Combination immunotherapy using adoptive T-cell transfer and tumor antigen vaccination based on hTERT and survivin following ASCT for myeloma

Aaron P. Rapoport, MD1*, Nicole A. Aqui, MD2*, Edward A. Stadtmauer, MD3*, Dan T. Vogl, MD3, Hong-Bin Fang, PhD1, Ling Cai, PhD1, Stephen Janofsky, BS2, Anne Chew, PhD3, Jan Storek, MD, PhD4, Gorgun Akpek, MD, MS1, Ashraf Badros, MD1, Saul Yanovich, MD1, Ming T. Tan, PhD1*, Elizabeth Veloso, JD3, Marcela F. Pasetti, PhD5, Alan Cross, MD1,5 Sunita Philip, MPH1, Heather Murphy3, Rita Bhagat, RN3, Zhaohui Zheng3, Todd Milliron, RN1, Julio Cotte3, Andrea Cannon3, Bruce L. Levine, PhD3*, Robert H. Vonderheide, MD, DPhil3*, and Carl H. June, MD3*

1University of Maryland, Marlene and Stewart Greenebaum Cancer Center, Baltimore, MD; 2Dept. of Pathology, Univ. of Pennsylvania, Philadelphia, PA; 3Abramson Cancer Center of the University of Pennsylvania, Philadelphia, PA; 4The University of Calgary, Calgary, AB; 5The University of Maryland Center for Vaccine Development, Baltimore, MD. *These authors contributed equally to the work. RHV and CHJ shared senior authorship.

Running Head: Vaccine responses after adoptive T-cell transfers in myeloma

Corresponding Author:
Aaron P. Rapoport MD
University of Maryland Greenebaum Cancer Center
University of Maryland School of Medicine and Department of Medicine
22 South Greene Street
Baltimore, MD 21201
P:  410-328-1230
F:  410-328-1975
E: Arapoport@umm.edu
Abstract

In a phase I/II 2-arm trial, 54 pts with myeloma received autografts followed by ex-vivo anti-CD3/anti-CD28 costimulated autologous T cells (ex-T) at day +2 posttransplant. HLA-A2+ study patients (Arm A, n = 28) also received pretransplant and posttransplant pneumococcal conjugate vaccine (PCV) immunizations and a multipeptide tumor antigen vaccine derived from the human telomerase reverse transcriptase (hTERT) and the anti-apoptotic protein survivin. HLA-A2 negative patients (Arm B, n = 26) received the PCV only. Patients exhibited robust T-cell recoveries by day +14 with supraphysiologic T-cell counts accompanied by a sustained reduction in regulatory T cells. The median EFS for all patients is 20 months [95% CI = 14.6 – 24.7 mos]; the projected 3-year OS is 83%. A subset of patients in Arm A (36%) developed immune responses to the tumor antigen vaccine by tetramer assays but this cohort did not exhibit better EFS. Higher posttransplant CD4+ T-cells counts and a lower percentage of FOXP3+ T-cells (Tregs) were associated with improved EFS. Patients exhibited accelerated polyclonal immunoglobulin recovery compared to patients without T cell transfers. Adoptive transfer of tumor antigen vaccine-primed and costimulated T-cells leads to augmented and accelerated cellular and humoral immune reconstitution, including anti-tumor immunity, after ASCT for myeloma. This study was registered at http://clinicaltrials.gov as NCT00499577.
Introduction

Autologous stem cell transplants (ASCT) for myeloma leads to complete responses and extended event-free survival (EFS) in about 20-40% of patients (1-3). However, even after tandem transplants, the 10-year EFS is < 20% and the frequency of cure is < 10% (4). Allogeneic stem cell transplants may increase the cure rate through a T-cell-mediated “graft-vs-tumor” effect, but at the expense of increased treatment-related morbidity and mortality from graft-vs-host disease (GVHD) and infection (5-8). Thus, novel strategies are needed to augment the efficacy of ASCT for myeloma and other hematologic malignancies. Efforts to improve upon the results of autotransplantation for myeloma and other hematological malignancies include the use of post-transplant consolidation chemotherapy and/or maintenance therapy based on targeted agents such as thalidomide and lenalidomide (9-12). These agents may increase the level of response and the time to progression but their impact on long-term survival and cure is unknown. Higher lymphocyte counts may predict better disease-free and overall survival for myeloma both early after autotransplantation and at diagnosis (13,14). Similar associations between higher lymphocyte counts and improved outcome have also been reported for lymphoma and myelodysplastic syndromes (15-18). Furthermore, the absolute lymphocyte count at the time of first relapse from large cell lymphoma predicted subsequent progression-free and overall survival (19).
Our objective has been to develop a strategy for inducing an effective antitumor immune response during the post-transplant period in order to control or eliminate residual disease. In theory, the post-transplant phase should be highly amenable to the application of immunotherapy due to a lower tumor burden. However, after high-dose therapy, the immune system is characterized by immune cell depletion and impaired function which may last for years (20,21).

We hypothesized that enforced T-cell recovery by adoptive transfer of ex vivo costimulated autologous T cells might improve event-free or overall survival after autotransplantation for hematologic neoplasms through augmentation or restoration of host anti-tumor immunity. In addition, enhanced numeric and functional recovery of T cells might provide a platform for post-transplant tumor vaccine immunotherapy. In our studies, ex-vivo costimulation involved coculture of autologous T cells with paramagnetic beads that deliver CD3 and CD28 signals designed to reverse T cell anergy (22–26). Based on this hypothesis, a randomized clinical trial was performed in which 54 patients with myeloma received costimulated autologous T cells after autotransplantation, along with immunizations using a 7-valent pneumococcal conjugate vaccine (PCV, Prevnar®) (27). One of the key observations from this earlier study was that transfers of ~10^10 ex-vivo costimulated autologous T cells on day +12 post-transplant led to significantly higher CD4 and CD8 T-cell counts at day +42 post-transplant. In addition, combined T cell/vaccine immunotherapy could induce vaccine-specific T cell and antibody immune responses early after transplant,
especially when patients were immunized prior to T-cell collection and ex-vivo expansion. The latter principle was recently reinforced by a parallel randomized study which showed that seroconversion to an influenza vaccine required pre-transplant in-vivo priming of autologous T cells prior to collection, expansion and adoptive transfer (28).

Using the strategy of combining pre- and post-transplant immunizations with early infusions of vaccine-primed and ex vivo costimulated T cells, a new trial was developed with two major objectives: i) to investigate the clinical effects of transferring T cells at day +2 post-transplant, which is 10 days earlier than in our previous study; and ii) to investigate whether the combination strategy could generate immune responses to a myeloma tumor antigen vaccine. The rationale for infusing cells at day +2 was to further exploit the stimulatory cytokine milieu induced by severe lymphopenia (e.g. free IL-15, IL-7) that may drive homeostatic lymphocyte expansion. In addition, earlier and more robust T cell recovery might help to promote immune responses to a tumor antigen/self-antigen vaccine, a significantly more challenging task than generating immune responses to a microbial vaccine. In the present study, 54 adults who were autografted for myeloma, received up to $5 \times 10^{10}$ ($\sim 10^9$/kg) T cells at day +2, along with pre and post-transplant immunizations with PCV. Also, patients who were HLA A2+ received a multipeptide tumor antigen vaccine based on peptides derived from hTERT and survivin, two “universal” tumor antigens that are often over-expressed in myeloma and may have prognostic relevance (29-32). Naturally
occurring CD8+ T-cells recognizing epitopes from these antigens have been described in myeloma patients (33, 34).

In our initial description of the day +2 T-cell transfer trial, we reported that the T-cell infusion induced robust lymphocyte recovery after autotransplantation with median CD3, CD4 and CD8 counts at day +14 post-transplant of 4198, 1545 and 2858 cells/μl, respectively (35). In addition, 16% of patients developed a T-cell “engraftment syndrome” with features of grade 1-3 graft-vs-host disease (GVHD). These lymphocyte levels were dramatically higher than were observed in our first trial after day +12 transfers, and the occurrence of clinically significant auto-GVHD was also new, suggesting an important schedule-dependent effect of early T-cell infusions on immune recovery. Here, we report the clinical outcomes of the 54 patients in this post-transplant immunotherapy trial as well as the immune responses to the hTERT/survivin multipeptide tumor antigen vaccine. By analysis of the biologic correlates of immune function, we also identified predictive biomarkers of event-free survival.

**Methods**

**Patients**

Study participants were at least 18 years old with symptomatic multiple myeloma. Patients received first-line therapy using at least 3 cycles of standard regimens (typically bortezomib, thalidomide or lenalidomide plus dexamethasone) by their referring oncologist. For enrollment, patients were required to have measurable disease (based on serum/urine electrophoresis studies or serum free light chain
studies); patients in complete remission (CR) were not eligible unless they had high-risk cytogenetic features (e.g., chromosome 13 or 17 deletions, 4;14 or 14;16 translocations, or complex karyotypes). All patients had adequate organ function as defined by serum creatinine levels < 3.0 mg/dl, left ventricular ejection fraction > 45% and lung function parameters > 40% predicted. All participants gave written informed consent in accordance with the Declaration of Helsinki; study approval was obtained from the Institutional Review Boards (IRBs) of the University of Maryland and the University of Pennsylvania and the FDA.

**Trial design**

The design of the trial is depicted in Figure 1. Briefly, patients were first tested for HLA A2 status: HLA A2+ patients (including any A2 allele) were assigned to ARM A and A2- patients were assigned to ARM B. Patients who were A2+ (ARM A) were immunized with 100 $\mu$g of each of the following peptides: i) hTERT I540 peptide [ILAKFLHWL] (36); ii) hTERT R572Y peptide [YLFFYRKSV] (37); iii) hTERT D988Y peptide [YLQVNSLQTV] (37); iv) survivin Sur1M2 peptide [LMLGEFLKL] (38); and v) CMV control peptide N495 [NLVPMVATV] (39). Immunizations consisted of aqueous solutions of peptide (each peptide >92% pure and good manufacturing grade; Merck Biosciences AG, Laufelfingen, Switzerland) emulsified in the adjuvant Montanide ISA 51 (Seppic Inc., Paris, France) and delivered subcutaneously in the thigh (right thigh, hTERT I540, hTERT R572Y and hTERT D988Y peptide emulsion; left thigh, Sur1M2 and CMV N495 peptide emulsion). Sargramostim (clinical grade GM-CSF; Berlex
Laboratories, Inc., Richmond CA) was also given subcutaneously at each of the two peptide-injection sites (70 μg per vaccination). Patients in ARM A also received an intramuscular (IM) injection of the pneumococcal conjugate vaccine (PCV, Prevnar®, Wyeth) into the non-dominant deltoid. HLA A2- patients (ARM B), received the PCV immunization only along with one injection of GM-CSF (70 μg) into each thigh.

About 10 days after the first set of immunizations all patients had steady-state apheresis to collect approximately 1 x 10^8 mononuclear cells per kilogram body weight. Patients then proceeded to stem cell mobilization using one of several regimens (most commonly cyclophosphamide at a dose of 1.5 – 4.5 g/m²) followed by subcutaneous injections of G-CSF (10 μg/kg). High-dose therapy was melphalan (200 mg/m²) followed by infusions of autologous stem cells (> 2 x 10^6 CD34+ cells/kg body weight) at day 0. Costimulated autologous T cells were infused on day +2. Supportive care measures included antibiotic prophylaxis and administration of G-CSF starting on day +5. Three additional sets of immunizations were given at days +14, +42, and +90 using the same ARM-specific vaccine composition and procedures that were used for the first immunization.

**T cell expansion and adoptive transfer**

The mononuclear cell apheresis product was monocyte-depleted by counter flow centrifugal elutriation (CaridianBCT Elutra™ Cell Separation System) since
monocytes may inhibit lymphocyte proliferation. Monocyte-depleted mononuclear cells were cryopreserved until 9-12 days before the scheduled re-infusion date (day +2 post-transplant). Cells were thawed and co-cultured with Dynal paramagnetic M-450 beads (DynalInvitrogen, Oslo, Norway) coated with anti-CD3 (OKT3, Ortho Biotech, Bridgewater, NJ)/anti-CD28 (clone 9.3) at a ratio of 3 beads per cell first in a Baxter Lifecell flask, and subsequently in the WAVE® bioreactor system (GE Healthcare Biosciences, Piscataway, NJ) (40). Additional details of T-cell expansion and harvesting are described elsewhere (35) and in Supplemental Methods. The harvested cells were transported by courier from the cell production facility to the patient and infused on the same day (day +2 of transplant). The cells were infused over 20–60 minutes without a leukocyte filter, after premedication with acetaminophen and diphenhydramine. The target number of costimulated T cells for infusion was ~ 5 x 10^{10}, which was 5-fold greater than that used in our previous trial. Of the 54 expansions, one product failed to meet release criteria due to bacterial contamination; after a second expansion, cells were successfully infused at day +16.

**Immunoassays and phenotyping**

In vitro stimulation. In vitro peptide stimulation of PBMCs to assess immune response was performed as previously described (41). Peptide/MHC class I tetramer analysis was performed using soluble peptide/HLA-A2 tetramers purchased from Beckman Coulter Immunomics (San Diego, CA), as previously described (41).
Proliferation assays. T cell responses to the CRM197 and hTERT/survivin peptides (Merck Biosciences AG, Laufelfingen, Switzerland) were measured by CFSE staining of responder T cells as previously described (27). Briefly, following six days in culture with media alone (negative control), staphylococcal enterotoxin B (SEB; positive control) (100ng/ml, EMD Chemicals, Gibbstown, NJ), CRM197 (20μg/ml, List Biological Laboratories, Campbell, CA), hTERT/survivin peptide mix (5μg/ml), or irrelevant peptide (Tax, 5μg/ml), the percentage of CFSE\textsuperscript{dim} cells after gating on live CD8\textsuperscript{+} or CD4\textsuperscript{+} T-cells was measured by flow cytometry.

Flow cytometry reagents and other materials. Fluorochrome-conjugated mAb used were: PE-Cy7-CD3 clone SK7, APC-C7-CD4 clone RPA-T4, PerCP-CD4 clone SK3, APC-Cy7-CD8 clone SK1, PerCP-CD14 clone MφP9, FITC-CD16 clone 3G8, APC-CD19 clone HIB19, APC-CD25 clone MA-251, FITC-CD27 clone M-T271, APC-CD28 clone CD28.2, APC-CD45RA clone HI100, FITC-CD45RO clone UCHL1, PE-CD56 clone B159, APC-CD69 clone FN50, PE-CD127 clone hIL-7R-M21, PE-HLA-DR clone L243, PE-CCR7 clone 3D12; (BD Biosciences, San Jose, CA); APC-CD8 (clone B9.11) (Beckman Coulter, Brea, CA); Alexa Fluor 488-FOXP3 clone 259D (Biolegend, San Diego, CA). Flow cytometry was performed using a custom FACSCanto cytometer and FACSDiva software (BD Biosciences Immunocytochemistry Systems, San Jose, CA). Data were analyzed using FlowJo software (Tree Star Inc., Ashland, OR). Intracellular staining for FOXP3 was performed using a fixation/permeabilization kit (eBioscience).
Antibody responses to the Prevnar® (pneumococcal conjugate vaccine) were assessed by ELISA binding assays for serotypes 6B, 14, 19F, 23F as previously described (27). Titers were reported in µg/mL using the reference standard serum 89SF.

**Statistical methods**

The Chi-square test (for categorical variables) and Wilcoxon test (for continuous variables) were used to compare patient characteristics between the two study arms. Pearson’s correlation coefficient was used for correlation analyses. Linear mixed models were used to analyze the effects of infused T-cell # on post-transplant T-cell recovery. Event-free survival (EFS) and overall survival (OS) were calculated from the date of transplant (day 0) according to the Kaplan-Meier product limit method. The log-rank test and Cox proportional hazards models were used to analyze the relationships of patient characteristics and immune parameters with EFS. The observation times were day 14, day 60, day 100, and day 180 as specified per protocol. Since the observation times were slightly different between the current trial and previous trials and some observations were missing, to compare patient immune responses between the trials, the generalized t-test was used based on the expectation-maximization algorithm (42). A p-value of <.05 was used for statistical significance.
Results

Patient characteristics

From 12/2006 - 2/2009, 56 patients were enrolled at the two participating institutions. After the initial priming immunization, two patients (both ARM A) did not mobilize adequate numbers of stem cells for transplant and did not remain on study. Table 1 shows the major clinical characteristics for the 54 patients (ARM A = 28, ARM B = 26) who proceeded to transplantation (see Supplemental Table S1 for more details). The mean age of study participants was 55 years with 52% males and 39% African-Americans. This cohort of higher risk patients had a mean marrow plasmacytosis of 27% (range, 1-95%) at enrollment despite extensive prior treatment with thalidomide/lenalidomide and/or bortezomib and 39% had abnormal cytogenetic studies (of which 15/21 or 71% had complex or other high-risk abnormalities). In addition, 50% of patients had at least two courses of prior therapy. The two arms were well balanced except that 18% of ARM A patients were African-American vs 62% of ARM B patients (p = 0.003) reflecting the known lower frequency of HLA-A2 alleles in African-Americans compared to other ethnicities.

Toxicities from T-cell infusions and immunizations

Table 2 shows the early and late toxicities that were possibly, probably or definitely related to the T-cell transfers. The most common early effects were mild to moderate chills and rigors (57% of all patients), low-grade fevers and mild nausea/vomiting. Arms A and B had similar patterns of early infusion-related
toxicities except for an excess of mild nausea/vomiting in Arm B patients (p = 0.02). Late effects of T-cell transfers included grade I/II rashes in more than 85% of the entire cohort of patients. These rashes primarily developed on the face, neck, upper chest and upper back. Late effects following T-cell infusions were also similar between Arrows A and B except for a significant excess of diarrhea (p=0.001) and anorexia/nausea (p=0.04) in Arm A. Table 3 depicts the injection site and constitutional reactions after hTERT/survivin multipeptide vaccine that were reported by Arm A patients in self-assessment symptom diaries. All reactions were mild or moderate in degree, with injection site pain, induration/redness, loss of energy and myalgias being the most common. There were no discernible trends in reactivity patterns over time, except for a significant increase in the frequency of injection site induration or redness zones of >50 mm post-transplant, likely due to development of delayed-type hypersensitivity (DTH) reactions in a proportion of vaccinated patients. In patient MD-012, the day 90 immunization with the hTERT/survivin multipeptide vaccine, elicited a 50 mm zone of redness and induration at the previous, day 42 injection site.

Event-free and overall survival
The 3-year projected overall survival (OS) for the entire cohort is 83% with no difference between the two Arrows (Fig. 2A). A total of 7 patients have died, all from relapse of myeloma: 4 in Arm A and 3 in Arm B. Clinical responses (defined as PR, VGPR, nCR and CR) at days 100 (n=40) and 180 (n=33) were predictive of better EFS (p=0.005 and <0.0001, respectively). Figure 2B shows the Kaplan-
Meier EFS for the entire cohort of 54 patients (black line) as well as the EFS curves for Arm A (red line) and Arm B (blue line). The median EFS for the entire cohort is 20 months (95% CI = 14.6 – 24.7 months). The EFS for Arm A and Arm B patients are significantly different (p = 0.0068). The two – year projected EFS is approximately 65% for Arm B patients (no hTERT/survivin vaccine) and 25% for Arm A patients. The major contributor to this difference is likely to be the differential usage of thalidomide maintenance between the two arms which was an optional intervention specified to begin on day 180 per protocol. Among the 46 patients whose EFS exceeded 180 days when the thalidomide maintenance could be started, 17/22 patients in Arm B received thalidomide versus only 7/24 patients in Arm A (p = 0.003). For the cohort of patients who remained event-free at 180 days post-transplant, Figure 2C shows the difference in EFS between those who received thalidomide maintenance (blue line) and those who did not (red line) (p = 0.0089). After adjustment for the effect of thalidomide maintenance using a stratified log-rank test, the p value for the difference in EFS between Arms A and B becomes non-significant (p = 0.21).

Immune responses to the hTERT/survivin tumor antigen vaccine

A major objective of the study was to determine whether adoptive transfer of vaccine-primed autologous T-cells could elicit early immune responses to the hTERT/survivin multipeptide vaccine. T-cell responses to the hTERT/survivin tumor antigens were assessed by tetramer analysis at serial timepoints following in vitro stimulation of mononuclear cells. The chief immunologic endpoint was
the frequency of positive tetramer responses, which was defined as both a staining level of > 0.1% and a > 3-fold increase versus the enrollment/baseline timepoint. Figure 3A shows the tetramer responses for the 10 patients (36%) who exhibited positive responses using a scale of 0–1% while Figure 3B shows the full spectrum of responses. Figure 4A is a deep skin biopsy of the DTH lesion from UMD-012 showing a perivascular CD3+/CD8+ T-cell infiltrate in the subcutaneous fat that also contained hTERT/survivin-specific CD8+ T cells by tetramer analysis (Figure 4, panel B). Figures 4C and 4D show the proliferative responses by CFSE dilution for one representative hTERT/survivin tetramer responder at day +180 post-transplant and a second patient at day +14 post-transplant. Both patients have substantial increases in CFSE<sup>dim</sup> cells after stimulation with the hTERT/survivin peptide mix, consistent with the significant frequency of tetramer-reactive cells shown in figure 3. However, we also observed an increased background proliferation in the cultures after incubation of the PBMCs with the irrelevant Tax peptide as well as in cultures of tissue culture media only.

**Immune responses to the pneumococcal conjugate vaccine (PCV)**

The relatively large number of patients (n = 54) who received the combination of PCV-primed autologous T-cells by early adoptive transfer followed by booster immunizations with the pneumococcal conjugate vaccine (Prevnar®) provided the opportunity to evaluate more accurately the frequency and magnitude of the T cell and B cell responses to this microbial conjugate vaccine. Figure 5A shows
the mean pneumococcal IgG levels for each of the 4 serotypes tested (6B, 14, 19F, 23F) as well as the sum of all 4 serotypes are shown at serial timepoints before and after stem cell transplantation. The mean antibody responses for all 4 serotypes increased progressively post-transplantation and far exceed the level of 0.5 μg/ml considered to be “protective” against infection. The % of patients who had antibody responses (> 4-fold increase in titers after vaccination compared to baseline) to > 1, > 2, > 3 or 4 of the 7 serotypes carried by the PCV were 94% (n = 50), 75% (n = 40), 58% (n = 31) and 47% (n = 25) respectively. The proliferative T-cell responses to the CRM-197 carrier protein were assayed by CFSE dilution studies (Figure 5B). In Figure 5B, the red circles represent “responders” who had > 3-fold increases in the %CFSEdim cells at one or more timepoints post-transplant, while the black circles represent “non-responders”. Using > 2, > 3, and > 4 – fold increases in the CFSEdim population compared to enrollment/baseline, 86%, 80% and 73% of the patients had positive T-cell responses to the CRM-197 carrier protein respectively.

**Immune cell recovery and improved EFS**

Although pre-transplant disease status did not influence post-transplant T-cell recovery, higher infused CD4+ T-cell doses significantly increased post-transplant CD4 counts (slope=0.66 in log scale, p=0.0001) and higher infused CD8+ T-cell doses significantly increased post-transplant CD8 recovery (slope=0.29, p<0.0001). The abundance of longitudinal phenotypic and functional immunologic data that were collected during the course of this study
also afforded the opportunity to analyze relationships between parameters of immune recovery and clinical outcome. Since thalidomide maintenance resulted in a significant improvement of EFS as mentioned above, Cox proportion hazards models stratifying on thalidomide maintenance were used to study correlations between immune parameters and EFS. Based on the univariate analysis, we found that higher absolute values of CD4+ T-cells were associated with improved EFS for the entire cohort of patients (p= 0.037 at day 14, 0.048 at day 60, 0.013 at day 100 and 0.034 at day 180, respectively). Higher % of CD4+ T-cells were also associated with improved EFS (p=0.034 at day 14, 0.017 at day 60 and 0.033 at day 100, respectively). Since % of CD8+ T cells were inversely correlated with % of CD4+ T cells, it was not surprising that lower % of CD8+ T cells led to improved EFS (p=0.017 at day 14, 0.015 at day 60 and 0.049 at day 100, respectively). Certain CD4 and CD8 T cell subsets (including day 180+ CD8+/CD45RO+ T cells) were correlated to EFS as well (see Supplemental Table S2). Furthermore, lower % of FOXP3+ T cells (Tregs) were associated with improved EFS for the entire cohort of patients (p=0.047 at TCH, 0.002 at day 14, 0.004 at day 60, <0.001 at day 100 and at day 180, respectively) along with a lower number of Tregs at enrollment (p = 0.01). The mean levels of these phenotypic variables over time for all patients on study are shown in Figures 6 and 7. The % Tregs was significantly lower (1.1% vs 5.5%,p<0.0001) and the loge Teff/Treg was significantly higher (5.92 vs 4.45, p=0.002) at day 60 when compared to a cohort of unselected patients (n=9) who were autografted for myeloma without T-cell transfers.
Accelerated immunoglobulin recovery after T-cell transfers

Post-transplant immunoglobulin levels at serial timepoints were compared between the current trial and an historical cohort of 102 myeloma patients who had standard autografts at the University of Maryland site without T-cell transfers (9). To eliminate the effect of the myeloma paraprotein, we compared IgG recovery for the non-IgG myeloma patients, IgA recovery for the non-IgA myeloma patients and IgM recovery for all patients. Early polyclonal recovery rates were significantly greater for the IgG, IgA and IgM fractions among the patients who received day +2 T-cell transfers as compared to the historical control patients (Table 4A). The estimated IgA levels among IgG myeloma patients who received day +2 T-cell transfers were significantly higher than in the historical control patients at days 60, 100 and 180, respectively (Table 4B). For the IgA patients, the estimated polyclonal IgG levels were significantly higher in the current trial at day 180 [810 mg/dl vs 611 mg/dl, p = 0.048]. The estimated IgM levels were also significantly higher at day 180 in the current trial [58 mg/dl vs 39 mg/dl, p = 0.029].

Discussion

Our long term goal is to improve antitumor immunity in patients after high dose chemotherapy, at a point when tumor burden may be reduced. The objective of this protocol was to build on our previous study (27), and to further define the schedule dependent effects of adoptive cell transfer. A secondary objective was to learn whether combination immunotherapy consisting of vaccine and adoptive
cell transfer could induce anti-self immunity, in the setting of the profound immunodeficiency following ASCT (20).

In our previous study, we found that T cell transfer on day +12 post stem cell infusion was significantly more effective than day +100 (27). Our current study has shown that day +2 T cell transfers result in a substantially accelerated reconstitution of T cell immunity, as revealed by a number of quantitative and functional measures. An unexpected finding was that T cell homeostasis was altered following day +2 T cell infusions, as a sustained T cell lymphocytosis was observed in most patients. To our knowledge, this is unprecedented, absent treatment of patients with cytokine infusions.

The lymphocytosis may have beneficial clinical consequences, as higher absolute values of CD4+ T cells were significantly predictive of improved EFS at all post-transplant timepoints tested as were higher levels of CD8+/CD45RO+ T cells at day +180. Patients with higher %CD4+ T-cells and correspondingly lower percentage of CD8+ T cells also exhibited better EFS. In addition to the schedule dependent lymphocytosis following day +2 T cell transfer, we recently reported that a subset of patients have an engraftment syndrome resembling autologous GVHD (35) that was not observed in our previous trial following day +12 nor day +100 T cell infusions (27).
In addition to the induction of lymphocytosis, we found that day +2 T cell transfer was associated with a reduction in %Tregs post-transplant compared to the baseline levels in the 54 patients and compared to a cohort of myeloma patients autografted without T-cell transfers. It should be recognized that FOXP3 expression is not entirely specific for Tregs as it can be transiently expressed in activated T-cells. The percentage of FOXP3+ T-cells (Tregs) was strongly and inversely associated with improved EFS at all post-transplant timepoints tested. This finding concurs with previous studies that indicate lower Treg levels and especially increased Teff/Treg ratios are associated with enhanced tumor necrosis in clinical trials of immune modulation (43). Our results indicating that myeloablative chemotherapy combined with immune reconstitution post T cell transfer are notable because to our knowledge other approaches have not led to a substantial reduction in Tregs in patients with advanced cancer. Uncovering the mechanisms leading to the increased Teff/Treg ratio will require further study and may involve a “purging effect” consequent to the relative depletion of Tregs in the adoptively transferred T cells, as well as host dependent effects during homeostatic expansion in that Tregs may be at a competitive disadvantage compared to effector T cells.

A major goal of our study was to test whether the combination of tumor vaccination and day +2 T cell infusions could induce anti-tumor immunity. Ten patients or 36% had immune responses to the hTERT/survivin multipeptide vaccine. This frequency exceeds the minimum of 7 responses that represented
the immunological efficacy endpoint for the study and compares favorably to the ~20% frequency of cellular or antibody immune responses to idiotype vaccines that were administered to myeloma patients following autotransplantation (44, 45). A subset of patients had a vigorous response to the vaccine, however the responses were generally modest compared to the robust cellular and humoral immunity that was nearly universally observed following the pneumococcal conjugate vaccine. Thus the immune response frequency to the multipeptide tumor antigen vaccine though higher than reported for idiotype vaccines is below what can be achieved with a microbial vaccine. A higher frequency and magnitude of immune response to cancer vaccines will likely be required for a significant long-term clinical impact.

The two-arm design of this study facilitated an assessment of the safety/toxicity profile of the hTERT/survivin immunizations and the vaccine-primed T-cells. Toxicity profiles were similar between the two arms except that a significant increase in diarrhea was observed among the Arm A patients (p = 0.001). Some of these cases of diarrhea (7/54 [13%] patients) were documented by colonic biopsy to be due to grade I-III autologous GVHD, but these events were evenly distributed between the two arms. The reason for the excess cases of diarrhea in the Arm A patients is unclear but both hTERT and survivin are known to be expressed in colonic crypt epithelial cells, suggesting a possible immunological basis for this observation (46, 47). Nonetheless, our data did not show evidence to correlate adverse effects such as diarrhea to hTERT/survivin tetramer
responses. The pattern of hTERT/survivin vaccine reactivities revealed no severe reactions. A significant increase in the frequency of > 50 mm of induration/redness developed during the course of immunizations consistent with a DTH (delayed-type hypersensitivity) response which was documented by immunoassays on a deep skin biopsy taken from 1 patient.

We anticipated that HLA-A2+ patients who received the hTERT/survivin vaccine (Arm A) might demonstrate better myeloma control. However, Arm A patients exhibited an inferior EFS vs Arm B patients with no difference in OS. However, the inferiority in EFS is unlikely due to the hTERT/survivin vaccine since only about a third of patients in Arm A developed an immune response. Furthermore, several differences in patient and treatment-related factors between the two arms likely contributed to a higher relapse rate in the Arm A cohort: i) the % patients with abnormal cytogenetics was 50% in Arm A but 27% in Arm B (p = 0.14); ii) the % plasma cells in the marrow at enrollment was 32% in Arm A vs 22% in Arm B; iii) and most importantly the % eligible patients who received thalidomide maintenance was 29% in Arm A and 77% in Arm B (p = 0.003). After adjustment for the difference in thalidomide usage alone, the difference in EFS between Arms A and B was no longer significant. The reason for differential thalidomide usage was that the study site where most of the Arm A patients enrolled, did not routinely use post-transplant thalidomide maintenance as a matter of practice. As the standard of care has evolved to include post-transplant maintenance, future studies should mandate such therapy for all patients. Emerging data suggests that post-transplant lenalidomide could play a dual role as a
maintenance treatment for myeloma and an immune modulator with the potential to augment and/or sustain cellular and antibody responses to tumor vaccines (11,12,48).

In summary, this study shows for the first time that adoptive transfer of vaccine-primed and costimulated autologous T-cells generates a rapid and schedule dependent recovery of the cellular and humoral immune system in patients with myeloma, and that immune responses to a cancer vaccine occur in a substantial proportion of patients early after autotransplantation. Whether T-cell transfers and/or tumor antigen immunizations enhanced EFS in this study is unknown. However, by a number of measures, the magnitude of the immune recovery was associated with improved EFS. Our results following the therapeutic induction of rapid lymphocyte recovery are consistent with previous studies showing that unmanipulated lymphocyte levels in myeloma patients correlate to EFS (13-16). Future studies will aim to improve the immunotherapeutic response by exploring vaccines for other tumor antigens (e.g. cancer/testis antigens) and incorporating more potent adjuvants.
Acknowledgments

We thank the apheresis centers and nurses of the BMT programs of the University of Maryland Greenebaum Cancer Center and the Abramson Cancer Center for outstanding clinical care provided to our patients. We specifically thank Sandra Westphal, Kathleen Ruehle RN and Carolynn Harris for cell processing, patient coordination and study regulatory support at the University of Maryland site. We thank Yiping Liu at the University of Calgary for providing post-transplant peripheral blood samples from a cohort of transplant patients who did not receive T-cell transfers for comparative analyses. This work was supported by NIH grant 5R21CA130293-02 and an ARRA supplemental grant (to APR), NIH grant 5K23A10675670-5 (to NAA), Leukemia and Lymphoma Society Grant 7414-07 (to CHJ, BLL and RHV) and Beckman Foundation Award (to RHV).

Author Contributions and Conflict of Interest Statement

A.P. Rapoport designed the research, performed the research, analyzed the data and wrote the paper. N.Aqui performed the research and helped write the paper. E.A. Stadtmauer designed the research, performed the research and helped write the paper. D. Vogl performed the research. H-B. Fang and L Cai performed statistical analysis. S. Janofsky performed research. A. Chew analyzed data. J Storek contributed vital reagents. E. Veloso performed research. M Pasetti performed research. G. Akpek performed research. A. Badros performed research. S. Yanovich performed research. MT Tan performed statistical
A. Cross performed research. S. Philip performed research. H. Murphy and R. Bhagat performed research. Z. Zheng performed research. T. Milliron performed research. J. Cotte performed research. A. Cannon performed research. R.H. Vonderheide designed research, performed research, contributed vital new reagents and helped write the paper. B.L. Levine designed research, performed research, contributed vital reagents, analyzed data and helped write the paper. C.H. June designed the research, contributed vital new reagents, analyzed the data and helped write the paper.

**Conflict of Interest Statement:** A.P. Rapoport (corresponding author) has no conflicts of interest to declare. R.H. Vonderheide declares a potential financial conflict of interest related to inventorship on a patent regarding hTERT as a tumor-associated antigen for cancer immunotherapy. C.H. June and B.L. Levine have patents and patent applications in the field of adoptive immunotherapy but have been divested of financial benefit from this technology. This arrangement is under compliance with the policies of the University of Pennsylvania. All other authors declare no conflicts of interest.
References


bacterial antigens in lymphoma patients 4-10 years after high-dose therapy with aBMT. Serological responses to revaccinations according to EBMT guidelines. *Bone Marrow Transplant.* 2001; 28: 681-7.


24. Levine BL, Ueda Y, Craighead N, Huang ML, June CH. CD28 ligands CD80 (B7-1) and CD86 (B7-2) induce long-term autocrine growth of CD4+ T cells and induce similar patterns of cytokine secretion in vitro. *International Immunology.* 1995; 7: 891-904.


28. Stadtmauer EA, Vogl DT, Prak EL. Transfer of influenza vaccine-primed co-stimulated autologous T-Cells after stem cell transplantation for multiple myeloma leads to reconstitution of influenza immunity: results of a


Table Legends

Table 1: Patient Characteristics. Shown are the patient characteristics in Arm A vs Arm B with statistically significant p-values shown in bold/red. Abbreviations: Thal Maint = thalidomide maintenance, for patients who remained event-free at 180 days post-transplant; β2M = β-2 microglobulin; EN=enrollment.

Table 2: T-cell Related Toxicities. Shown are toxicities which were possibly, probably or definitely related to adoptive T-cell transfers either early (0-48 hours) post-transfer or late (> 48 hours) post-transfer. P-values show significant differences between total # of events for Arms A and B for each type of toxicity (NS: p > 0.05). Severity grades of I,II and III are shown; no grade IV or V toxicities were observed. Other toxicities (not listed), such as hypertension, hypoxia/pulmonary, rash, and diarrhea (0-48 hours); cytopenias, neuropathy, hepatic(↑AST/ALT), eosinophilia, renal/edema, mucositis and mental status changes (> 48 hours) occurred in individual patients in both groups with no significant differences between the two groups.

Table 3: hTERT Vaccine Reactivities. Shown are # (and %) of reactions among 28 total Arm A patients who received hTERT/survivin vaccinations. Results are shown for each of the four immunization timepoints. Figures in bold are significantly higher than some of the earlier timepoints (P < 0.05), except for T > 100 which was significantly higher than a later timepoint, and nausea/vomiting which was significantly higher than a later timepoint and an earlier timepoint.
Table 4: Effect of Adoptive T cells on Immunoglobulin Recovery. (A) Estimated rates of initial recovery (mg/dl/day) of polyclonal IgA (in non-IgA myeloma patients), IgG (in non-IgG myeloma patients) and IgM for 54 patients in current trial who received expanded T cells (+ex-T) vs 102 total patients in historical database who did not receive expanded T cells (-exT). P values were significant for Ig recovery rates. (B) Estimated values for polyclonal IgA among 36 evaluable IgG (or light chain only) patients in current trial vs 64 IgG (or light chain only) patients in the historical data base at days 60, 100, 180 post-transplant. P-values were significant at each day analyzed.
Table 1. Patient Characteristics

<table>
<thead>
<tr>
<th></th>
<th>Range (Mean)</th>
<th>Arm A</th>
<th>Arm B</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong># of Patients</strong></td>
<td>54</td>
<td>28</td>
<td>26</td>
<td></td>
</tr>
<tr>
<td><strong>Age</strong></td>
<td>37~68 (55)</td>
<td>45 ~ 68 (55.6)</td>
<td>37 ~ 67 (54.3)</td>
<td>0.74</td>
</tr>
<tr>
<td><strong>Race</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>White: 32 (59%)</td>
<td>White: 22 (78%)</td>
<td>White: 10 (38%)</td>
<td>0.003</td>
<td></td>
</tr>
<tr>
<td>AA: 21 (39%)</td>
<td>AA: 5 (18%)</td>
<td>AA: 16 (62%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Asian: 1 (2%)</td>
<td>Asian: 1 (4%)</td>
<td>Asian: 0</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Gender</strong></td>
<td>Male: 28 (52%)</td>
<td>Male: 16 (57%)</td>
<td>Male: 12 (46%)</td>
<td>0.59</td>
</tr>
<tr>
<td>Female: 26 (48%)</td>
<td>Female: 12 (43%)</td>
<td>Female: 14 (54%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Myeloma subtypes</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IgA: 15</td>
<td>IgA: 9</td>
<td>IgA: 6</td>
<td>0.08</td>
<td></td>
</tr>
<tr>
<td>IgG: 35</td>
<td>IgG: 15</td>
<td>IgG: 20</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Light chains: 4</td>
<td>Light chains: 4</td>
<td>Light chains: 0</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Thal Maint (pts EFS&gt;180days)</strong></td>
<td>Yes: 24</td>
<td>Yes: 7</td>
<td>Yes: 17</td>
<td>0.003</td>
</tr>
<tr>
<td>No: 22</td>
<td>No: 17</td>
<td>No: 5</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>β-2 microglobulin levels at EN</strong></td>
<td>0.87 ~ 4.13 (1.91)</td>
<td>0.87 ~ 3.33 (1.70)</td>
<td>0.03</td>
<td>0.03</td>
</tr>
</tbody>
</table>
### Table 2 T cell-Related Toxicities (Arm A vs. Arm B)

<table>
<thead>
<tr>
<th>Event (0 to 48 hrs)</th>
<th>I A</th>
<th>I B</th>
<th>II A</th>
<th>II B</th>
<th>III A</th>
<th>III B</th>
<th>Totals</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rigors/Chills</td>
<td>7</td>
<td>6</td>
<td>11</td>
<td>7</td>
<td>0</td>
<td>0</td>
<td>18 (64)</td>
<td>NS</td>
</tr>
<tr>
<td>Nausea/Vomiting</td>
<td>1</td>
<td>7</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1 (4)</td>
<td>0.02</td>
</tr>
<tr>
<td>Fever</td>
<td>3</td>
<td>4</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>3 (11)</td>
<td>NS</td>
</tr>
<tr>
<td>Headache/Pain</td>
<td>0</td>
<td>3</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>3 (12)</td>
<td>NS</td>
</tr>
<tr>
<td>Event (onset &gt; 48 hrs)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rash</td>
<td>19</td>
<td>19</td>
<td>4</td>
<td>4</td>
<td>0</td>
<td>0</td>
<td>23 (82)</td>
<td>NS</td>
</tr>
<tr>
<td>Fever</td>
<td>6</td>
<td>4</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>8 (29)</td>
<td>NS</td>
</tr>
<tr>
<td>Gut GVHD</td>
<td>1</td>
<td>0</td>
<td>3</td>
<td>2</td>
<td>0</td>
<td>1</td>
<td>4 (14)</td>
<td>NS</td>
</tr>
<tr>
<td>Arthralgias</td>
<td>3</td>
<td>4</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>3 (11)</td>
<td>NS</td>
</tr>
<tr>
<td>Myalgias</td>
<td>5</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>5 (18)</td>
<td>0.05</td>
</tr>
<tr>
<td>Headache/Pain</td>
<td>4</td>
<td>1</td>
<td>3</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>7 (25)</td>
<td>0.05</td>
</tr>
<tr>
<td>Anorexia/Nausea</td>
<td>6</td>
<td>2</td>
<td>2</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>9 (32)</td>
<td>0.04</td>
</tr>
<tr>
<td>Conjunctivitis</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>3</td>
<td>0</td>
<td>0</td>
<td>3 (12)</td>
<td>NS</td>
</tr>
<tr>
<td>Fatigue</td>
<td>6</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>7 (25)</td>
<td>0.05</td>
</tr>
<tr>
<td>Diarrhea</td>
<td>10</td>
<td>3</td>
<td>6</td>
<td>1</td>
<td>2</td>
<td>1</td>
<td>18 (64)</td>
<td>0.001</td>
</tr>
</tbody>
</table>
Table 3. hTERT Vaccine Reactivities in Arm A

<table>
<thead>
<tr>
<th>Reaction</th>
<th>Immunization Timepoint</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pre-transplant</td>
</tr>
<tr>
<td>T ≥ 100° F</td>
<td>3(11)</td>
</tr>
<tr>
<td>Pain at injection site(≥Grade 2)</td>
<td>9(32)</td>
</tr>
<tr>
<td>Loss of Appetite (≥Grade 2)</td>
<td>2(7)</td>
</tr>
<tr>
<td>Loss of Energy (≥Grade 2)</td>
<td>6(21)</td>
</tr>
<tr>
<td>Headaches (≥Grade 2)</td>
<td>2(7)</td>
</tr>
<tr>
<td>Muscle Aches (≥Grade 2)</td>
<td>9(32)</td>
</tr>
<tr>
<td>Nausea/Vomiting (≥Grade 2)</td>
<td>1(4)</td>
</tr>
<tr>
<td>Redness or Induration(≥12mm)</td>
<td>13(46)</td>
</tr>
<tr>
<td>Redness or Induration(≥36mm)</td>
<td>11(39)</td>
</tr>
<tr>
<td>Redness or Induration(&gt;50mm)</td>
<td>7(25)</td>
</tr>
</tbody>
</table>
### Table 4A. Ig Recovery Rates

<table>
<thead>
<tr>
<th>Ig Subtype</th>
<th>Rate (-exT)</th>
<th>Rate (+exT)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>IgA</td>
<td>0.62</td>
<td>1.33</td>
<td>0.041</td>
</tr>
<tr>
<td>IgG</td>
<td>3.41</td>
<td>11.22</td>
<td>0.0006</td>
</tr>
<tr>
<td>IgM</td>
<td>-0.03</td>
<td>0.31</td>
<td>0.0001</td>
</tr>
</tbody>
</table>

### Table 4B. IgA Recovery of IgG patients

<table>
<thead>
<tr>
<th>Time</th>
<th>-exT (estimated) (N=64)</th>
<th>+exT (estimated) (N=36)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>IgA –day 60</td>
<td>71.7 mg/dl</td>
<td>129.8 mg/dl</td>
<td>0.004</td>
</tr>
<tr>
<td>IgA –day 100</td>
<td>59.4 mg/dl</td>
<td>114.4 mg/dl</td>
<td>0.008</td>
</tr>
<tr>
<td>IgA –day 180</td>
<td>48.7 mg/dl</td>
<td>98.4 mg/dl</td>
<td>0.004</td>
</tr>
</tbody>
</table>
Figure Legends

Figure 1: Trial Design.

Figure 2: Kaplan-Meier Survival Curves. (A) Overall survival. (B) Event-free survivals (EFS) according to study Arm assignment. EFS for Arm B is superior to EFS for Arm A (P = 0.0068). (C) EFS for patients who remained event-free at day 180 post-transplant and received thalidomide maintenance had improved EFS compared to patients who did not receive thalidomide (P = 0.0089).

Figure 3: Tetramer Immune Responses to hTERT/survivin Vaccine. (A) Bar graph showing 10 patients (out of 28, 36%) with positive tetramer responses defined as > 3-fold increase in tetramer staining compared with enrollment/baseline and minimum level of 0.1%. (B) Same patients depicted in panel A but using an expanded Y-axis to show the full spectrum of responses.

Figure 4: Functional Immune Responses in Specific Patients. (A) Deep skin biopsy of 5 cm+ area of induration (from patient MD012) showing CD8+/CD3+ T-cell infiltrate. (B) Tetramer analysis of T-cells extracted from the skin biopsy depicted in panel A; 2.1% of the cells analyzed exhibited hTERT/survivin tetramer staining vs 0.1% in the control (tetramer negative) sample. (C) Proliferative response of CD8+ T-cells (from patient UPCC/13406-11) after stimulation with hTERT/survivin peptide mix by CFSE dilution analysis; % CFSE$^{\text{dim}}$ was 11.2% in the hTERT/survivin – stimulated cells (lower panel) vs
6.67% in cells stimulated with an irrelevant peptide derived from the Tax protein (upper panel) and 6.2% in cells exposed to medium only (data not shown). (D) Proliferative response of CD8+ T-cells from a second immunized patient (UPCC/13406-22) after stimulation with hTERT/survivin peptide mix by CFSE dilution analysis; % CFSE\textsuperscript{dim} was 33.2% in the hTERT/survivin – stimulated cells (lower panel) vs 11.7% in cells which were incubated in media only without peptide stimulation (upper panel).

Figure 5: B and T cell Responses to PCV Vaccine. (A) Log-transformed plot of mean serum IgG antibody responses for each of the 4 PCV serotypes tested over the course of the study. (B) CD4+ T-cell responses to the CRM-197 carrier protein based on proliferation assays using CFSE dilution; %CFSE\textsuperscript{dim} cells after CRM-197 stimulation at various timepoints for 49 total patients are shown. Black circles: Non-responders; Red circles: Responders. A responder is defined as a patient having at least a 3-fold increase of the enrollment measurement at one or more post-transplant timepoints.

Figure 6: Immune Reconstitution with Sustained Lymphocytosis of Multiple T-cell Subsets Post-transplant. (A) Mean CD3+, CD4+, and CD8+ counts at various timepoints before and after transplant. EN = enrollment; TCH = T-cell harvest (apheresis); TR = transplant date. (B) Mean levels of CD4+ subsets pre- and post-transplant. Bars denote the standard errors.
Figure 7: %CD4+FOXP3+ T-cells and Teff/Treg ratio Pre- and Posttransplant.

(A) %CD4+FOXP3+ T-cells at various timepoints before and after transplant. Bars denote 1 standard deviation. (B) Log$_e$ of Teff/Treg ratio at various timepoints before and after transplant.
Figure 1

HLA-A2+ (Arm A)
- Mobilization
- Stem Cell Collection
- High-dose Melphalan
- Stem Cell Transplant
- T Cell In Vitro Activation and Expansion to Infuse ~5x10^10 Cells
- T Cell Collection
- Immune Assessment Studies at Day 60, 100, and 180

HLA-A2- (Arm B)
- PCV
- T Cell Collection
- Mobilization
- Stem Cell Collection
- High-dose Melphalan
- Stem Cell Transplant
- T Cell In Vitro Activation and Expansion to Infuse ~5x10^10 Cells
- T Cell Collection
- TERT, Survivin, CMV, PCV + PCV boosters at Day +14, 42, and 90

Study Day
- -42
- -30
- -15
- -1
- 0
- 12
- 30
- 90
- 100
Figure 2

A

Overall survival probability

Time (days)

B

Event free survival probability

Between Arm A and Arm B: p = 0.0068

Arm A
Arm B
Both Arms

Time (days)

C

Event free survival probability

p = 0.0089

Thai Maint
No Thal Maint

Time (days)
Figure 3

A

hTERT/Survivin Responders

% tetramer+ CD8+ cells

B

hTERT/Survivin Responders

% tetramer+ CD8+ cells
**Figure 4**

(A) Image of tissue with hTERT/survivin tetramers.

(B) Flow cytometry plots showing CD8+ T cell populations with Neg. tetramer and hTERT/survivin tetramers.

(C) Flow cytometry plots showing CD8+ T cell populations with Tax peptide and hTERT/Survivin peptides.

(D) Flow cytometry plots showing CD8+ T cell populations with Media and hTERT/Survivin peptides.

- **Tax Peptide**
  - Neg. tetramer: 0.1%
  - hTERT/survivin tetramers: 2.1%
  - CD8+ T cells with 6.7% CFSE.

- **hTERT/Survivin Peptides**
  - Neg. tetramer: 11.2%
  - Media: 11.7%
  - CFSE: 32.2%
Figure 5

A

Mean pneumococcal polysaccharide (µg/mL)

Time points

EN   TCH   D14  D60  D100  D180

B

% CFSE<sup>dim</sup> cells

Time points

EN   TCH   D14  D60  D100  D180

Legend:
- 6B
- 14
- 19F
- 23F
- sum
Figure 6

A

Counts

EN TCH TR D14 D60 D100 D180

Time points

B

Immunologic parameter values (absolute counts)

EN TCH Day 14 Day 60 Day 100 Day 180

Time points

Combination immunotherapy using adoptive T-cell transfer and tumor antigen vaccination based on hTERT and survivin following ASCT for myeloma

Aaron P. Rapoport, Nicole A. Aqui, Edward A. Stadtmauer, Dan T. Vogl, Hong-Bin Fang, Ling Cai, Stephen Janofsky, Anne Chew, Jan Storek, Gorgun Akpek, Ashraf Badros, Saul Yanovich, Ming T. Tan, Elizabeth Veloso, Marcela F. Pasetti, Alan Cross, Sunita Philip, Heather Murphy, Rita Bhagat, Zhaohui Zheng, Todd Milliron, Julio Cotte, Andrea Cannon, Bruce L. Levine, Robert H. Vonderheide and Carl H. June

Information about reproducing this article in parts or in its entirety may be found online at: http://www.bloodjournal.org/site/misc/rights.xhtml#repub_requests

Information about ordering reprints may be found online at: http://www.bloodjournal.org/site/misc/rights.xhtml#reprints

Information about subscriptions and ASH membership may be found online at: http://www.bloodjournal.org/site/subscriptions/index.xhtml

Advance online articles have been peer reviewed and accepted for publication but have not yet appeared in the paper journal (edited, typeset versions may be posted when available prior to final publication). Advance online articles are citable and establish publication priority; they are indexed by PubMed from initial publication. Citations to Advance online articles must include digital object identifier (DOIs) and date of initial publication.