13-valent Pneumococcal Conjugate Vaccine in children with acute lymphoblastic leukaemia: protective immunity can be achieved on completion of treatment

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SUMMARY:

Children with acute lymphoblastic leukaemia can develop invasive pneumococcal disease. This study demonstrated that the immunogenicity of a 13-valent pneumococcal conjugate vaccine on completion of ALL treatment is equivalent to its immunogenicity when given six months later, thus providing a rationale for providing earlier protection.

ABSTRACT (max: 250 words):

Background:

Children with acute lymphoblastic leukaemia (ALL) are at increased risk of invasive pneumococcal disease. This study describes the immunogenicity of 13-valent pneumococcal conjugate vaccine (PCV13) during and after chemotherapy.

Methods:

Children with ALL were allocated to study groups and received a single dose of PCV13: Group 1 - maintenance chemotherapy; Group 2 - end of chemotherapy; Group 3 - 6 months after chemotherapy. A protective vaccine response was defined as at least 10 of 12 serotypes (or greater than 83% of serotypes with data) achieving post-vaccination serotype-specific IgG ≥ 0.35 µg/mL and ≥ 4 fold rise, compared to pre-vaccination at 1 and 12 months.

Results:

One hundred and eighteen children were recruited. Only 12.8% (5/39; 95% CI 4.3% to 27.4%) of patients vaccinated during maintenance (Group 1) achieved a protective response at 1 month post-vaccination and none had a protective response at 12 months. For Group 2 patients, 59.5% (22/37; 95% CI 42.1% to 75.3%) achieved a response at 1 month and 37.9% (11/29; 95% CI 20.7% to 57.7%) maintained immunity at 12 months. For Group 3 patients, 56.8% (21/37; 95% CI 39.5% to 72.9%) achieved a protective response at 1 month and 43.3% (13/30; 95% CI 25.5% to 62.6%) maintained immunity at 12 months.

Conclusion:

This study demonstrated the earliest time point at which protective immunity can be achieved in children with ALL is on completion of chemotherapy. This is earlier than current recommendations and may improve protection during a period when children are most susceptible to infection.

Keywords: Acute Lymphoblastic Leukaemia, Pneumococcal Conjugate Vaccine, Immunisation, Immunocompromised

Title: Immunogenicity of 13-valent Pneumococcal Conjugate Vaccine in children with acute lymphoblastic leukaemia: protective immunity achievable on completion of treatment

BACKGROUND:

Outcomes for children with acute lymphoblastic leukaemia (ALL) have steadily improved over time with recent trials demonstrating survival rates of > 90% in standard risk patients (1, 2). The excellent survival rates have been achieved through enhanced risk stratification with increased chemotherapy intensity and duration together with improvements in supportive care. However, the risk of treatment-related mortality remains a significant concern in the context of low relapse risk. The most common cause of treatment-related mortality is infection. During the UK ALL 2003 trial, for children in the low-risk group, deaths from infection were almost as common as death from ALL itself (3). As mortality from vaccine-preventable infections is potentially preventable, it is important to consider how vaccination may be optimised to minimise the risk to immunocompromised children both during and after completion of treatment.

The prolonged period of chemotherapy of up to three years for children with ALL renders them significantly immunosuppressed and at risk of infection. Studies in children with ALL have shown that they develop persistent deficits in their immune function that can last for months after completion of chemotherapy and that their immunity to vaccines received prior to diagnosis declines after completion of treatment (4-7).

One of these vaccine-preventable pathogens is *Streptococcus pneumoniae*, or pneumococcus. Invasive pneumococcal disease (IPD) is a major cause of hospitalisation and mortality in the immunocompromised child (8-11). For children with cancer, the risk of IPD is significantly higher than in healthy children and other immunocompromised groups such as those with human

immunodeficiency virus (12). Studies have reported a specific pneumococcal immune defect in children with ALL which persists after completion of chemotherapy (13, 14).

Traditional polysaccharide pneumococcal vaccines are ineffective in infants and in immunocompromised children (15, 16). However, pneumococcal conjugate vaccines (PCV) are much more immunogenic by eliciting a T-cell–dependent immune response. T cells provide the signals required for the generation of B-cell memory (17, 18). Conjugate vaccines have the potential to elicit a memory response on subsequent natural exposure to vaccine-type strains and PCV have been shown to be immunogenic in immunocompromised hosts (19-23). In the United Kingdom (UK), the introduction of 7-valent PCV in 2006 and 13-valent PCV in 2010 has had a significant effect in reducing the burden of invasive pneumococcal disease. (24, 25)

Despite the known increased risk of IPD in children with ALL and the reported immunogenicity of PCV, there is no consensus as to when the optimum time for vaccination should be and international practice is variable (26-28). Current published guidelines recommend that children with cancer should receive pneumococcal vaccine 3 to 6 months after completion of treatment (29-33) (6 months in UK). However, delaying vaccination until 3 or 6 months after completion of cancer therapy potentially results in a prolonged period during which patients remain at risk of VT IPD. It is possible that PCV may be effective during chemotherapy or as soon as chemotherapy is complete. The aim of this study therefore was to examine the immunogenicity of PCV13 in children with ALL at 3 time points during / after their treatment, to identify the earliest time point when protective immunity can be generated. The study also provided an opportunity to characterise the immune function of children receiving, and recovering from, current ALL treatment regimens.

METHODS:

Study Design:

This non-randomised open-label study was conducted at 8 centres in the UK between September 2010 and July 2015. Children with ALL who fulfilled all study eligibility criteria were identified and then allocated to study groups on the basis of their time point in treatment. The following study groups were used:

Study group 1: Vaccine administered during 3rd cycle of maintenance chemotherapy, 6 months from last intensive chemotherapy;

Study group 2: Vaccine administered 4 weeks after last oral maintenance chemotherapy, at the end of treatment;

Study group 3: Vaccine administered 6 months after last oral maintenance chemotherapy

Study population

Children between the ages of 2 to 18 years with ALL confirmed by immunophenotyping and receiving maintenance treatment or who had completed treatment within last 6 months as per UK ALL 2003 protocol were eligible for recruitment (1). Exclusion criteria included children with concomitant acquired or congenital immunodeficiency, those receiving immunosuppressive medication other than UK ALL 2003 chemotherapy, previous severe or anaphylactic reaction to PCV and/or diphtheria toxoid and any children with a contraindication to receipt of any vaccine as per UK guidelines (33). Receipt of PCV vaccines as part of the routine schedule prior to diagnosis was not an exclusion criteria.

Study vaccine

The vaccine used in the study was PCV13 (Prevenar-13™, Pfizer) which contains pneumococcal serotypes (1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F and 23F). A single 0.5 mL intramuscular dose of vaccine was administered to each study patient as allocated to the study groups 1, 2 or 3 described above.

Safety and tolerability assessment

All patients were observed for 30 minutes following vaccine administration with appropriate medical treatment and supervision readily available in case of an anaphylactic event. Parents were given a report card to document any reactions to the vaccine.

Study procedures

Baseline blood samples were obtained pre-vaccination and follow-up samples were taken at 1 and 12 months post-vaccination. Serum concentrations of IgG anti-capsular polysaccharide antibodies to pneumococcal serotypes 1, 3, 4, 5, 6B, 7F, 9V, 14, 18C, 19A, 19F and 23F were assayed by Public Health England, Manchester. Serotype-specific pneumococcal IgG antibodies were quantified for 12 serotypes using a multiplex microsphere assay as previously described (34). Functional antibodies to 13 serotypes were measured in a multiplexed opsonophagocytic assay (OPA) as previously described in assays conducted at the WHO Reference laboratory at Great Ormond Street Institute of Child Health, University College London (35). Values are expressed as an OPA titre, equivalent to the reciprocal of the serum dilution required to produce 50% killing of the relevant serotype.

Immunogenicity assessment

In line with World Health Organization (WHO) guidance, both antibody concentrations and functional assays were used to define protective levels of anti-pneumococcal immunity at 1 and 12 months post-vaccination (36). IgG serotype-specific geometric mean concentrations (GMCs) and the proportion of children with concentrations ≥ 0.35 µg/mL and with a ≥ 4 fold rise compared to pre-vaccination were calculated at 1 and 12 months post-vaccination for 12 of the 13 serotypes (1, 3, 4, 5, 6B, 7F, 9V, 14, 18C, 19A, 19F and 23F). A protective response was classified as having at least 10 out of 12 serotypes (or greater than 83% of serotypes with data) meeting this definition.

Functionality of anti-pneumococcal antibodies was assessed in 4 of the 13 serotypes (1, 4, 5 and 19A). Protective immunity was defined if all 4 serotypes achieved opsonophagocytosis assay titres ≥ 8 at 1 and 12 months post-vaccination (37). A patient was defined as having an overall composite response if they had an antibody response, as defined above, plus protective immunity, at 1 and 12 months.

Samples collected from study participants for analysis in secondary assays were were peripheral blood lymphocyte subsets, serum concentrations of total immunoglobulins and IgG subclasses and nasopharyngeal swabs for culture of pneumococci, serotyping and multi-locus sequence typing (MLST) of any pneumococcal isolates.

Statistical methods

Power calculations were based on the premise that ≥70% of children will develop protective levels of anti-pneumococcal antibodies following one dose of PCV. However, if a ≥ 50% response rate was

observed then this would still be considered clinically useful for this group given their increased susceptibility to IPD.

The study required 40 participants per group (120 in total). With a response rate of ≥70% of subjects developing protective levels of anti-pneumococcal antibodies following one dose of PCV, the true response rate was estimated to within a confidence interval of ± 14% (i.e lower confidence interval of 56%), with 95% confidence.

Patient demographics and leukaemia and immunisation history information was summarised by study group. For the primary outcomes of overall response in concentrations of anti-pneumococcal antibodies at 1 and 12 months following a single dose of PCV13, point estimates (proportions) with 95% confidence intervals were calculated separately for the three study groups. Point estimates (proportions) with 95% confidence intervals were also calculated for the secondary outcomes of response in absolute concentration of anti-pneumococcal antibodies at 1 and 12 months post-vaccination, response in functionality of antibodies measured at 1 and 12 months post-vaccination, response in concentration of antibodies to each of the 12 serotypes measured at 1 and 12 months post-vaccination, and response in functionality of antibodies to each of the 4 serotypes measured at 1 and 12 months post-vaccination.

Logistic regression adjusting for leukaemia regimen and number of delayed intensifications was applied to the response data. Analysis of covariance (ANCOVA) was used to model the absolute concentration and absolute functionality data values of each serotype separately at 1 and 12 months post-vaccination.

Mann-Whitney tests were used to compare the concentration of anti-pneumococcal antibodies pre-vaccination and at 1 and 12 months post-vaccination respectively between PCV-7 subset and non-PCV-7 subset by serotype in PCV-7 for each study group.

Box and whisker plots were used to visualise the absolute values of the PCV-7 serotypes from the concentration assay at 1 and 12 months post-vaccination.

All statistical analyses were carried out using SAS 9.4 on records with complete data.

Ethical approval and compliance with guidelines.

The study was conducted in accordance with the principles of the Declaration of Helsinki, the standards of Good Clinical Practice (as defined by the International Conference on Harmonisation), and United Kingdom regulatory requirements. Ethics approval was obtained from National Research Ethics Service (NRES) Committee South Central (REC reference number 09/H0504/112)

RESULTS:

Study population

A total of 224 children were screened, of which 118 (52.7%) were recruited to the study. Of the 118 children recruited, 39 (33.1%) were allocated to Group 1, 40 (33.9%) were allocated to Group 2, and 39 (33.1%) were allocated to Group 3 (Figure 1, Consort diagram). Details on baseline patient characteristics, leukaemia and immunisation history are shown in Table 1. There were 84 patients

(71.8%) who had previously been immunised with PCV7, with the mean number of previous PCV7 immunisations highest in study group 1, followed by study group 2 and study group 3 (2.5, 1.8 and 1.4, respectively). The mean age in years at the time of PCV13 vaccination was 6.3 years (standard deviation [SD] 4.13) in Group 1, 8.4 years (SD 3.95) in Group 2 and 9.3 years (SD 4.40) in Group 3.

Rates of seroprotection

At baseline, only 2/118 patients (1.7%) had protective antibody concentrations against at least 10 serotypes (all in study group 1 (2/39 [5.1%]). An overall protective response at 1 month post-vaccination (i.e. concentrations of serotype specific anti-pneumococcal antibody ≥ 0.35 µg/ml and 4-fold rise in ≥ 10 serotypes) was achieved in 12.8% of Group 1 (5/39; 95% CI 4.3% to 27.4%), 59.5% of Group 2 (22/37; 95% CI 42.1% to 75.3%) and 56.8% of Group 3 (21/37; 95% CI 39.5% to 72.9%) (Table 2). At 12 months post-vaccination, protective responses were achieved in 0% in Group 1 (0/37), 37% in Group 2 (11/29; 95% CI 20.7% to 57.7%) and 43.3% of Group 3 (13/30; 95% CI 25.5% to 62.6%) (Table 3).

The proportion of patients achieving protective responses to individual serotypes at 1 and 12 months post-vaccination is shown in Figures 2 and 3. These individual serotype responses were higher than the overall protective response proportions where the threshold was defined as 10 of 12 serotypes reaching ≥ 0.35 µg/ml and ≥4-fold rise. Protective responses were not maintained at 12 months in a proportion of patients, particularly those who had been vaccinated during chemotherapy.

Functional assay responses

The proportion of patients in each treatment group with an OPA titre ≥ 8 at 1 and 12 months post-vaccination by serotype 1, 4, 5 and 19A and for all 4 serotypes is shown in Figures 4 and 5. For each treatment group, the proportion of patients who achieved a functional response was greater or equal to the number with concentrations of anti-pneumococcal antibodies considered to be protective both at 1 and 12 months post-vaccination.

Overall composite immune responses

Patients who had completed chemotherapy (Groups 2 and 3) had a significantly higher overall composite response rate compared to those children in Group 1, with no patients in Group 1 achieving the definition of both response in concentration of anti-pneumococcal antibodies and a response in functionality of anti-pneumococcal antibodies at 1 and 12 months (Table 4 and 5). Furthermore, logistic regression modelling confirmed no association between the intensity of treatment regimen (A, B or C) nor the number of delayed intensifications (1 or 2) and the response rate to immunisation (Tables 4 and 5).

The results of the immune function of the study population will be reported separately.

Impact of previous vaccination with PCV7

There were 84 patients (71.8%) who had received PCV7 vaccine prior to study entry. Of these, none had protective anti-pneumococcal antibody concentrations at baseline to the PCV7 serotypes (4, 6B, 9V, 14, 18C, 19F and 23F).

There was no significant difference between the antibody response achieved after PCV13 vaccination in the PCV7 subset and the non-PCV7 subset at 1 and 12 months post-vaccination.

Safety and tolerability

For the 7 days following vaccination, a significant fever (≥ 38°C but ≤ 40°C) was reported in 11/109 (10.1%) and there were no reports of fever duration longer than 2 days (Supplementary Data: Table 1). The incidence of pain and/or redness at the injection sites reported after vaccination is shown in Supplementary Table 2. In 4 of 118 (3.4%) of patients, fever or injection site reaction was significant enough to warrant hospital admission. In total there were 11 serious adverse events (SAEs) in the 118 patients over the study period. The majority of these (7/11 [63.6%]) were considered to be unrelated or unlikely to be related to the vaccine.

There were no cases of proven pneumococcal disease reported during the study period.

Nasopharyngeal carriage of pneumococcal serotypes

Pneumococcal carriage was documented in 14 of 55 subjects (25.5%) at study entry and 8 of 43 (18.6%) at 12 months post-vaccination. The results of the molecular characterisation of these isolates will be reported separately.

DISCUSSION:

It is well-established that children with ALL are at particular risk of IPD during and after completion of treatment (9). This is the first immunogenicity study of PCV13 in a cohort of children with ALL. Our study demonstrates that while it is safe to administer PCV13 vaccine during therapy for ALL, immune responses are suboptimal. However, our data show that children can achieve protective immunity to PCV13 at the end of treatment which is equivalent to that achieved when PCV is given at 6 months after the completion of treatment, which is the current recommendation for such

children. Almost identical results were found when carrying out post hoc analyses for the binomial

confidence intervals using the modified Wald test (38).

Hung et al. recommend a single dose of PCV13 as soon as possible after cancer diagnosis followed by

an additional dose of PCV13 after the completion of treatment (23). This study showed that children

who had received previous PCV7 vaccination had similar rises in antibody titres for the 7 serotypes

when comparing children who were receiving chemotherapy with those who had completed

chemotherapy and concluded that children receiving chemotherapy could benefit from a single dose

of PCV13. This finding was not replicated in our study. This is perhaps because the Hung et al. study

recruited a number of children with solid tumours, including brain tumours, who do not have the

same degree of immunosuppression as children with ALL.

Our study supports the safety and tolerability of PCV13, in keeping with published data in other

immunocompromised populations with comparable rates of fever, pain and/or redness at the

injection site. In 4 of 118 (3.4%) of patients fever or injection site reaction was significant enough to

warrant hospital admission. This is higher than reported in healthy populations, but most likely

reflects a lower threshold for triggering hospital admission in these children, rather than increased

toxicity (39,40). The majority of the 11 SAEs were considered to be unrelated to the vaccine and

reflect the frequent hospital admission rate in this population of children while they are receiving

chemotherapy.

The threshold for defining an overall protective response was high (10 out of 12 serotypes achieving

WHO definition protection of ≥ 0.35 μg/mL and ≥4- fold rise). This definition is considered the

serological correlate of protection against IPD. Of note, this WHO definition was derived from

healthy infants and it therefore may not be appropriate to apply the same threshold to children who are immunosuppressed both from ALL itself and their immunosuppressive therapy.

Immunocompromised patients may potentially mount an adequate serological response but lack functional anti-pneumococcal protection. Our study shows that the functional OPA assays performed demonstrated that the number of patients with functional protective immunity was equal or greater to the number with concentrations of serotype specific IgG considered to be protective. However, while OPA assays are a useful surrogate marker of vaccine efficacy, it is unlikely that a uniform threshold can be applied for all serotypes for children with varying immune function.

It should be noted that for children in clinical risk groups, vaccine-mediated protection from invasive pneumococcal disease caused by the vaccine serotypes can be significantly lower than in healthy children (41). Furthermore, children with co-morbidities are infected with non-vaccine serotypes more often than healthy children. Therefore the overall protection from pneumococcal disease in a high-risk patient population is likely to be suboptimal but vaccination at an earlier time point in treatment may help to counter this.

Although there were no documented cases of pneumococcal infection during the study period, a study limitation is that this surveillance did not extend further. While our data have shown the immunogenicity of PCV13, we were unable, in this study, to demonstrate its effect on the incidence of pneumococcal infections before and after vaccination with PCV13. Another potential limitation of this study was the slightly lower number of patients recruited than planned. The number of patients required for the study was 40 patients per group (120 in total), but only 113 patients had primary endpoint information at both baseline and 1 month.

Further studies in children receiving chemotherapy are required to ascertain whether other vaccines in the routine schedule could induce similar protective immunity as demonstrated in our study.

Conclusion

This study demonstrates the vaccination with PCV13 in children with ALL at the end of chemotherapy is similar in immunogenicity to vaccination at 6 months after completion of chemotherapy. We propose that relevant guidelines should now reflect this in order to protect such children against pneumococcal disease.

Novartis, Alios, J&J, and Merck; he also reports fees paid to institution from AstraZeneca/Medimmune, Sanofi, Pfizer, Sequerius, Sandoz, and Merck, outside the submitted work. Professor Goldblatt's laboratory at Great Ormond Street Institute of Biomedical Research has previously performed contract research on behalf of GSK, Merck and Sanofi Pasteur. Dr. Goldblatt has also received advisory board fees from GSK, Merck, and Sanofi Pasteur, outside the submitted work.

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TABLES:

Table 1 - Patient Demographics, and leukaemia and immunisation history

Characteristic	Group 1 (n=39)	Group 2 (n=40)	Group 3 (n=39)	Total (n=118)
Age (years) –				
n	39	40	39	118
Mean	6.3	8.4	9.3	8.0
Standard deviation	4.13	3.95	4.40	4.32
Range	2.0 to 17.0	4.0 to 17.0	4.0 to 17.0	2.0 to 17.0
Gender – n (%)				
Female	16 (41.0%)	17 (42.5%)	14 (35.9%)	47 (39.8%)
Male	23 (59.0%)	23 (57.5%)	25 (64.1%)	71 (60.2%)
Total	39 (100%)	40 (100%)	39 (100%)	118 (100%)
Treatment Protocol – n (%)				
UKALL 2003 Regimen A	22 (56.4%)	25 (62.5%)	21 (53.8%)	68 (57.6%)
UKALL 2003 Regimen B	7 (17.9%)	10 (25.0%)	11 (28.2%)	28 (23.7%)
UKALL 2003 Regimen C	10 (25.6%)	5 (12.5%)	7 (17.9%)	22 (18.6%)
Total	39 (100%)	40 (100%)	39 (100%)	118 (100%)
Number of Delayed Intensifications – n (%)				
None	0	0	0	0
One	15 (38.5%)	19 (47.5%)	16 (41.0%)	50 (42.4%)
Two	24 (61.5%)	21 (52.5%)	23 (59.0%)	68 (57.6%)
Total	39 (100%)	40 (100%)	39 (100%)	118 (100%)

Previous PCV-7
immunisations – n (%)

Characteristic	Group 1 (n=39)	Group 2 (n=40)	Group 3 (n=39)	Total (n=118)
None	3 (7.9%)	14 (35%)	16 (41%)	33 (28.2%)
At least one	35 (92.1%)	26 (65%)	23 (59%)	84 (71.8%)
Total	38 (100%)	40 (100%)	39 (100%)	117 (100%)
Missing	1 (2.6%)	0 (0%)	0 (0%)	1 (0.8%)
Number of previous PCV-7 immunisations				
N	38	40	39	117
Mean	2.5	1.8	1.4	1.9
Standard deviation	0.95	1.41	1.35	1.33
Range	0.0 to 4.0	0.0 to 4.0	0.0 to 4.0	0.0 to 4.0

Table 2 - Response in concentration of   anti-pneumococcal   antibodies   at   1   month post-vaccination

Statistic	Group 1 (n=39)	Group 2 (n=40)	Group 3 (n=39)	Total (n=118)
Proportion of patients who had a response	12.8% (5/39)	59.5% (22/37)	56.8% (21/37)	42.5% (48/113)
95% CI of the proportion	(4.3%, 27.4%)	(42.1%, 75.3%)	(39.5%, 72.9%)	(33.2%, 52.1%)
Missing response information	*0% (0/39)*	*7.5% (3/40)*	*5.1% (2/39)*	*4.2% (5/118)*

CI = Confidence Interval (Exact Binomial).

A patient is defined as having a response if their results at 1 month post vaccination for at least 10 out of the 12 serotypes (or greater than 83% of serotypes with data) satisfy the following condition:

serotype-specific IgG ≥ 0.35 μg/ml AND ≥ 4 fold rise in serotype-specific IgG concentrations compared to pre-vaccination.

Table 3 - Response in concentration of anti-pneumococcal antibodies at 12 months post-vaccination

Statistic	Group 1 (n=39)	Group 2 (n=40)	Group 3 (n=39)	Total (n=118)
Proportion of patients who had a response	0% (0/37)	37.9% (11/29)	43.3% (13/30)	25.0% (24/96)
95% CI of the proportion	N/A	(20.7%, 57.7%)	(25.5%, 62.6%)	(16.7%, 34.9%)
Missing response information	*5.1% (2/39)*	*27.5% (11/40)*	*23.1% (9/39)*	*18.6% (22/118)*

N/A=Result not available due to small numbers.

CI = Confidence Interval (Exact Binomial).

A patient is defined as having a response if their results at 12 months post vaccination for at least 10 out of the 12 serotypes (or greater than 83% of serotypes with data) satisfy the following condition: serotype-specific IgG ≥ 0.35 µg/ml AND ≥ 4 fold rise compared to pre-vaccination.

Table 4 - Logistic regression model results for overall composite response in antibodies at 1 month post-vaccination

Characteristic	Response rate n / N (%)		
Study group 1	4/39 (10.3%)		
Study group 2	17/36 (47.2%)		
Study group 3	17/34 (50.0%)		
Leukaemia regimen A	24/64 (37.5%)		
Leukaemia regimen B	10/24 (41.7%)		
Leukaemia regimen C	4/21 (19.0%)		
1 delayed intensification	19/44 (43.2%)		
2 delayed intensifications	19/65 (29.2%)		

Comparison	Odds ratio[1]	95% CI	p-value
Study group: 1 vs. 2	0.13	(0.04 to 0.46)	0.001
Study group: 1 vs. 3	0.11	(0.03 to 0.40)	<0.001
Study group: 2 vs. 3	0.84	(0.32 to 2.20)	0.725
Leukaemia regimen: A vs. B	0.94	(0.33 to 2.66)	0.900
Leukaemia regimen: A vs. C	1.85	(0.48 to 7.03)	0.369
Leukaemia regimen: B vs. C	1.97	(0.43 to 9.03)	0.381
No. of delayed intensifications: 1 vs. 2	1.68	(0.66 to 4.25)	0.274

Characteristic	Response rate
	n / N (%)

CI = Confidence Interval.

A patient is defined as having an overall composite response if they have a response in concentration of anti-pneumococcal antibodies and a response in functionality of anti-pneumococcal antibodies at 1 month.

[1] Odds ratios above 1 represent a favourable outcome for the first category in the comparison.

Table 5 - Logistic regression model results for overall composite response in antibodies at 12 months post-vaccination

Characteristic	Response rate n / N (%)
Study group 1	0/37 (0%)
Study group 2	5/27 (18.5%)
Study group 3	8/30 (26.7%)
Leukaemia regimen A	7/53 (13.2%)
Leukaemia regimen B	5/21 (23.8%)
Leukaemia regimen C	1/20 (5.0%)
1 delayed intensification	6/39 (15.4%)
2 delayed intensifications	7/55 (12.7%)

Comparison	Odds ratio[1]	95% CI	p-value
Study group: 1 vs. 2	NR	NR	NR
Study group: 1 vs. 3	NR	NR	NR
Study group: 2 vs. 3	0.58	(0.16 to 2.11)	0.406
Leukaemia regimen: A vs. B	0.48	(0.12 to 1.93)	0.298
Leukaemia regimen: A vs. C	2.58	(0.26 to 25.97)	0.421
Leukaemia regimen: B vs. C	5.42	(0.50 to 59.21)	0.166
No. of delayed intensifications: 1 vs. 2	0.98	(0.26 to 3.64)	0.971

Characteristic	Response rate
	n / N (%)

CI = Confidence Interval.

A patient is defined as having an overall composite response if they have a response in concentration of anti-pneumococcal antibodies and a response in functionality of anti-pneumococcal antibodies at 12 months.

[1] Odds ratios above 1 represent a favourable outcome for the first category in the comparison.

Figure Legends:

Figure 1- Consort Diagram

Figure 2 - Forest plot of the proportion of patients with a response in concentration of anti-pneumococcal antibodies at 1 month post-vaccination by serotype for each treatment group[1, 2]

[1] For each serotype, a patient is defined as having a response if their results at 1 month for the corresponding serotype satisfy the following condition:

1 month post-vaccination serotype-specific IgG ≥ 0.35 µg/ml and ≥ 4 fold rise compared to pre-vaccination.

[2] For the total, a patient is defined as having a response if their results at 1 month for at least 10 out of the 12 serotypes (or greater than 83% of serotypes with data) satisfy the following condition: serotype-specific IgG ≥ 0.35 µg/ml and ≥ 4 fold rise compared to pre-vaccination.

Figure 3 - Forest plot of the proportion of patients with a response in concentration of anti-pneumococcal antibodies at 12 months post-vaccination by serotype for each treatment group[1, 2, 3]

1 For each serotype, a patient is defined as having a response if their results at 12 months for the corresponding serotype satisfy the following condition: serotype-specific IgG ≥ 0.35 µg/ml and ≥ 4 fold rise compared to pre-vaccination.

2 For the total, a patient is defined as having a response if their results at 12 months for at least 10 out of the 12 serotypes (or greater than 83% of serotypes with data) satisfy the following condition: serotype-specific IgG ≥ 0.35 µg/ml and ≥ 4 fold rise compared to pre-vaccination.

3 In total there was no patient with response in study group 1, therefore no result is shown for the proportion of patients achieving response in the "total" row of the figure above.

Figure 4 - Forest plot of the proportion of patients with a functional anti-pneumococcal antibody response at 1 month post-vaccination by serotype for each treatment group[1, 2]

[1] For each serotype, a patient is defined as having a response if their results at 1 month for the corresponding serotype satisfy the following condition:

Opsonophagocytosis assay (OPA) titre for the serotype ≥ 1:8 dilution.

[2] For the total, a patient is defined as having a response if their results at 1 month for all 4 serotypes satisfy the following condition:

Opsonophagocytosis assay (OPA) titre ≥ 1:8 dilution.

Figure 5 - Forest plot of the proportion of patients with a functional antibody response at 12 months post-vaccination by serotype for each treatment group[1, 2]

1 For each serotype, a patient is defined as having a response if their results at 12 months for the corresponding serotype satisfy the following condition:

Opsonophagocytosis assay (OPA) titre for the serotype ≥ 1:8 dilution.

2 For the total, a patient is defined as having a response if their results at 12 months for all 4 serotypes satisfy the following condition:

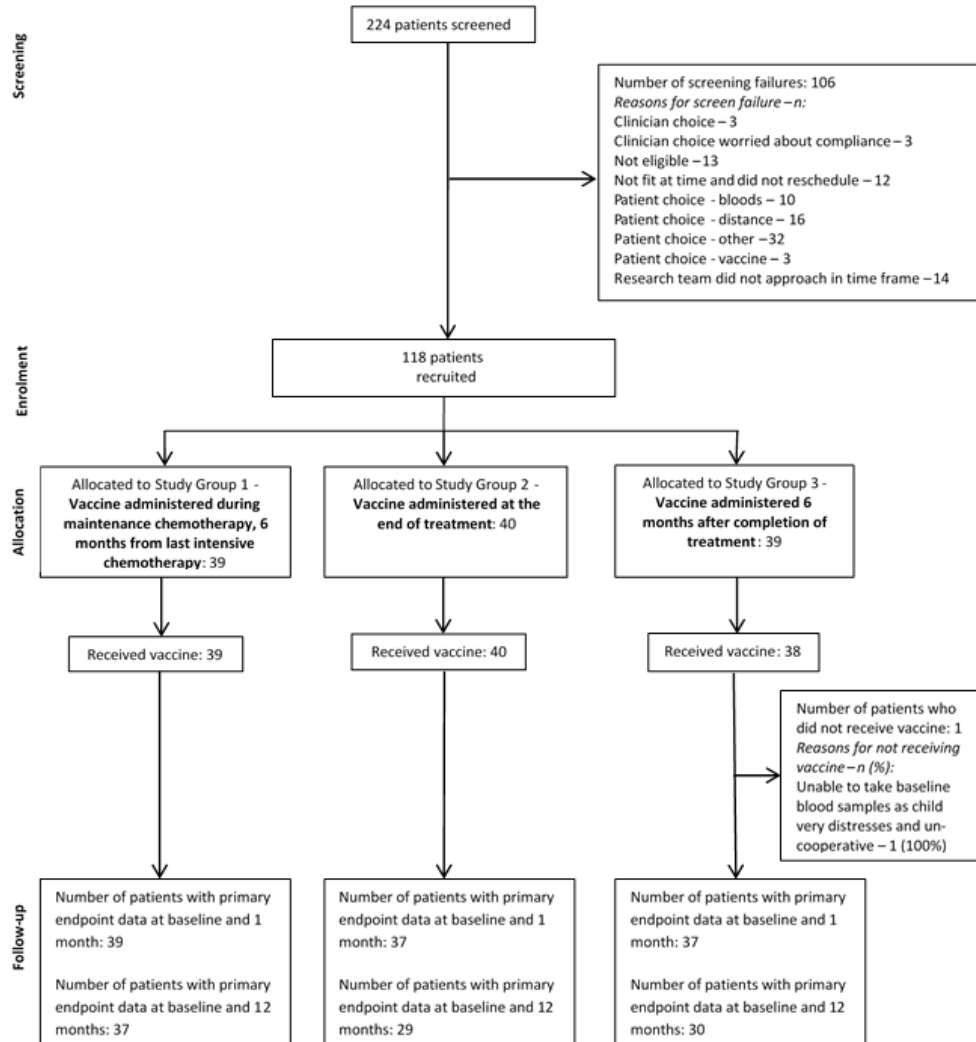Opsonophagocytosis assay (OPA) titre ≥ 1:8 dilution.

Figure 1

Figure 2 - Forest plot of the proportion of patients with a response in concentration of anti-pneumococcal antibodies at 1 month post-vaccination by serotype for each treatment group
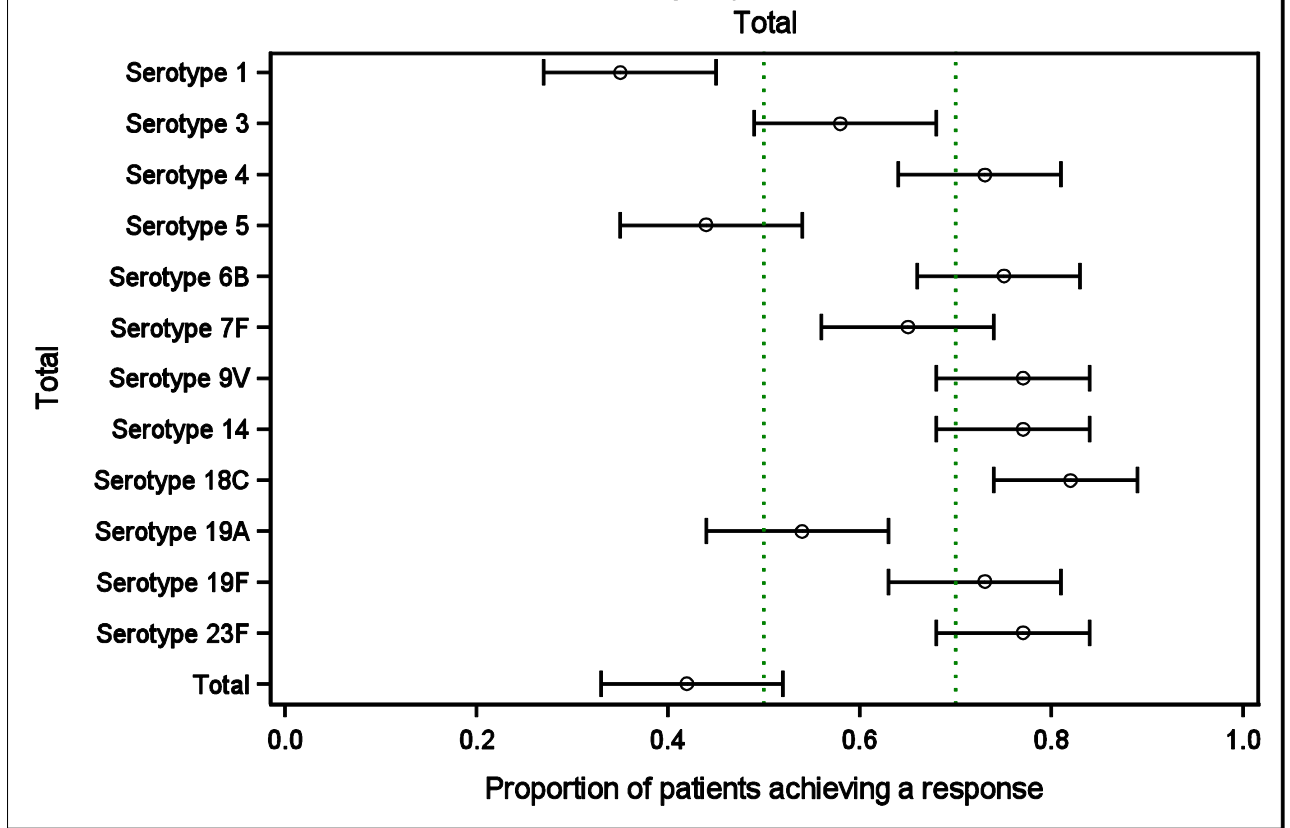
Figure 3 - Forest plot of the proportion of patients with a response in concentration of anti-pneumococcal antibodies at 12 months post-vaccination by serotype for each treatment group
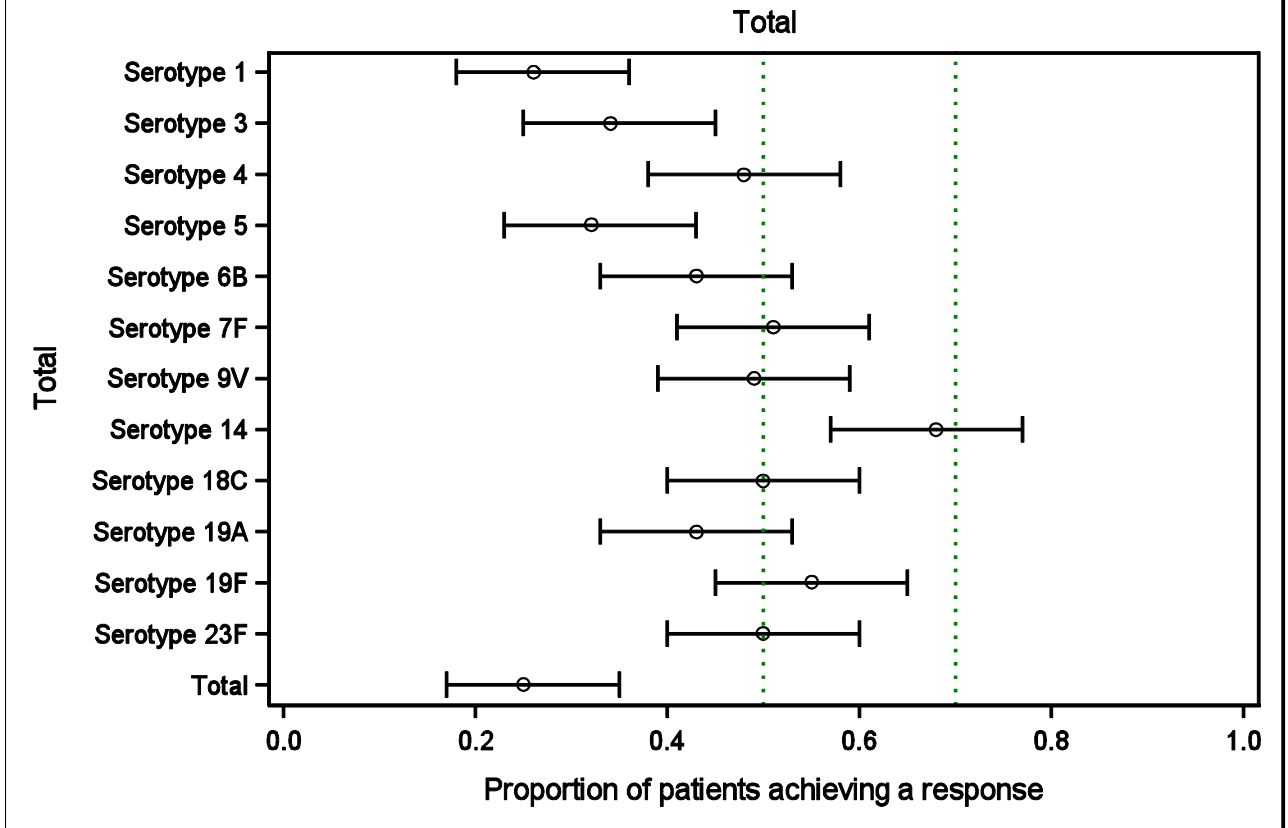
Figure 4 - Forest plot of the proportion of patients with a response in functionality of anti-pneumococcal antibodies at 1 month post-vaccination by serotype for each treatment groupnti-pneumococcal antibodies at 12 months post-vaccination (Study Group 1=&all_a. Study Group 2=&all_b. Study Group 3=&all_c. Total=&all_t.)
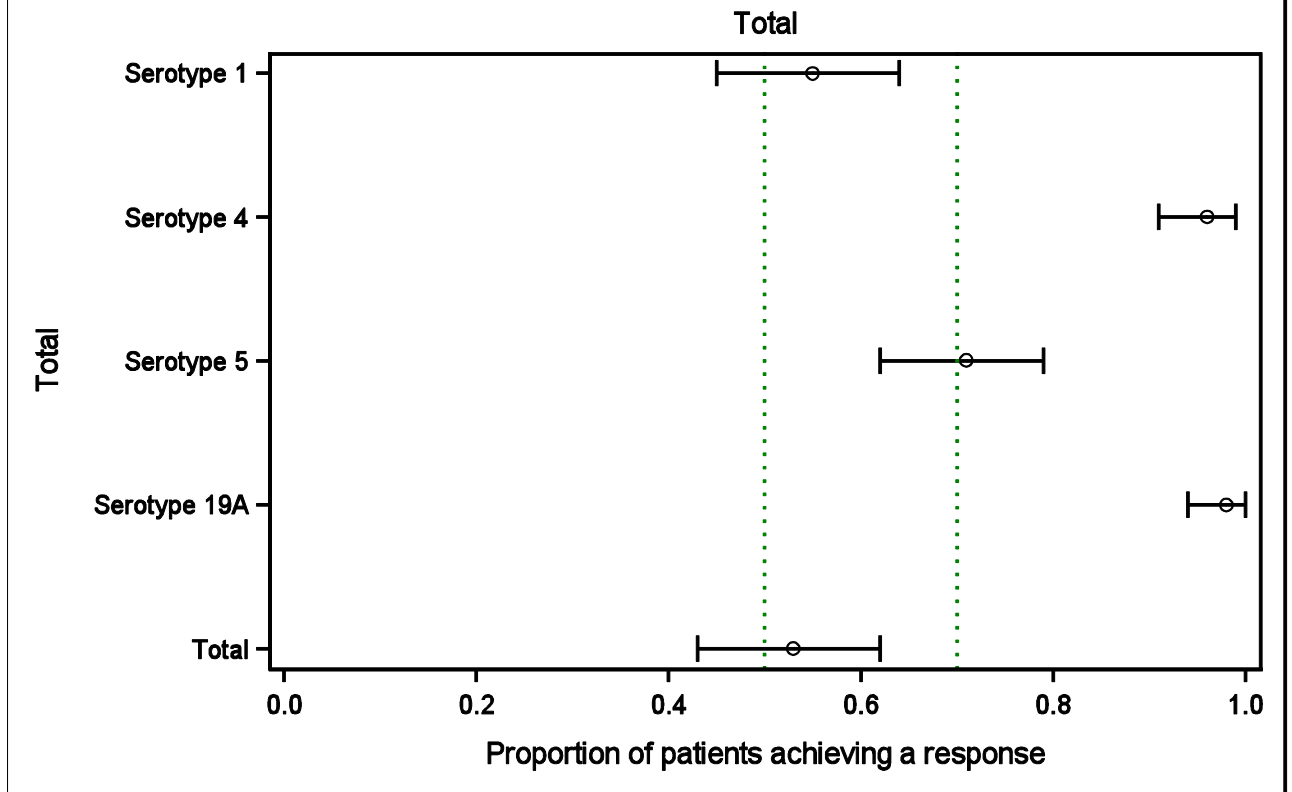
Figure 5 - Forest plot of the proportion of patients with a response in functionality of anti-pneumococcal antibodies at 12 months post-vaccination by serotype for each treatment groupnti-pneumococcal antibodies at 12 months post-vaccination (Study Group 1=&all_a. Study Group 2=&all_b. Study Group 3=&all_c. Total=&all_t.)