

PART 1: SCIENTIFIC ABSTRACT

Over the last several years there has been increasing interest in immunotherapeutic approaches to melanoma. Researchers are using vectors to deliver melanoma-associated antigens which are known cytotoxic T cell targets and are expressed by a high proportion of melanoma tumors (Rivoltini et al., 1995) (Kawakami et al., 1995). Two Phase I studies were recently conducted at the National Cancer Institute (NCI), in collaboration with Genzyme, in which patients received adenoviral vectors expressing the melanoma-associated antigens MART-1 or gp100. These studies demonstrated that these adenoviral vectors, which are produced by Genzyme, are safe and well tolerated. Additionally, several patients had significant clinical responses.

Genzyme and other researchers also have been working to understand the role that dendritic cells play in provoking a cytotoxic T lymphocyte (CTL) response against autologous tumor cells. Dendritic cells (DCs), a trace population of leukocytes derived from either the myeloid or early lymphoid lineage, are potent antigen-presenting cells (APCs). Several studies have demonstrated the safety of *ex vivo* culture and peptide-pulsing of DCs with tumor-associated antigens followed by infusion into patients (Hsu et al., 1996) (Nestle et al., 1998) (Salgaller et al., 1998). Genzyme pre-clinical data produced the following observations:

- adenoviral vectors can transduce human DCs *ex vivo* with high efficiency,
- transduction of DCs with adenoviral vectors encoding tumor-associated antigens, such as gp100, results in the appropriate presentation of gp100 epitopes in a MHC Class I-restricted manner *in vitro*,
- antigen presenting activity of DCs is longer lasting following transduction by an adenoviral vector encoding gp100 compared to pulsing with a gp100 peptide,
- using a murine B16 melanoma active treatment model (i.e., animals were inoculated with the tumor subcutaneously prior to treatment initiation), administration of DCs transduced with an adenoviral vector encoding a melanoma-associated antigen inhibited tumor growth and prolonged survival,
- the appropriate presentation of multiple antigens is more effective than the presentation of a single antigen.

Genzyme believes that the appropriate presentation of multiple tumor-associated antigens by professional APCs may result in a cellular immune response of such magnitude as to provide clinical benefit in melanoma patients. Further, based on literature demonstrating that low dose recombinant human Interleukin-2 (rhIL-2) therapy promotes a type 1 cytokine profile (e.g., γ -interferon) in vivo (Bernstein et al, 1995), Genzyme hypothesizes that low dose rhIL-2 may enhance this cellular immune response.

In this proposed study, Genzyme believes the investigational product will optimize the appropriate presentation of the MART-1 and gp100 tumor antigens, provoking a CTL response and inhibiting tumor growth. The investigational product, herein referred to as Modified Dendritic Cells, consists of a combination of autologous dendritic cells in which equal parts of the following are pooled:

- DCs transduced with the adenovirus Ad2/MART-1v2 encoding MART-1
- DCs transduced with the adenovirus Ad2/gp100v2 encoding gp100
- DCs pulsed with a Hepatitis B (HepB) virus core antigen peptide

The Modified DCs will be administered by subcutaneous (s.c.) injection into the patient, with or without low dose rhIL-2.

This Phase I/II, open label trial will evaluate the safety, immunogenicity, and potential therapeutic efficacy of human DCs transduced with the Ad2/MART-1v2 and Ad2/gp100v2 viruses. HLA-A2 positive patients with measurable, stage IV melanoma expressing both the gp100 and MART-1 antigens will be sequentially enrolled to receive 6 vaccinations at one of three DC dose levels with or without rhIL-2 by s.c. administration. DCs pulsed with a hepatitis-B virus core antigen peptide, a strong foreign antigen, will be used as a control and administered along with the adenovirus-transduced DCs at each vaccination to assess its ability to evoke an immune response.

A maximum of 36 patients will be treated on this study. Patients will be treated for a total of six 21 day treatment cycles (total of 18 weeks). The Modified DC vaccine will be administered on Day 1 of each treatment cycle. Patients assigned to receive rhIL-2 will receive a s.c. injection on days 4 through 19 of each of the six 21 day treatment cycles. Patients will return 3 weeks following their 6th vaccination for Study Completion tests and procedures. A post completion

Follow-Up will be performed 3 months following Study Completion. Patients will then be followed every six months until the time of death to evaluate overall survival (OS).

Primary endpoints are establishing 1) a safety profile, 2) dose limiting toxicity (DLT), and 3) a maximum tolerated dose (MTD). Secondary endpoints are observing and evaluating 1) a cellular immune response and 2) tumor regression.