

PHASE I STUDY OF TUMOR-INFILTRATING LYMPHOCYTES DERIVED FROM IN VIVO HLA-B7 GENE MODIFIED TUMORS IN THE ADOPTIVE IMMUNOTHERAPY OF MELANOMA

1.0 Background and Rationale

1.1 Overview

Cancer is a disease in which certain cells grow uncontrolled by the body's normal self-regulatory mechanisms. Traditional chemotherapy seeks to control cancer by killing rapidly dividing cells or by preventing cells from entering cell cycle and dividing. However, a number of non-malignant cells in the body such as bone marrow cells and intestinal epithelium cells, are also rapidly dividing and hence are highly susceptible to the toxicity of chemotherapy. Doses sufficient to induce remission in the cancer cannot be administered without life-threatening side effects in 50% of the patients and the overall mortality from chemotherapy is 0.5%. A therapeutic approach that selectively kills tumor cells with high efficacy would theoretically be far superior to currently available therapies.

Immunotherapy has shown promise as an approach to the treatment of malignancy. The goal of immunotherapy is to stimulate the immune system to recognize and kill cancer cells. This is achieved either by modifying the tumor cells or the host response causing various lymphocyte populations particularly cytotoxic T lymphocytes, to respond specifically to tumor cell antigens. In fact, cancers such as melanoma, renal cell carcinoma and colon adenocarcinoma are responsive to immunotherapy because the immune system can be induced to recognize tumor-associated and tumor-specific antigens in these cells.

In some instances, the immune system appears to contribute to the surveillance and destruction of neoplastic cells, either by mobilization of cellular or humoral immune effectors. Cellular mediators of antitumor activity include MHC-restricted cytotoxic T cells (CTLs), natural killer (NK) cells (1, 2) and lymphokine-activated killer (LAK) cells (3). Cytolytic T cells which infiltrate tumors have been isolated and characterized (4). These tumor infiltrating lymphocytes (TIL) selectively lyse cells of the tumor from which they were derived (5, 6). Macrophages can also kill neoplastic cells through antibody-dependent mechanisms (7, 8), or by activation induced by substances such as Bacillus Calmette-Guerin (BCG) (9).

Cytokines can also participate in the antitumor response, either by a direct action on cell growth or by activating cellular immunity. The cytostatic effects of tumor necrosis factor- α (TNF- α), interferon α (IFN- α), interferon- γ (IFN- γ) (10) and lymphotoxin (11) can result in neoplastic cell death. Interferon- γ markedly increases class I and II MHC cell surface expression (12, 13) and synergizes with TNF- α in producing this effect (14). Colony stimulating factors such as G-CSF and GM-CSF activate neutrophils and macrophages to lyse tumor cells directly (15), and interleukin-2 (IL-2) activates Leu-19+ NK cells to generate lymphokine activated killer cells (LAK) capable of lysing autologous, syngeneic or allogeneic tumor cells but not normal cells (3, 16, 17). The LAK cells lyse tumor cells without preimmunization or MHC restriction (18). Interleukin-4 (IL-4) also generates LAK cells and acts synergistically with IL-2 in the generation of tumor-specific killer cells (19).

Since most malignancies arise in immunocompetent hosts, it is likely that tumor cells have evolved mechanisms to escape host defenses, perhaps through evolution of successively less immunogenic clones (20). Deficient expression of class I MHC molecules is one of the factors that limits the ability of tumor cells to present antigens to cytotoxic T cells. Freshly isolated cells from naturally occurring tumors frequently lack class I MHC antigen completely or show decreased expression (21-25). Reduced class I MHC expression could also facilitate growth of these tumors when transplanted into syngeneic recipients. Several tumor cell lines which exhibit low levels of class I MHC proteins become less oncogenic when expression vectors encoding the relevant class I MHC antigen are introduced into them (26-30). In some experiments, tumor cells which express a class I MHC gene confer immunity in naive recipients against the parental tumor (27-28).

Recently, molecular genetic interventions have been designed in an attempt to improve the efficacy of immunotherapy. Nabel and colleagues at the University of Michigan laid the groundwork for the molecular genetic approach that enhances the immune response to tumors by *in vivo* gene transfer. This immunotherapeutic approach based on animal model work (29, 30) uses a gene encoding a transplantation antigen, an allogeneic class I major histocompatibility complex (MHC) antigen, HLA-B7. This gene is introduced into human tumors *in vivo* by direct injection of plasmid DNA that expresses the HLA-B7 on the surface of the tumor cells. Expression of allogeneic MHC antigens on tumor cells stimulates immunity against both the transfected cells as well as previously unrecognized antigens present in unmodified tumor cells (13). The introduction of an allogeneic MHC gene directly into tumors *in vivo* has induced partial tumor regressions, as well as specific cytotoxic T cell responses to other antigens (13).

In a preliminary trial with 5 malignant melanoma patients, Nabel has demonstrated: 1) Evidence of gene transfer on biopsy of the injected tumor by measuring plasmid mRNA and cell surface expression, 2) Two patients, where cell lines were established from the tumor, showed an immune response by lysing autologous tumor cells 3) a partial remission which involved cutaneous and visceral disease and 4) no adverse effects from injecting plasmid DNA (complexed with a cationic/neutral lipid combination) into human melanoma (13).

These data suggest that tumor cells modified with the HLA-B7 gene not only stimulate CTLs and potentially other immune system cells to recognize tumors expressing HLA-B7, but they may also provide a stimulus to immune cells to eliminate tumor cells at other sites which express tumor associated antigens in association with the patient's own HLA antigens.

The induction- of tumor reactive cells by the intratumoral inoculation of Allovectin-7 was further evaluated by the adoptive transfer of TIL in a single patient at the University of Michigan. In this case, a patient with multiple subcutaneous nodules had achieved a partial regression of HLA-B7 inoculated tumors. Analysis of TIL retrieved by core biopsies after therapy demonstrated very specific immunological reactivity to autologous tumor in cytolytic assays as well as cytokine release studies. Because of this high degree of specificity and the response to HLA-B7 inoculation, the injected subcutaneous nodules were re-injected and removed 5 days later for generation of TIL cells. The subsequent infusion of TIL along with the concomitant administration of IL-2 resulted in complete regression of all residual disease. This experience suggested that tumor reactive T cells may have been induced by the intratumoral inoculation of HLA-B7. This establishes the rationale for the proposed study.

The discovery that HLA-B7 when expressed on the surface of melanoma tumor cells stimulates an immune response to both the transfected antigen and other tumor antigens inspired the systematic development of Allovectin-7 as a candidate for cancer immunotherapy. Allovectin-7 is the product name. VCL-1005 is the code name for the specific DNA plasmid construct currently under development. The plasmid is formulated with a cationic/neutral lipid complex (DMRIE/DOPE), which in turn complexes with the DNA.

The safety of Allovectin-7 was evaluated in pre-clinical animal studies by i.v. injection into mice and a primate model at a substantial multiple to the anticipated human intratumoral dose. Even high doses of the DNA/lipid complex did not have an adverse effect in the animal studies. Allovectin-7 was then evaluated in a Phase I/II clinical trial at three sites in melanoma, renal cell carcinoma and colon adenocarcinoma. The trial was designed to compare the effect of multiple 10 µg doses (1, 2, and 3 doses) with single doses of 50 and 250 µg. Although the trial is ongoing and the data is still being evaluated, the 10 µg dose appears to be as effective as the 50 and 250 µg dose in transfecting the tumor cells to express HLA-B7. The search for drug-related adverse events has been so far uneventful. Although difficult to measure objectively, the clinical impression from the investigators has been favorable. The three investigators that performed the trial, as well as oncology professionals assembled to review the trial information, agree that further investigation is warranted.

In this protocol, we propose to administer Allovectin-7 in subcutaneous melanoma tumors which will be surgically excised for the generation of TIL [...].