggestions of clinical effectiveness. Basic research on T-cell biology has now revealed that activation of T-cells depends on delivery of two signals. The first signal is derived from processed antigens that are presented by tumor cells or professional antigen-presenting cells. The second signal is provided by a distinct set of cell surface proteins collectively referred to as co-stimulatory molecules. Several co-stimulatory molecules have now been described, including B7.1, ICAM-1, LFA-3, and others. The combined expression of B7.1, ICAM-1, and LFA-3 in both vaccinia and fowlpox viruses have demonstrated superior activity in the initiation of T-cell proliferative responses and the combination was superior to single gene vectors in eradicating animal tumor in pre-clinical studies. The expression of co-stimulatory molecules with tumor antigens is easily accomplished in poxviruses and several trials have demonstrated the safety and potential increased clinical effectiveness of using this approach. Previous trials have tested vaccinia and fowlpox vectors expressing PSA in a prime/boost approach and conformed pre-clinical data supporting this as a strategy for enhancing immune and clinical responses. The goal of this protocol is to determine the safety and dosing of a prime/boost approach using an admixture of vaccinia viruses expressing CEA and a triad of co-stimulatory molecules (B7.1, ICAM-1, and LFA-3, designated rV-CEA-TRICOM) and vaccinia virus expressing MUC-1 (designated rV-MUC-1) followed by three booster doses of fowlpox virus expressing CEA and the same triad of co-stimulatory molecules (designated rF-CEA-TRICOM). An open label, pilot clinical trial is proposed. Recent reports of antigen spreading suggest that an

immune response against one antigen may enhance, or "spread" to other antigens also expressed by established tumors. Thus, this clinical trial is supported by extensive pre-clinical and prior early phase clinical trials and will be able to examine the phenomenon of antigen spreading in a more specific manner by the inclusion of two antigenic targets. The incorporation of several important methods into this vaccine trial builds on previous knowledge of tumor vaccines and immunology, including the prime/boost strategy with different vectors, the inclusion of multiple co-stimulatory molecules, the CEA modification for enhanced recognition, and the combination of two distinct antigens. Further clinical development of the colorectal cancer vaccine program will depend on the safety profile and evidence of enhanced CEA- and MUC-1-specific T-cell responses, as well as clinical efficacy, with this approach.