

A PHASE II STUDY OF ACTIVE IMMUNOTHERAPY WITH PANVAC™ OR AUTOLOGOUS, CULTURED DENDRITIC CELLS INFECTED WITH PANVAC™ AFTER COMPLETE RESECTION OF HEPATIC METASTASES OF COLORECTAL CARCINOMA

SCIENTIFIC ABSTRACT

Although resection of localized colon cancer can be curative, 25-40% of patients will have a recurrence in the liver. Unfortunately, many of these individuals will not be candidates for resection of the metastases, but for those with small volume disease located exclusively in the liver, hepatectomy can be offered with an attempt to cure the disease with a 5 year survival of 16-28%. Recent clinical trials have suggested the benefit for post-operative chemotherapy, typically including intra-hepatic FUDR along with systemic chemotherapy, in reducing recurrences. Nonetheless, intra- and extra-hepatic recurrences remain common. Therefore, the evaluation of additional strategies such as immunotherapy is indicated.

Numerous approaches to inducing immune responses against colon cancer have been attempted including immunization with autologous tumor, viral vectors encoding tumor antigens such as CEA, peptides, and dendritic cell based vaccines. We have previously performed a phase I/II study of autologous dendritic cells loaded with CEA mRNA following hepatic resection of colon cancer metastases. The immunizations were well tolerated without toxicity, but immune responses were difficult to detect and the recurrence-free survival was no better than results reported for patients who undergo hepatic resection and did not receive adjuvant therapy. One explanation for these results was that the antigen-specific T cell response induced by the vaccine was of very low frequency. Recently, recombinant pox vectors including vaccinia (rV) and fowlpox (rF) encoding CEA have been tested in patients with advanced colon cancers. An intriguing observation was that the group of patients who received the prime boost strategy of rV-encoding CEA followed by rF-encoding CEA had greater CEA-specific T cell responses and prolonged survival compared with those who received the reverse order of administration. This suggests that immunization with pox-based vectors may in fact be able to provide a clinical benefit. Nonetheless, the magnitude of the immune responses was still fairly low. Modifications to these vectors have been made to increase their immunogenicity and include the addition of a triad of co stimulatory molecules (CD54, CD58, CD80 referred to as TRICOM) and modification of the amino acid sequence of the HLA A2 restricted epitope of CEA, CAP-1, to yield a sequence called CAP-1(6D) with better T cell receptor binding characteristics. The fowlpox vector is called rF-CEA(6D)/TRICOM and its vaccinia counterpart is called rV-CEA(6D)/TRICOM. A phase I study has identified that rV-CEA(6D)/TRICOM followed by three doses of rF-CEA(6D)/TRICOM administered with GM-CSF is tolerable and induces the most potent immune responses. In order to increase immune responses further, we have been studying alternative "adjuvants." Because dendritic cells (DC) loaded with tumor antigens induce the most potent immune responses in animal models, we initiated a phase I study of *ex vivo* cultured DC infected with rF-CEA(6D)/TRICOM. Of 12 patients who have completed their enrollment in the study, one patient experienced a minor response and 3 had stable disease for at least one cycle of immunization. Using ELISpot analysis, we have observed the highest level of antigen-specific immune responses yet observed in our clinical trials with levels ranging from 10 to >500 antigen-specific T cells per 100,000 peripheral blood mononuclear cells following

immunization. The magnitude of T cell response for the responder/stable disease group was higher than for those with progressive disease (mean: 241 vs 50 T cells/100,000 PBMC). Because the greatest clinical benefit for immunization strategies is expected in the setting of less advanced disease, we now propose to test the use of pox-vector infected DC in the setting of minimal residual disease following hepatic resection of colon cancer metastases and adjuvant chemotherapy. Because it is more complicated to generate DC for use in vaccine strategies, an important question is whether DC actually augment the activity of the pox-vectors. We therefore propose to allocate patients to ex vivo cultured DC infected with CEA expressing pox vectors or to the pox vectors themselves. Recently, a modification to the vaccinia and fowlpox vectors has been made in which the gene encoding the tumor antigen MUC-1 has been inserted into these vectors in addition to the CEA(6D) and TRICOM molecules. MUC-1 is a tumor antigen also frequently expressed by colon cancers. These vectors are called PANVAC-V and PANVAC-F and have begun phase I testing where they have demonstrated similar safety to the non-MUC-1 containing vectors. DC infected ex vivo with the PANVAC vectors display similar levels of expression of CEA and the TRICOM molecules and similar in vitro function. We therefore propose to use these new vectors in the immunization strategies to be tested in this study.

We hypothesize that the addition of dendritic cells to the prime boost regimen of PANVAC-V followed by PANVAC-F injections will reduce the rate of relapse and improve the survival rate for resected hepatic metastases.

Our primary objective is to choose between two immunization strategies, dendritic cells infected with PANVAC-V followed by dendritic cells infected with PANVAC-F or PANVAC-V followed by PANVAC-F, in terms of which is associated with a better rate of disease-free survival at 2 years following hepatic metastasis resection and adjuvant chemotherapy.

Secondly, we will describe the rate and magnitude of immune response, as determined by the ELISpot assay for each of the two immunization strategies. In those with positive immunologic responses, characterize the response further by tetramer analysis, intracellular cytokine release, cytotoxicity assays, and proteomic analysis.