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Response to Pneumococcal (PNCRM7) and Haemophilus Influenzae Conjugate Vaccines (HIB) in Pediatric and Adult Recipients of an Allogeneic Hematopoietic Cell Transplantation (alloHCT)

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ABSTRACT

Young children and allogeneic hematopoietic cell transplantation (HCT) recipients respond poorly to polysaccharide antigens, rendering them susceptible to severe infections because of encapsulated bacteria. This study evaluated the responses of 127 HCT patients, median age 23.0 years, vaccinated with PNCRM7 and Haemophilus influenzae (HIB) conjugate, 2 conjugate vaccines highly immunogenic in healthy children. Median time to vaccination was 1.1 years after HCT. Sixty-two percent of patients responded to PNCRM7 (45 of 51 children, 34 of 76 adults, $P < .001$). Overall response to HIB was 86%, including 77% of PNCRM7 nonresponders. Although PNCRM7 response was adversely affected by older age ($P < .001$), individuals ≥50 years old responded significantly better if vaccinated following acquisition of specific minimal milestones of immune competence, CD4 >200/μL, IgG >500 mg/dL, PHA within 60% lower limit of normal (11 of 19 versus 0 of 8, $P < .006$). A similar trend was observed in patients with limited chronic graft-versus-host disease (cGVHD). In all patients, higher levels of circulating $CD4^+CD45RA$ cells correlated with improved PNCRM7 response. These data demonstrate that PNCRM7 is immunogenic in allogeneic HCT patients, including older adults, but suggest that vaccination at fixed intervals after HCT, irrespective of immune competence, may limit its effectiveness. Prospective, multicenter trials assessing the best strategy to administer this vaccine and its impact on pneumococcal infections following transplantation are warranted.

KEY WORDS

Vaccination • Pneumococcus • Haemophilus influenzae

INTRODUCTION

Invasive pneumococcal infections (IPIs) remain a significant, potentially vaccine preventable cause of morbidity and mortality late after successful allogeneic transplantation [1-4]. Although IPIs are highest in patients with chronic graft-versus-host disease (cGVHD) [1-5], the incidence in patients without GVHD is over 20 times that reported in the general population [1-5]. Using data prospectively collected by the Toronto Invasive Bacterial Diseases Network from 1995 through 2005, Kumar et al. [6] compared the incidence of IPIs in adults 18 to 65 years of age who did or did not receive a hematopoietic cell transplant. The calculated incidence of IPIs was 590 per 100,000 allogeneic transplant recipients per year compared with 11.5/100, 000 persons per year in nontransplanted individuals 18 to 65 years of age ($P < .0001$). All pneumococcal strains isolated from transplant patients were serotypes included in the pure pneumococcal polysaccharide vaccine, PPV23. Despite recommendations to use this vaccine after HCT [7,8], it is poorly immunogenic in transplant recipients [9-11], contributing to a false sense of security in PPV23 recipients of the vaccine and inconsistent prescribing by health care professionals [5,6].

Table 1. *Patient Characteristics*

	All N = 127	Matched Related n = 67	Unrelated n = 53	Mismatched Related n = 7
Sex, % female	58 (45.7)	29 (43.3)	26 (48.2)	3 (42.9)
Age at transplant, median, y (range)	23 (0.1-64)	32 (0.1-64)	28 (0.2-62)	6 (0.8-9)
Diagnosis (%)				
Acute Leukemia	64 (50.4)	38 (56.7)	20 (38.5)	2 (28.6)
Chronic Leukemia	6 (4.72)	4 (6.0)	2 (3.9)	0
Aplastic Anemia/MDS	26 (20.5)	8 (11.9)	11 (21.2)	3 (42.9)
NHL/HD	13 (10.2)	6 (9.0)	7 (13.5)	0
Others*	18 (14.2)	11 (16.4)	3 (5.8)	2 (28.6)
Stem-Cell product (%)				
Unmodified Transplant	56 (44.1)	35 (52.2)	20 (38.5)	1 (14.3)
T cell depleted transplant	71 (55.9)	32 (47.8)	32 (61.5)	6 (85.7)
TBI (%)	59 (46.5)	33 (49.3)	24 (46.2)	2 (28.6)
Acute GVHD (%)	10 (7.9)	5 (7.5)	5 (9.6)	0
Chronic GVHD (%)	25 (19.7)	14 (20.9)	11 (20.7)	0
Donor Leukocyte Infusion (%)	12 (9.4)	11 (16.4)	1 (1.9)	0
CD4+ CD45RA+ Median, (range)	119 (0-1199)	110 (0.3-1029)	115 (0-1077)	360 (128-1199)

In 2000, the FDA licensed the 7-valent protein-conjugated pneumococcal vaccine (Prevnar [PNCRM7®]) containing 7 serotypes (4, 6B, 9V, 14, 18C, 19F, 23F) that cause 70% to 80% of invasive pneumococcal infections in North America [12,13]. Five of the serotypes (6B, 9V, 14, 19F, 23F) are responsible for the majority of antibiotic resistant pneumococcal infections worldwide [12-14]. Conjugation of pneumococcal saccharides to a carrier protein (CRM_{197}, a nontoxic variant of diphtheria toxin) results in T cell-dependent B cell generation of memory B cells that respond to the attached sugars [12,13]. In contrast, PPV23, elicits a T cell-independent B cell response, and fails to induce antigenic memory, precluding the development of an anamnestic response following antigenic reexposure [14]. PNCRM7 vaccination has dramatically decreased the incidence of pneumococcal disease in healthy infants and children [15], populations who possess circulating B cells that are phenotypically ($CD20^+CD5^+CD38^+CD1c^+$) and functionally similar to those of HCT recipients [16].

To determine the response of allogeneic HCT recipients to PNCRM7 compared with HIB, the immunization records and pre- and postvaccine titers of all patients transplanted at Memorial Sloan Kettering Cancer Center (MSKCC) between 2002 and 2005, were analyzed for immunization against pneumococcus. The effect of stem cell and donor type, use and method of T cell depletion, presence of GVHD, and receipt of donor leukocyte infusion (DLI) on vaccine responses were assessed. The effect of PPV23 administration following the conjugate vaccine was also analyzed.

PATIENTS, MATERIALS, AND METHODS

A waiver of authorization to conduct this study was approved by the MSKCC institutional review board. The medical records of all patients who remained disease free for 1 year after an allogeneic transplant performed at this center from January 1, 12002 through December 31, 2005 were reviewed for immunization against pneumococcus. Dates of vaccination and pre- and post vaccine titers were obtained from a prospectively maintained database and confirmed by retrospective chart review. Of 189 patients who survived disease free ≥1 year after HCT, 80% were vaccinated against pneumococcus. Because of preliminary data demonstrating poor vaccine responses in patients immunized prior to the development of a CD4 cell count >200 cells/μL or IgG level >500 mg/dL, 75% of patients were vaccinated after achieving these milestones. One hundred thirty-two patients received a series of 3 PNCRM7 immunizations administered 4 to 8 weeks apart. Because of PNCRM7 shortages during the study period, 21 patients (median age: 43.1 year) received PPV23 as their primary pneumococcal vaccine. Pre- and postvaccine titers were available in 127 of 132 PNCRM7 recipients (Table 1) and 18 of 21 patients PPV23 recipients. Pre- and postvaccine titers were also analyzed in 36 of the 127 recipients of PNCRM7 who received PPV23 as their secondary pneumococcal vaccine. Ninety-four percent of patients vaccinated with PNCRM7 received a concomitant series of Haemophilus influenza b conjugate vaccines. Ninety percent of patients received at least 2 vaccines at MSKCC. All patients were evaluated at MSKCC before and after completing vaccinations,

including assessment of acute GVHD (aGVHD) and cGVHD using established criteria [17,18].

Serotype-specific pneumococcal capsular polysaccharide IgG was measured by enzyme-linked immunsorbant assay (ELISA). The U.S. Food and Drug Administration (FDA) standard reference serum 89-S was used as the calibration standard. Titers against pneumococcal serotypes 1, 3, 14, 19F, 23F, and 7F were measured to distinguish responses specific to PPV23 (1, 3, and 7F) from responses common to both vaccines (14, 19F, and 23F). Response to PNCRM7 or PPV23 was defined as seroconversion or >3-fold rise in titer against serotypes 14, 19F, and 23F, 3 significant causes of antibiotic resistant pneumococcal infections in HCT recipients [2,5]. Initial antibody response was measured at a median of 3.0 months (range: 2-12 months) after completion of PNCRM7 and HIB immunization. PPV23 response was measured at a median of 3.2 months (range: 1.5-12 months) following immunization. HIB response, measured by ELISA, was defined as seroconversion (>1 μg/mL) or >3-fold increase in antibody titer. The lower limits of quantification for pneumococcal and H flu antibody levels were 0.5 μg/mL and 0.15 μg/mL, respectively.

Immunofluorescence Studies

Two color immunofluorescence was performed on the circulating lymphocytes all patients within 3 months of vaccine initiation. Directly fluoresceinated (FITC) antibodies, including CD45 (common leukocyte antigen, positive control), MsIgG, CD3 (pan T cell), CD20 (pan B cell), CD14 (monocyte marker), and phycoerythrin-conjugated (PE) antibodies MsIgG, CD8 (cytotoxic or suppressor T cell marker), CD4 (helper T cell), CD45RA (naïve T cell subset), and CD56 (natural killer cells) were purchased from Becton Dickinson (Mountain View, CA). Immunofluorescence was performed on whole blood as previously described [19]. Immunofluorescence samples were analyzed on a FACScan (Becton Dickinson). The lymphoid populations to be analyzed were gated on using log 90 degree and forward-angle scatter characteristics. The leukocyte-specific monoclonal antibody CD45 was used to gate out any residual red cells. Monocyte contamination was ruled out by lack of reactivity (<1%) of the gated lymphoid cells with the monocyte-specific marker CD14.

Patient and Transplant Characteristics

The demographics of the 127 patients vaccinated with PNCRM7 are shown in Table 1. The median age at HCT was 23.0 years (range: 0.1-64.0 years). Thirty-three percent of the population was ≥40 years old at HCT. The stem cell donor was an HLA-A, B, DRβ1 identical sibling, an HLA-mismatched family member, or unrelated donor in 53%, 5.5%, and 42% of cases, respectively. Fifty-six percent of patients received a T cell-depleted bone marrow or peripheral blood stem cell (PBSC) product. T cells were depleted from bone marrow by soybean lectin agglutination, followed by rosetting with sheep erythrocytes (n = 16) [20], and from peripheral blood by CD34 positive selection followed by rosetting with sheep erythrocytes (n = 55) [21]. The majority (84%) of recipients of an unmodified HCT received either short-course methotrexate (MTX) combined with tacrolimus or cyclosporine (Cy; n = 33) or Cy and alemtuzumab (n = 14) for GVHD prophylaxis. Of the 56 patients who received an unmodified HCT, 26% developed cGVHD, of whom 32% were on immunosuppression at the time of revaccination.

Twelve patients received adoptive immunotherapy in the form of unfractionated donor lymphocytes for the treatment (n = 2) or prevention (n = 1) of an EBV-LPD, to prevent recurrent malignancy (n = 6), or to treat mixed T cell chimerism (n = 3). Twelve patients received posttransplant rituximab for the treatment or prevention of an EBV-LPD (n = 9), treatment of an autoimmune cytopenia (n = 2), or prevention of recurrence of a $CD20^+$ malignancy [1]. Twelve patients received the CD20 monoclonal antibody, rituximab, for the treatment of an autoimmune hemolytic anemia (n = 1), treatment (n = 2), or prevention (n = 7) of an EBV lymphoproliferative disorder, or prevention of recurrence of a $CD20^+$ malignancy (n = 2). Rituximab was administered at a median (range) of 252 (135-728) days prior to revaccination.

Biostatistics

Frequency distributions for characteristics of patients by donor type and response to vaccination was summarized in Tables 1-3. Fisher's exact test and the Wilcoxon rank sum test were used to examine covariate differences between responders and nonresponders. Subsequent to the univariate analyses, a logistic regression model was developed to determine the set of factors that independently predicted vaccine response. The Hosmer and Lemeshow goodness-of-fit statistic was used to assess how close the model-predicted values were to the corresponding observed values [22]. The statistical packages SAS (9.1) and R (2.3.1) were used to generate the test statistics and build the regression model.

RESULTS

There were no serious adverse reactions in any patient attributable to vaccination with PNCRM7. PPV23, or HIB. Prior to revaccination, over 80% of patients lacked detectable titers against any pneumococcal serotype and Haemophilus influenzae. The

Table 2. *Characteristics of Prevnar responders and nonresponder*

Characteristics	Responder N = 79	Nonresponder N = 48	Univariate	Multivariate
Median age (range)	15 (0.1-59)	40.5 (0.7-64)	<0.001	0.001
Sex, male, no. (%)	39 (49.4)	30 (62.5)	0.20	
*Diagnosis, no. (%)			0.03	0.27
Acute Leukemia	37 (46.8)	27 (56.3)		
Chronic Leukemia	4 (5.1)	2 (4.2)		
Aplastic Anemia/MDS	19 (24.1)	7 (14.6)		
NHL/HD	4 (5.1)	9 (18.8)		
Others	15 (19.0)	3 (6.3)		
Graft type, no. (%)			0.46	
Unmodified Transplant	37 (46.8)	19 (39.6)		
T Cell depleted Transplant	42 (53.2)	29 (60.4)		
Donor, no. (%)			0.09	
HLA-matched related	39 (49.4)	28 (58.3)		
HLA-mismatched related	7 (8.8)	0		
Unrelated	33 (41.8)	20 (41.7)		
TBI regimen, no. (%)	36 (45.6)	23 (47.9)	0.85	
DLI, no. (%)	2 (2.6)	10 (20.9)	0.001	0.06
Acute GVHD, no (%)	9 (11.4)	1 (2.1)	0.08	
Chronic GVHD, no. (%)	18 (22.8)	7 (14.6)	0.35	
Natural Log CD4+/CD45RA+ Median (range)	5.0 (-4.6-7.1)	4.4 (-4.6-6.4)	0.004	0.21

median time to initiate vaccination with PNCRM7 and HIB was 1.1 years post-HCT. Forty percent of patients were immunized within 1 year and 81% within 2 years of transplant. There was no significant difference in the time to PNCRM7 vaccination in recipients of a T cell-depleted versus an unmodified (T cell replete) transplant (median 1.0 year versus 1.14 years, $P = .3$). The median time to vaccination in children and adults was 1 year and 1.3 years, respectively ($P < .001$). The shorter interval between HCT and vaccination of children was in part because of more rapid immune reconstitution in patients <18 years of age combined with the tendency of pediatricians to vaccinate immediately after the milestones were met. In the 25 patients with cGVHD, the median time to vaccination was 1.8 years compared to 1.1 years in patients without GVHD ($P < .001$).

Sixty-two percent (79 of 127) of patients responded to PNCRM7 (Table 2a, 2b). A dichotomy existed between the response observed in children (median age: 9 years (0.1-17.9) and adults (median age: 41 years [range: 18-64]). Eighty-eight percent of children responded to the heptavalent pneumococcal conjugate vaccine compared to 44% of adults ($P < .001$). In contrast, only 1 of 18 patients, median age 42.5 years, immunized with PPV23 responded to serotypes 14, 19F, and 23F. Of the 16 adult recipients of PPV23, only 1 of 16 responded compared to 34 of 76 adults given the conjugated vaccine ($P = .003$). PNCRM7 response in children and adults was not affected by the use of T cell depletion or donor type (Table 2b). Five of 12 patients who received rituximab responded to PNCRM7, including 2 of 4

Table 2b. *PNCRM7 response in children (upper) and adults (lower)*

Children <18 yrs Median age: 9 years Range: 0.1-17.9		
Overall Response	45/51 (88%)	
Transplant type		P = 0.67
Unmodified	23/27 (85%)	
T-cell depleted	22/24 (92%)	
Donor Type		P = 0.40
HLA-matched Related	22/24 (92%)	
Unrelated	16/20 (80%)	
HLA-MM related	7/7 (100%)	
HLA-matched related		P = 0.53
Unmodified	14/16 (88%)	
T-cell depleted	8/8 (100%)	
Unrelated donor		P = 1.00
Unmodified	8/10 (80%)	
T-cell depleted	8/10 (80%)	
Adults >18yrs, Median 41 yrs Range: 18.0-64.0		
Overall response	34/76 (44%)	
Transplant type		P = 0.63
Unmodified	14/29 (48%)	
T-cell depleted	20/47 (43%)	
Donor Type		P = 0.35
Related	17/43 (40%)	
Unrelated	17/33 (52%)	
HLA-matched related		P = 0.20
Unmodified	10/19 (53%)	
T-cell depleted	7/23 (30%)	
Unrelated donor		P = 0.46
Unmodified	4/10 (44%)	
T cell depleted	13/23 (57%)	

children and 3 of 8 adults. For PNCRM7 vaccination, results from multivariate logistic regression model indicated that age at transplant significantly predicted response ($P = .0002$). DLI and disease diagnosis were associated with response, but did not significantly predict response after adjusting for age at transplant (Table 2a).

HIB response was evaluable in 115 of 127 recipients of PNCRM7 and 13 of 18 recipients of PPV23. Overall, 86% of PNCRM7 recipients responded to HIB, including 96% of children (48 of 50) and 79% (51 of 65) of adults ($P = .006$). Response to the HIB conjugate vaccine was observed in 77% and 100% of patients who failed PNCRM7 or PPV23, respectively. Because age was the only prognostic factor associated with response to the HIB conjugate vaccine, a single variable logistic regression model was used to predict the probability of response based on age at transplant (Table 3). A graph depicting the probability of response to HIB and PNCRM7 based on the age at transplant is shown in Figure 1.

Correlation of PNCRM7 response with in vitro parameters of immune reconstitution including the PHA response and circulating numbers of CD3, CD4, $CD4^+CD45RA$ naïve T cells, CD8, and CD19 cells/μL was evaluated. In patients >50 years of age, 11 of 19 patients vaccinated after reaching minimal milestones of immune reconstitution (CD4 >200/μL, IgG >500 mg/dL, PHA within 60% lower limit of normal) responded to PNCRM7, compared with 0 of 8 vaccinated prior to reaching these milestones ($P = .006$). Among patients vaccinated after achieving minimal milestones of immune reconstitution, there was no correlation between response and the absolute level of CD3 T cells, $CD8^+$ T cells, CD4, or $CD19^+$ B cells (data not shown). In contrast, the level of circulating $CD4^+CD45RA^+$ cells/μL, $P < .01$, correlated with response (Figure 2).

Twenty-seven patients who did not respond to an initial series of PNCRM7 were subsequently vaccinated with PPV23 (n = 16) or received a second series of PNCRM7 (n = 11). Patients were vaccinated at a median of 270 days following their last pneumococcal vaccine. Four (25%) patients responded to PPV23 and 7 (44%) to a second series of PNCRM7 ($P = .06$). Ten patients who failed PPV23 as their initial pneumococcal vaccine received a second PPV23 (n = 4) or were immunized with a series of PNCRM7 (n = 6). Five of 6 patients responded to PNCRM7 compared with 0 of 4 given a second PPV23. Response to

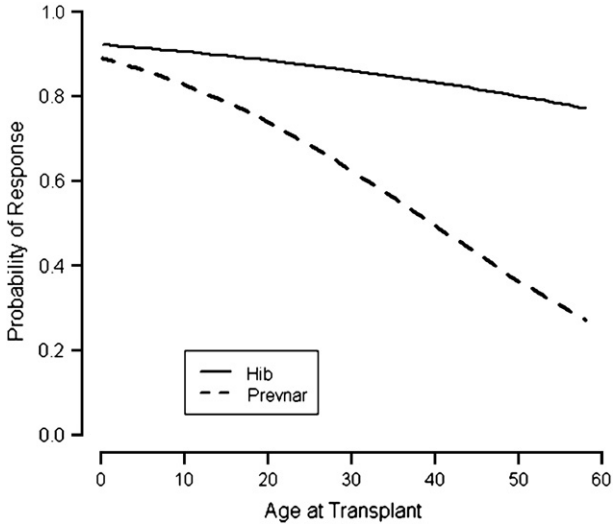

Figure 1. Predicted probability of response to PNCRM7 and HIB from logistic regression model by age at transplant.

Table 3. *Characteristics of Hib responders and nonresponder*

Characteristic	Responder N = 99	Nonresponder N = 16	*P* Value
Median age (range)	18 (0.1-64)	34.5 (0.7-62)	0.07
Sex, male, no. (%)	54 (54.6)	10 (62.5)	0.60
Diagnosis, no. (%)			0.22
Acute Leukemia	51 (51.5)	6 (37.5)	
Chronic Leukemia	4 (4.0)	2 (12.5)	
Aplastic Anemia/MDS	21 (21.2)	4 (25)	
NHL/HD	8 (8.1)	3 (18.8)	
Graft type, no. (%)			0.78
Unmodified Transplant	43 (43.4)	8 (30.0)	
T Cell depleted Transplant	56 (56.6)	8 (50.0)	
Donor, no. (%)			0.75
Matched related	49 (49.5)	9 (56.2)	
Mismatched related	7 (7.1)	0	
Unrelated	43 (43.4)	7 (43.7)	
TBI regimen, no. (%)	45 (45.5)	8 (50.0)	0.79
DLI, no. (%)	7 (7.1)	1 (6.3)	1.0
Acute GVHD, no (%)	8 (8.1)	1 (6.3)	1.0
Chronic GVHD, no. (%)	20 (20.2)	3 (18.8)	1.0

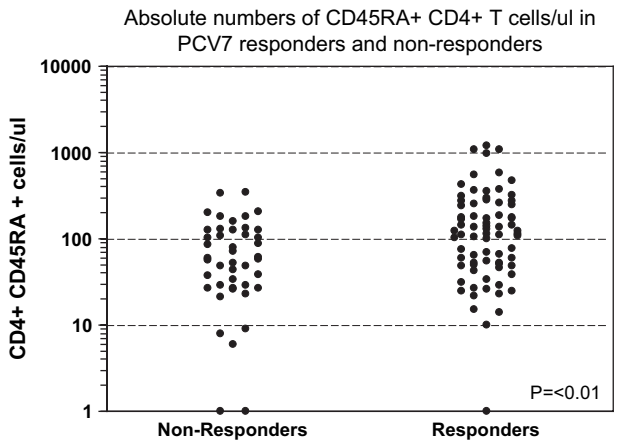

Figure 2. The figure demonstrates the relationship between circulating numbers of $CD4^+CD45RA^+$ cell counts and PNCRM7 response in 127 recipients of an allogeneic HCT.

the PPV23-specific serotypes (1, 3, 7F) was observed in <20% of patients who received PPV23 either as a primary or secondary vaccine (data not shown).

DISCUSSION

The development of more tolerable and effective cytoreductive regimens and better supportive care has increased not only the number of surviving transplant patients returning to school and the workplace, but the age of surviving population [21,23-30], individuals particularly vulnerable to severe invasive pneumococcal infections even in the absence of HCT [14]. Although the current CDC and EBMT vaccination guidelines recommend the HIB conjugate vaccine following HCT [7,8], both recommend the use of PPV23 either as a single dose of PPV23 at 12 months (EBMT) or sequential doses at 12 months and 24 months (CDC). Nevertheless, several studies have shown a dichotomy between the responses of allogeneic transplant recipients to pure polysaccharide vaccines compared with protein-conjugated vaccines. Barra et al. [31] demonstrated that only 4 of 20 (25%) adult allogeneic transplant recipients given the pure polysaccharide H flu vaccine developed a specific IgG response compared with 11 of 20 (55%) patients given the H flu conjugate vaccine ($P < .05$). Guinan et al. [9] studied the responses of 21 allogeneic and 14 autologous HCT recipients immunized with H flu conjugate vaccine and the polysaccharide pneumococcal vaccine, Pnu-immune (Lederle laboratory Division, American Cyanamid Co., Pearl River, NY). Following the 24-month immunization, only 19% of patients developed protective titers against the 6 measured pneumococcal serotypes (1, 3, 6A, 7F, 8, 9N), whereas 80% responded the HIB-conjugate vaccine. Our study similarly demonstrates that the majority of patients who fail the pneumococcal polysaccharide vaccine are capable of responding to HIB. In addition, we show that 77% of patients unable to respond to PNCRM7 responded to the conjugated HIB vaccine. The greater response to the HIB conjugate versus the pneumococcal conjugate vaccine may reflect the fact that the former vaccine contains a single protein-saccharide conjugate, is inherently more immunogenic, and/or expands memory T and B cell populations persisting from prior childhood immunization or natural infection with Haemophilus influenzae.

To date, this is the largest study evaluating PNCRM7 responses following allogeneic HCT and the first to evaluate PNCRM7 responses in adult recipients of an unmodified or T cell-depleted unrelated HCT or adults following a T cell-depleted HCT from any donor type. Although retrospective, it includes 80% of all patients transplanted during a defined period (2002-2005) who survived disease-free >1 year. Molrine et al [32] evaluated PNCRM7 response in 65 unmodified HLA-matched related HCT recipients (median age: 40 years) immunized with PNCRM7 at 3, 6, and 12 months, 30 of whom received grafts from donors immunized before bone marrow harvest. After the first immunization, antibody responses in the immunized donor group were significantly higher for 5 of the 7 vaccine serotypes. Although response after the third PNCRM7 immunization was not evaluable in approximately 30% of the starting population because of relapse (n = 8), death from other causes (n = 5), thrombocytopenia (n = 3), missed vaccines (n = 2), or DLI (n = 1), 60% of the remaining patients in both groups demonstrated significant pneumococcal titers to all 7 serotypes. In a prospective, randomized double-blind trial in adult recipients of an HLA-matched related HCT, Kumar and colleagues [33] compared the effect of vaccinating donor-recipient pairs with a single PPV23 or PNCRM7. Donors were vaccinated at least 2 weeks prior to stem cell harvest; patients at 6 months post HCT. At 12 months post-HCT, the mean number of serotypes for which a response was observed was 1.8 in the PPV23 group and 3.1 in the PNCRM7 group. Similar to the study of Storek et al. [34], no advantage to pre-HCT immunization of the donor and/or host with PPV23 was observed. Meisel et al. [35] studied the response of 43 patients <17 years of age after a related (45%) or unrelated (55%) HCT, demonstrating that 74% of 43 patients responded to all 7 vaccine serotypes following the third immunization. In a study of 30 children vaccinated according to the Royal College of Paediatrics and Child Health guidelines, Patel and colleagues [36,37] demonstrated that 80% responded to each of the serotypes contained in PNCRM7 when given 2 monthly doses initiated at 15 months or 21 months following an HLA-matched sibling (n = 10) or unrelated HCT (n = 20), respectively. Considering the poor response of transplant recipients to PPV23 [9-11,31,33,34] and its failure to reduce the incidence of pneumococcal pneumonia even in healthy middle-aged and elderly adults [38], this and the above studies [33,35] support further trials of PNCRM7 in adults and children following HCT.

The EBMT [4] and CDC guidelines [2] advocate revaccination at fixed intervals following transplant, irrespective of patient age, donor or hematopoietic cell type, intensity of conditioning, presence of cGVHD, or use of posttransplant rituximab. Nevertheless, many of these variables affect the kinetics of T and B cell reconstitution following HCT [19,39], and likely the ability of patients to respond and maintain protective titers following vaccination [40]. Only the Royal College of Paediatrics and Child Health guidelines stratify the timing of vaccination on the basis of donor type (autologous and HLA matched sibling HCT versus all other donors) because of the delayed recovery of

immune competence often associated with receipt of an unrelated or HLA mis-matched donor transplant [19,39]. Our study demonstrates that in older patients response to PNCRM7 is significantly better in those vaccinated after acquisition of minimal milestones of immune reconstitution, milestones are reached at different time points post-HCT, and depend on age and presence of cGVHD [19,39]. It also shows that even within a population of patients who have achieved minimal milestones of immune reconstitution, not all patients respond, suggesting that qualitative and/or quantitative differences in effector and memory helper T and B cells are likely to differentiate responders and nonresponders. It is possible that certain vaccines such as tetanus and polio may elicit responses in the majority of patients early post-HCT because of expansion of memory T and B cells transferred with the graft. In contrast, vaccines such as recombinant Hepatitis B [41] and pneumococcus may constitute neoantigens requiring generation of T and B cells from donor lymphoid progenitors developing within the host thymus and other tissues of the lymphoid system. Our study demonstrated a relationship between higher numbers of circulating $CD4^+CD45RA^+$ T cell numbers and improved PNCRM7 response, suggesting that responders had achieved a significant level of reconstitution from precursor populations developing within the host. The correlation, however, was not absolute. Thus, impaired reconstitution of B cell precursors or antigen-presenting cells may persist in some patients despite de novo T cell reconstitution.

Although invasive pneumococcal infections occur more frequently in patients with cGVHD, deaths from these infections occur in patients with and without cGVHD [1-5], as well as in autologous transplant recipients [5]. This study, as well as the majority of studies evaluating PNCRM7 response to date, have not included large numbers of patients with cGHVD, either by design or early death from cGVHD in enrolled patients [32,33,35,36]. Clearly, there is a need to design multicenter prospective trials that do include patients with limited and potentially extensive cGVHD to identify the most effective vaccination strategy to elicit protection against pneumococcal disease in the highest proportion of patients at the earliest time possible. Inclusion in such trials of correlative studies analyzing in vitro immune reconstitution should identify immune characteristics most predictive of early and durable immune responses.

Prior to the introduction of the conjugated pneumococcal vaccine, 3000 cases of meningitis, 50,000 cases of bacteremia, 500,000 cases of pneumonia, and 7 million cases of otitis media because of pneumococcus occurred annually in this country, causing 40,000 deaths per year (reviewed in [7]). Following widespread vaccination of infants with PNCRM7, rates of invasive pneumococcal disease because of penicillin resistant strains decreased from 6.3 cases in 1999 to 2.7 cases/100,000 in 2004 [42]. The reduction in pneumococcal infections in children vaccinated with PNCRM7 [13] and the seroconversion rates noted in this and other studies, suggest that the impact of PNCRM7 should be prospectively studied in a large multicenter trial. To improve responses in older patients, strategies such as a primary series of 4 doses of PNCRM7, the use of B and T cell immunomodulatory agents, such as interleukin-7 [43] and/or the addition of an adjuvant as incorporated in the recombinant Hepatitis B vaccine, Fendrix, should be explored [44]. To provide even more comprehensive coverage in this at-risk population, future studies assessing the PCV13 vaccine, currently being tested in healthy adults and children [45] should be undertaken.

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