

Appendix 1

Scientific Abstract

Over the last several years there has been increasing interest in immunotherapeutic approaches to melanoma. A distinguishing feature of melanoma is the identification of several tumor-associated antigens (Robbins and Kawakami, 1996). Early immunotherapy trials with IL-2 demonstrated that regressing lesions exhibited infiltration by T cells. Purification of T lymphocytes from tumor specimens yielded specific cellular reagents known as tumor infiltrating lymphocytes (TIL). Boon and associates succeeded in cloning MAGE-1 (melanoma antigen-1) from a melanoma cDNA library by virtue of its recognition by a cytotoxic T lymphocyte (CTL) clone (Van der Bruggen et al., 1991). Further efforts identified a nine amino acid fragment of MAGE-1 that is restricted by HLA-A1. Similar approaches using CTL and TIL to screen cDNA libraries have yielded three important lineage-restricted, melanocyte-specific antigens: tyrosinase, gp100/Pmel17 (Bakker et al, 1994) (Kawakami et al., 1994), and Melan-A/MART-1 (Coulie et al., 1994) (Kawakami et al., 1994). Dominant epitopes contained within 9-10 amino acid fragments from each of the three antigens have been defined (Robbins and Kawakami, 1996).

The tumor antigen gp100 has been particularly well studied. This is a 661 amino acid transmembrane glycoprotein expressed uniformly in cultured melanocytes and in melanoma cell lines, but not in non-melanoma tumors. gp100 expression is established by the diagnostic monoclonal antibody HMB-45. Five epitopes presented by the class I allele, HLA-A2.1, have been identified and three of these peptides (G209, G280, G457) bind with low to intermediate affinity to HLA-A2 (Kawakami et al., 1995). The remaining peptides bind with higher affinity; however, they are not recognized by most TILs with demonstrated reactivity to gp100. For example, in one study (Kawakami et al., 2000) of 35 HLA-A2+ restricted TILs from melanoma patients, thirteen recognized gp100 while twenty-two recognized Melan-A/MART-1. In contrast, a high affinity epitope (G476) was recognized by only one of the eight TIL lines tested (Kawakami et al., 1995).

The factors affecting binding of these epitopes to the human leukocyte antigen (HLA) complex have been studied in detail. Parker et al. (1994) and Sette (Sette and Sidney, 1999) have independently shown that peptide binding affinity is influenced principally by the rate of dissociation of the HLA/peptide/ β 2-microglobulin complex. Amino acids at peptide position 2 (P2) and 9 (P9) are primary anchor residues which serve to form hydrogen bonds in the major binding groove (B and F pockets) of the HLA-A2.1 molecule. Residues at P1 and P3 are secondary anchor residues that also influence peptide stability for HLA-A2.1. Modification of these residues to generate an anchor modified peptide (AMP) is predicted to alter the binding affinity of the peptide for HLA-A2.1, but not to affect T cell receptor engagement (Garboczi et al., 1996). Thus, substitution of certain residues offers the prospect of improving the immunogenicity of gp100 epitopes without interfering with T cell recognition of the native antigen present

on tumor cells. Recent trials have investigated immunization against multiple peptides from the melanoma associated antigens Melan-A/MART-1, gp100, and tyrosinase as well as the use of modified peptides designed to increase binding to MHC molecules (Kawakami et al., 1995) (Parker et al, 1994) (Sette and Sidney, 1999). They have demonstrated that the native epitopes or anchor-modified peptides elicit immune responses.

Genzyme has pursued an alternative strategy, developing replication-deficient modified adenoviral constructs which express either the melanoma-associated antigen Melan-A/MART-1 or gp100. The general safety of these adenoviral vectors and their ability to elicit an immune response have been demonstrated in extensive pre-clinical *in vitro*, *in vivo*, and toxicology studies and in several human clinical trials. Two Phase I studies were conducted at the National Cancer Institute (NCI), in collaboration with Genzyme, in which patients received first generation adenoviral vectors Ad2/MART-1 (OBA # 9610-163) or Ad2/gp100 (OBA # 9604-151) (Rosenberg et al., 1998). These vectors were well tolerated when administered individually to patients.

More recently, Genzyme has sponsored two Phase I/II studies using second generation modified adenoviral vectors (Ad2/MART-1v2 and Ad2/gp100v2) administered in combination. In the first, both vectors are transfected *ex vivo* into autologous dendritic cells (OBA # 9901-281; IND 8055) and delivered to patients as a series of subcutaneous vaccinations. This Phase I/II, dose escalation, open label *ex vivo* trial was designed to evaluate the safety, immunogenicity, and clinical efficacy of Ad2/MART-1v2 and Ad2/gp100v2 viruses administered in this manner. To date, this study has demonstrated that these adenoviral vectors administered via this vaccination strategy are well tolerated.

In the second study, Genzyme is evaluating the same vectors (Ad2/MART-1v2 and Ad2/gp100v2) administered directly to patients with resected stage II-IV malignant melanoma, without evidence of disease, via intradermal injection. This Phase I/II, dose escalation, open label *in vivo* trial is designed to administer Ad2/MART-1v2 and Ad2/gp100v2 viruses either sequentially or concurrently for up to 6 vaccinations. Thirty-nine patients have been enrolled in this study.

The primary objective of this study is to evaluate the safety, dose limiting toxicity (DLT), and maximum tolerated dose (MTD). Secondary endpoints are to:

- assess the dose-response changes in the frequency of Melan-A/MART-1 and gp100 reactive T cells (CD4+ and CD8+) in response to multiple ID injections of each antigen encoded in an adenoviral vector.
- assess the body's ability to mount a T cell response to one melanoma antigen encoded in an adenoviral vector following 3 prior treatments with the adenoviral vector encoding the other melanoma antigen.
- assess the effect of concomitant vaccinations with Ad2/MART-1v2 and Ad2/gp100v2 on the potency of the T cell response to each (to assess antigen competition).

- assess time to disease recurrence and overall survival in evaluable patients (patients who completed at least three vaccinations) for up to 5 years (extended follow up) following last vaccination.

This clinical design outlines a Phase I/II *in vivo* dose ranging study that Genzyme believes will further the understanding of these modified serotype 2 adenoviral vectors when administered intradermally and begin to establish an optimal dose and treatment regimen for future clinical studies.