OFFICIAL JOURNAL OF THE AMERICAN ACADEMY OF PEDIATRICS
PEDIATRICS

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Journal:	*Pediatrics*
Manuscript ID	2017-4250.R1
Article Type:	Regular Article
Date Submitted by the Author:	n/a
Complete List of Authors:	Martinón-Torres, Federico; Hospital Clinico Universitario de Santiago, Pediatric Emergency and Critical Care Division; Universidad de Santiago de Compostela, Pediatrics Bernatowska, Ewa; The Children's memorial Health Institute, Department of Immunology Shcherbina, Anna; Research and Clinical Centre of Pediatric Hematology, Oncology and Immunology named after Dmitry Rogachev Esposito, Susana; Universita degli Studi di Perugia, Department of Surgical and Biomedical Sciences Szenborn, Leszek; Medical University, Department of Pediatric Infectious Diseases Campins, Magda; Hospital Universitari Vall d'Hebron, Preventive Medicine Hughes, Stephen; Royal Manchester Children's Hospital, Paediatric Immunology Faust, Saul; University of Southampton, Professor of Paediatric Immunology & Infectious Diseases Yu, Ly-Mee; University of Oxford, Centre for Statistics in Medicine D'Agostino, Diego; GSK, Vaccines Calabresi, Marco; GSK, Vaccines Toneatto, Daniela; GSK, Vaccines Snape, Matthew; University of Oxford, Paediatrics
Keyword/Topic:	Vaccine/Immunization < Infectious Diseases, Immunologic Disorders < Allergy/Immunology

Meningococcal B vaccine immunogenicity in children with defects in complement and splenic function

Federico Martinón-Torres, PhD,[1] Prof Ewa Bernatowska, MD,[2] Prof Anna Shcherbina, MD,[3] Prof Susanna Esposito, MD,[4] Prof Leszek Szenborn, MD,[5] Prof Magda Campins Marti, PhD,[6] Stephen Hughes, PhD,[7] Prof Saul N Faust, FRCPCH,[8] Ly-Mee Yu PhD[9], Diego D'Agostino, MSc,[10†] Marco Calabresi, PhD,[11*] Daniela Toneatto, MD,[11] Matthew D Snape, MD[12, 13]

1. Translational Pediatrics and Infectious Diseases, Hospital Clinico Universitario de Santiago de Compostela, Santiago de Compostela, Spain
2. Department of Immunology, The Children's Memorial Health Institute, Warsaw, Poland
3. Research and Clinical Centre of Pediatric Hematology, Oncology and Immunology named after Dmitry Rogachev, Moscow, Russian Federation
4. Pediatric Clinic, Department of Surgical and Biomedical Sciences, Università degli Studi di Perugia, Perugia, Italy
5. Department of Pediatric Infectious Diseases, Medical University, Wroclaw, Poland
6. Hospital Universitario Vall d'Hebron, Barcelona, Spain
7. Royal Manchester Children's Hospital, Manchester, UK
8. NIHR Welcome Trust Clinical Research Facility, University of Southampton and Southampton, University Hospital NHS Foundation Trust, Southampton, UK
9. Nuffield Department of Primary Care Health Sciences, University of Oxford, Oxford, UK.
10. GSK, Amsterdam, The Netherlands
11. GSK, Siena, Italy
12. Oxford Vaccine Group, University of Oxford Department of Paediatrics Oxford, UK
13. Oxford Biomedical Research Centre, Oxford, UK

† Current affiliation: Document IT BeNeLux B.V., RKranenburg 21, 1083 JM Amsterdam, the Netherlands

* Current affiliation: Covance Inc., the drug development business of Laboratory Corporation of America Holdings (LabCorp), Rome, Italy

Corresponding author: Dr Matthew Snape, Oxford Vaccine Group, Department of Paediatrics, University of Oxford, OX3 7LE, United Kingdom

Electronic address: matthew.snape@paediatrics.ox.ac.uk.
Tel: +44(0) 1865 572873

Short title: **Meningococcal B vaccine in Complement Deficiency**

Financial Disclosure
The institution of Dr Martinón-Torres received clinical trial fees from Novartis during the conduct of this study, and he received personal fees/non-financial support/grants/other from Novartis, Pfizer, SPMSD, GSK group of companies and/or Merck, outside the submitted work. Prof Esposito reports grant from Novartis, GSK groups of companies, Pfizer, Sanofi-Pasteur and Valeas, and personal fees from Novartis, GSK groups of companies, Pfizer, Sanofi-Pasteur, Novavax and Vifor, outside

the submitted work. Prof Szenborn received personal fees from Novartis and GSK groups of companies during the conduct of the study. The institution of Prof Campins Marti received clinical trial fees from Novartis during the conduct of the study, and she received grants from Novartis, GSK and Pfizer outside of the submitted work. The institution of Prof Faust received grant from GSK group of companies during the conduct of the study. The institution of Prof Faust received grant for his participation in advisory boards (Astra Zeneca and Cubist) and speaking engagements (Pfizer). Prof Faust acts as an investigator for clinical studies from both non-commercial funding bodies and commercial sponsors (i.e. some all of Novartis Vaccines, GSK groups of companies, MedImmune/AstraZeneca and Pfizer Vaccines) conducted on behalf of Southampton University Hospital NHS Foundation Trust and the University of Southampton. Dr Calabresi and Mr D'Agostino were, and Dr Toneatto is employees of the GSK group of companies. Dr Toneatto owns stock/stock options in the GSK group of companies. Dr Snape acts as an investigator for clinical studies from both non-commercial funding bodies and commercial sponsors (i.e. some or all of Novartis Vaccines, GSK group of companies, Sanofi-Aventis, Sanofi-Pasteur MSD, MedImmune and Pfizer Vaccines) conducted on behalf of the University of Oxford and Oxford University Hospitals NHS trust. The NIHR Oxford Biomedical Research Centre provides salary support for Dr Snape, who is a Jenner Investigator. Prof Bernatowska, Dr Hughes, Dr Yu and Prof Shcherbina have nothing to disclose.

Conflicts of Interest
The authors have no additional conflicts of interest other than those described in the financial disclosure statement.

Funding
This study was funded by Novartis Vaccines and Diagnostics (now GlaxoSmithKline Biologicals SA).

Clinical Trial Registration
(NCT02141516).

Abbreviations:

4CMenB – 4 component Meningococcal B vaccine (Bexsero)
MenB – capsular group B meningococcus
IMD – Invasive meningococcal disease
fHBP – factor H binding protein
NadA – Neisserial adhesion A
PorA – Porin A
NHBA – Neisserial heparin binding antigen
CI – confidence intervals
GMT – Geometric mean Titers
GMR – Geometric Mean Ratio
SAE – serious adverse event
hSBA – serum bactericidal activity using exogenous human complement
end-hSBA – serum bactericidal activity using endogenous complement
betalac-hSBA – serum bactericidal activity using exogenous human complement in the presence of beta-lactamase

Summary:
Children with complement deficiency respond less well to immunization with 4CMenB than healthy controls; surveillance for vaccine failures is required to determine the significance of this.

What's known on this subject:
4CMenB immunization of infants is known to effectively prevent group B meningococcal disease. No data are available on the immunogenicity of 4CMenB in children with complement deficiency, who are at increased risk of disease, nor in children with splenic dysfunction.

What this study adds:

This study shows that most children with complement deficiencies develop an immune response following immunization with 4CMenB, although this is reduced in children with terminal chain complement deficiencies and on eculizumab. Ongoing surveillance for vaccine failures is required.

Word count: Abstract 249 (max 250)
Manuscript 3036 (max 3000)

Contribution of authors

All authors had full access to all the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis. All authors reviewed and commented critically drafts of the manuscript for important intellectual content and gave final approval to submit for publication.

Matthew Snape and Federico Martinón-Torres contributed to the acquisition of data, the analysis of the data and prepared the first draft of the manuscript.

Daniela Toneatto and Marco Calabresi contributed to the study conception and design and interpretation of the data.

Susanna Esposito, Leszek Szenborn, Ewa Bernatowska, Anna Shcherbina, Magda Campins Marti, Saul Faust and Stephen Hughes conducted the study, contributed to the acquisition of data and the interpretation of the data.

Diego D'Agostino, Ly-Mee Yu provided statistical expertise and contributed to the analysis and interpretation of the data.

Abstract (249 words/250 words limit)

Background
The capsular group B meningococcal vaccine (4CMenB) is recommended for children with complement deficiencies, asplenia and splenic dysfunction, however, data on the immunogenicity of 4CMenB in these 'at-risk' children are missing.

Methods
Participants aged 2-17 years in Italy, Spain, Poland, UK and Russia with complement deficiencies, asplenia or splenic dysfunction received two doses of 4CMenB two months apart, as did healthy controls. Exogenous and endogenous human complement serum bactericidal activity (hSBA) was determined at baseline and one month following the second immunization against four test strains: H44/76 (assessing vaccine antigen fHbp), 5/99 (NadA), NZ98/254 (PorA) and M10713 (NHBA).

Results
Of 239 participants (mean age 10·3 years, 45% female), 40 were complement-deficient (9 eculizumab therapy, 4 terminal-chain deficiencies, 27 'other'), 112 children had asplenia/splenic dysfunction (8 congenital asplenia, 8 functional asplenia, 96 splenectomy) and 87 were healthy. Following immunization the proportions of complement-deficient participants with exogenous complement hSBA titers ≥ 1:5 were 87% (H44/76), 95% (5/99), 68% (NZ98/254) and 73% (M10713), compared to 97%, 100%, 86% and 94% respectively for asplenic children, and 98%, 99%, 83% and 99% (controls). When testing with endogenous complement, strain-specific bactericidal activity was evident in only one eculizumab treated participant and one terminal-chain complement deficient participant.

Conclusion
4CMenB administration is similarly immunogenic in healthy children and those with asplenia/splenic dysfunction. The significance of the trend to lower responses SBA titers in complement deficient children (especially those with terminal-chain complement deficiency or those on eculizumab therapy) must be determined by ongoing surveillance for vaccine failures.

Introduction

The licensure of the capsular group B meningococcal vaccine 4CMenB in Europe ,[1] Australia[2] and the Americas[3-5] offers the potential for vaccine prevention of invasive disease due to this bacterium. This vaccine is now recommended for use in healthy infants in the United Kingdom,[6] Ireland[7] and some regions of Germany, Italy, Canada.[8,9]

In addition, these and many other countries recommend (and re-imburse) the use of 4CMenB in children, teenagers and adults at increased risk of invasive meningococcal disease (IMD); in the USA this recommendation applies only to pre-teens, teens and adults.[10-13] Such 'at-risk' groups include those with terminal chain complement deficiencies, known to have rates of IMD 7000 to 10 000 higher than the general population,[14] and those receiving the monoclonal antibody eculizumab, which acts by binding complement 5.[15] In view of reports of overwhelming sepsis and elevated mortality,[16,17] children with asplenia or splenic dysfunction are also included in these recommendations, although the incidence of IMD in these populations may not be higher than their healthy peers.[11]

While previous studies have shown immunization of complement-deficient patients with polysaccharide capsular group A, C, W and Y meningococcal vaccines to be effective against invasive disease,[18] the use of 4CMenB in these populations has not previously been studied. Accordingly, we evaluated here the immunogenicity and tolerability of 4CMenB in children with congenital or acquired complement deficiencies, and with asplenia or splenic dysfunction, and compared this with healthy controls.

Methods

This was an open-label phase IIIb study conducted at 18 sites (four in Italy, four in Spain, three in Poland, four in the United Kingdom and three in Russia) between May 2014 and March 2015.

The study recruited children aged 2 to 17 years in one of the three categories: 'complement deficient', 'asplenia/splenic dysfunction' or 'healthy controls'. Children in the 'complement deficient' category were those diagnosed by their clinician as having a primary deficiency (a congenital deficiency leading to a reduced concentration of one or more proteins in the complement cascade, including C1, C2, C3, C4, factor D, properdin, C5, C6, C7, C8, C9, factor H or homozygous factor I) or a secondary deficiency (conditions indirectly leading to a reduced concentration of one or more proteins in the complement cascade, including those treated with eculizumab). Children in the 'asplenia/splenic dysfunction' category were those with congenital anomalies of the spleen, surgical splenectomy or autosplenectomy (e.g. in patients with sickle cell disease). Healthy controls were healthy age-matched children. Children were excluded if they had previously received any capsular group B meningococcal vaccine, or if they had suffered IMD within the last year (complement deficient or asplenia/splenic dysfunction groups) or at any time previously (healthy controls). Participants could not be on any antibiotics within three days prior to enrollment other than those prescribed for prophylaxis, and immunization was deferred until at least six hours following the administration of analgesics or antipyretics. Full inclusion and exclusion criteria are presented in supplementary materials.

The study intervention was two doses of 4CMenB (Bexsero, GSK, Italy) administered intramuscularly two months apart. As previously described[19] each 0·5 ml dose of vaccine contains 50 µg each of purified factor H binding protein (fHBP), Neisserial adhesion binding antigen (NHBA), Neisserial adhesion A (NadA) antigens, and 25 µg of outer membrane vesicle component with immunodominant porin protein (PorA).

The primary immunogenicity objective was to evaluate the immunogenicity of two doses of 4CMenB in participants with increased risk of meningococcal disease because of complement deficiency or asplenia and in healthy age-matched children, at one month after the second vaccination. Immunogenicity for this primary objective was determined by using exogenous human complement assays measuring serum bactericidal activity (hSBA) against four test strains: H44/76 (assessing vaccine antigen fHBP), 5/99 (NadA), NZ98/254 (PorA) and M10713 (NHBA). hSBA assays were performed at the Clinical Sciences Laboratory, Novartis Vaccines, (now GlaxoSmithKline Biologicals SA) Marburg. Exogenous human complement was added to serial diluted sera following the addition of bacteria solution. For assays with complement-independent bactericidal activity, suggestive of antibiotic killing, the above assays were repeated in the presence of beta-lactamase (betalac-hSBA). For hSBA and betalac-hSBA assays the threshold of response was 1:5, as previously reported.[19]

In an additional exploratory analysis serum bactericidal assays were also performed using endogenous complement alone (end-hSBA). For these assays, dilution was only performed to a 1:8 titer, in view of the inherent limitations of complement dilution in the absence of exogenous complement, and threshold of response was 1:4.

The primary safety objective was to assess the safety and tolerability of two doses of 4CMenB in these children. During the week following a vaccination, parents recorded the local injection site and systemic symptoms. Separate classification systems were used for children aged 2 to 5 years old and 6 to 17 years olds as shown in supplementary Figures 1 and 2. Adverse event recording was enhanced by telephone contact during the week after study vaccination. All serious adverse events (SAEs) reported during the study were recorded.

The primary population for immunogenicity analysis was the full analysis set, consisting of all participants who received a study vaccination and provided post-immunization serum that was evaluable for at least one of the study strains. The primary immunogenicity endpoints were, for each of the strains at baseline and one month after the second dose of vaccine, the percentages of participants with hSBA titers ≥ 1:5, hSBA ≥ 1:8 and with a four-fold rise in hSBA titers above baseline, along with 95% Clopper confidence intervals (CIs). Between-group comparisons were performed through computation of the 2-sided 95% CI for the differences in percentages between the study groups. For end-hSBA titers the percentage of participants with titers ≥ 1:4 and hSBA ≥ 1:8 was calculated.

The hSBA geometric mean titers (GMTs) and associated 2-sided 95% Clopper-Pearson CIs were constructed by exponentiating the least square means of the corresponding log-transformed hSBA titers and their 95% CIs obtained from an analysis of variance with study group ('complement deficient' and asplenia/splenic dysfunction) and study site as model factors. For healthy controls, raw unadjusted GMTs and 95% CIs were calculated by exponentiating the means of the logarithmically transformed titers and their 95%. Geometric mean ratios (GMRs) of

hSBA titers post/pre immunization were calculated, and ratios of post-immunization hSBA GMTs between groups and their 95% CIs were calculated for comparisons of immune responses between the 'at-risk' groups and healthy controls. Titers below the limit of detection were set to half that limit for the purpose of analysis. The percentage of participants experiencing vaccine reactions were analysed for each dose separately according to the age groups 2 to 5 years and 6 to 17 years.

The planned sample size was approximately 240 participants, with approximately 160 in the 'at-risk' groups (with at least 15 from each of the complement-deficient group and the asplenia/splenic dysfunction group), and approximately 80 healthy controls. Recruitment of healthy controls occurred in a 1:2 ratio for 'at-risk' children, stratified according to site and to the age groups 2 to 5 years, 6 to 10 years and 11 to 17 years. A sample size of 80 participants in a group was calculated to provide a 95% CI of 81·2% to 95·6% around an observed percentage 'response' of 90·0%.

Results

Across 18 sites 239 participants were enrolled (mean age 10·35 years, 45% female), 40 of whom were complement-deficient (nine eculizumab therapy, four terminal-chain deficiencies, one with factor H deficiency, four with factor I deficiency, two with C1 deficiency, five with C2 deficiency, 14 with C3 and/or C4 deficiency and one with alternative pathway deficiency). 112 participants had asplenia/splenic dysfunction (eight congenital asplenia, eight functional asplenia and 96 splenectomy) and 87 were healthy controls. Further details of participant demographics, their underlying diagnoses, and the study flow chart are shown in Table 1, Figure 1 and supplementary table 1.

Immunogenicity

When tested with SBAs using exogenous human complement, no complement-deficient participants had baseline hSBA titers ≥ 1:5 for strains H44/76, 5/99 and NZ98/254 (and no more than 12% of asplenics and 6% of controls). Baseline seropositivity rates for M10713 were 56% (complement-deficient) 79% (asplenics) and 78% (controls). Following immunization the proportions of complement-deficient participants with hSBA titers ≥ 1:5 (using exogenous complement) rose to 87% (H44/76), 95% (5/99), 68% (NZ98/254) and 73% (M10713), compared to 97%, 100%, 86% and 94% respectively for the asplenic group, and 98%, 99%, 83% and 99% for controls (Table 2 and Figure 2). Between group comparisons revealed the point estimate of the percentage of 'responders' was 4 to 26% lower in complement deficient participants than in healthy controls; but this was statistically significant for strains H44/76 and M10713 only. No such trend to a lower response was seen for asplenic/splenic dysfunction participants compared to healthy controls. GMTs and percentage of participants with SBA titers ≥ 1:8 showed a broadly similar pattern (supplementary Table 2 and 3). The analyses of the percentage of participants developing hSBA titers ≥ 1:5 and hSBA GMTs by complement deficiency type, revealed that of eight participants on eculizumab therapy with hSBA results only four (H44/76), six (5/99), two (NZ98/254) and one (M10713) had hSBA titers ≥ 1:5 post immunization (supplementary table 4 and 5). By contrast, all four participants with terminal chain complement deficiencies achieved these thresholds for each strain.

Fewer than half of complement-deficient and asplenic/splenic dysfunction participants reported taking antibiotics during the study, and only 1 to 2·8% of participants per group had evidence of complement independent killing on hSBA

assays that would be suggestive of antibiotic activity. The bactericidal assays on these participants were repeated using pre-treatment with beta-lactamase (additional exploratory analyses), but given the low numbers this had minimal impact on overall response rates or GMTs (data not shown).

When using endogenous complement, post-immunization bactericidal activity at 1:4 dilution was observed in 68% (H44/76), 60% (5/99), 41% (NZ98/254) and 60% (M10713) of participants, compared with 98%, 100%, 85% and 100% respectively in healthy controls (supplementary Table 6). These percentages varied according to diagnosis, with only one of the four terminal-chain complement deficient participants (reported as having a C7 deficiency), and only one of the eight eculizumab recipients, displaying any bactericidal activity post-immunization (Figure 3 and supplementary Table 7). For asplenic children, when using endogenous complement, bactericidal activity at 1:4 dilution was seen in 11% (H44/76), 35% (5/99), 5% (NZ98/254) and 95% (M10713), rising to 100%, 100%, 88% and 100% respectively post immunization.

Reactogenicity

Fever rates per-immunization amongst 2-5 year olds were 17% in complement-deficient children after each dose, 11% after each dose in asplenics and 31% (first dose) and 8% (second dose) for controls. Amongst 6-17 years olds these rates were 18% after the first dose, and 4% after the second dose in complement deficient participants; per-dose fever rates were 3-4% for all other participants. Full details of solicited systemic and local adverse events are shown in supplementary figures 1 and 2. There were seven SAEs in six participants (appendicitis, gastroenteritis, two

respiratory infections, concussion, tonsillitis and intracardiac thrombus), none of which were considered related to immunization.

Discussion

This study provides the first data to inform existing recommendations for use of 4CMenB in children at increased risk of meningococcal disease. Following two doses of 4CMenB vaccine, children with asplenia or splenic dysfunction had bactericidal activity against four test strains that was very similar to healthy controls. Immunization with 4CMenB was also able to generate bactericidal activity in the presence of exogenous complement in the majority of children with complement deficiency, although this response was lower when tested using endogenous complement and among those with terminal chain complement deficiencies or on eculizumab treatment.

The central role for complement in '*in vivo*' killing of meningococci (by generation of the terminal membrane complex) is reflected in the use of the serum bactericidal assay as the accepted correlate of protection against IMD in clinical trials of meningococcal vaccines[16]. Rather than only testing the quantity of antigen specific antibodies induced by immunization, SBA assays provide a qualitative test of the ability of these antibodies to kill meningococci in the presence of complement. Assessing the immunogenicity of meningococcal vaccines in children with acquired or congenital complement deficiencies is therefore problematic, as testing the immune response in these children with exogenous complement from 'healthy' donors could overestimate the protection that immunization is likely to afford.

While bearing this important caveat in mind, an increase in bactericidal activity was observed in most children with non-terminal chain complement deficiencies, whether measured with exogenous or endogenous complement. By contrast, and not

unexpectedly, children on eculizumab had poor responses even when tested with exogenous complement, presumably secondary to binding of the exogenous complement. Similarly, it is surprising that even a single participant with a terminal-chain complement deficiency or undergoing treatment with eculizumab demonstrated an increase in bactericidal activity when tested with endogenous complement.

Of relevance to this study is the experience with the use in immunocompromised patients of other meningococcal vaccines based on capsular antigens. The best direct evidence of the ability for antibodies against capsular antigens to provide protection, even in the absence of functional (endogenous) complement, comes from an observational study of 45 patients with terminal chain complement deficiency, all of whom were offered the plain polysaccharide capsular A, C, W and Y meningococcal vaccine.[18] Of the 31 patients receiving the vaccine, six episodes of IMD were observed over a two-year observation period, compared with six out of 14 who did not receive the vaccine; mean time to first episode of IMD was approximately eight years in vaccine recipients compared with approximately five in non-recipients, a difference that was statistically significant. Sera from these individuals displayed a post-immunization increase in killing of serogroup A and W meningococci that was dependent on the presence of polymorphonuclear leukocytes, suggesting that some protection was afforded by opsonophagocytosis rather than complement mediated killing.[20] While these data are based on immunity against capsular polysaccharides, rather than sub-capsular proteins, Plested et al provide encouraging evidence that sera from adults immunized with a precursor to 4CMenB is able to kill meningococci *ex vivo* (in both passive protection and opsonophagocytic assays) even in the absence of complement dependent bactericidal activity.[21] This again suggests a role for

opsonophagocytosis in protecting patients with primary complement deficiency against IMD, an aspect of the immune response that was not evaluated in this study. Of note is that this additional mode of killing may not apply to patients treated with eculizumab; recent data suggests that opsonophagocytic dependent killing of meningococcci in a whole blood assay is blocked in the presence of eculizumab.[22]

Reassuringly, children with asplenia/splenic dysfunction generate an immune response that is at least as good as healthy controls. This contrasts with the reduced immunogenicity of the serogroup C meningococcal protein –polysaccharide vaccines in adults with surgical asplenia reported by Balmer et al.[23] These contrasting findings may reflect the older age group in the later study or potential differences in the immune response of asplenic patients to capsular polysaccharides rather than sub-capsular proteins. Given this robust immune response to 4CMenB it is reasonable to expect that this vaccine will be as effective in children with asplenia/splenic deficiency as in healthy controls. It is also reassuring, though not unexpected, that the reactogenicity profile in the 'at-risk' children was broadly similar to healthy controls, and to previously reported studies in these age groups.[24-26]

Ultimately, the best understanding of the role of 4CMenB in immunocompromised patients will come from surveillance for vaccine failures. To date there are two published case reports of group B IMD in eculizumab-treated patients who had received a full course of 4CMenB[27,28] and one fatal case of IMD due to an unencapsulated strain bearing fHBP and NHBA proteins similar to those in 4CMenB.[29] Together with reports of group C, W and Y IMD in such patients following immunization with MenACWY vaccines[29-31], these cases may support the

above concerns regarding the ability of immunization to generate protective immunity against IMD in the presence of eculizumab.

More formal calculations of vaccine effectiveness will include a comparison of rates of disease in immunized versus non-immunized 'at risk' individuals on eculizumab and with other 'at-risk' conditions, and comparing these to the vaccine failure rate observed in age matched healthy controls. Ongoing surveillance is crucial, and will also inform whether specific schedules for 'at-risk' vaccine recipients may be required, e.g. with additional booster doses. Given the incomplete coverage of 4CMenB for all capsular group B strains, and the uncertainty regarding the effectiveness of the 4CMenB vaccine in the most at-risk populations, it should be emphasized that immunization with 4CMenB does not remove the need for ongoing antibiotic prophylaxis in those for whom it is currently recommended. The low rates of antibiotic use in this study, and lack of evidence of antibiotic activity in the serum of the vast majority of study participants does raise the possibility that adherence to this recommendation is poor.

This study had a number of limitations, among them the relatively small number of children with terminal chain complement deficiencies and on eculizumab therapy. Additionally, the diagnosis of complement deficiency was based on local clinical diagnosis, with no formal standardised diagnostic criteria or central laboratory testing of complement function; this is potentially of relevance to the participant with reported C7 deficiency who developed bactericidal activity post-immunization. Also, this study did not include those with HIV infection, known to be at increased risk of disease,[32,33] nor did the study address the immunogenicity of the licensed vaccine

schedules for immunocompromised children under 2 years of age. Finally, as previously described many participants had SBA titers ≥5 pre-immunization for strain M10713 (evaluating the NHBA component of 4CMenB).[34] Nevertheless the increase in GMTs observed does provide some support for the immunogenicity of this vaccine antigen in the populations studied. .

Conclusion

The data from this study provide provisional support for the existing guidelines for immunization against capsular group B disease in children with complement deficiencies and splenic dysfunction. Ongoing surveillance for vaccine failures is required to determine the significance of the trend to reduced immune response in children with terminal chain complement deficiencies or undergoing treatment with eculizumab. In the meantime it is important that these patients are identified, receive education about sepsis management plans and are prescribed prophylactic antibiotics according to local guidelines, along with vaccination, to provide every chance for them to be protected against this deadly disease.

References

1. Committee for Medicinal Products for Human Use. European Medicines Agency. *Assessment report: Bexsero.* 2013.
2. Australian Register of Therapeutic Goods. ARTG ID 190719 - BEXSERO. 2013; http://search.tga.gov.au/s/search.html?collection=tga-artg&profile=record&meta_i=190719. Accessed 25th June 2016.
3. US Food and Drug Administration. BEXSERO. 2015; https://www.fda.gov/BiologicsBloodVaccines/Vaccines/ApprovedProducts/ucm431374.htm. Accessed 25th June 2016.
4. Bianchi A, Fantoni S, Prugnola A. Meningococcal B vaccine and the vision of a meningitis free world. *Journal of preventive medicine and hygiene.* Aug 31 2015;56(3):E140-143.
5. Health Canada. 2014; http://www.hc-sc.gc.ca/dhp-mps/prodpharma/sbd-smd/drug-med/sbd_smd_2014_bexsero_147275-eng.php. Accessed 09 February 2018, 2018.
6. JCVI. JCVI position statement on use of Bexsero meningococcal vaccine in the UK. 2014; https://www.gov.uk/government/publications/meningococcal-b-vaccine-jcvi-position-statement. Accessed 25th June 2016.
7. National Immunisation Advisory Committee. Immunisation Guidelines for Ireland Chapter 13 Meningococcal infection. 2016; http://www.hse.ie/eng/health/immunisation/hcpinfo/guidelines/chapter13.pdf. Accessed 25th June 2016.
8. Dube E, Gagnon D, Hamel D, et al. Parents' and adolescents' willingness to be vaccinated against serogroup B meningococcal disease during a mass vaccination in Saguenay-Lac-St-Jean (Quebec). *The Canadian journal of infectious diseases & medical microbiology = Journal canadien des maladies infectieuses et de la microbiologie medicale.* May-Jun 2015;26(3):163-167.
9. Signorelli C, Chiesa V, Odone A. Meningococcal serogroup B vaccine in Italy: state-of-art, organizational aspects and perspectives. *Journal of preventive medicine and hygiene.* Aug 31 2015;56(3):E125-132.
10. Public Health England. Immunisation against Infectious Disesase. Chapter 7, Immunisation of Individuals with Underlying Medical Conditions. 2014; http://www.gov.uk/government/uploads/system/uploads/attachment_data/file/309218/Green_Book_Chapter_7_v1_3.pdf. Accessed 25th June 2016.
11. Hellenbrand W, Koch J, Harder T, et al. Background Paper for the update of meningococcal vaccination recommendations in Germany: use of the serogroup B vaccine in persons at increased risk for meningococcal disease. *Bundesgesundheitsblatt, Gesundheitsforschung, Gesundheitsschutz.* Nov 2015;58(11-12):1314-1343.
12. Pinto MV, Bihari S, Snape MD. Immunisation of the immunocompromised child. *The Journal of infection.* Jul 5 2016;72 Suppl:S13-22.
13. Center for Disease Control and Prevention. Meningococcal: who needs to be vaccinated? 2015; https://www.cdc.gov/vaccines/vpd/mening/public/index.html. Accessed 25th June 2016.

14. Figueroa J, Andreoni J, Densen P. Complement deficiency states and meningococcal disease. *Immunologic research.* 1993;12(3):295-311.
15. Hillmen P, Muus P, Roth A, et al. Long-term safety and efficacy of sustained eculizumab treatment in patients with paroxysmal nocturnal haemoglobinuria. *British journal of haematology.* Jul 2013;162(1):62-73.
16. Holdsworth RJ, Irving AD, Cuschieri A. Postsplenectomy sepsis and its mortality rate: actual versus perceived risks. *The British journal of surgery.* Sep 1991;78(9):1031-1038.
17. Holmes FF, Weyandt T, Glazier J, Cuppage FE, Moral LA, Lindsey NJ. Fulminant Meningococcemia after splenectomy. *Jama.* Sep 4 1981;246(10):1119-1120.
18. Platonov AE, Vershinina IV, Kuijper EJ, Borrow R, Kayhty H. Long term effects of vaccination of patients deficient in a late complement component with a tetravalent meningococcal polysaccharide vaccine. *Vaccine.* Oct 1 2003;21(27-30):4437-4447.
19. Gossger N, Snape MD, Yu LM, et al. Immunogenicity and tolerability of recombinant serogroup B meningococcal vaccine administered with or without routine infant vaccinations according to different immunization schedules: a randomized controlled trial. *Jama.* Feb 8 2012;307(6):573-582.
20. Platonov AE, Vershinina IV, Kayhty H, Fijen CA, Wurzner R, Kuijper EJ. Antibody-dependent killing of meningococci by human neutrophils in serum of late complement component-deficient patients. *International archives of allergy and immunology.* Apr 2003;130(4):314-321.
21. Plested JS, Welsch JA, Granoff DM. Ex vivo model of meningococcal bacteremia using human blood for measuring vaccine-induced serum passive protective activity. *Clinical and vaccine immunology : CVI.* Jun 2009;16(6):785-791.
22. Konar M, Granoff DM. Eculizumab treatment and impaired opsonophagocytic killing of meningococci by whole blood from immunized adults. *Blood.* Aug 17 2017;130(7):891-899.
23. Balmer P, Falconer M, McDonald P, et al. Immune response to meningococcal serogroup C conjugate vaccine in asplenic individuals. *Infection and immunity.* Jan 2004;72(1):332-337.
24. McQuaid F, Snape MD, John TM, et al. Persistence of bactericidal antibodies to 5 years of age after immunization with serogroup B meningococcal vaccines at 6, 8, 12 and 40 months of age. *The Pediatric infectious disease journal.* Jul 2014;33(7):760-766.
25. McQuaid F, Snape MD, John TM, et al. Persistence of specific bactericidal antibodies at 5 years of age after vaccination against serogroup B meningococcus in infancy and at 40 months. *CMAJ : Canadian Medical Association journal = journal de l'Association medicale canadienne.* Apr 21 2015;187(7):E215-223.
26. Santolaya M, ORyan M, Valenzuela M, Prado V, Vergara R, Muñoz A. Immunogeni city and tolerability of an investigational multicomponent meningococcal serogroup B (4CMenB) vaccine in healthy adolescents. Paper presented at: Presentado en: 29th Annual Meeting of the European Society for Paediatric Infectious Diseases2011; The Hague, Netherlands.

27. Hernando Real S, Vega Castano S, Pajares Garcia R. Meningococcemia in vaccinated patient under treatment with eculizumab. *Enfermedades infecciosas y microbiologia clinica.* Mar 2017;35(3):200-201.

28. Parikh SR, Lucidarme J, Bingham C, et al. Meningococcal B Vaccine Failure With a Penicillin-Resistant Strain in a Young Adult on Long-Term Eculizumab. *Pediatrics.* Sep 2017;140(3).

29. McNamara LA, Topaz N, Wang X, Hariri S, Fox L, MacNeil JR. High Risk for Invasive Meningococcal Disease Among Patients Receiving Eculizumab (Soliris) Despite Receipt of Meningococcal Vaccine. *American journal of transplantation : official journal of the American Society of Transplantation and the American Society of Transplant Surgeons.* Sep 2017;17(9):2481-2484.

30. Cullinan N, Gorman KM, Riordan M, Waldron M, Goodship TH, Awan A. Case report: Benefits and challenges of long-term eculizumab in atypical hemolytic uremic syndrome. *Pediatrics.* Jun 2015;135(6):e1506-1509.

31. Struijk GH, Bouts AH, Rijkers GT, Kuin EA, ten Berge IJ, Bemelman FJ. Meningococcal sepsis complicating eculizumab treatment despite prior vaccination. *American journal of transplantation : official journal of the American Society of Transplantation and the American Society of Transplant Surgeons.* Mar 2013;13(3):819-820.

32. Miller L, Arakaki L, Ramautar A, et al. Elevated risk for invasive meningococcal disease among persons with HIV. *Annals of internal medicine.* Jan 7 2014;160(1):30-37.

33. Simmons RD, Kirwan P, Beebeejaun K, et al. Risk of invasive meningococcal disease in children and adults with HIV in England: a population-based cohort study. *BMC medicine.* Dec 9 2015;13:297.

34. Snape MD, Voysey M, Finn A, et al. Persistence of Bactericidal Antibodies After Infant Serogroup B Meningococcal Immunization and Booster Dose Response at 12, 18 or 24 Months of Age. *The Pediatric infectious disease journal.* Apr 2016;35(4):e113-123.

Acknowledgements

The authors thank Richard Sewell, Hannah Robinson, Tim Sell, Austen Worth, Paola Durando, Caterina Cancrini, Fernando Baquero Artigao, Annaelisa Tasciotti, Vasundhara Dindore, Gema Ariceta, Maurizio De Martino, Jose Sachez de Toledo, Sonia Maria Uriona and JoAnne Welsch for their contributions to the study. The authors acknowledge the support of Tiberia Pop for the preparation of the immunogenicity and reactogenicity figures, and Maria Ana de la Grandiere for manuscript coordination (both XPE Pharma and Science C/O GSK).

Figure legends

Figure 1. Study design and subject disposition flowchart.

Figure 2. Reverse cumulative distribution curves of hSBA titers displayed by group pre and post-immunization with 2 doses of 4CMenB for strains 44/76 (A), 5/99 (B), NZ98/254 (C) and M10713 (D) (Full analysis set).

Figure 3. Percentage of subjects with bactericidal serum activity at 1:4 dilution using endogenous human complement (end-hSBA) by group pre (Day 1) and post-immunization (Day 91) with two doses of 4CMenB for strains 44/76 (A), 5/99 (B), NZ98/254 (C) and M10713 (D) (Full analysis set).

Table 1: Participant demographics.

	Complement Deficient N = 40	Asplenia/splenic dysfunction N = 112	Healthy N = 87	Total N = 239
Age* (years):	8.5±4.4	11.1±3.7	10.2±4.1	10.3±4.1
Sex:				
Male	23 (58%)	66 (59%)	43 (49%)	132 (55%)
Female	17 (43%)	46 (41%)	44 (51%)	107 (45%)
Country of Enrollment:				
Italy	7 (18%)	14 (13%)	12 (14%)	33 (14%)
Poland	8 (20%)	29 (26%)	20 (23%)	57 (24%)
Russia	4 (10%)	24 (21%)	17 (20%)	45 (19%)
Spain	13 (33%)	29 (26%)	24 (28%)	66 (28%)
United Kingdom	8 (20%)	16 (14%)	14 (16%)	38 (16%)
Race:				
Asian	1 (3%)	0	1 (1%)	2 (1%)
Black or African American	0	4 (4%)	1 (1%)	5 (2%)
White	36 (90%)	105 (94%)	84 (97%)	225 (94%)
Other	3 (8%)	3 (3%)	1 (1%)	7 (3%)

* Age is shown as mean +/- standard deviation

Table 2. Percentage of analysed subjects (and 95% confidence intervals) with hSBA titers ≥5. Results are for participants with valid results pre and post immunization.

Strain	Timing	Complement deficient	Asplenia/splenic dysfunction	Healthy
		N = 39	**N = 106**	**N = 85**
H44/76	Baseline	0 (0%) (0.0% to 9.0%)	7 (7%) (2.7% to 13.4%) N = 104	5 (6%) (2.0% to 13.3%) N = 84
	1 Month after second vaccination	34 (87%) (72.6% to 95.7%)	101 (97%) (91.8% to 99.4%) N = 104	83 (98%) (91.8% to 99.71%)
	Study group differences 1 month after second vaccination	Complement deficient to healthy -10% (-24.6% to -1.6%)	Asplenia/splenic dysfunction to healthy -1% (-6.1% to 5.6%)	
5/99	Baseline	0 (0%) (0.0% to 9.5%) N = 37	12 (12%) (6.2% to19.5%) N = 103	5 (6%) (2.0% to 13.7%) N = 82
	1 Month after second vaccination	36 (95%) (82.3% to 99.4%) N = 38	106 (100%) (96.6% to100.0%)	82 (99%) (93.5% to 99.97%) N = 83
	Study group differences 1 month after second vaccination	Complement deficient to healthy -4% (-16.3% to 2.2%)	Asplenia/splenic dysfunction to healthy 1% (-2.3% to 6.5%)	
NZ98/254	Baseline	0 (0%) (0.0%-9.7%) N = 36	4 (4%) (1.0%-9.5%) N = 105	2 (2%) (0.29%-8.4%) N = 83
	1 Month after second vaccination	26 (68%) (51.3% to 82.5%) N = 38	91 (86%) (77.7% to 91.9%)	70 (83%) (73.6%-90.6%) N = 84
	Study group differences 1 month after second vaccination	Complement deficient to healthy -15% (-32.4% to 0.8%)	Asplenia/splenic dysfunction to healthy 3% (-7.8% to13.5%)	
M10713	Baseline	20 (56%) (38.1%-72.1%) N = 36	81 (79%) (70.3%-86.8%) N = 102	64 (78%) (67.5%-86.4%) N = 82
	1 Month after second vaccination	27 (73%) (55.9% to 86.2%) N = 37	97 (94%) (87.8%-97.8%) N = 103	82 (99%) (93.5%-99.97%) N = 83
	Study group differences 1 month after second vaccination	Complement deficient to healthy -26% (-42.0% to−13.7%)	Asplenia/splenic dysfunction to healthy -5% (-11.1% to1.3%)	

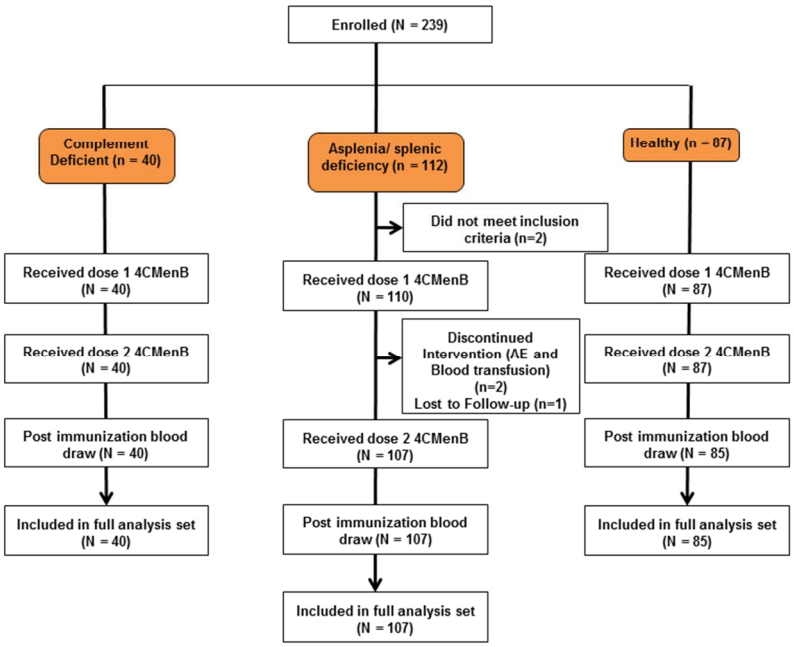

Figure 1. Study design and subject disposition flowchart.

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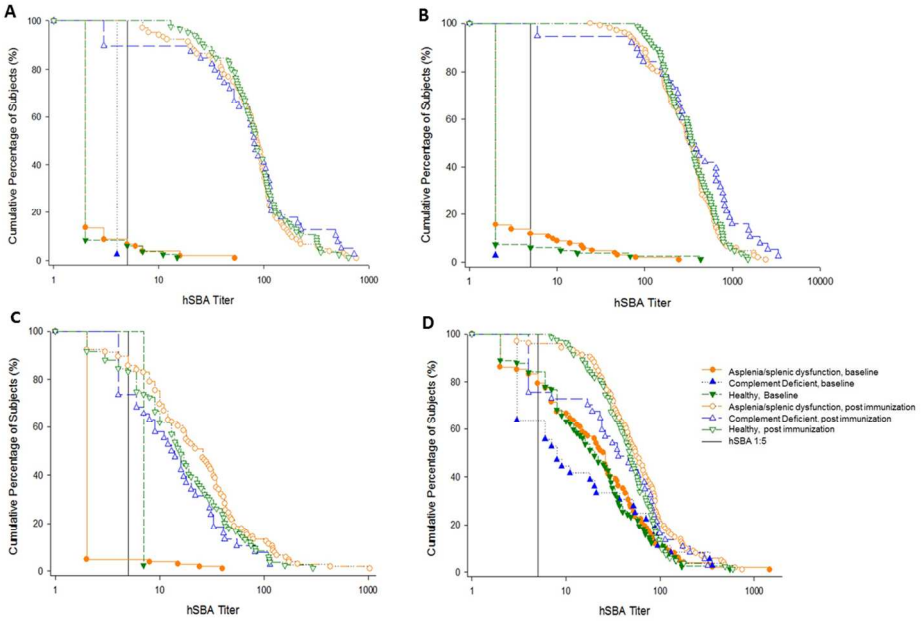

Figure 2. Reverse cumulative distribution curves of hSBA titers displayed by group pre and post-immunization with 2 doses of 4CMenB for strains 44/76 (A), 5/99 (B), NZ98/254 (C) and M10713 (D) (Full analysis set).

359x229mm (96 x 96 DPI)

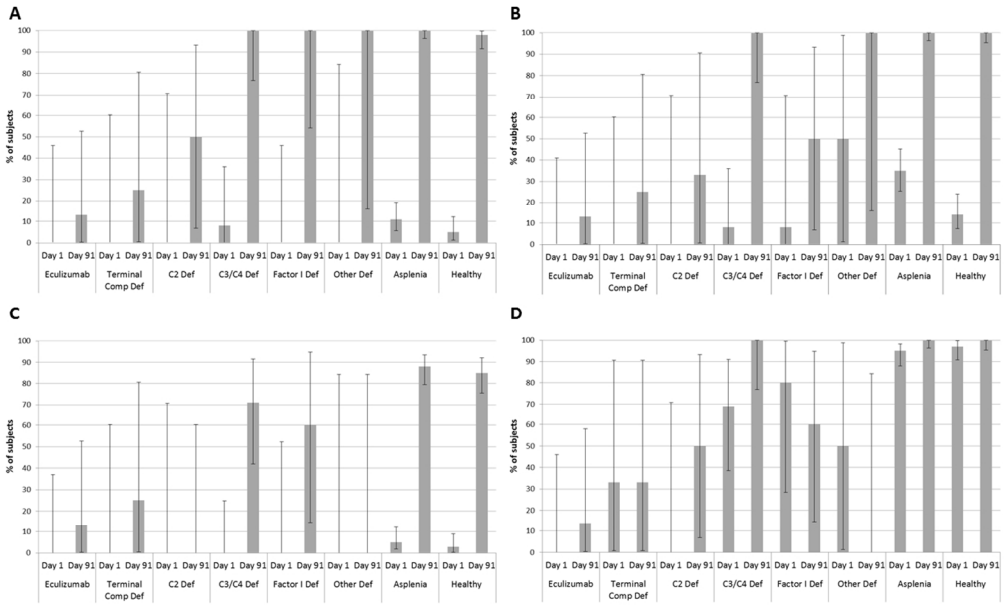

Figure 3. Percentage of subjects with bactericidal serum activity at 1:4 dilution using endogenous human complement (end-hSBA) by group pre (Day 1) and post-immunization (Day 91) with two doses of 4CMenB for strains 44/76 (A), 5/99 (B), NZ98/254 (C) and M10713 (D) (Full analysis set).

359x229mm (96 x 96 DPI)

Supplementary material: Inclusion and Exclusion Criteria

Inclusion Criteria

In order to participate in this study, all subjects had to meet all of the inclusion criteria applicable to the relevant group.

Inclusion criteria applicable to all groups

1. Individuals aged 2 to 17 years (inclusive) at enrollment;
2. Individuals who had given written informed assent (if appropriate) and whose parent/legal guardian had given written informed consent after the nature of the study had been explained;
3. Available for all the visits scheduled in the study;
4. Weighing at least 13 kg at the time of enrollment.

Inclusion criterion applicable to Complement Deficient children

5. Subjects at risk of meningococcal disease because of primary or secondary complement deficiencies:
    a. Primary deficiencies: patients with a congenital condition leading to a reduced concentration of one or more proteins in the complement cascade, including C1 (q,r,s), C2, C3, C4, Factor D, Properdin, C5, C6, C7, C8, C9, Factor H, Factor I (homozygous)
    b. Secondary deficiencies: patients with a condition indirectly leading to a reduced concentration of one or more proteins in the complement cascade, including patients who were already in treatment with eculizumab at the time of enrollment and have been diagnosed with PNH or with aHUS

For patients with a secondary complement deficiency the investigator had to include in the source documentation evidence of the increased risk of meningococcal disease based on reduced complement protein concentrations or on previous meningococcal infection. This was to be documented in the medical records.

Inclusion criterion applicable to Asplenia/spelenic dysfunction

6. Subjects at risk of meningococcal disease because of functional or anatomic asplenia:
    a. Congenital anomalies of the spleen, isolated or in association with other splenic anomalies
    b. Surgical splenectomy, which may occur after significant splenic trauma or other clinical disorders, such as idiopathic (autoimmune) thrombocytopenic purpura
    c. Autosplenectomy (ie, infarction) which may occur in patients with sickle cell disease or other hemoglobinopathies

For patients with functional asplenia the investigator was to collect medical documentation for reduced splenic function diagnosed with an appropriate technique.

Patients with anatomic asplenia or sickle-cell disease did not require assessment of the splenic function.

Inclusion criterion applicable to Health Controls

7. Healthy immunocompetent subjects, in good health as determined by medical history, physical examination and clinical judgment of the investigator.

Exclusion Criteria

In order to participate in this study, all subjects had to meet none of the exclusion criteria applicable to the relevant group.

Exclusion criteria applicable to all groups

1. History of any previous immunization with a meningococcal B vaccine at the time of enrollment;
2. History of severe allergic reaction after previous vaccinations, or hypersensitivity to any component of the vaccine;
3. Known HIV infection;
4. History of any progressive or severe neurologic disorder, or seizure disorder (exception: one self-limited febrile seizure is acceptable);
5. Contraindication to intramuscular (IM) injection or blood drawn;
6. Females who were pregnant, planning a pregnancy or nursing (breastfeeding);
7. Females of childbearing potential who had not used or did not plan to use acceptable birth control measures, for the three months duration of the study. Oral, injected or implanted hormonal contraceptive, barrier methods (diaphragm or condom with spermicide), intrauterine device or sexual abstinence are considered acceptable forms of birth control. If sexually active the subject had to have been using one of the accepted birth control methods for at least 60 days prior to study entry;
8. Child's parent(s)/legal guardian(s) were not able to comprehend and to follow all required study procedures for the whole period of the study;
9. Intent to participate in another clinical study during this study;
10. Family members or household members of site research staff;
11. History or any illness/condition that, in the opinion of the investigator, might interfere with the results of the study or pose additional risk to the subjects due to participation in the study.

Exclusion criterion applicable to both Complement deficiency and Asplenia/ Splenic dysfunction groups

12. Previous known or suspected disease caused by *N meningitidis* in the last year.

Exclusion criteria applicable to Healthy Controls

13. Previous known or suspected disease caused by *N meningitidis*;
14. Known or suspected impairment/alteration of the immune system resulting from, for example, receipt of immunosuppressive/immunostimulant therapy.

There might have been instances when individuals met all entry criteria except one that related to transient clinical circumstances (eg, body temperature elevation or recent use of excluded medication or vaccine). Under these circumstances, a subject might be considered eligible for study enrollment if the appropriate window for delay had passed, inclusion/exclusion criteria had been rechecked, and if the individual was confirmed to be eligible.

Criteria for Delay of Enrollment

Subjects meeting any one or more of the criteria below may not have been assigned a subject ID and enrolled until the specified time period had passed as detailed below:

Enrollment delay criteria applicable to all groups

- Receipt of blood, blood products and/or plasma derivatives or any parenteral immunoglobulin preparation in the previous 12 weeks;
- Household contact with and/or intimate exposure to an individual with any laboratory confirmed *N meningitidis* infection within 60 days of enrollment;
- Significant acute illness within the previous seven days or body temperature ≥ 38·0°C within the previous three days;
- Receipt of any analgesic/antipyretic medication within the previous six hours;
- Receipt of, or plan to receive, any other vaccine(s), within the previous 14 days (or 28 days for live vaccines);
- Participation in any clinical trial with another investigational product within the previous 30 days.

Enrollment delay criterion applicable to both Complement deficiency and Asplenia/ Splenic dysfunction groups

- Receipt of systemic antibiotics other than the one administered for prophylactic purpose within the previous three days.

Enrollment delay criterion applicable to group Healthy

- Receipt of systemic antibiotics within the previous three days.

Criteria for delay of vaccination and/or blood sampling

After enrollment, subjects might have encountered clinical circumstances that warranted a delay in subsequent study vaccination. These situations are listed below. In the event that a subject met a criterion for delay of vaccination, the subject could receive the study vaccination once the window for delay had passed as long as the subject was otherwise eligible for study participation.

Vaccination delay criteria applicable to all groups

- Receipt of blood, blood products and/or plasma derivatives or any parenteral immunoglobulin preparation in the previous 12 weeks;
- Significant acute illness within seven days or body temperature ≥ 38·0°C within three days of intended study vaccination;
- Receipt of any analgesic/antipyretic medication within the previous six hours;
- Receipt of, or plan to receive, any other vaccine(s), within 14 days (or 28 days for live vaccines) of intended study vaccination.

There were clinical circumstances that warranted delay of blood collection for immunogenicity assessments in this study. These situations are listed below. In the event that a subject met a criterion for delay of blood collection, blood collection would have proceeded once the window for delay had passed.

Blood sampling delay criterion applicable to groups CompDef and Asplenia

- Receipt of systemic antibiotics, other than the one administered for prophylactic purpose, within the previous three days.

Blood sampling delay criterion applicable to Healthy Controls

- Receipt of systemic antibiotics within the previous three days.

Supplementary Table 1: summary of complement deficiencies

Category	Number	Comments
Eculizumab treatment	9*	Underlying conditions of atypical haemolytic uraemic syndrome (n=7), proliferative glomerulonephritis (n=2)
Terminal complement deficiencies	4	Primary C7 deficiency (n=2), C5 deficiency and C6 deficiency.
C2 deficiency	5	Presentations primarily with recurrent bacterial sepsis
C3 and/or C4 deficiency	14	
Factor I deficiency	6	
Factor H mutation	1	
Alternative pathway deficiency	1	

* one participant with terminal complement deficiency did not have analyzable sample at any point in the study, and did not contribute to immunogenicity data

Supplementary Table 2: Serum Bactericidal Activity Geometric Mean Titres (SBA GMTS); exogenous complement

Strain		Complement deficient	Asplenia/splenic dysfunction	Healthy
		N = 39	N = 106	N = 85
H44/76	Baseline	1·08 (0·87-1·33)	1·17 (1·03-1·32), N = 104	1·15 (1·03-1·28), N = 84
	1 Month after second vaccination	48 (29-79)	65 (48-88), N = 104	76 (61-94)
	Study group differences 1 month after second vaccination	Complement deficient to healthy 0·69 (0·42-1·13)	Asplenia/splenic dysfunction to healthy 0·90 (0·63-1·29)	
5/99	Baseline	0·87 (0·61-1·26), N = 37	1·43 (1·16-1·78), N = 103	1·24 (1·02-1·52), N = 82
	1 Month after second vaccination	263 (166-415), N = 38	300 (230-392)	307 (250-376), N = 83
	Study group differences 1 month after second vaccination	Complement deficient to healthy 0·81 (0·51-1.28)	Asplenia/splenic dysfunction to healthy 0·97 (0·70-1·35)	
NZ98/254	Baseline	0·95 (0·78-1·16), N = 36	1·10 (0·98-1·24), N = 105	1·05 (0·98-1·12), N = 83
	1 Month after second vaccination	8·46 (4·85-15), N = 38	18 (13-24)	14 (10-18), N = 84
	Study group differences 1 month after second vaccination	Complement deficient to healthy 0·64 (0·36-1·14)	Asplenia/splenic dysfunction to healthy 1·33 (0·88-2·01)	
M10713	Baseline	8·57 (4·43-17) N = 36	15 (10-22) N = 102	16 (11-22) N = 82
	1 Month after second vaccination	20 (11-34), N = 37	45 (33-62), N = 103	42 (34-52), N = 83
	Study group differences 1 month after second vaccination	Complement deficient to healthy 0·47	Asplenia/splenic dysfunction to healthy 1·20	

Strain		Complement deficient	Asplenia/splenic dysfunction	Healthy
		(0·28-0·78)	(0·83-1·73)	

Supplementary Table 3. **Percentage of analysed subjects (and 95% confidence intervals) with hSBA titres ≥8; exogenous complement**. Results are for participants with valid results pre and post immunization.

Strain		Complement deficient	Asplenia/splenic dysfunction	Healthy
		N = 39	N = 106	N = 85
H44/76	Baseline	0 (0%) (0·0% to 9·0%)	2 (2%) (0·23% to 6·8%) N = 104	2 (2%) (0·29% to 8·3%) N = 84
	1 Month after second vaccination	34 (87%) (72·6% to 95·7%)	99 (95%) (89·1% to 98·4%) N = 104	83 (98%) (91·8% to 99·71%)
	Study group differences 1 month after second vaccination	Complement deficient to healthy -10% (-24·6% to -1·6%)	Asplenia/splenic dysfunction to healthy -2% (-8·8% to 3·9%)	
5/99	Baseline	0 (0%) (0·0% to 9·5%) N = 37	11 (11%) (5·5% to 18·3%) N = 103	4 (5%) (1·3% to 12·0%) N = 82
	1 Month after second vaccination	35 (92%) (78·6% to 98·3%) N = 38	106 (100%) (96·6% to 100·0%)	82 (99%) (93·5% to 99·97%) N = 83
	Study group differences 1 month after second vaccination	Complement deficient to healthy -7% (-19·8% to 0·21%)	Asplenia/splenic dysfunction to healthy 1% (-2·3% to 6·5%)	
NZ98/254	Baseline	0 (0%) (0·0%-9·7%) N = 36	4 (4%) (1·0%-9·5%) N = 105	0 (0%) (0·0%-4·3%) N = 83
	1 Month after second vaccination	24 (63%) (46·0% to 78·2%) N = 38	84 (79%) (70·3% to 86·5%)	61 (73%) (61·8%-81·8%) N = 84
	Study group differences 1 month after second vaccination	Complement deficient to healthy -9% (-27·8% to 7·8%)	Asplenia/splenic dysfunction to healthy 7% (-5·5% to 19·1%)	
M10713	Baseline	17 (47%) (30·4%-64·5%) N = 36	69 (68%) (57·7%-76·6%) N = 102	56 (68%) (57·1%-78·1%) N = 82
	1 Month after second vaccination	26 (70%) (53·0% to 84·1%) N = 37	97 (94%) (87·8%-97·8%) N = 103	81 (98%) (91·6%-99·7%) N = 83
	Study group differences 1 month after second vaccination	Complement deficient to healthy -27% (-43·7% to−14·3%)	Asplenia/splenic dysfunction to healthy -3% (-10·1% to 3·2%)	

Supplementary Table 4: Serum Bactericidal Activity Titres ≥ 1:5 by complement deficiency; exogenous complement. Results are for participants with valid results pre and post immunization.

Strain		Eculizumab	Terminal complement deficiency	C2 deficiency	C3 +/- C4 deficiency	Factor I deficiency	Other*
		N = 8	N = 4	N = 5	N= 14	N=6	N=2
H44/76	Baseline	0 (0%) (0.0%-36.9%)	0 (0%) (0.0%-60.2%)	0 (0%) (0.0%-52.2%)	0 (0%) (0.0%-23.2%)	0 (0%) (0.0%-45.9%)	0 (0%) (0.0% - 84.2%)
	1 Month after second vaccination	4 (50%) (15.7%-84.3%)	4 (100%) (39.8%-100.0%)	5 (100%) (47.8% - 100.0%)	14 (100%) (76.8%- 100.0%)	6 (100%) (54.1 – 100.0%)	1 (50%) (1.3% - 98.7%)
5/99	Baseline	0 (0%) (0.0%-36.9%)	0 (0%) (0.0%-60.2%)	0 (0%) (0.0% - 60.2%) N=4	0 (0%) (0.0% - 23.2%)	0 (0%) (0.0% - 52.2%) N=2	0 (0%) (0.0% - 84.2%)
	1 Month after second vaccination	6 (75%) (39.4%-96.8%)	4 (100%) (39.8%-100.0%)	5 (100%) (47.8%-100.0%)	14 (100%) (76.8%-100%)	5 (100%) (47.8% - 100.0%)	2 (100%) (15.8%-100.0%)
NZ98/254	Baseline	0 (0%) (0.0%-36.9%)	0 (0%) (0.0%-60.2%)	0 (0%) (0.0%-60.2%) N=4	0 (0%) (0.0%-24.7%) N=13	0 (0%) (0.0%-52.2%) N=5	0 (0%) (0.0%-84.2%)
	1 Month after second vaccination	2 (25%) (3.2%-65.1%)	4 (100%) (39.8%-100.0%)	3 (60%) (14.7% - 94.7%)	13 (93%) (66.1%-99.82%)	4 (80%) (28.4% -99.5%) N=5	0 (0%) (0.0%-84.2%)
M10713	Baseline	0 (0%) (0.0%-41.0%) N=7	3 (75%) (19.4%-99.4%)	3 (75%) (19.4%-99.4%) N=4	11 (79%) (49.2%-95.3%)	3 (60%) (14.7% -94.7%) N=5	0 (0%) (0.0% - 84.2%)
	1 Month after second vaccination	1 (14%) (0.36%-57.9%) N=7	4 (100%) (39.8%-100.0%)	5 (100%) (47.8%-100.0%)	13 (93%) (66.1%-99.8%)	4 (80%) (28.4% - 99.5%) N=5	0 (0%) (0.0%-84.2%)

* One participant with heterologous Factor H deficiency, one with alternative pathway deficiency

Supplementary Table 5: Serum Bactericidal Activity Geometric Mean Titres (SBA GMTS) by complement deficiency; exogenous complement

Strain		Eculizumab	Terminal complement deficiency	C2 deficiency	C3 +/- C4 deficiency	Factor I deficiency	Other*
		N = 8	N = 4	N = 5	N= 14	N=6	N=2
H44/76	Baseline	1.0 (0.85 – 1.18)	1.0 (0.79 – 1.27)	1.0 (0.81-1.24)	1.10 (0.97 – 1.25)	1.0 (0.90-1.11)	1.00 (0725-1.40)
	1 Month after second vaccination	11 (3.81-30)	69 (16-296)	81 (22-297)	84 (38-182)	180 (55-589)	9.80 (1.25-77)
5/99	Baseline	1.09 (1.0-1.19)	1.0 (0.89-1.13)	1.0 (0.89-1.13) N=4	1.0 (0.94-1.07)	1.0 (0.90-1.11) N=5	1.0 (0.85-1.18)
	1 Month after second vaccination	146 (42-505)	762 (132-4392)	226 (47-1081)	278 (109-709)	951 (198-4555) N=5	40 (3.37-478)
NZ98/254	Baseline	1.00 (1.00-1.00)	1.00 (1.00-1.00)	1.00 (1.00-1.00) N=4	1.00 (1.00-1.00) N=14	1.00 (1.00-1.00) N=5	1.00 (1.00-1.00)
	1 Month after second vaccination	1.77 (0.74-4.21)	25 (7.33-85)	6.25 (2.09-19)	19 (9.95-37)	14 (4.51-41)	2.00 (0.35-11)
M10713	Baseline	1.0 (0.27-3.72) N=7	14 (2.49-81)	8.87 (1.56-51) N=4	16 (6.33-41)	23 (4.87-109) N=5	1.73 (0.15-20)
	1 Month after second vaccination	1.87 (0.66-5.31) N=7	44 (11-177)	28 (8.17-97) N=4	58 (28-121)	34 (9.81-116) N=5	1.00 (0.14-7.07)

* One participant with heterologous Factor H deficiency, one with alternative pathway deficiency

Supplementary Table 6: Serum Bactericidal Activity Titres at 1:4 using endogenous complement (end-SBA) Results are for participants with valid results pre and post immunization.

Strain		Complement deficient	Asplenia/splenic dysfunction	Healthy
		N = 39	N = 106	N = 85
H44/76	Baseline	1 (3%) (0·07%-14·9%) N = 35	11 (11%) (5·7%-19·2%) N = 98	4 (5%) (1·4%-12·2%) N = 81
	1 Month after second vaccination	26 (68%) (51·3% to 82·5%)	102 (100%) (96·4% to100·0%)	80 (98%) (91·5%-99·70%)
	Study group differences 1 month after second vaccination	Complement deficient to healthy -29% (-45·4% to −15·8%)	Asplenia/splenic dysfunction to healthy 2% (-1·3%-8·5%)	
5/99	Baseline	2 (6%) (0·8%-20·8%) N = 32	33 (35%) (25·3%-45·2%) N = 95	11 (14%) (7·4%-24·1%) N = 77
	1 Month after second vaccination	21 (60%) (42·1%-76·1%) N = 35	101 (100%) (96·4%-100%) N = 101	78 (100%) (95·4%-100·0%) N = 78
	Study group differences 1 month after second vaccination	Complement deficient to healthy -40% (-56·5% to −25·5%)	Asplenia/splenic dysfunction to healthy 0% (-3·7%-4·7%)	
NZ98/254	Baseline	0 (0%) (0·0%-10·0%) N = 35	5 (5%) (1·8%-12·1%) N = 93	2 (3%) (0·31%-9·0%) N = 78
	1 Month after second vaccination	15 (41%) (24·8%-57·9%) N = 37	86 (88%) (79·6%-93·5%) N = 98	69 (85%) (75·6%-92·1%) N = 81
	Study group differences 1 month after second vaccination	Complement deficient to healthy -45% (-60·7% to −26·4%)	Asplenia/splenic dysfunction to healthy 3% (-7·6%-13·3%)	
M10713	Baseline	15 (47%) (29·1%-65·3%) N = 32	89 (95%) (88·0%-98·3%) N = 94	73 (97%) (90·7%-99·68%) N = 75
	1 Month after second vaccination	21 (60%) (42·1%-76·1%) N = 35	99 (100%) (96·3%-100·0%) N = 99	79 (100%) (95·4%-100·0%) N = 79

Strain		Complement deficient	Asplenia/splenic dysfunction	Healthy
	Study group differences 1 month after second vaccination	Complement deficient to healthy -40% (-56·5% to −25·5%)	Asplenia/splenic dysfunction to healthy 0% (-3·8%-4·7%)	

Supplementary Table 7: Serum Bactericidal Activity Titres ≥ 1:4 using endogenous complement (end-SBA) by complement deficiency. Results are for participants with valid results pre and post immunization.

Strain		Eculizumab	Terminal complement deficiency	C2 deficiency	C3 +/- C4 deficiency	Factor I deficiency	Other*
		N = 8	N = 4	N = 5	N= 14	N=6	N=2
H44/76	Baseline	0 (0%) (0.0%-41.0%) N=7	0 (0%) (0.0%-60.2%)	0 (0%) (0.0%-70.8%) N=3	1 (8%) (0.19%-36.0%) N=13	0 (0%) (0.0%-45.9%)	0 (0%) (0.0% - 84.2%)
	1 Month after second vaccination	1 (13%) (0.32-%-52.7%)	1 (25%) (0.6%-80.6%)	2 (50%) (6.8% - 93.2%) N=4	14 (100%) (76.8%- 100.0%)	6 (100%) (54.1 – 100.0%)	2 (100%) (15.8% - 100.0%)
5/99	Baseline	0 (0%) (0.0%-41.0%) N=7	0 (0%) (0.0%-60.2%)	0 (0%) (0.0% - 70.8%) N=3	1 (8%) (0.19% - 36.0%) N=13	0 (0%) (0.0% - 70.8%) N=3	1 (50%) (1.3% - 98.7%)
	1 Month after second vaccination	1 (13%) (0.32%-52.7%)	1 (25%) (0.6%-80.6%)	1 (33%) (0.8%-90.6%) N=3	14 (100%) (76.8%-100%)	2 (50%) (6.8% - 93.2%) N=4	2 (100%) (15.8%-100.0%)
NZ98/254	Baseline	0 (0%) (0.0%-36.9%)	0 (0%) (0.0%-60.2%)	0 (0%) (0.0%-70.8%) N=3	0 (0%) (0.0%-24.7%) N=13	0 (0%) (0.0%-52.2%) N=5	0 (0%) (0.0%-84.2%)
	1 Month after second vaccination	1 (13%) (0.32%-52.7%)	1 (25%) (0.6%-80.6%)	0 (0%) (0.0% - 60.2%) N=4	10 (71%) (41.9.1%-91.6%)	3 (60%) (14.7% -94.7%) N=5	0 (0%) (0.0%-84.2%)
M10713	Baseline	0 (0%) (0.0%-45.9%) N=6	1 (33%) (0.8%-90.6%) N=3	0 (0%) (0.0%-70.8%) N=3	9 (69%) (38.6%-90.9%) N=13	4 (80%) (28.4% -99.5%) N=5	1 (50%) (1.3% - 98.7%)
	1 Month after second vaccination	1 (14%) (0.36%-57.9%) N=7	1 (33%) (0.8%-90.6%) N=3	2 (50%) (6.8%-93.2%) N=4	14 (100%) (76.8%-100.0%)	3 (60%) (14.7% - 94.7%) N=5	0 (0%) (0.0%-84.2%)

* One participant with heterologous Factor H deficiency, one with alternative pathway deficiency

TREND Statement Checklist

Paper Section/ Topic	Item No	Descriptor	Reported?	
			√	Pg #
Title and Abstract				
Title and Abstract	1	Information on how unit were allocated to interventions	√	1, 3
		Structured abstract recommended	√	5
		Information on target population or study sample	√	1,3,5
Introduction				
Background	2	Scientific background and explanation of rationale	√	6
		Theories used in designing behavioral interventions	NA	NA
Methods				
Participants	3	Eligibility criteria for participants, including criteria at different levels in recruitment/sampling plan (e.g., cities, clinics, subjects)	√	7
		Method of recruitment (e.g., referral, self-selection), including the sampling method if a systematic sampling plan was implemented	√	7, suppl materia
		Recruitment setting	√	7
		Settings and locations where the data were collected	√	7
Interventions	4	Details of the interventions intended for each study condition and how and when they were actually administered, specifically including:		
		○ Content: what was given?	√	8
		○ Delivery method: how was the content given?	√	8
		○ Unit of delivery: how were the subjects grouped during delivery?	√	8
		○ Deliverer: who delivered the intervention?	/	/
		○ Setting: where was the intervention delivered?	√	8
		○ Exposure quantity and duration: how many sessions or episodes or events were intended to be delivered? How long were they intended to last?	√	7-8
		○ Time span: how long was it intended to take to deliver the intervention to each unit?	√	7-8
		○ Activities to increase compliance or adherence (e.g., incentives)	NA	NA
Objectives	5	Specific objectives and hypotheses	√	8-9
Outcomes	6	Clearly defined primary and secondary outcome measures	√	8-9
		Methods used to collect data and any methods used to enhance the quality of measurements	√	7-10
		Information on validated instruments such as psychometric and biometric properties	/	/
Sample Size	7	How sample size was determined and, when applicable, explanation of any interim analyses and stopping rules	√	10
Assignment Method	8	Unit of assignment (the unit being assigned to study condition, e.g., individual, group, community)	√	6
		Method used to assign units to study conditions, including details of any restriction (e.g., blocking, stratification, minimization)	√	6-9
		Inclusion of aspects employed to help minimize potential bias induced due to non-randomization (e.g., matching)	√	6-9

TREND Statement Checklist

Blinding (masking)	9	Whether or not participants, those administering the interventions, and those assessing the outcomes were blinded to study condition assignment; if so, statement regarding how the blinding was accomplished and how it was assessed.	NA	NA
Unit of Analysis	10	Description of the smallest unit that is being analyzed to assess intervention effects (e.g., individual, group, or community)	√	10
		If the unit of analysis differs from the unit of assignment, the analytical method used to account for this (e.g., adjusting the standard error estimates by the design effect or using multilevel analysis)	NA	NA
Statistical Methods	11	Statistical methods used to compare study groups for primary methods outcome(s), including complex methods of correlated data	√	10
		Statistical methods used for additional analyses, such as a subgroup analyses and adjusted analysis	√	10
		Methods for imputing missing data, if used	NA	NA
		Statistical software or programs used	√	8-10
Results				
Participant flow	12	Flow of participants through each stage of the study: enrollment, assignment, allocation, and intervention exposure, follow-up, analysis (a diagram is strongly recommended)	√	Figure 1
		○ Enrollment: the numbers of participants screened for eligibility, found to be eligible or not eligible, declined to be enrolled, and enrolled in the study	√	
		○ Assignment: the numbers of participants assigned to a study condition	√	
		○ Allocation and intervention exposure: the number of participants assigned to each study condition and the number of participants who received each intervention	√	
		○ Follow-up: the number of participants who completed the follow-up or did not complete the follow-up (i.e., lost to follow-up), by study condition	√	
		○ Analysis: the number of participants included in or excluded from the main analysis, by study condition	√	
		Description of protocol deviations from study as planned, along with reasons	√	Figure 1
Recruitment	13	Dates defining the periods of recruitment and follow-up	√	7
Baseline Data	14	Baseline demographic and clinical characteristics of participants in each study condition	√	11, Table 1
		Baseline characteristics for each study condition relevant to specific disease prevention research	√	Table 1
		Baseline comparisons of those lost to follow-up and those retained, overall and by study condition	√	Figure 1
		Comparison between study population at baseline and target population of interest	√	Table 1
Baseline equivalence	15	Data on study group equivalence at baseline and statistical methods used to control for baseline differences	/	/

TREND Statement Checklist

Numbers analyzed	16	Number of participants (denominator) included in each analysis for each study condition, particularly when the denominators change for different outcomes; statement of the results in absolute numbers when feasible	√	11, Tables 2 and 3, +suppl
		Indication of whether the analysis strategy was "intention to treat" or, if not, description of how non-compliers were treated in the analyses	√	Figure 1
Outcomes and estimation	17	For each primary and secondary outcome, a summary of results for each estimation study condition, and the estimated effect size and a confidence interval to indicate the precision	√	11-14
		Inclusion of null and negative findings	√	11-14
		Inclusion of results from testing pre-specified causal pathways through which the intervention was intended to operate, if any	NA	NA
Ancillary analyses	18	Summary of other analyses performed, including subgroup or restricted analyses, indicating which are pre-specified or exploratory	√	8, 12
Adverse events	19	Summary of all important adverse events or unintended effects in each study condition (including summary measures, effect size estimates, and confidence intervals)	√	13, 14
DISCUSSION				
Interpretation	20	Interpretation of the results, taking into account study hypotheses, sources of potential bias, imprecision of measures, multiplicative analyses, and other limitations or weaknesses of the study	√	14-18
		Discussion of results taking into account the mechanism by which the intervention was intended to work (causal pathways) or alternative mechanisms or explanations	√	14-18
		Discussion of the success of and barriers to implementing the intervention, fidelity of implementation	√	14-18
		Discussion of research, programmatic, or policy implications	√	14-18
Generalizability	21	Generalizability (external validity) of the trial findings, taking into account the study population, the characteristics of the intervention, length of follow-up, incentives, compliance rates, specific sites/settings involved in the study, and other contextual issues	√	17
Overall Evidence	22	General interpretation of the results in the context of current evidence and current theory	√	17, 18

From: Des Jarlais, D. C., Lyles, C., Crepaz, N., & the Trend Group (2004). Improving the reporting quality of nonrandomized evaluations of behavioral and public health interventions: The TREND statement. *American Journal of Public Health*, 94, 361-366. For more information, visit: http://www.cdc.gov/trendstatement/

List word counts below (do not paste the text here). Please see the Decision Letter Attachment for allowances as they pertain to your manuscript type.

# of words in Abstract: **249** (250 words allowed)
# of words in Manuscript Body: **3036** (3000 allowed for Regular Articles/Quality Reports; 4000 Reviews/Special Articles; 800 Commentaries; 1200 Perspectives)
# of characters in Main Title: **98** characters (97 characters allowed, including spaces)
# of characters in Short Title: **48** (55 characters allowed, including spaces)
# of words in "Table of Contents Summary": **26** (25 words allowed; this section appears in all articles with abstracts)
# of words in "What's Known on this Subject": **40** (40 words allowed; this section appears in Regular Articles only)
# of words in "What this Study Adds": **38** (40 words allowed; this section appears in Regular Articles only)

2017-4250.RX – Meningococcal B vaccine immunogenicity in children with defects in complement and splenic function -- by Martinon Torres et al

EDITOR/REVIEWER COMMENTS *Paste each of the editor and reviewer queries here.*	**AUTHOR'S RESPONSE** *Paste your answer to the editor and reviewer queries here. If you alter your manuscript to address this query, you MUST paste the relevant altered text here – verbatim as it appears in the manuscript.*	**REFERENCE PAGE** *State where* the change now appears in your newly revised manuscript.*	**CHANGE APPROVED? FOR EDITORIAL USE ONLY**
EXAMPLE: Reviewer 1's comment	EXAMPLE: A brief response to this reviewer's comment. The text now states: "insert relevant changed text here"	EXAMPLE 1: Page 7, lines 10-22 EXAMPLE 2: No change	
Request to change short title	Short title changed	**Page 1**	
Reviewer 1			
Page 5, abstract, line 23, 8 not 88 with functional asplenia plus numbers in parentheses do not add up to 112).	These numbers have been corrected to include 5 participants (4 surgical splenectomy, 1 congenital asplenia) who were enrolled but not immunized).	**Page 5, lines 19-20**	
Results, page 10, line 44, the number of total participants with asplenic/splenic dysfunction is recorded as 112 but the numbers in parentheses on the next line adds up to 107	These numbers have been corrected to include 5 participants (4 surgical splenectomy, 1 congenital asplenia) who were enrolled but not immunized).	**Page 10, line 20**	
Discussion, page 16, line 18. asplenia/splenic typos	Corrected	**Page 16, line 7**	

References, page 21, line 27. Journal name missing and volume incorrect.	Corrected	**Page 20, line 31**	
Page 29, supplemental material, line 55. The specifics of what was considered acceptable for documenting splenic dysfunction need to be noted-ie Howell-Jolly Bodies on peripheral smear, abnormal spleen scan, etc.	This information is not available as these diagnoses were made according to local clinical practices, which varied between sites.	**No change**	
Reviewer 2			
The discussion is fairly long and could be shortened. While most of the information is important. I think the paper may benefit from an understanding of the data from the polysaccharide vaccine experience (Platanov) in the introduction rather than the discussion, so the reader can have that as background while reading this study.	We have deleted the following sections from the discussion: • , possibly due to binding of C5a. • (although the relatively small numbers of healthy age-matched vaccine recipients could be problematic in this regard) • Finally, the immunogenicity of the vaccine was tested against only 4 reference strains and recent data from Giutini et al demonstrate that bactericidal titers against other strains may be lower[34]. Nevertheless, the vaccine was licensed in Europe and elsewhere on the basis of immunogenicity against these four reference strains, and preliminary data from the UK suggest routine immunization of infants with 2 doses of 4CMenB is 82.9% effective against group B IMD In addition, we have added the following sentence to the introduction as suggested • While previous studies have shown immunization of complement-deficient patients with polysaccharide capsular group A, C, W and Y meningococcal vaccines to be effective against invasive disease,	**Page 16, line 6** **Page 17, lines 8-9** **Page 18, lines 7-13** **Page 6, lines 18-20**	
An explanation of why the baseline SBA against the M10703 strain were higher in this population would be helpful.	We have added the following sentence to clarify the results for M10703 at baseline: Finally, the previously observed tendency to higher SBA titres against strain M10713 than other strains prior to immunization	**Page 17, lines 1-5**	

	creates some limitations in the interpretation of the results for this strain, nevertheless the increase in GMTs observed does provide some support for the immunogenicity of the NBHA component of 4CMenB in the populations studied		
Add the reference for the MenC studies in children with asplenia Further to the introductory comment that : *The result are quite reassuring for the asplenic population, which is different than for the conjugate vaccine studies with MenC vaccine.*	We have amended the relevant section of the discussion to (additions/changes underlined): Reassuringly, children with asplenia/splenic dysfunction generate an immune response that is at least as good as healthy controls. This contrasts with the reduced immunogenicity of the serogroup C meningococcal protein –polysaccharide vaccines in adults with surgical asplenia reported by Balmer et al. These contrasting findings may reflect the older age group in the later study or potential differences in the immune response of asplenic patients to capsular polysaccharides rather than sub-capsular proteins. Given this robust immune response to 4CMenB it is reasonable to expect that this vaccine will be as effective in children with asplenia/splenic deficiency as in healthy controls.	**Page 16, lines 8-15**	
In the summary conclusion paragraph, while it is implied at the start of the paragraph that vaccine is recommended, I suggest adding "vaccination" to the last sentence along with antibiotic prophylaxis and increased awareness of sepsis.	This sentence has been amended to: In the meantime it is important that these patients are identified, receive education about sepsis management plans and are prescribed prophylactic antibiotics according to local guidelines, along with vaccination, to provide every chance for them to be protected against this deadly disease.	**Page 18, line 15**	
Authors			
References	References were renumbered through the text and in the references section following to the addition of 2 references	**Page 6, line 11** **Page 8, lines 2 and 20** **Page 14, line 15** **Page 15, lines 13, 21 and 25** **Page 16, lines 5 and 10**	

Typos	The following typos were found in the text: • Meningo~~co~~coccal • occur**r**ed	**Page 6, line 3** **Page 10, line 10**	
Change in the affiliation	DDA left GSK – new affiliation was added Document IT BeNeLux B.V., RKranenburg 21, 1083 JM Amsterdam	**Page 1, lines 7, 31-32**	
Change in the financial disclosure	DDA left GSK Dr Calabresi and Mr D'Agostino were~~as~~, ~~DDA~~ and Dr Toneatto is~~are~~ employees of the GSK group of companies.	**Page 2, lines 10-11**	

Instructions:

Please use this table format to answer the questions posed by the editors and reviewers of your paper. Copy and paste the editor/reviewer's question in the "Comments" column and your answer to that question in the corresponding "Response" column. Be sure to ALSO paste the corrected text along with your response. For minor copyediting changes such as spelling and grammar corrections, you may simply state that the error was corrected, without pasting the altered text.
* Use the page/line numbers from your revised .doc, .rtf, or .txt file; do *not* use the page/line numbers from the submission system's auto-generated PDF.

For clarity, use one row per question. Make sure to list the page and line reference where your change can be found. If no change was made, please make sure to note that in your response in addition to your reasoning. You may delete the sample row and insert rows to this table as needed.