**SUPPLEMENTAL METHODS**

**Manufacture of NY-ESO-1 TCR transgenic lymphocytes**

The clinical grade retroviral vector expressing NY-ESO-1 TCR was manufactured at the Indiana University Viral Production Facility based on a master cell bank that was provided by Drs. Steven A. Rosenberg and Paul F. Robbins from the Surgery Branch, National Cancer Institute (NCI). The 1G4 high- affinity TCR pair specific for NY-ESO-1157-165 presented by HLA-A\*0201 was originally cloned from a patient with metastatic melanoma (1). The TCR was engineered for higher specificity for NY-ESO-1 by modifying the CDR of the alpha chain of the TCR, leading to the current version of the 1G4 TCR used in this study (2). This MSGV1-A2aB-1G4A-LY3H10 TCR contains two amino acid substitutions in the third complementarity determining region of the native 1G4-TCR alpha chain that conferred an increased ability of CD4+ and CD8+ T cells to recognize the HLA-A\*0201/NY-ESO-1157-165 complex while maintaining antigen specificity (2). This vector was previously used in a clinical trial run by the NCI (3). Moreover, there have been multiple clinical trials using adoptively transferred T cells specific for NY-ESO-1 in the setting of advanced malignancies (e.g. NCT00670748, NCT01343043, NCT00871481). Both the recent NCI clinical trial and this trial at UCLA are based on the same murine stem cell gamma retroviral vector (MSGV) expressing the NY-ESO-1 TCR used to transduce leukapheresis-derived PBMCs and re-infused to patients after a non-myeloablative but lymphodepleting chemotherapy conditioning regimen.

A non-mobilized leukapheresis processing two plasma volumes from study patients was processed in the UCLA Human Gene and Cell Therapy Facility. PBMC were isolated by Ficoll gradient centrifugation and cultured in AIM V media (Gibco, Invitrogen, Chicago, IL) supplemented with 5% human AB serum (Omega Scientific, Tarzana, CA) in the presence of 50 ng/ml anti-CD3 (OKT3, Miltenyi Biotec, Auburn, CA) and 300 IU/mL IL-2 in order to stimulate T-cell growth to prepare for viral vector transduction. Activated PBMC were then infected by the clinical grade MSGV1-A2aB-1G4A-LY3H10 retroviral vector supernatant using retronectin-coated plates (Retronectin, Takara, Otsu, Shiga, Japan) for two consecutive days, maintained in culture for 4 days from the start of transduction in IL-2, and then infused fresh into trial subjects. Aliquots of these cells were used to fulfill the lot release criteria.

In-process and final product testing included Gram stain, fungal stain, sterility culture for bacteria and fungus, mycoplasma assay (MycoAlert assay, Lonza, Walkersville, MD), and endotoxin assay (Endosafe PTS system, Charles River, Charleston, SC). Transduction efficiency was tested with MHC dextramer analysis for NY-ESO-1 (Immudex, Copenhagen, Denmark) gated in CD3 (BD Bioscience, Franklin Lakes, NJ) positive lymphocytes. The potency of the NY-ESO-1 TCR transgenic cells was assessed using a co-culture system to detect NY-ESO-1- specific IFN-γ production by ELISA. Briefly, transduced and control non-transduced T cells were co-cultured with M257A2.1 melanoma cell line positive for NY-ESO-1 and HLA-A\*0201. M257, NY-ESO-1 positive, HLA-A\*0201 negative cell line, was used as a control. The IFN- γ cytokine levels were quantified in the supernatants collected after 24h of co-culture by human IFN-γ ELISA (eBioscience, San Diego, CA).

**Manufacture of NY-ESO-1 peptide-pulsed dendritic cells**

Autologous DC were differentiated from adherent peripheral blood monocytes in a one-week *in vitro* culture in media containing 5% heat-inactivated autologous plasma supplemented with GM-CSF and IL-4 as previously described (4-6). The functionality of IL-4 and GM-CSF was measured in healthy PBMC by phosphor activation of STAT-6 and STAT-5, respectively, by phosphoflow, as previously described (7). DC were pulsed with the NY-ESO-1157-165 anchor-modified immunodominant peptide (Biosynthesis Lewisville, TX) in the HLA-A2\*0201 haplotype and administered intradermally. Three preparations of freshly manufactured and lot release tested DC vaccines were administered starting on day +1, day +14, and day +30. In-process and final product testing included Gram stain, fungal stain, sterility culture for bacteria and fungus, mycoplasma assay and endotoxin assay.

**Detection of Replication Competent Retrovirus (RCR)**

A GALV quantitative PCR (qPCR) assay, developed and optimized at the IU VPF (8), was used to rule out the presence of replication competent retrovirus (RCR). This assay was performed retrospectively in PBMC processed from post-infusion peripheral blood samples at 3, 6, 12 months. Annual samples were archived thereafter.

**Serum cytokine analysis**

Serum cytokine levels over the course of the study were assessed using the Luminex xMAP® Immunoassay, which was performed in the UCLA Immune Assessment Core. Human 38-plex magnetic cytokine/chemokine (EMD Millipore) were used per manufacturer's instructions. Briefly, 25 μL undiluted plasma samples were mixed with 25 μL magnetic beads, and allowed to incubate overnight at 4°C while shaking. After washing the plates twice with wash buffer in a Biotek ELx405 washer, 25 μL of biotinylated detection antibody was added and incubated for 1 hour at room temperature. 25 μL streptavidin-phycoerythrin conjugate was then added to the reaction mixture and incubated for another 30 minutes at room temperature. Following two washes, beads were resuspended in sheath fluid, and fluorescence was quantified using a Luminex 200™ instrument.

**Mass Cytometry Data Analysis**

Cytometry data files were manually gated in FlowJo for stability of time, cells with no beads (Ir193+/ Ce140-), cleanup (double positive for DNA), singlets (Ir193+), live (195Pt-/CD45+), CD45+/CD33-/CD56-/CD19-/CD3+/TCR-Vβ13.1+ cells. These events were then exported for analysis utilizing the cytofkit software package (9), where they were arcsinh transformed with a coefficient of 5. Individual sample data were subsampled to 5,000 events. We set this cut-off based on the reasoning that 5,000 events would be sufficient for phenotypic clustering and dimension reduction analyses in the context of many total events. t-Distributed Stochastic Neighbor Embedding (t-SNE) dimension reduction and RPhenoGraph clustering analyses were performed using cytofkit using all T cell-specific markers not utilized for manually gating. For t-SNE plots overlaid with expression of individual parameters, signal intensity is displayed in arcsinh transformed values. For generation of heatmap displays, median marker expression was normalized to a mean of 0 and standard deviation of 1. Clusters of less than 0.5% average frequency (of the input population; i.e., transgenic T cells) were not displayed in frequency plots or heatmaps to increase the clarity of the figures, and were excluded from further analysis. Based on this metric, two low frequency transgenic T cell subsets were not displayed in the heatmaps or frequency plots. Of note, the marker expression patterns of these subsets suggested they were likely technical artifacts. Remaining clusters were then manually annotated based on varying intensities of the different markers measured, as previously described (10, 11).

For analysis of the dendritic cell subsets, manual gating of individual PBMC samples was performed with FlowJo to gate for single, live, CD45+, lineage-negative (CD3-/CD19-/CD56-/CD68-) cells, which were further classified as CD14+DCs (CD14+/HLA-DR+/CD11c+), myeloid DCs (mDCs; CD14-/HLA-DR+/CD11c+/CD123-), and plasmacytoid DCs (pDCs; CD14-/HLA-DR+/CD11c-/CD123+) (12).

**SUPPLEMENTAL REFERENCES**

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