

Restoration of immunity in lymphopenic individuals with cancer by vaccination and adoptive T-cell transfer

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Immunodeficiency is a barrier to successful vaccination in individuals with cancer and chronic infection. We performed a randomized phase 1/2 study in lymphopenic individuals after high-dose chemotherapy and autologous hematopoietic stem cell transplantation for myeloma. Combination immunotherapy consisting of a single early post-transplant infusion of *in vivo* vaccine-primed and *ex vivo* costimulated autologous T cells followed by post-transplant booster immunizations improved the severe immunodeficiency associated with high-dose chemotherapy and led to the induction of clinically relevant immunity in adults within a month after transplantation. Immune assays showed accelerated restoration of CD4 T-cell numbers and function. Early T-cell infusions also resulted in significantly improved T-cell proliferation in response to antigens that were not contained in the vaccine, as assessed by responses to staphylococcal enterotoxin B and cytomegalovirus antigens ($P < 0.05$). In the setting of lymphopenia, combined vaccine therapy and adoptive T-cell transfer fosters the development of enhanced memory T-cell responses.

A prolonged and severe immune deficiency has prevented successful vaccination after high-dose chemotherapy and autologous stem cell transplantation^{1,2}, leading to impaired immune responses against tumor and microbial antigens. Retrospective studies suggest that better clinical outcomes may be associated with more rapid lymphocyte recovery after transplant³. In addition, myeloma-reactive T cells have been detected at low frequencies in the marrow or blood of untreated individuals with myeloma^{4,5}. Thus, strategies to augment the recovery and function of autologous T cells after transplant may be beneficial.

We hypothesized that combination immunotherapy consisting of the adoptive transfer of *in vivo* vaccine-primed T cells and post-transplant vaccination might provide a platform for post-transplant immunotherapy in the setting of chemotherapy-induced lymphopenia. We stimulated autologous T cells by coculture with immunomagnetic beads to which CD3-specific and CD28-specific monoclonal antibodies had been conjugated, based on studies showing that signals through CD3 and CD28 can prevent T-cell anergy^{6–10}. For purposes of vaccination, we used the 7-valent vaccine pneumococcal conjugate vaccine (PCV) because it invokes T cell-dependent antibody responses and is approved by the US Food and Drug Administration for use in children¹¹. Using randomized design in which participants were assigned to receive T-cell infusions either on day 12 or day 100

after transplant, and either one pre- and two post-transplant PCV immunizations or two post-transplant immunizations only, we sought to determine: (i) whether adoptive transfer of costimulated autologous T cells could accelerate or improve recovery of acquired immunity, and (ii) whether vaccine-primed T cells administered in conjunction with immunizations could augment the development of pneumococcal immunity.

RESULTS

Subjects and treatment

We carried out a randomized phase 1/2 trial in individuals with advanced myeloma to determine whether combination immunotherapy consisting of vaccination and adoptive T-cell transfer could correct the immunodeficiency and lymphopenia induced by high-dose chemotherapy (Fig. 1). We enrolled a total of 54 participants. We first carried out a single arm run-in phase to show the safety and feasibility of T-cell collection and expansion in this participant population and to work out logistical details. Twelve individuals participated in this phase. After the run-in phase, we enrolled 42 participants in a second phase of the protocol in which *ex vivo*-costimulated T-cell infusions were combined with immunizations using the 7-valent pneumococcal conjugate vaccine (PCV, Prevnar). We randomly assigned the 42

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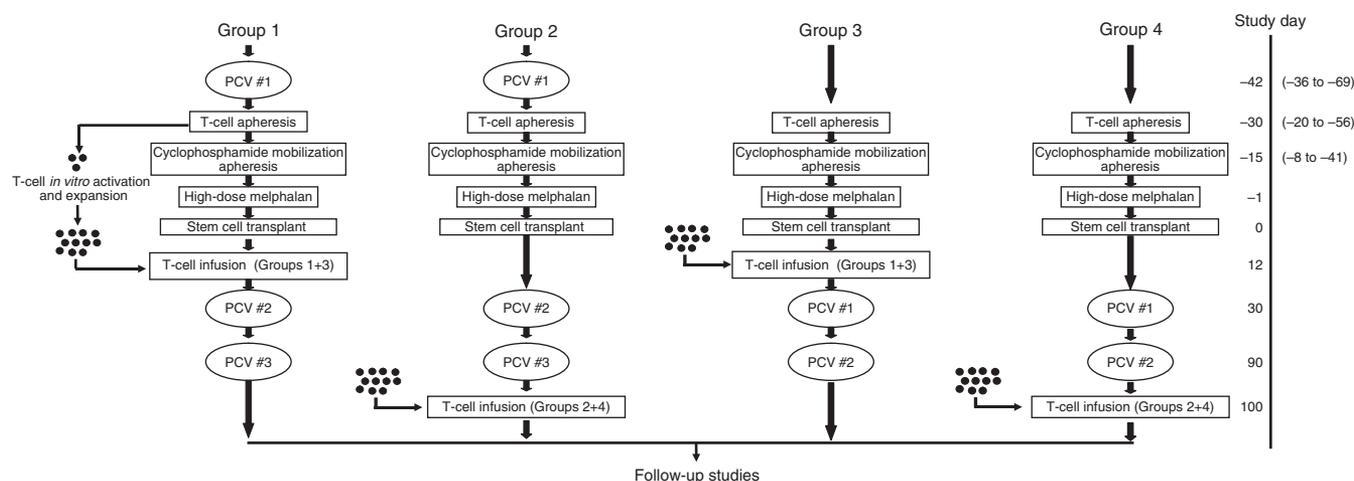


Figure 1 Protocol design. Study day 0 is designated as the day of hematopoietic stem cell transplantation. We performed two apheresis procedures to provide T cells for culture and CD34⁺ progenitor cells for transplantation.

participants to one of four different treatment groups using a random number generator (**Fig. 1**). Participants assigned to Group 1 received three PCV immunizations including: (i) about 10 d before the steady-state collection of mononuclear cells, (ii) at day 30 after transplant and (iii) at day 90 after transplant and also an infusion of costimulated T cells ‘early,’ at day 12 after transplant. Participants assigned to

Group 2 received three PCV immunizations as for the Group 1 participants, but received the T cells at study day 100 after transplant. Participants assigned to Group 3 received two PCV immunizations, one each at day 30 and day 90 after transplant only, along with an infusion of T cells at day 12 after transplant. Participants assigned to Group 4 received two PCV immunizations as for the Group 3 participants, but received T cells at ~day 100 after transplant. We randomly assigned 10 subjects each to Groups 1 and 2, and 11 subjects each to Groups 3 and 4. Characteristics of the randomized groups were well balanced (**Tables 1** and **2**). There was no statistical difference, as determined by two-tailed *t*-test, among the groups in the total number of adoptively transferred T cells (**Supplementary Fig. 1** online).

Table 1 Subject characteristics

Characteristic	Value
Total subjects enrolled	54
	<div style="display: flex; align-items: center;"> <div style="border-left: 1px solid black; border-right: 1px solid black; width: 10px; height: 10px; margin-right: 5px;"></div> <div style="margin-right: 10px;">12 nonrandomized run-in phase</div> </div> <div style="display: flex; align-items: center;"> <div style="border-left: 1px solid black; border-right: 1px solid black; width: 10px; height: 10px; margin-right: 5px;"></div> <div>42 randomized subjects</div> </div>
Median age	56 years (range, 38–71)
Gender	
Male	36 (67%)
Female	18 (33%)
Ethnicity	
White	44 (81%)
Black	10 (19%)
Subtypes	
IgG	31 (58%)
IgA	12 (22%)
Light chain only	11 (20%)
Median β 2-microglobulin (at diagnosis)	3.3 (1.09–73.7)
Median number of prior regimens	2 (range 1–5)
Median percent plasmacytosis (at diagnosis)	40% (range 2–100%)
Cytogenetics:	
Normal	41 (76%)
Unknown	5 (9%)
Abnormal	8 (15%)
del 13/13q:	1
ch. 11 abnormality:	2 (+ other abnormalities)
Complex	3
Other	2

T-cell recovery

The randomized design of this trial (**Fig. 1**) afforded the opportunity to determine whether adoptive transfer of *ex vivo* costimulated polyclonal T cells could accelerate recovery of CD4⁺ or CD8⁺ T-cell

Table 2 Subgroup characteristics

Characteristic	Group				<i>P</i> value
	1	2	3	4	
Number	10	10	11	11	
Median age	54.5	57.5	55	54	0.43
Median marrow plasmacytosis (at diagnosis)	40.5	40	40	65	0.74
Median β 2-microglobulin (at diagnosis)	3.9	3.1	3.5	3.6	0.88
Gender (male/female)	6/4	5/5	9/2	8/3	0.45
Abnormal cytogenetics (number)	1	2	2	2	0.99
Number of prior regimens (median, range)	2 (1–4)	1 (1–4)	2 (1–3)	2 (1–3)	0.85
Time from diagnosis (median in months, range)	5.4 (2.3–8.4)	5.5 (0.8–9.6)	4.3 (0.9–7.4)	4.4 (1.2–10.0)	0.63

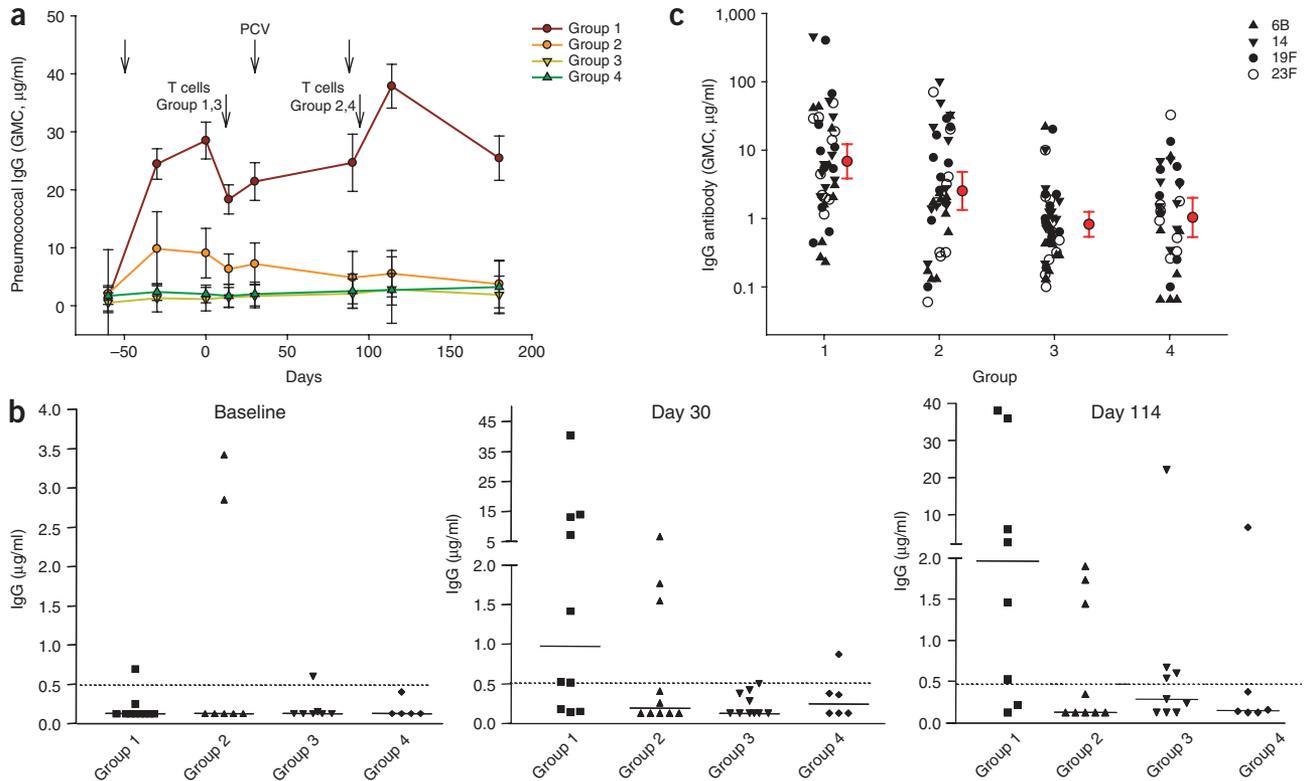


Figure 2 Time course and magnitude of total serum IgG antipneumococcal antibody responses. **(a)** Kinetics of response to serotypes 6B, 14, 19F and 23F in Groups 1–4. Individual ELISA assays specific for the indicated serotypes at each of the time points were performed, and the results were summed for each participant. The geometric mean antibody concentration is plotted (GMC \pm 95% confidence interval (CI)). Data represent 7–10 participants per data point in Groups 1–3, and 5–7 participants per data point in Group 4. Group 1 is superior to Groups 2, 3 and 4 at days 42–180 after transplant ($P < 0.002$). **(b)** Scattergrams of serum IgG levels to pneumococcal serotype 6B at baseline, and study day 30 and 114 for Groups 1–4, with a lower limit of detection of 0.13 $\mu\text{g/ml}$. The GMC is indicated for each group; Group 1 is superior to Groups 2–4 at day 30 ($P < 0.03$, $P < 0.01$ and $P = 0.02$, respectively) and at day 114 ($P = 0.02$, $P = 0.07$ and $P = 0.05$, respectively). Reference line indicates the threshold level that is potentially protective ($> 0.5 \mu\text{g/ml}$). **(c)** The peak serum antibody responses and the GMC \pm 95% CI (in red) of the peak responses during the first 180 d after transplant are plotted for serotypes 6B, 14, 19F and 23F for Groups 1–4. Group 1 is superior to Group 2 ($P = 0.02$) and Groups 3 and 4 ($P < 0.001$).

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populations after chemotherapy-induced lymphopenia. We compared the geometric means of the CD4⁺ and CD8⁺ T-cell counts at transplant day +42 between these groups using the *t*-test with the Satterthwaite approximation method. The differences were significant ($P = 0.004$ for CD4⁺ T-cell counts; $P = 0.04$ for CD8⁺ T-cell counts; **Supplementary Note** online). These results indicate that an infusion of polyclonal costimulated T cells early after transplant can accelerate the recovery of CD4⁺ and CD8⁺ T cells after chemotherapy-induced lymphodepletion. The randomized T-cell infusion groups did not differ with respect to the number of CD34⁺ progenitor cells or passenger lymphocytes that were infused at the time of autotransplantation.

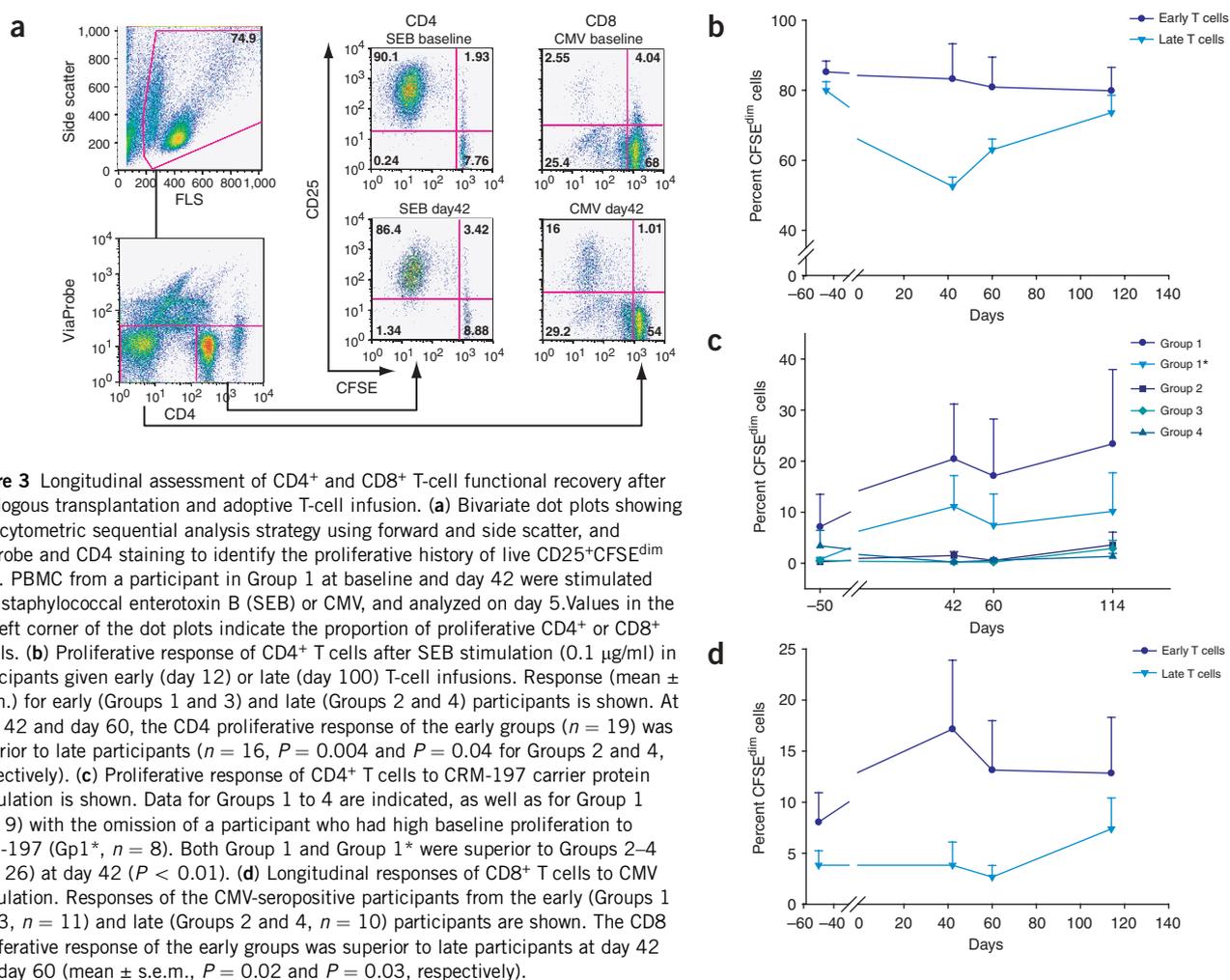
Studies of B-cell function

We aimed to determine whether administration of a T cell-dependent conjugate vaccine for *Streptococcus pneumoniae* (PCV) combined with adoptively transferred T cells could enhance recovery of B- and T-cell function. We measured T-cell dependent antipneumococcal IgG antibody titers to serotypes 6B, 14, 19F and 23F for all study participants (**Fig. 2a**). The geometric mean responses of Groups 1 and 2 to the initial priming immunization were similar ($P = 0.33$), peaking between 12 and 28 $\mu\text{g/ml}$ on day -30. In contrast, the response of Group 1 was superior to Groups 2–4 at days 42 to 180 after transplantation ($P < 0.002$). In the period after transplant, the

antipneumococcal antibody response rapidly waned nearly to baseline levels in Group 2, whereas Group 1 responded to post-transplant booster immunizations and had sustained antibody titers for the duration of the study. Groups 3 and 4 did not respond to post-transplant immunizations and had low or absent antibody levels throughout the study, confirming previous reports of severe post-transplant immunosuppression^{1,12,13}.

There was considerable heterogeneity among subjects in the responses to the various capsular antigens contained in the vaccine (**Fig. 2b**). But the magnitude of the antipneumococcal responses to serotype 6B in Group 1 subjects was superior to Groups 2–4, both early and late after transplantation ($P \leq 0.02$). We selected serotype 6B for analysis because it is considered to be poorly immunogenic¹⁴ and may thus be more representative of responses to tumor-antigens.

The robust magnitude and the kinetics of the antibody responses in some Group 1 subjects were notable. Although there is a lack of definitive serological correlates of protection for most of the pneumococcal serotypes included in polyvalent pneumococcal vaccines, in children antipneumococcal antibody levels of $> 0.5 \mu\text{g/ml}$ have been shown to correlate with clinical protection from invasive disease¹⁵. As a reference level, this is a somewhat more conservative threshold than suggested in other studies¹⁶. At day 30, 6 of 10 (60%) subjects in Group 1 were potentially protected against serotypes 6B, 14, 19F and



23F, whereas only 5 of 27 (18%) subjects in Groups 2–4 were protected against all four serotypes ($P = 0.02$, chi-squared test). The cumulative peak antibody responses to serotypes 6B, 14, 19F and 23F were higher in Groups 1 and 2 than in Groups 3 and 4 (Fig. 2c). Given that the peak levels were < 1 μg/ml in many subjects in Groups 3 and 4, these subjects were unlikely to be protected from invasive infection, indicating that the pre-transplant vaccine regimen schedule was superior to the post-transplantation-only schedule. Furthermore, when considering the subjects who received three vaccines (*i.e.*, Groups 1 and 2), the peak titers were higher in Group 1 than in Group 2 ($P < 0.02$), consistent with the augmentation in mean titers by early T-cell infusion (Fig. 3a). Finally, it is notable that the peak antibody levels were generally higher than has been reported in elderly and normal adult volunteers after PCV immunization^{17–21}. Because the Group 1 treatment schedule differed from the Group 2 schedule only in the timing of the T-cell infusion, these results indicated that infusion of antigen-primed costimulated T cells early after transplant followed by booster immunizations leads to more robust and sustained T cell-dependent antibody responses.

Studies of T-cell function

The function of T cells, in particular CD4⁺ cells, is impaired for months to years after peripheral blood stem cell transplantation². The accelerated numerical T-cell reconstitution combined with the augmented T cell-dependent IgG antibody responses to vaccination in

Group 1 subjects suggested that T-cell function was improved by early adoptive T-cell transfer after transplant. To address this issue, we isolated T cells from subjects at baseline and after vaccination and transplantation and stimulated them with a variety of antigens and mitogens to assess CD4⁺ and CD8⁺ T-cell function. We labeled the cells with a fluorescent dye and measured cell division by the halving of fluorescence with each generation (Fig. 3a). The T cell-proliferative responses were not concordant, with examples of maintained, improved or impaired responses observed in the same subject sample when pre-transplant and post-transplant T cell-proliferative responses were compared; for example, a subject from Group 1 maintained a robust CD4⁺ T cell-proliferative response to staphylococcal enterotoxin B (SEB) on day 42, whereas in the same subject the day 42 CD8⁺ T cell-proliferative response to cytomegalovirus (CMV) showed an increase (Fig. 3a).

We used the superantigen SEB to measure the general proliferative capacity of CD4⁺ T cells after transplantation, as the response to this mitogen should be independent of the relatively constricted T-cell receptor repertoire after transplantation²². All subjects had robust T-cell proliferation before transplant, as $85 \pm 3\%$ of CD4⁺ T cells had diluted carboxyfluorescein succinimidyl ester (CFSE) content by day 5 of culture after SEB stimulation (Fig. 3b). But only the subjects given early T-cell infusions in Groups 1 and 3 had intact proliferative capacity on day 42 after transplant, whereas the subjects who had not yet received adoptively transferred T cells in Groups 2 and 4

Table 3 T-cell infusion events

Toxicity	I	II	III	IV	Total
Chills or rigors	7 (13%)	21 (40%)	0	0	28 (54%)
Fever	9 (17%)	3 (6%)	2 (4%)	0	14 (27%)
Nausea or vomiting	7 (13%)	1 (2%)	0	0	8 (15%)
Hypertension	3 (6%)	0	1 (2%)	0	4 (8%)
Hypotension	2 (4%)	0	0	0	2 (4%)
Pain or headache	3 (8%)	0	0	0	3 (8%)
Dyspnea or hypoxia	1 (2%)	1 (2%)	1 (2%)	0	3 (6%)

had impaired proliferation ($P < 0.02$). After the late T-cell infusion on study day 100, Groups 2 and 4 subjects experienced restoration of responsiveness to SEB.

We next assessed CD4⁺ T-cell responses to the CRM-197 protein. The pneumococcal vaccine is a 7-valent vaccine that contains the capsular antigens for *S. pneumoniae* conjugated to a carrier protein, CRM-197 (ref. 23). At baseline, most subjects had low but detectable levels of CD4⁺ T-cell proliferation to CRM-197, consistent with a central memory response (Fig. 3c). But one subject had high-level proliferation to both CRM-197 (64.5%) and diphtheria toxoid (30.9%, data not shown) at baseline, consistent with past infection with diphtheria or recent tetanus or diphtheria vaccine. Therefore, the data for Group 1 are presented with and without the inclusion of this subject. At 42 d after transplant, subjects in Group 1 had high levels of CD4⁺ T-cell proliferation in response to CRM-197 stimulation, whereas all other groups were at baseline levels ($P < 0.01$). Thus, by comparing Groups 1 and 2, we concluded that a pre-transplant immunization with the protein conjugate vaccine alone was insufficient to confer restoration of antigen-specific CD4⁺ T-cell function, whereas a pre-transplant immunization in combination with an early infusion of costimulated polyclonal T cells obtained after *in vivo* vaccine priming resulted in the generation of robust antigen-specific CD4⁺ T-cell proliferation in the early period after transplant. The elevated level of proliferation was maintained for at least several months in the Group 1 subjects, consistent with the generation of memory T cells. All subjects received T cell–replete peripheral blood apheresis products for the stem cell graft on day 0 of the study; the grafts contained an average of 3×10^9 passenger T cells. Thus, the carryover of vaccine-primed resting T cells during the infusion of peripheral blood T cells collected during the stem cell mobilization apheresis was insufficient to prime this process, as we could not detect CD4⁺ T-cell proliferation in Group 2 subjects above baseline until day 114 after transplant (*i.e.*, 2 weeks after the late infusion of costimulated and primed T cells; Fig. 3c).

To determine whether the costimulated T-cell infusions had an effect on CD8⁺ T-cell function, we evaluated the proliferative response to CMV in the subjects who were CMV seropositive. Beginning as early as day 42 after transplantation (*i.e.*, ~30 d after early T-cell infusion), there was an increased proliferative response of the CD8⁺ T cells to CMV stimulation in subjects receiving early T-cell infusions that was superior to the response seen in the late T-cell infusion group (Fig. 3d, $P = 0.02$). We did not detect CMV viremia or clinical infection in any of the subjects. Together, these results indicate that T-cell function in subjects improved in response to antigens in the vaccine, as assessed by CRM-197 and T cell–dependent antibody titers, after early T-cell infusion. But it is notable that the responses of T cells to antigens not contained in the vaccine improved as well, as shown by the responses to SEB and CMV stimulation. This indicates general improvement of T-cell proliferative capacity in the subjects, as it

occurred independent of vaccination and was associated with early T-cell infusions. It is possible that homeostatic T-cell expansion contributed to the enhanced SEB and CMV responses, because the T-cell levels were higher in the early group (Supplementary Fig. 2 online).

Clinical outcomes

The Kaplan-Meier plots of the overall and event-free survivals for the entire cohort are shown in Supplementary Fig. 3 online. See Supplementary Note online for details. Using laboratory parameters at diagnosis for baseline levels, the following maximal responses were obtained: 12 participants (22%) had a complete response, 38 participants (72%) had a partial response and 3 participants (6%) had a minimal response in accordance with standard criteria. At last update, 41 participants remained alive at a median follow-up time point of 16 months, and 23 participants were surviving event-free at a median follow-up time point of 13 months.

DISCUSSION

High-dose therapy followed by autologous stem cell transplantation is an effective means to achieve a minimal disease state and improve survival for myeloma. But relapses are frequent and cures are rare because of the lack of an effective antitumor immune response to eradicate minimal residual disease. In principle, the early post-transplant state is an ideal setting for immunotherapy given the reduction of tumor burden. But immunotherapy in the early post-transplant period has been unsuccessful to date, as the immune system is typically characterized by low lymphocyte counts and impaired immune-cell function during this time². Animal models suggest that the early post-transplant period may be most appropriate for the induction of effective antitumor immune responses^{24,25}, and furthermore that the combination of vaccine and adoptive immunotherapy may augment antitumor responses²⁶. These animal models, along with prior clinical studies that showed poor post-transplant immune responses to antimicrobial vaccines^{13,27}, suggested that combination immunotherapy to accelerate immune reconstitution might result in more rapid and potent induction of immunity. In the absence of an established myeloma vaccine, we first evaluated our strategy for reconstitution of immunity in the context of a randomized controlled study by testing a licensed pneumococcal vaccine.

Constriction of the T-cell repertoire and a variety of functional impairments^{2,22} contribute to the immunodeficiency that can persist 4–10 years after autologous stem cell transplantation²⁸. We found that a single infusion of costimulated autologous T cells in the early post-transplant period accelerated the numerical and functional recovery of T cells. Robust numerical T-cell recovery has been previously observed in smaller nonrandomized studies of costimulated autologous T-cell infusions^{9,10}, but this is the first randomized study to show accelerated T cell reconstitution, and the first demonstration of improved immune function. A corollary of our findings is that the large numbers of passenger T lymphocytes in the autologous peripheral blood stem cell product (range, 0.29 – 10.6×10^9 in this protocol) are not sufficient for rapid immune reconstitution and do not provide help for vaccination in the early post-transplant period. In addition, our results confirm previous studies showing a profound reduction of vaccine responses in recipients of autologous peripheral blood stem cell and bone marrow transplants within the first 2 years after transplant^{12,13}.

Costimulated autologous T-cell infusions have been well tolerated in previous nonrandomized studies^{9,10}. The early T-cell infusion subjects had a delayed 23% incidence of rash at a median of 13 d (range, 3–48) after T-cell infusion that was not observed in the early post-transplant period in Groups 2 and 4. The etiology of the rash

remains to be determined, but the timing and appearance was typical of previous reports of autologous graft-versus-host disease^{1,29}. It is not clear from our study whether T-cell costimulation is necessary for the augmentation of cellular and humoral immunity that was observed in Group 1. For practical matters, it was necessary to have an efficient culture system in order to achieve the target dose of 10^{10} adoptively transferred T cells. But it is possible that the increased T-cell numbers rather than their activated or costimulated state were primarily responsible for the enhanced immune responses.

Preclinical studies suggested that adoptive transfer would increase vaccine responses in individuals who had undergone transplants³⁰, and mathematical modeling suggests that adoptive cell transfers could improve tumor immunotherapy³¹. This is among the first studies to show that adoptive transfer of antigen-primed, *ex vivo* costimulated T cells leads to accelerated immune reconstitution and enhanced antigen-specific CD4⁺ and CD8⁺ T-cell function *in vivo*. This conclusion is based on two lines of evidence: first, antipneumococcal antibody responses to the T cell-dependent PCV were equivalent in Groups 1 and 2 after the pre-transplant immunization; however, only the response in Group 1 subjects was sustained and boosted by subsequent immunizations after transplant. The transient response in Group 2 subjects probably represents insufficient CD4⁺ T helper cell effects to sustain the B-cell expansion and differentiation, and is consistent with recent studies showing that age-related reductions in the cognate helper function of CD4⁺ T cells contribute substantially to defects in B-cell function and impaired humoral responses observed in aged individuals³². Second, the proliferative T-cell responses to the CRM-197 carrier protein of the PCV were significantly greater in Group 1 subjects ($P < 0.01$). In contrast, CD4⁺ T cells in subjects from Groups 2–4 were anergic throughout the entire post-transplant period.

Our results agree with those from previous studies of PCV immunizations in individuals who have undergone autologous or allogeneic transplants^{33,34}, which show that pre-transplant immunization results in higher pneumococcal antibody responses during the post-transplant period. T-cell responses were not examined in these studies.

An unexpected result from our study was that the proliferative response of CMV-specific CD4⁺ and CD8⁺ T cells was enhanced in recipients of early T-cell infusions. Further studies will be required to determine the mechanism of this effect, but it probably reflects contributions of accelerated numerical T-cell reconstitution as well as enhanced function of the T cells after early adoptive T-cell transfer. It is possible that relative depletion of regulatory T cells during the *ex vivo* expansion of the T cells to be infused also contributes to the robust immunity that we have observed. Lymphopenia in the early post-transplant period has been shown to promote homeostatic T-cell proliferation, a state in which naive and memory T cells compete with each other for expansion at the ultimate expense of naive T cells. This process can lead to enhanced function of T cells in animal models³⁵. Given that none of our subjects experienced activation of CMV infection, it is reasonable to assume that the increased function and/or numbers of CMV-specific T cells was antigen independent, and perhaps related to CD4⁺ T helper cells that can prevent the development of 'helpless' CD8⁺ T cells, memory T cells that are unable to proliferate upon antigenic stimulation³⁶. Our observation that early T-cell infusions prevented impaired CD4⁺ T-cell proliferation in response to SEB in the early post-transplant period is consistent with this hypothesis, although other interpretations are possible. Previous studies have documented profound CD4⁺ T-cell dysfunction after autologous stem cell transplantation^{2,37}.

Our protocol consisting of pre-transplant immunization followed by a single early post-transplant infusion of costimulated T cells resulted

in rapid immunologic reconstitution, as assessed by potentially protective levels of pneumococcal antibody within 30 d after transplant, and normal or above-normal antigen-specific T-cell proliferation in the early post-transplant period. We believe that the magnitude of immune reconstitution was clinically meaningful, as protective levels of antibody are estimated to be about 0.5 $\mu\text{g/ml}$ ^{15,16}. In addition, the observation that many subjects in Group 1 had antibody levels that were equivalent to or higher than those reported in previous PCV studies of normal older adults^{17–19}, adults in general^{20,21} and children^{11,38–41} indicates the robustness of the antibody responses obtained in this cohort of immunosuppressed subjects. There was substantial intersubject variability in the magnitude of the antibody responses observed, and this could reflect a number of factors, including previous clinical or subclinical pneumococcal infections, the type and cumulative amounts of previous cytotoxic chemotherapy, and the immunosuppressive effects from previous corticosteroid therapy.

The mechanism for the robust immunity in Group 1 subjects remains to be determined. It will be crucial to determine the relative contributions of antigen-dependent expansion of PCV-specific memory T cells during the *ex vivo* polyclonal stimulation or to the antigen dependent homeostatic expansion that may occur *in vivo* in the lymphopenic recipient. Alternatively, indirect mechanisms such as cytokine release from the infused expanded polyclonal population contributing to homeostatic expansion *in vivo* could be relevant.

Our long-term goal is to rebuild immunity early after cytotoxic therapy, decreasing infections and preventing relapse. It is hoped that this study may provide a platform for the design of future studies using T cells and tumor vaccines to eradicate minimal residual disease after transplant. The combination of active and passive immunotherapy evaluated in this study is a safe and potentially effective method for inducing post-transplant immune responses. Further studies are required to determine whether the principles observed here can be applied to tumor immunity.

METHODS

For details, see **Supplementary Methods** online.

Subjects and treatment. Study participants were at least 18 years old, with newly diagnosed myeloma that required systemic treatment. Most of the participants had already received induction therapy for myeloma by their referring oncologist. Untreated participants received one or two courses of pulse dexamethasone⁴². All participants gave written informed consent upon enrollment, which was performed in accordance with the Declaration of Helsinki; we obtained approval for the study from the institutional review boards of the University of Pennsylvania and the University of Maryland and from the US Food and Drug Administration.

From February 2002 to January 2004, we enrolled 54 participants at two centers (12 in the run-in phase, and 42 in the randomized phase). Eighteen of the 20 participants in Groups 1 and 2 received the three intended PCV immunizations; one participant in Group 1 did not receive the third (day 90) immunization because of a strong local reaction to the day 30 post-transplant immunization, and one participant in Group 2 did not receive the third (day 90) immunization because of mortality at day +85 after transplant. All 22 participants who were assigned to Groups 3 and 4 received the two intended post-transplant PCV immunizations at days 30 and 90. The median age was 56 years, 67% were male, and 19% were black. IgG and IgA paraproteins were present in 58% and 22% of participants respectively, whereas 20% had light-chain excretion only. There were no significant differences between the groups with respect to participant age, marrow plasmacytosis, diagnostic β_2 microglobulin levels, gender, number of prior treatments, proportion with abnormal cytogenetic studies and paraprotein subtypes. We thawed and cultured autologous T cells as specified in an US Food and Drug Administration-approved investigational new drug application^{9,43}.

Safety and toxicity. The costimulated T-cell infusions were well-tolerated. For T-cell infusion-related events, see **Table 3**.

Study design and treatment. We carried out a single nonrandomized arm run-in phase to show the safety and feasibility of T-cell collection and expansion in this subject population and to finalize logistical details. Twelve subjects participated in this phase. We enrolled 42 participants in a second phase of the protocol in which *ex vivo* costimulated T-cell infusions were combined with PCV (Pevnar) immunizations.

Laboratory end points. Primary end points for the study were numeric and functional B and T-lymphocyte recovery. We assessed antibody responses to four of the seven vaccine serotypes (6B, 14, 19F, 23F) by ELISA binding assays⁴⁴. We evaluated numeric T-cell recovery by enumeration of CD4⁺ and CD8⁺ lymphocytes at days 30, 42, 90 and 180 after transplant, with day 42 chosen as the primary endpoint to evaluate the efficacy of T-cell infusions, as Groups 1 and 3 had been infused at this point, whereas Groups 2 and 4 had not. We assessed T-cell responses to the CRM-197 carrier protein of the PCV by CFSE staining on day 6 of culture of peripheral blood mononuclear cells as described⁴⁵. We used flow cytometry to gate first by forward and side scatter, then on viable cells (ViaProbe negative, BD Biosciences) to identify CD4⁺ or CD8⁺ T cells, and finally on the CD25⁺CFSE^{dim} quadrant to quantify the antigen-specific proliferative responses for each treatment group because previous studies have shown that all activated human T cells are CD25⁺ at this point in culture⁴⁶. Because responses to conjugate vaccines reflect T-cell help, we also evaluated functional T-cell reconstitution by measurement of IgG antibody responses to the PCV vaccine as described above.

Clinical end points. Secondary end points included myeloma responses and the projected overall and event-free survival rates. We evaluated the maximal myeloma responses as specified⁴⁷.

Statistical analysis. We performed time-to-event analyses according to the Kaplan-Meier method. We used the Kruskal test or chi-squared test for comparisons of participants' characteristics in the randomized groups. We carried out pairwise comparisons of the ELISA-binding antibody levels at each timepoint using the Mann-Whitney nonparametric test or Student *t*-test after logarithmic transformation of the data. We examined the reproducibility of the ELISA by determination of the interassay percent coefficient of variation. The percent coefficient of variation differed by pneumococcal serotype and ranged from 13.3% to 22.6% for the various serotypes, with a mean of 18.6% for all serotypes. For samples below threshold, ELISA values were assigned a value of 50% of the lower limit of detection. We compared results from T-cell responses of CFSE dye-dilution experiments using two-sided *t*-test after logarithmic transformation of the data.

Note: Supplementary information is available on the Nature Medicine website.

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COMPETING INTERESTS STATEMENT

The authors declare competing financial interests (see the *Nature Medicine* website for details).

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