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 randomized clinical trial

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Running Head: Primed T cells stimulate influenza immunity

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Abstract

Severe immune deficiency follows autologous stem cell transplantation for multiple myeloma and is associated with significant infectious morbidity. This study was designed to evaluate the utility of a pre-transplant vaccine and infusion of a primed autologous T cell product in stimulating specific immunity to influenza. Twenty one patients with multiple myeloma were enrolled from 2007-2009. Patients were randomized to receive an influenza-primed autologous T cell product or a non-specifically primed autologous T cell product. The study endpoint was the development of hemagglutination inhibition titers to the strain-specific serotypes in the influenza vaccine. ELISPOT assays were performed to confirm the development of influenza-specific B cell and T cell immunity. Patients who received the influenza-primed autologous T cell product were significantly more likely to seroconvert in response to the influenza vaccine (p=0.001). Seroconversion was accompanied by a significant B cell response. There were no differences in the global quantitative recovery of T cell and B cell subsets nor in global T cell and B cell function. The provision of a primed autologous T cell product significantly improved subsequent influenza vaccine responses. This trial was registered at www.clinicaltrials.gov as NCT00499577.
Introduction

Epidemics of influenza A virus strains have been associated with hospitalization of approximately 200,000 people per year in the United States with 30,000-50,000 deaths per year. The case fatality rates have been estimated to be 0.5/100,000 in the 0-49 year age range and as high as 100/100,000 in the >65 year age range. The primary method to combat influenza is the administration of a vaccine appropriate to the seasonal infecting strains.

Patients with compromised immunity are at particularly high risk of complications from influenza infection and yet have less protection from vaccination. Patients with malignancy have an increased attack rate of influenza and data suggest that 10-40% of oncology patients are infected during each seasonal epidemic. This is higher than in the general population where the case rate is usually 5-15%.

Multiple myeloma is one example of a population particularly at risk for severe influenza. Patients have severe humoral and cellular immune deficiency. This is associated with impaired responses against both tumor, microbial, and vaccine antigens. Furthermore, therapy with high dose melphalan and autologous stem cell transplantation (ASCT) is used frequently for the treatment of relapsed or refractory disease. Though hematopoietic recovery after ASCT occurs within 3 weeks, full recovery of T- and B-cell function may take months to years and vaccine responses are typically poor. Patients post-
ASCT have increased morbidity associated with respiratory viruses in general\textsuperscript{22,23}. This, coupled with a higher attack rate, the potential for prolonged shedding, and the emergence of resistant viruses, mandates that improved preventive strategies be developed\textsuperscript{24,25}.

The altered number, function and dynamics of immune cell recovery after ASCT for myeloma increases patient risk for serious infections such as VZV, CMV, \textit{S. pneumoniae}, and influenza\textsuperscript{26}. In particular, influenza accounts for 20\% of respiratory virus infections in the transplant patients\textsuperscript{27}. The ability to respond to influenza vaccination is impaired well beyond the quantitative repletion of lymphocytes\textsuperscript{13,28}. Strategies to accelerate and augment the recovery and function of autologous T-cells after transplant for myeloma may be beneficial.

In this study, we examined whether immunization with the seasonal influenza vaccine would be immunogenic in patients with myeloma undergoing ASCT and whether sustained influenza-specific protective responses could be elicited after ASCT. Our data demonstrate that vaccine-primed autologous T cell infusions are associated with restoration of antigen-specific antibody production. Combined vaccination and adoptive transfer represents a powerful strategy to improve host defenses post-ASCT.
Methods

Study Design

Patients for this randomized, controlled study were recruited from the Abramson Cancer Center at the University of Pennsylvania in Philadelphia, PA. This study was distinct from a prior study investigating the timing of an autologous T cell product and pneumococcal vaccine responses\textsuperscript{29}. The study protocol was approved by the University of Pennsylvania institutional review board and informed consent was obtained from patients in accordance with the Declaration of Helsinki. The analysis plan was determined before study initiation and laboratory assays were performed in a blinded fashion.

Eligible patients were patients with symptomatic multiple myeloma scheduled to receive an ASCT on a companion study (UPCC 13406, ClinicalTrials.gov Identifier NCT00499577), which biologically assigned patients by HLA type to peptide vaccination against hTERT and survivin (HLA-A2 patients) or no hTERT vaccination (all other HLA types); all patients received peptide vaccines against CMV and the pneumococcal polyvalent conjugated vaccine as well as GM-CSF injected at the sites of peptide vaccines. Patients were required to have high risk disease as defined by either cytogenetics or measurable disease (less than complete remission (CR)) at time of transplantation. Patients had adequate organ function as defined by serum creatinine < 3.0 mg/dl, Left Ventricular
Ejection Fraction > 45%, and DLCO > 40% predicted. Patients could not have had an influenza vaccination within 2 months prior to study entry.

Patients were randomly assigned 1:1 to the Vaccine-Transfer-Vaccine group or the Transfer-Vaccine group (Figure 1). No stratification variables were used. Vaccine-Transfer-Vaccine patients received seasonal influenza vaccine prior to T cell harvest while the Transfer-Vaccine group did not. All patients then received the seasonal influenza vaccination on day +14. Pre-transplant, both groups underwent steady state T-cell harvest (14 days after the first vaccine for the Vaccine-Transfer-Vaccine group) by 1 hour apheresis followed by administration of cyclophosphamide 4.5 g/m² over 12 hours and G-CSF-stimulated blood stem cell harvest 2 weeks later. Patients then received melphalan 200mg/m² over 1 hour followed by infusion 24-48 hours later of at least 2 x 10⁶ CD34+ cell/kg on day 0. All patients received an infusion of co-stimulated autologous T-cells on day +2. Thus, Vaccine-Transfer-Vaccine patients received a pre-harvest influenza vaccine and will be referred to as the Primed Group, and both patient groups received a post-ASCT influenza vaccine. The Transfer-Vaccine patients will be referred to as the Non-primed Group.

The commercially available seasonal influenza vaccine, (Fluzone, Sanofi-Pasteur) was given IM. Throughout the course of the study 3 seasonal vaccines were used: 2006–2007 season A/New Caledonia/20/99 (H1N1)-like virus; A/Wisconsin/67/2005 (H3N2)-like virus; B/Malaysia/2506/2004-like virus, 2007–
2008 season A/Solomon Islands/3/2006 (H1N1)-like virus; A/Wisconsin/67/2005 (H3N2)-like virus; B/Malaysia/2506/2004-like virus and 2008-2009 season A/Brisbane/59/2007 (H1N1)-like virus; A/Brisbane/10/2007, (H3N2)-like virus; B/Florida/4/2006-like virus. The seven valent conjugated pneumococcal vaccine was given to all subjects and responses to four serotypes were measured. For analytic purposes, the four serotype responses were summed to a single microgam per milliliter value at each time point.

Blood samples from patients undergoing ASCT were obtained at baseline, prior to steady-state T-cell harvest, and on days +14, + 60, +100 and +180 after stem cell infusion. Patients were examined during these time points and clinical events recorded.

**T cell Infusions**

Patients underwent apheresis to collect >1 X 10^9 mononuclear cells. For the Primed Group, this procedure was scheduled 14 days after the first vaccine. The cells were depleted of monocytes and cryopreserved. The T cell manufacturing process has been described previously and consisted of CD3 and CD28 bead-based stimulation. All infused T cell products were required to meet FDA-specified safety and release criteria prior to infusion. The target number of T cells for infusion was ≈5 X 10^{10} T cells.
Immunologic Assays

The assessment of the primary endpoint, influenza vaccine response, utilized a standard hemagglutination inhibition (HAI) assay optimized for the vaccine administered each year. Flow cytometry for T cell and B cell subsets was performed to characterize those compartments after ASCT and T cell infusion. Analysis of T cells utilized fixation with 1% paraformaldehyde and were run on an LSR II (BD Biosciences) and analyzed using FlowJo software (TreeStar). Approximately 200,000 to 500,000 events were collected per sample. CD4 Naïve cells were defined as CD45RA+CD31+, CD4 Central Memory T cells were defined as CD27+CD45RO+CCR7+, CD4 Effector Memory T cells were defined as CD45RO+/CD27+/CCR7-, and CD4 Reverted Memory T cells were defined as CD45RA+/CD31-/CCR7+. CD8 Naïve cells were defined as CD45RA+CD31+, CD8 Central Memory T cells were defined as CD27+CD45RO+CCR7+, CD8 Effector Memory T cells were defined as CD45RO+/CD27+/CCR7-, and CD8 Reverted Memory T cells were defined as CD45RA+/CD31-/CCR7+.

For B cell analysis, fresh venous whole blood anti-coagulated with EDTA was prepared and stained with antibodies (all from BD Pharmingen, San Diego, CA), as described previously. B cells were defined as CD19+ lymphocytes. Analyses were performed on a FACSCalibur with CellQuest software (Version 5.2.1, Becton Dickenson, San Jose, CA). B cells were identified on the basis of CD19 expression and forward and side scatter characteristics consistent with lymphocytes. CD19+ lymphocytes were analyzed for CD27 and IgM. A
minimum of 10,000 CD19+ events were generally analyzed per tube. The absolute B cell count was obtained by multiplying the absolute lymphocyte count by the CD19+ fraction. Five samples were excluded from the analyses in Figure 3 due to failure to meet quality control standards (primarily inadequate cell counts).

To examine functional responses to the vaccine, T cell ELISPOTs and B cell ELISPOTs were performed. A cocktail of influenza proteins (Protein Sciences, Meriden, CT) based on the described vaccines and matched to the year of inoculation was used as specific antigen (at 5 µg/mL) in a standard γ-interferon T cell ELISPOT assay \(^{33}\). PMA and ionomycin (combined at 5 µg/mL each) were used as a positive control. This assay examined a range of epitopes, and was HLA-dependent. Foreign antigen sources were avoided to minimize background. The B cell ELISPOT defined the frequency of memory B cells activated by influenza to produce antibody \(^{34}\). PBMC were stimulated for 6 days with pokeweed mitogen at 1:100,000, Staphylococcus aureus (SAC) at 1:10,000 and CpG-2006 at 6mg/ml (Sigma Aldrich St. Louis, MO). Following the stimulation period, cells were treated for six hours with the influenza protein cocktail described above (at 0.5 µg/mL). Finally, IgG production assessed by quantification of effector cells using ImmunoSpot (CTL, version 4) software.

**Statistical analysis**
The comparison of the study’s endpoints measured repeatedly over time was carried out using the mixed effects models and/or the generalized estimating equations (GEE) method. The longitudinal assessments of the outcomes were statistically tested using a repeated measures model with the following three main effects: The overall group differences, the overall changes over time, and the interaction effect. Baseline measurements for both groups were used as covariates to adjust for potential group differences at baseline. The independent t-test or the Mann-Whitney test was used for the comparisons of responder frequency and seroconversion. The geometric mean titers were calculated using the standard formula: n-th root of \( (X_1)(X_2)..(X_n) \). The 95% confidence intervals of the geometric mean titers were calculated by taking the anti-log of the 95% confidence intervals of the arithmetic means of the log-transformed values. Due to the study design and the large number of needed comparisons, corrections for multiple comparisons were not performed. Significance was set at \( p<0.05 \).
Results

Patients
A total of 21 patients with multiple myeloma were enrolled between December 2007 and February 2009. As shown in Figure 1, eleven were randomly assigned to the Primed Group (Vaccine-Transfer-Vaccine, pre and post-transplant influenza vaccination) and ten were assigned to the Non-primed Group (Transfer-Vaccine, post-transplant influenza vaccine only). All patients received the post-transplant influenza vaccination and were evaluable for the immunological assessments. The patients in each group did not differ significantly in terms of age, race, sex, immunoglobulin subtype, HLA A2 status, International Staging System (ISS), prior therapies (including dexamethasone and bortezomib), baseline organ function, CD19 count, CD3 count, or absolute lymphocyte counts (Table 1).

Response to high-dose melphalan and survival was not a primary endpoint of this study, which included a high-risk patient population. Nevertheless, with a median follow-up of 12 months, overall survival was 83% (18/21) with event free survival of 48% (10/21). Response assessment at day 180 after ASCT was 6 Complete Response or Near Complete Response with positive immunofixation (CR/nCR), 3 Very Good Partial Response (VGPR) (43% >VGPR), 4 Partial response (PR) (63% > PR) 1 Stable Disease (SD), 4 Progressive Disease (PD),
and 3 unevaluable. There was no difference in response or survival between the Primed and Non-primed Groups.

Hemagglutination Inhibition (HAI) Assay Results
The primary endpoint of this study was antibody response to influenza as measured by the serotype-specific influenza hemagglutinin inhibition antibody responses. HAI titer is the parameter with strongest correlation to protection from wild type infection. H3N2, and H1N1 HAI geometric mean titers were higher at all time points after vaccination in the Primed patients compared to the Non-primed Patients (Figure 2). Titers in the Non-primed Group remained near baseline throughout all time points. A mixed effects model was used to define the differences in geometric mean titers over time between the two arms. A significant difference was seen comparing the Primed and the Non-primed patients’ responses to the H1N1 (p=0.006) and H3N2 (p<0.0001) components of the vaccine. Influenza B HAI geometric mean titers exhibited no difference between the two groups (p=NS) and this may reflect its weaker immunogenicity.

We defined seroconversion as the percentage of patients with a 4-fold or greater increase in HAI titer after immunization. Seroconversion for H1N1 occurred in 36% of the Primed Group and 10% of the Non-primed Group (p=0.31). Seroconversion for H3N2 occurred in 72% of the Primed Group and 0% of the
Non-primed Group (p<0.01). Seroconversion for influenza B occurred in 45% of the Primed Group and 20% of the Non-primed Group (p=0.36). Seroconversion to any serotype occurred 73% of the time in the Primed Group and 30% of the time in the Non-primed Group (p=0.08). Therefore, by multiple criteria, Influenza vaccine responses were superior in the vaccine-primed recipients compared to those with adequate T cell recovery but without priming.

**B-cell and T-cell recovery kinetics**

ASCT usually leads to a sustained immune deficiency. We examined B-cell and T-cell recovery kinetics to understand the landscape of the immune system after ASCT. It is clear that the provision of the autologous T cell product led to rapid quantitative reconstitution of both the B cell and T cell compartments (Figures 3 and 4). The kinetics of recovery were similar in both groups. At baseline, the majority of B cells had a naïve phenotype (IgM+, CD27-). At day 14 post ASCT, B cells reached a nadir and the majority had a switched memory phenotype (IgM-, CD27+). The GEE method was used to define group and time effects in this repeated measure study design. There was a significant change in total B cell counts (p=0.01) and naïve B cell counts over time (p=0.001). For switch memory B cells (CD27+IgM-), there was no significant change in the absolute counts over time.
The provision of an autologous T cell infusion has been previously shown to quantitatively restore both CD4 and CD8 T cells. In this study, we examined functionally distinct T cell subsets. T cell recovery differed dramatically between CD4 and CD8 T cells, however, there were no differences between the Primed and Non-primed Groups. CD4 Effector Memory T cells were the most highly retained cell type, while CD4 Naïve, CD4 Central Memory, and CD4 Reverted Memory cells remained depressed for a sustained period of time. In contrast, CD8 Naïve, CD8 Central Memory, and CD8 Reverted Memory all increased after the infusion of the autologous T cell product. None of the CD8 T cell subsets exhibited a group effect when analyzed by GEE. The Naïve CD8 and Reverted Memory CD8 T cells exhibited a significant time effect (p<0.001 and p=0.05, respectively). The apparent difference in CD8 Effector Memory populations between the Primed Group and the Non-primed Group is not significant and is due to three outliers with engraftment syndrome. Therefore, there were no differences in T cell subsets between the two arms.

Autologous T cell infusions have been demonstrated previously to retain a diverse and consistent T cell repertoire after in vitro expansion, however, the retention of the repertoire after infusion has not been previously investigated. We identified the fraction of abnormal Vβ families (oligoclonal or <10% of the average signal in controls) after spectratyping. Approximately half of the Vβ families were abnormal and there were no differences between the two groups or across time points (data not shown). Approximately 60% of the Vβ families
exhibited no change from time point to time point. Therefore, the repertoire appears to be largely stable within the host.

Studies of T- and B- cell responsiveness

In principle, the mechanism of enhanced vaccine response after a priming dose could be due to either recruitment of antigen-specific memory T cells carried over from the priming at the time of the post-ASCT vaccine or persistence of memory B cells generated at the time of the priming dose followed by expansion and maturation after the post-ASCT vaccine. To examine this question, B-cell responsiveness was assessed by a B cell ELISPOT and the T cell responsiveness was assessed by a T cell ELISPOT (Figure 5). Both assays utilized the season-specific antigen to match the vaccine administered. The anti-influenza antigen responsive B cells increased in the primed recipients after the priming dose, however, the responses subsequently fell. This may be due to the lower frequency of effector cells once the naïve B cell population has expanded (Figure 3). The B cell responses to influenza did not change over the time course in the Non-primed patient group. The differences between the two groups did not reach statistical significance. The global capacity of the B cells to produce any antibody was measured by a total IgG ELISPOT and there were no differences between the two groups.
To assess the T cell responses, γ-interferon-producing T cells were measured after stimulation with a cocktail of T cell epitope peptides. The patients in the Primed Group trended towards a higher CD8 T cell response than the patients in the Non-primed Group, however, the difference did not reach statistical significance. The inactivated influenza vaccine stimulates predominantly CD4 T cell responses. We therefore measured T cell ELISPOT results after depletion of CD8 T cells and found that CD4-specific responses were significantly better in the Primed group at the T cell harvest time point (p=0.02) (Figure 5). Finally, to ensure that there were no global differences between the ability of the two groups of patients to respond to stimuli, responses to PMA and ionomycin were measured. There were no differences between the two patient groups (Figure 5).

We then analyzed whether early T cell and B cell responses were associated with subsequent specific antibody production independently of the study group. Influenza-specific B cell ELISPOT responses analyzed on the day of T cell harvest was very strongly associated with subsequent anti-H1N1 and anti-H3N2 responses. The Spearman correlation for influenza-specific B cell ELISPOT responses on the day of T cell harvest ranged from 0.03 to 0.006 for H1N1 responses at each subsequent time point. The Spearman correlation for influenza-specific B cell ELISPOT responses on the day of T cell harvest was 0.05 for H3N2 responses at the final time point. There were no statistically significant associations between B cell ELISPOT results and Influenza B titers. We similarly investigated the relationship of T cell ELISPOT results with
subsequent titer production and found no consistent relationship. Therefore, although the T cell infusion quantitatively restores the T cell compartment, the most significant effect in this study was on the restoration of B cell functional responses.

**Clinical Correlates of influenza vaccine responses**

There were no differences between the Primed and Non-primed groups in terms of demographics, medications, hospitalizations, or baseline CD3, CD19, ALC, or ANC. Four patients developed engraftment syndrome and all were in the Non-primed group. Two of these four received short courses of steroids. No subject developed community acquired influenza and sought medical attention. We considered whether any of the clinical variables might influence HAI responses. Age, gender, race, dexamethasone prior to enrollment, bortezomib prior to enrollment, baseline CD3, baseline CD19, baseline ANC, and baseline ALC were examined using the Wilcoxon test for association with HAI seroconversion status. None of the variables were statistically associated with seroconversion. Study subjects also received the conjugated pneumococcal vaccine as a control and we evaluated the concordance between pneumococcal responses and HAI seroconversion. We defined a pneumococcal responder by summing the responses to four serotypes and defining a four-fold increase from baseline. There was no association between HAI responders and pneumococcal responders. Overall, 21% of enrolled subjects (all primed with the conjugated
pneumococcal vaccine) were pneumococcal responders, much lower than the 73% overall responder frequency seen in the Primed group for influenza seroconversion.
Discussion

This study demonstrates that seasonal influenza vaccination priming prior to autologous T cell collection, ASCT, infusion of autologous T cells and repeat influenza vaccination leads to superior influenza vaccine responses in patients with multiple myeloma compared to the Non-primed patients. The importance of this result is two-fold. Infection is one of the most common causes of death after transplantation for multiple myeloma\textsuperscript{26,27}. This is thought to be a consequence of the age at the time of transplant and the natural immune suppression arising after high-dose melphalan. The provision of an autologous co-stimulated and expanded T cell product may improve the outcome by augmenting host responses to infection. In this study, the provision of the autologous T cell product did not restore vaccine responses unless the patient and the T cell product had been primed by prior antigen exposure. Even though both groups of patients had full T cell recovery after melphalan, the Non-primed group did not respond to the post-transplant vaccine. Therefore, this strategy could be broadly applicable in transplant patients where influenza is a common pathogen with high morbidity\textsuperscript{27}. The second implication of this work is that this strategy could be harnessed for improved tumor vaccine responses, where responses to date have been compromised by poor T cell function post-ASCT\textsuperscript{18}.

In a prior study, we explored an approach of combination immunotherapy with the 7-valent pneumococcal conjugate vaccine and adoptive T-cell\textsuperscript{29}. We
immunized patients with PCV before T-cell collection and after ASCT and infusion of the autologous co-stimulated and expanded T-cells product \(^{46}\). This corrected the immunodeficiency and lymphopenia and improved the responsiveness to the pneumococcal vaccine when compared to similar groups of patients that did not receive this approach \(^{29}\). The combined results of the current influenza study and the pneumococcal study suggest broad applicability of co-stimulated and expanded T cells to boost vaccine-induced immunity.

When analyzed without stratifying by study group, B cell ELISPOT results were significantly associated with subsequent HAI titers. This assay measures only bloodstream B cells responding to antigen and may underestimate the potential in the secondary lymphoid organs where the memory B cells are located. Nevertheless, B cells were clearly impacted by the primed autologous T cell product because antigen-specific antibody production was enhanced and B cell responses were increased after the priming dose and the B cell ELISPOT results were strongly associated with subsequent antibody production.

The mechanism underlying this effect remains to be determined. The conditioning regimen and lymphodepletion leads to high levels of IL-15 in the serum, which probably contributes to the significant expansion of CD8 T cells \(^{30}\). Similarly, high levels of IL-6 could support B cell recovery \(^{47}\). The global expansion of central memory CD8 T cells generically (Figure 3) may explain the previously observed increase in CMV responsiveness seen after autologous T
cell infusion but does not account for the enhanced response to the inactivated influenza vaccine seen exclusively in the primed patients 29,48. The CD4 T cells were less tolerant of melphalan and in the absence of a primed post-ASCT infusion, may not have been able to supply sufficient help for B cell differentiation. At the time of the study design, follicular helper T cells (T_{FH} cells) were not well-defined and we did not perform flow cytometric identification of these cells 49. We hypothesize that the autologous T cell product contained antigen-specific T_{FH} cells, which contributed to the B cell maturation and subsequent production of HAI antibodies.

This study contributes to the growing body of knowledge regarding the provision of antigen-specific defenses to immune compromised patients. Achieving the goal broadly could markedly improve the survival of patients with multiple myeloma who undergo ASCT. In this patient population, infection is the most common cause of morbidity 7,8,18,27. In addition, harnessing antigen-specific immunity for tumor vaccines will become increasingly important as immune-based therapies become a reality 50.
Acknowledgements

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Authorship Contributions and Conflict of Interest

Drs. Stadtmauer, Rapoport, Sullivan, Vogl and June conceived and designed the study. Drs. Jawad, Luning Prak, and Boyer provided statistical and analytical support for the study. The autologous T cells were produced by Drs. Levine and Vonderheide. Clinical trials management, enrollment and database analyses were provided by Mss. Veloso, McDonald, Hou, Murphy, Bhagat, Mangan, and Chew. Dr. Aqui provided T cell analyses.

Conflict of Interest:

Edward A. Stadtmauer, Dan T. Vogl, Aaron P. Rapoport, Bruce L. Levine, Eline Luning Prak, Jean Boyer, Nicole A. Aqui, Xiaoling Hou, Heather Murphy, Rita Bhagat, Patricia A. Mangan, Anne Chew, Elizabeth A. Veloso, Kenyetta R. McDonald, Abbas Jawad, and Kathleen E. Sullivan- No conflict of interest.

Dr. Vonderheide is an inventor on a patent that relates to hTERT immunotherapy.

Dr. June is an inventor on a patent related to the T cell manufacturing process that was used for this protocol. This conflict has been disclosed and was managed in accordance with the policies of the University of Pennsylvania.
References


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Figure 1. The study protocol. A) The clinical protocol is shown schematically. Primed patients received both a pre- and post-ASCT vaccine, while the Non-primed group received only the post-ASCT vaccine. B) The CONSORT diagram of the study enrollment.

Figure 2. Influenza titers. HAI influenza titers are induced to high levels in the Primed Group. Geometric mean titers and 5-95% confidence intervals are displayed. The mixed effects model for repeated measures demonstrated a p value of 0.006 for H1N1 and p<0.0001 for H3N2.

Figure 3. B cells are depleted after melphalan conditioning. Plotted are the average absolute B cell counts (top panel), absolute numbers of naive B cells (IgM+, CD27-, middle panel) or percentage of B cells with a switched memory phenotype (IgM-, CD27+, bottom panel) as a function of time. At most time points, the majority of the B cells are naïve B cells. During the time of maximal B cell depletion (Day 14), switch memory B cells predominate.

Figure 4. Effect of melphalan and T cell infusion of T cell subsets. T cell subsets are differentially affected by conditioning and autologous T cell infusions. The subsets were identified as described in the Methods section and the fraction of each subset within the CD3 population is plotted on the Y-axis.
Figure 5. Functional analysis of T cell and B cell responses. Functional responses to antigen are augmented in the Primed Group. T cell and B cell responses to influenza antigens were measured by antigen-specific ELISPOTS. CD4 T cell-antigen-specific responses (γ-interferon responses to intact protein after CD8 T cell depletion) were increased in the Primed Group compared to the Non-primed Group at the T cell harvest time point (p=0.02). There were no statistically significant differences between the two groups for the CD8-specific responses (responses to influenza peptides) and the global responses (PMA and ionomycin and total IgG) also did not differ between the two groups at any time point.
Figure 1

A

V-T-V Primed → Influenza vaccine → T cell harvest → Stem cell harvest → Megablock → Stem cell infusion Day 2 → Stem cell infusion Day 14 → Assays

T-V Non-primed → Trial Enroll → Cyclophosphamide

B

Assessed for eligibility for companion transplant study (n=43)

Excluded (n=19)
- Not meeting inclusion criteria (n=4)
- Declined to participate (n=14)
- Died prior to enrollment (n=1)

Participated in companion transplant study (n=24)

Excluded, influenza vaccine not available (n=3)

Enrolled (n=21)

Allocated to 2-vaccine group (n=11)
- Received both vaccines (n=11)
  Did not complete 180 days of follow-up (n=2)
  - Died on day +37 of transplant from transplant-related toxicity (n=1)
  - Died on day +164 of transplant from progressive disease (n=1)
  Included in analysis (n=11)

Allocated to 1-vaccine group (n=10)
- Received day +14 vaccine (n=10)
  Did not complete 180 days of follow-up (n=1)
  - Hospice care started on day +168 for progressive disease (n=1)
  Included in analysis (n=10)
Figure 2

HAI H1N1 Geometric Mean

HAI H3N2 Geometric Mean

HAI Influenza B Geometric Mean
Figure 4

% Naive CD4

% Naive CD8

% CD4 Central Memory

% CD8 Central Memory

% CD4 Effector Memory

% CD8 Effector Memory

% CD4 Reverted Memory

% CD8 Reverted Memory
Figure 5

PMA Ionomycin T Cell ELISPOT

B cell ELISPOT Total IgG

CD8-Specific T Cell ELISPOT

B cell ELISPOT Anti-flu

CD4-Specific T Cell ELISPOT

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