

Phase II Study of Metastatic Melanoma Using Lymphodepleting Conditioning Followed by Infusion of Anti-MART-1 F5 TCR-Gene Engineered Lymphocyte

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Scientific Abstract:

The low response rate in our prior MART-1 TCR gene transfer protocol led us to attempt to identify MART-1 reactive TCR with higher avidity than the MART-1 F4 TCR used in the prior gene therapy clinical trial. The highest avidity T cell clone identified in this analysis was the DMF5 clone. In this overnight co-culture assay measuring interferon-gamma release, the DMF5 clone had the highest recognition of T2 cells pulsed with the native MART-1 peptide. This clone secreted 17,161pgm/ml of interferon gamma compared to 1,987pgm/ml secreted by the DMF4 clone used in prior trials. In addition, the recognition of the mel 526 and the mel 624 A2⁺ MART-1⁺ cell lines was also higher utilizing the DMF5 clone than the DMF4 clone (5806 and 10,865 pgm/ml compared to 1780 and 2397 pgm/ml). Neither clone had significant recognition of the control A2⁻ MART-1⁺ mel 888 line.

The DMF5 TCR, when electroporated into the same activated donor CD8⁺ PBMC, was the most reactive TCR when assessed against the MART-1 peptide pulsed onto T2 cells as well as to A2⁺ MART-1⁺ melanoma cell lines. The reactivity of the T cell receptors recapitulated the reactivity seen in the original clones. We next checked lysis in a four hour chromium release assay, and again the DMF5 TCR outperformed all of the other TCR and was approximately 10 times more avid compared to the DMF4 TCR used in our prior gene therapy clinical trial.

In summary, the new anti-MART-1 F5 TCR appears to have excellent reactivity against both the MART-1 peptide as well as melanoma cell lines. We have thus developed a GMP quality retroviral vector encoding the alpha and beta chains of the MART-1 F5 TCR, which recognized the MART-1:27-35 epitope, for use in this clinical trial. This TCR is at least ten times more reactive with melanoma cells than the MART-1 TCR that mediated tumor regression in three patients with metastatic melanoma. Based on these observations the following clinical protocol is proposed.

Patients with metastatic melanoma who are HLA-A2 positive will receive a nonmyeloablative but lymphocyte depleting preparative regimen consisting of cyclophosphamide and fludarabine, and then will be treated by the adoptive transfer of autologous peripheral blood lymphocytes that have been genetically engineered to be reactive with melanoma tumor antigen MART-1. Following adoptive cell transfer, all patients receive high-dose IL-2. The primary objective will be to determine whether patients with metastatic melanoma who are HLA-A2+ and who receive PBL transduced with the anti-MART-1 F5 TCR plus HD IL-2 are able to produce modest numbers of clinical responses. Additional objectives include determining the *in vivo* survival of TCR gene-engineered cells, and the toxicity profile of this treatment regimen.