Brief Report

CLINICAL TRIALS AND OBSERVATIONS

Pneumococcal Conjugate Vaccine Provides Early Protective Antibody Responses in Children After Related and Unrelated Allogeneic Hematopoietic Stem Cell Transplantation*

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Abstract

Following allogeneic hematopoietic stem cell transplantation (alloHSCT) children are at risk for life-threatening pneumococcal infections. Whereas vaccination with polysaccharide vaccines fails to elicit protective immunity in most alloHSCT recipients, pneumococcal conjugate vaccines may effectively prevent invasive disease by eliciting T-cell-dependent antibody responses. Here we report safety and immunogenicity in fifty-three children immunized with a regimen of three consecutive doses of a heptavalent pneumococcal conjugate vaccine (7vPCV) in monthly intervals starting 6-9 months after alloHSCT. Immunization was well tolerated with no vaccine-related serious adverse events. Serologic response rates evaluable in forty-three patients ranged from 41.9-86.0% and 58.1-93.0% after two and three vaccinations respectively, with 55.8 and 74.4% of patients achieving protective antibody levels to all seven vaccine serotypes. Our study provides first evidence, that vaccination with 7vPCV is safe and elicits protective anti-pneumococcal antibody responses in pediatric recipients of related or unrelated donor alloHSCT within the 1st year following transplantation.
Introduction

Patients following allogeneic hematopoietic stem cell transplantation (alloHSCT) are at significant risk for life-threatening infections in spite of leukocyte engraftment.\textsuperscript{1,2} This is based on the loss of protective immunity to vaccine-preventable diseases and the relative immaturity of the emerging immune system.\textsuperscript{3-9} In this setting, infections with encapsulated bacteria, particularly pneumococci, are associated with significant morbidity and mortality.\textsuperscript{10,11} Epidemiological studies have shown a cumulative incidence of invasive pneumococcal disease between 1 and 10\% with a median onset at one year following transplantation.\textsuperscript{12,13} Therefore, effective prevention including chemoprophylaxis and active immunization is warranted. This is particularly true for children who upon reintegration into day care and school become highly exposed to pathogens.

Current guidelines of the Centers for Disease Control and Prevention (CDC) and the European Blood and Marrow Transplantation group (EBMT) generally recommend vaccination of all alloHSCT recipients with the standard 23-valent pneumococcal polysaccharide vaccine starting at 12 months after transplant.\textsuperscript{14,15} Due to immaturity of the immune system, however, vaccination with polysaccharide vaccines fails to elicit protective immunity in the majority of pediatric alloHSCT recipients, with response rates ranging from 20-30\% in the 1\textsuperscript{st} to 50\% in the 2\textsuperscript{nd} year post alloHSCT.\textsuperscript{16,17} In contrast, in the heptavalent pneumococcal conjugate vaccine (7vPCV), serotype-specific polysaccharides are conjugated to an immunogenic protein carrier and hence elicit T-cell dependent antibody responses. 7vPCV contains the seven most prevalent pneumococcal serotypes and provides effective protection against invasive disease in infants and young children who, like alloHSCT recipients, only weakly respond to polysaccharide vaccines due to immunologic immaturity.\textsuperscript{18-20} Currently, there is a dearth of data on the use of 7vPCV in pediatric alloHSCT recipients. Here we report our data on immunogenicity and tolerability of 7vPVC.
vaccination in 53 pediatric alloHSCT recipients and show for the first time, that 7vPCV is well tolerated and provides early protective anti-pneumococcal antibody responses in children following alloHSCT from both related and unrelated donors.

Study design

Following alloHSCT from a related or unrelated donor with standard intensity conditioning children and adolescents up to 16 years of age with stable engraftment and remission from underlying malignant disease were recruited into the prospective, multi-center IKAST trial (ClinicalTrials.gov number NCT00169728) after informed consent was obtained. Approval for the IKAST trial was obtained from the ethics committees of all participating study sites. Patients transplanted for primary immunodeficiency or having uncontrolled GvHD (Lansky score <60%) or known intolerance to study vaccines were excluded. Patient-, disease- and transplant-characteristics are detailed in Table 1. Patients were immunized with a primary series of three doses of the 7-valent pneumococcal conjugate vaccine (7vPCV; Prevenar™, Wyeth Pharma, Münster, Germany) in conjunction with the hexavalent combination vaccine (Infanrix hexa™; GlaxoSmithKline, Munich, Germany) in monthly intervals starting at 6-9 months following alloHSCT with a minimum interval of 2 months from last intravenous immunoglobulin application or transfusion. Serum was drawn for assessment of antibody concentrations prior to the 1st and 4-6 weeks after the 2nd and 3rd vaccine. Serotype specific antibody concentrations to the pneumococcal antigens 4, 6B, 9V, 14, 18C, 19F and 23F were determined by an ELISA technique as previously described using the reference serum lot 89-SF (Center for Biologics, Rockville, MD) for assay standardization. An antibody concentration of \( \geq 0.5 \mu g/ml \) was considered protective as suggested by efficacy data from a large trial of 7vPCV vaccination in healthy children. Local and systemic adverse events
were prospectively collected by a standardized patient’s/guardian’s diary and physician’s assessment for the duration of one month following each administration of study vaccine.

The primary study endpoint was a serologic response to primary immunization defined as seroconversion to protective antibody levels or, in patients exhibiting protective antibody levels prior to first vaccination, a >2-fold increase from baseline antibody concentration. Accordingly, the primary study aim employed for sample size calculation was defined as detection of serologic response to ≥3 pneumococcal serotypes in >60% of study participants. Secondary endpoints included serologic responses to booster immunization scheduled one year after baseline immunization as well as safety of the vaccine with a particular focus on tolerability in the older age group (6-16 years) for which 7vPCV is currently not licensed. Geometric mean antibody concentrations (GMCs) were calculated after log-transformation and compared by 2-tailed t test. Frequency of adverse events and proportions of patients exhibiting protective antibody concentration to all seven vaccine serotypes were compared using Fisher exact test. The projected total enrollment was 100 patients with a pre-specified interim analysis scheduled after complete primary vaccination of 50 patients. For the interim analysis, 53 patients were recruited since 3 patients dropped out during the primary vaccination series due to relapse (n=2) or thrombocytopenia (n=1). Of the 50 patients who completed the primary vaccination series, 7 patients were not evaluable for serologic analysis (Table 1). As in the interim analysis the primary study aim was prematurely achieved at a high significance level (p<0.001) further recruitment to the study was concluded in agreement with the ethics committee and the data safety and monitoring board.

Results and discussion
The age of study participants ranged from 1.4 to 16.9 years (median: 8.3), thus including a considerable number of older children and adolescents. According to general trends in pediatric alloHSCT, the majority of patients (55%) were transplanted from unrelated donors and in 68% of patients T cells were depleted either in vivo (64%) or in vitro (4%).

Serologic response rates to the different pneumococcal serotypes ranged from 41.9 to 86.0% after the 2nd vaccine at a median time of 10 months (range: 8-12) following transplantation. The lowest response rate was observed against serotype 9V (Figure 1A). Following the 3rd vaccine, 58.1 to 93.0% of patients responded to the individual vaccine serotypes. This is paralleled by a significant rise in geometric mean antibody concentration for each serotype (3.2- to 34.1-fold increase from baseline after 3rd vaccination) with a major increase observed after two vaccinations (p<0.001) and a further substantial increase after the 3rd (p<0.05; Figure 1B). Thus, following the 3rd vaccination, mean pneumococcal antibody concentrations for all serotypes were at least as high as those observed in a large 7vPCV vaccination trial in healthy infants where efficient protection from invasive pneumococcal disease has been convincingly demonstrated.19 Of note, even the weakest serological response against serotype 9V provided a mean antibody concentration well above the presumed protective threshold. As the clinically most critical parameter, we determined the proportion of patients exhibiting protective antibody levels against the individual vaccine serotypes: 69.8 to 100% and 81.4 to 100% of patients showed antibody concentrations above the protective threshold of ≥ 0.5 µg/ml following two and three vaccination, respectively, demonstrating a similar response as 7vPCV vaccination in healthy children.20 Complete seroprotection against all 7 vaccine serotypes was achieved in 55.8% (95% confidence interval [95%-CI]: 41.1–69.6%) of patients after the 2nd and 74.4 % (95%-CI: 59.6–85.2%) of patients after the 3rd vaccine independent of donor type (Figure 1C), recipient age (1-5 years vs. 6-16 years; p>0.25) and time from transplantation to first vaccination (< vs. ≥ median; p>0.5). This rate of protective
titers was achieved in spite of the fact that prior to vaccination, only 11.6% (95%-CI: 4.6–24.9%) of patients had already titers associated with protection and this rate again was irrespective of donor type (Figure 1C). In children receiving immunosuppressive therapy (IST) at vaccination 3/7 (42.9%) evaluable patients achieved protective antibody levels to all seven vaccine serotypes compared to 29/36 (80.6%) patients without immunosuppression (p=0.06). However, even in the small subgroup of children receiving IST, 5/7 (71.4%) patients attained protective antibody concentrations to ≥6/7 pneumococcal serotypes.

Our results compare well with data from a prospective study evaluating the use of 7vPCV in predominantly adult patients following alloHSCT from related donors: In the 25 patients completing the entire series of three 7vPCV vaccinations at 3, 6 and 12 months after transplant without prior donor vaccination, a significant rise in antibody concentration was observed only after the 3rd vaccine with 64% of patients protected to all seven vaccine serotypes.23 Our report extends these observations to an entirely pediatric cohort with the typical spectrum of transplant indications for this age group. It demonstrates that an early intensive vaccination schedule with three vaccinations administered in monthly intervals provides protective antibody responses not only to recipients of related but also unrelated donor transplantation, in whom immune reconstitution is often delayed due to in vitro or in vivo T cell depletion.2 With an intensified vaccination regimen analogous to newborn vaccination schemes, protective antibody levels were achieved after the 2nd vaccine as early as 10 months (median; range: 8–12) post transplant; protection was further improved by the 3rd vaccine resulting in seroprotection in 74% of patients at 11 (9-13) months. In the above mentioned study of 7vPCV vaccination following matched-related donor transplantation, this level of protection was achieved as early as 6 months after transplantation when, in addition to patients, sibling donors were immunized before stem cell harvest.23 As there is a continuous increase in unrelated donor transplantation, our vaccination schedule offers a
strategy to achieve early protection in the majority of pediatric patients without the need for donor vaccination.

While a total of four serious adverse events were observed, none of them was related to the study vaccine. The frequency of injection site reaction was 13.4 to 44.7% for redness and swelling and 66.7 to 75.0% for pain (Figure 2A). Advanced local reactions as well as pain interfering with limb movement were exceedingly rare events. The same is true for fever as a generalized adverse event with 10.5 to 20.8% of patients showing febrile reactions (Figure 2B). Only one single case of high grade fever (≥ 39.5°C) was observed in spite of the fact that 7vPCV was administered in conjunction with the hexavalent combination vaccine Infarix hexa™. Of note, febrile reactions following 2nd and 3rd vaccine were mostly limited to the younger age group (< 6 years) for which 7vPCV is currently licensed.

In summary, our data provide first evidence that in pediatric alloHSCT recipients, vaccination with 7vPCV elicits early protective antibody responses within the 1st year following transplantation from both related and unrelated donors. Furthermore, 7vPCV was well tolerated in all pediatric age groups. Thus, early vaccination with 7vPCV should be introduced into routine reimmunization programs for pediatric alloHSCT recipients. The long-term course of antibody levels and functionality as well as the response to booster immunization with 7vPCV or the 23-valent polysaccharide vaccine are the subject of further study. As the introduction of 7vPCV vaccination into routine reimmunization programs may lead to a shift in pneumococcal serotype distribution, continuous and comprehensive surveillance of pneumococcal serotype distribution in alloHSCT recipients developing invasive pneumococcal disease is warranted.24,25
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Appendix

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References

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Table 1. Characteristics of the study cohort

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Data</th>
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<tr>
<td>Patients, no.</td>
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<tr>
<td>Median patient age, y (range)</td>
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<tr>
<td>1-5 years, no. (% )</td>
<td>18 (34)</td>
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<tr>
<td>6-16 years, no. (%)</td>
<td>35 (66)</td>
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<td>Male, no. (%)</td>
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<td>Diagnosis, no. (%)</td>
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<tr>
<td>ALL</td>
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<tr>
<td>AML</td>
<td>8 (15)</td>
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<tr>
<td>MDS/JMML</td>
<td>9 (17)</td>
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<td>CML</td>
<td>4 (8)</td>
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<tr>
<td>SAA</td>
<td>4 (8)</td>
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<tr>
<td>Other*</td>
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<td>Conditioning regimen, no. (%)</td>
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<td>Busulfan-based</td>
<td>25 (47)</td>
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<tr>
<td>Cyclophosphamide only†</td>
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<tr>
<td>Donor type, no. (%)</td>
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<tr>
<td>Related</td>
<td>24 (45)</td>
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<tr>
<td>Unrelated</td>
<td>29 (55)</td>
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<td>Stem cell source, no. (%)</td>
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<td>BM</td>
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<tr>
<td>PBSC</td>
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<td>T cell depletion, no. (%)</td>
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<td>no</td>
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<tr>
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<td>in vitro (CD34-positive selection)</td>
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GvHD prophylaxis, no. (%)  
CSA 9 (17)  
CSA + MTX 42 (79)  
CSA + MTX + MMF 2 (4)  

IVIG after transplantation, no. (%) 46 (87)  
Ongoing IST at vaccination‡, no. (%) 10 (19)  

Evaluable§, no. (%)  
Safety 53 (100)  
Efficacy 43 (81)

ALL indicates acute lymphoblastic leukemia; AML, acute myeloblastic leukemia; MDS, myelodysplastic syndrome; JMML, juvenile myelomonocytic leukemia; CML, chronic myelogenous leukemia; SAA, severe aplastic anemia; TBI, total body irradiation; BM, bone marrow; PBSC, peripheral blood stem cells; ATG, anti-thymocyte globulin; GvHD, graft-versus-host disease; CSA, cyclosporin A; MTX, methotrexate; MMF, mycophenolate mofetil; IVIG, intravenous immunoglobulin; IST, immunosuppressive therapy.

* Other diagnoses are beta-thalassemia (n=2), Fanconi anemia (n=1), unspecified myeloproliferative disease (n=1), congenital amegakaryocytic thrombocytopenia (n=1), familial hemophagocytic lymphohistiocytosis (n=1), mucopolysaccharidosis type I (n=1), and Diamond-Blackfan anemia (n=1).

† Patients with SAA undergoing BM transplantation from identical sibling donors received cyclophosphamide + ATG for conditioning.

‡ Indications for IST at vaccination were chronic GvHD (n=9) and mixed hematopoietic chimerism (n=1). Patients received IST at all three vaccinations (n=8) or at 1st and 2nd vaccination only (n=2).

§ All patients receiving at least one dose of study vaccine were evaluated for toxicity. For analysis of efficacy, patients with complete data on pneumococcal serotype-specific antibody concentrations were included. Reasons for incomplete serologic data were (i) dropping out during the primary vaccination series due to relapse (n=2) or persistent thrombocytopenia (n=1), (ii) insufficient or delayed serum sampling (n=6), and (iii) administration of intravenous immunoglobulin for varicella-zoster-virus contact during primary vaccination series (n=1).
Figures Legends

Figure 1. Efficacy of 7vPCV vaccination in pediatric alloHSCT recipients.
(A) Serologic response rates given in % of patients (n=43) either showing seroconversion or achieving a 2-fold increase of pre-vaccination antibody level against the 7 vaccine serotypes (4, 6B, 9V, 14, 18C, 19F and 23F) following two and three 7vPCV vaccinations. Bars indicate 95% confidence intervals (95%-CI). (B) Geometric mean antibody concentrations (GMCs) for the 7 vaccine serotypes before, after two, and after three 7vPCV vaccinations. Increases of GMCs following two vaccinations (p<0.001) and further rises of GMCs following the 3rd vaccination (p<0.05) are statistically significant for all serotypes as determined by 2-tailed t test. The presumed threshold of seroprotection (≥ 0.5 µg/ml) is indicated by the horizontal line. (C) Rates of patients exhibiting protective antibody concentrations (≥ 0.5 µg/ml) to all seven vaccine serotypes before, after two, and after three 7vPCV vaccinations for the entire study cohort as well as recipients of related (n=21) and unrelated (n=22) hematopoietic stem cell transplantation. Differences between the related and unrelated donor group were not statistically significant (Fisher exact test).

Figure 2. Safety of 7vPCV vaccination in pediatric alloHSCT recipients.
(A) Frequency of local reactions and advanced local reactions (≥ 5 cm in diameter, pain interfering with limb movement) following 7vPCV vaccination. Bars indicate 95%-CIs. Frequencies were significantly different for redness and swelling between 1st and 3rd vaccination (p<0.05) as determined by Fisher exact test. No significant differences were found between age groups except for swelling after first vaccination, which was more frequent in the younger (1-5 years) age group (27.8% vs. 5.9%; p=0.04) (B) Frequency of fever as the most common systemic adverse event. The left panel shows the overall rate of fever (body temperature ≥ 38.0°C) and high grade fever (body temperature ≥ 39.5°C).
following the 1\textsuperscript{st}, 2\textsuperscript{nd} and 3\textsuperscript{rd} 7vPCV vaccine for the entire group. The right panel compares fever rates between the younger (1-5 years; n=18) and older (6-16 years; n=35) age group with significant differences following the 2\textsuperscript{nd} and 3\textsuperscript{rd} vaccination (p ≤ 0.05) as determined by Fisher exact test. No other significant differences were found. Additional systemic adverse events considered potentially related to study medication by the investigators include gastroenteritis (n=2), unspecific exanthema (n=1), herpes labialis (n=1) and persistent thrombocytopenia leading to drop out from the study (n=1).
Figure 1

(A) Serologic response rate [% of patients] after 2nd and 3rd vaccination for different pneumococcal serotypes.

(B) Geometric mean antibody concentration [µg/ml] pre, after 2nd, and after 3rd vaccination, with p-values indicated.

(C) Rate of patients exhibiting complete seroprotection [%] for different vaccination schedules and donor transplant types.
Figure 2

(A) Incidence of local reactions:
- Redness
- Swelling
- Pain

- Any local reaction
- Advanced local reaction

(B) Incidence of fever:
- Temp. $\geq 38.0^\circ$C
- Temp. $\geq 39.5^\circ$C

Age groups:
- 1 - 5 years
- 6 - 16 years

Statistical significance:
- $p < 0.01$
- $p = 0.05$
- $p = 0.02$

Legend:
- $1^{st}$, $2^{nd}$, $3^{rd}$ vaccination

Figure 2
Pneumococcal conjugate vaccine provides early protective antibody responses in children after related and unrelated allogeneic hematopoietic stem cell transplantation