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B Part of It: A cluster randomised controlled trial to assess the impact of 4CMenB vaccine on nasopharyngeal carriage of *Neisseria meningitidis* in adolescents

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ABSTRACT

Introduction:

South Australia (SA) has the highest notification rate of invasive meningococcal disease in Australia with the majority of cases due to serogroup B. *Neisseria meningitidis* is carried in the pharynx of up to 24% of adolescents. A vaccine designed to offer protection against serogroup B (4CMenB) was licensed in Australia in 2013. The SA MenB vaccine carriage study, aims to assess the impact of 4CMenB on carriage of *N. meningitidis* in adolescents.

Methods and Analysis:

This is a parallel cluster randomised controlled trial enrolling year 10, 11 and 12 school students throughout SA, in metropolitan and rural/remote areas. Schools will be randomised to intervention (vaccinated with 4CMenB) or control (wait-listed group for vaccination in 2018) with randomisation stratified by school size and socio-economic status, as measured by the Index of Community Socio-Educational Advantage. Oropharyngeal swabs will be taken from all students at the first visit and then 12 months later from year 11 and 12 students. Students unvaccinated in 2017 will receive vaccine at the 12 month follow-up. Carriage prevalence of *N. meningitidis* will be determined by PCR at baseline and 12 months following 4CMenB vaccination and compared to carriage prevalence at 12 months in unvaccinated students. A questionnaire will be completed at baseline and 12 months to assess risk factors associated with carriage.

The primary outcome of carriage prevalence of disease causing *N. meningitidis* at 12 months will be compared between groups using logistic regression, with generalised estimating equations used to account for clustering at the school level. The difference in carriage prevalence between groups will be expressed as an odds ratio with 95% confidence interval.

Ethics and dissemination:

The study was approved by the Women's and Children's Health Network Human Research Ethics Committee. Results will be published in international peer review journals and presented at national and international conferences.

Trial registration number: The study is registered with the Australian and New Zealand Clinical Trials ACTRN12617000079347 and Clinical Trials.GOV NCT03089086 registries.

Strengths and limitations of this study

- A parallel cluster randomised controlled trial will allow a causal determination of the impact of meningococcal B vaccine on oropharyngeal carriage of *N. meningitidis*.
- This clinical trial will be the largest interventional population study of its kind.
- Attrition of participants over the 12 month follow-up may compromise group comparisons.
- It is not known what percentage reduction in pharyngeal carriage will be sufficient to provide herd immunity.

INTRODUCTION

Neisseria meningitidis infection is an important cause of morbidity (~500,000 – 1,200,000 cases/year) and mortality (50,000 – 135,000 deaths/year) worldwide.(1, 2) Clinically the most important serogroups are A, B, C, W, X and Y. The global serogroup distribution is dynamic over time and there are regional variations in disease epidemiology.(3)

Carriage of N. meningitidis

Exposure to *N. meningitidis* is common in the general population, leading to asymptomatic nasopharyngeal carriage which may be transient, temporary, or long term. Age influences carriage, with a rapid rise from 15 years of age to a peak in carriage at around 19 years, likely due to increases in the number and closeness of social contacts. (4, 5) Other factors that influence carriage are male gender, concomitant or predisposing respiratory infections, active and passive smoking, and low socioeconomic status.(6) Disease is a rare outcome of infection and the relationship between carriage and disease incidence is not fully understood.(4, 7) Given that carriage and transmission rates are significantly higher in adolescents than other members of the population and very low in infants, a reduction of carriage in adolescents has the potential to provide indirect protection to unvaccinated individuals, including infants.(8)

Epidemiology in Australia and South Australia

As in many countries, the incidence of invasive meningococcal disease (IMD) in Australia is highest in children under 1 year of age (3.7/100,000), followed by adolescents between the ages of 15 to 19 years (2.6/100,000).(9) In 2016, 262 cases of IMD were notified nationally (1.1/100,000), with 28 notifications in South Australia (SA) including one death.(10) SA has a

population of 1.7 million and has the highest notification rate of IMD in Australia (1.65/100,000), with serogroup B predominating (n=23/28, 82%; 2016).(10) The most common serogroup causing IMD nationally between 1999 and 2015 was serogroup B. In 2016, serogroup W notifications exceeded serogroup B notifications nationally (110 versus 93 cases, respectively).(10)

Meningococcal vaccines and herd protection

Since the early 2000s, countries that offer universal vaccination against meningococcal serogroup C (MenC) have seen a dramatic decrease in the incidence of serogroup C disease.(11-13) Aligned to this, where adolescents have been targeted for vaccination, carriage of serogroup C in adolescents has reduced, resulting in indirect protection through reduced transmission and herd protection, with disease rates reduced across all age groups as a consequence.(12, 13) The ability of a meningococcal vaccine to impact colonisation and transmission of meningococci and, in turn, provide indirect effects through herd protection, has important implications for evaluating the population impact and risk/benefit of the vaccine and for determining vaccine policy. As a result, there is high interest in assessing meningococcal B vaccines in relation to their impact on carriage, ideally in a large post-licensure population study.(14)

In Australia, 4CMenB is registered for use in persons ≥2 months of age for the prevention of invasive disease caused by serogroup B meningococci and is recommended by the Australian Technical Advisory Group on Immunisation for children <2years of age and adolescents 15-19 years of age.(15) However, 4CMenB is only available through purchase on the private market in Australia as it has not been included on the National Immunisation

Program due to lack of data on effectiveness in a population program and herd protection to inform cost-effectiveness estimates.(16)

In contrast to serogroups A, C, W and Y, the poor immunogenicity of the meningococcal serogroup B polysaccharide capsule, coupled with the marked genetic variability of the immunodominant serogroup B surface proteins, has prevented the development of a universal serogroup B vaccine. As the meningococcal B vaccines have been developed with novel technologies, their ability to induce herd protection is unknown.(14) In Australia, based on the Meningococcal Antigen Typing System (MATS) data, approximately 76% of 373 MenB isolates from invasive disease collected from 2007-2011 were predicted to be covered by this vaccine with the predicted coverage for SA at that time being 90%. A recent longitudinal study covering the past 15 year (2000-2014) history of meningococcal disease in Western Australia, a neighbouring state, indicates that although there was fluctuation over time in MenB vaccine coverage, the overall 15 year average remained high (60% with an annual range of 40% to 82%).(17)

Vaccine effectiveness in an infant 4CMenB population program in the UK has been reported as 82.9% (95%CI 24.1, 95.2).(18)

In the UK, a randomised, multi-centre controlled study was conducted to examine carriage in 18-24 year old university students pre-vaccination and at serial follow-up points post-vaccination with 4CMenB.(19) From 3 months after dose 2, 4CMenB vaccination resulted in significantly lower carriage of any meningococcal genogroup (18.2% (95% CI 3.4-30.8) carriage reduction), and 26.6% (95%CI 10.5, 39.9) reduction in genogroups BCWY. A significant carriage reduction for disease-associated sequence types of capsular B meningococci compared to controls was not observed (12.6% (95%CI -15.9-34.1). This non-

significant finding may in part be attributable to low acquisition of meningococcal strains, a slower than expected enrolment, and limited vaccination prior to or during the period of maximal carriage acquisition.(19)

The SA MenB vaccine carriage study "B Part of it" aims to assess the impact of 4CMenB on carriage of disease causing *N. meningitidis* by comparing carriage prevalence at 12 months post implementation of a MenB vaccine program in schools, with participating schools randomised to intervention or control.

METHODS AND ANALYSIS

Study Design

This parallel cluster randomised controlled trial (RCT) will measure the impact of 4CMenB on carriage prevalence in adolescents in SA. All 260 schools in metropolitan and rural/remote SA are invited to participate with immunisation provided through the school immunisation program, managed by the Immunisation Branch, SA Health, in SA. For the purposes of the study, a school is defined as an educational institution at which students in years 10, 11, 12 physically attend school during the week. Each school year level in SA has a cohort of 19,000-20,000 students.

As carriage of the meningococcus is temporary and fluctuates over time and the adolescent years, a control group is essential to assess a causal relationship between the intervention, MenB vaccination, and any change in carriage prevalence during this study. Two doses of 4CMenB will be given with a 2 month interval to all students attending school in years 10, 11, and 12. Individuals eligible to be enrolled into this study are South Australian secondary school students in years 10, 11, and 12 in 2017, who provide informed consent, are available at school for at least the first pharyngeal swab and willing to comply with study procedures.

Students are ineligible if they have previously received any doses of Bexsero® (4CMenB) or had an anaphylactic reaction to any component of the vaccine or are known to be pregnant.

All students will undergo baseline oropharyngeal swab sampling, with schools randomised for students to receive either 4CMenB in 2017 (Group A) or 4CMenB in 2018 (Group B)(Figure 1). The latter will receive 4CMenB at the 12 month follow-up swab visit. As follow-up swabs will only be available for year 10 and 11 students, the primary outcome is PCR positivity in year 10 and 11 students enrolled in the study. Year 12 students will undergo baseline posterior pharyngeal swabs only. Year 12 students in Group B will be offered 4CMenB vaccine in 2018 at designated immunisation clinics as the majority will have completed school in 2017. The advantages of conducting a study in school rather than university students include the opportunity to vaccinate prior to rapid carriage acquisition and the relatively closed accessible environment with an existing vaccination program infrastructure. Year 12 students are included as they are likely to have the highest carriage rates and to avoid any impact on any vaccine effect due to mixing of year levels.

Primary Objective

- Estimate the difference in overall carriage prevalence of disease causing genogroups of *N. meningitidis* (A, B, C, W, X, Y) following the 12 month pharyngeal swab in year 10 and 11 students who received two doses of Bexsero®, compared to unvaccinated students.

Secondary objectives

- Estimate the difference in carriage prevalence of each disease causing genogroup of *N. meningitidis* (A, B, C, W, X, Y) following the 12 month pharyngeal swab in year 10 and 11 students who received two doses of Bexsero®, compared to unvaccinated students.

- Estimate the difference in carriage prevalence of all genogroups of *N. meningitidis* following the 12 month pharyngeal swab in year 10 and 11 students who received two doses of Bexsero ®, compared to unvaccinated students.
- Estimate the difference in acquisition (negative at baseline, positive at 12 month followup) of carriage of disease causing genogroups of *N. meningitidis* (A, B, C, W, X, Y) over a 12 month period in students who received two doses of Bexsero ®, compared to unvaccinated students.
- Estimate the difference in acquisition (negative at baseline, positive at 12 month followup) of carriage of all genogroups of *N. meningitidis* over a 12 month period in students who received two doses of Bexsero ®, compared to unvaccinated students.
- Identify characteristics associated with carriage prevalence of all genogroups *N. meningitidis* in South Australian school students at baseline and 12 months.
- Identify characteristics associated with carriage prevalence of disease causing genogroups of *N. meningitidis* (A, B, C, W, X, Y) in South Australian school students at baseline and 12 months.

Randomisation

Randomisation will take place at the school level and will be stratified by school size (<60, 60 to 119, and ≥120 students per year level) and school socio-economic status, as measured by the Index of Community Socio-Educational Advantage (ICSEA); (ICSEA <970, 970 to 1020, >1020).(20) All schools agreeing to participate will be randomised to intervention (4CMenB vaccine) in 2017 or control (vaccination at the follow-up visit in 2018) (Figure 1). The

randomisation schedule will be generated by an independent statistician not otherwise involved in the study using Stata version 14.

Study Processes

Immunisation providers will be trained in all aspects of the study processes, including collection of a posterior oropharyngeal swab, using a standardised technique. A flocculated swab will be wiped across the posterior oropharynx from one tonsillar area to the other and the swab placed immediately in STGG (skim milk, typtone, glucose, glycerine; Thermo-Fisher Scientific Australia) transport medium.(21) Swab vials will be labelled and placed in a portable cooler and delivered to the nearest SA Pathology collection centre.

School immunisation providers and the study team will approach all schools in SA to confirm their involvement in the study. Consent forms and information sheets will be sent home to parents and both parental consent and student assent will be obtained. Consent forms will be collected from the schools by the immunisation nurses, checked for completeness and data entered into the designated "B Part of It" study web based database established by Adelaide Health Technology Assessment, The University of Adelaide.

Immunisation providers will explain the process of swab collection and immunisation to each student prior to any procedures being performed. All students will have an oropharyngeal swab taken and complete the risk factor questionnaire from 01 April – 30th June 2017. All Group A students will be administered the first dose of 4CMenB (Figure 1).

Participants will be asked to complete a one page de-identified questionnaire to collect information on characteristics that may be relevant to carriage of *N. meningitidis* (smoking

history, household size, recent antibiotic use) at each swab visit. The questionnaire will be re-identified by subject number to link questionnaire data with carriage data.

Participants will be offered a A$20 iTunes card for completion of the questionnaire and oropharyngeal swabs to compensate them for their time. A SMS reminder will be sent 2 days prior to the school visits to notify parent/participants of the first and follow up school visits.

Stakeholder Engagement and Communication

The three Education Sectors will provide information to schools and support the study within schools. A communications officer will work with stakeholders on establishing appropriate and accessible avenues of communication. Involving students in the planning and delivery of communication strategies is expected to facilitate communication and provide opportunities for students to engage in research. A multi-media strategy will be overseen by the University of Adelaide, with the support of a public relations/communications company and SA Health. Key activities include website development *www.bpartofit.com.au*,(22) brand identity "B Part of It", advertising and creation of supporting materials, ambassador engagement, public relations management and media training, social media strategy and amplification and bespoke content development.

Vaccine Safety Plan and Surveillance

Vaccine safety will be monitored through the South Australian Vaccine Safety Surveillance (SAVSS), an enhanced passive surveillance system used for timely detection of signals suggestive of an increase in adverse events following immunisation. Serious adverse events

(SAE) considered possibly or probably related to administration of 4CMenB vaccine will be reported to the Research Ethics Committee (REC) and the vaccine manufacturer within 72 hours of the site becoming aware of the SAE. Monthly summaries of all adverse events reported will be provided to the International Scientific Advisory Committee (ISAC), and the vaccine manufacturer. A Study vaccine safety committee including independent vaccine safety experts has been established and has prepared a vaccine safety surveillance protocol.

Training of immunisation providers

Training for the study has been conducted in metropolitan Adelaide and major rural locations. A detailed training manual and standard medication order has been provided to all immunisation providers. Nurses are trained in and practice swab collection at the scheduled training days to ensure standardized and adequate posterior oropharyngeal swab collection technique. Schools will be randomly selected for monitoring of protocol related study processes including throat swab technique.

Laboratory Processes

On receipt of samples, DNA will be extracted using an automated extraction on the Roche MagnaPure system and subjected to PCR screening for the presence of specific meningococcal DNA (using PorA gene detection). Further molecular analysis will be used to determine the capsular group (A, B, C, W, X, Y). Any samples yielding a positive PCR will be identified and cultured for Neisseria species on selective and non-selective agar and incubated overnight in CO2 at 35 ° C. Plates will be examined daily for isolates for up to 72 hours. *N. meningitidis* will be identified by standard diagnostic laboratory bacteriological methods using oxidase reaction and MALDI ToF with further PCR testing to determine the capsular group.

Quantitative PCR will be applied to the positive screen samples for estimation of the density of carriage of the Neisseria species.(23) A standard curve will be generated allowing comparison of crossing point values from the specimen analysis with the standard curve allowing the estimation of Neisseria density in the specimen. Samples will be stored long term in STGG broth at -80◦C for future whole genome sequencing.(24)

Sample size and analysis plan

Students attending school have been chosen as the study population, as carriage of *N. meningitidis* increases from around 15 years of age (4) and a funded program for adolescents would likely be introduced in this age group. Study results will then predict the likelihood of indirect effects of 4CMenB in a national immunisation program which includes adolescents.

Consistent with previous published carriage rates in school students,(25, 26) we estimate the carriage prevalence in unvaccinated South Australian adolescents will be 6-8 %.

With around 80% uptake and 20% attrition, we anticipate 12160 vaccinated and 12160 unvaccinated year 10 and 11 students with a 12 month pharyngeal swab. Assuming the carriage rate among the unvaccinated cohort is 8%, this sample size will provide 90% power to detect a 20% relative reduction in carriage to 6.4% in vaccinated participants (two tailed alpha = 0.05). These calculations incorporate a design effect of 2.19, based on an average of 120 students per school providing 12 month swab data and an intra-class correlation coefficient estimate of 0.01 as reported in other studies involving students in schools.(27) Should uptake or study completion be suboptimal, the study will still have 80% power provided that at least 8,970 participants per arm contribute 12 month swab results.

All analyses will be undertaken according to a pre-specified statistical analysis plan. Available outcome data for students will be analysed according to the randomised group of their school (intention to treat principle). A sensitivity per-protocol analysis of the primary outcome will also be conducted in vaccine group students that followed a 2 dose schedule of 4CMenB and control group students that did not receive 4CMenB before the 12 month follow-up.

The primary outcome of carriage of disease causing *N. meningitidis* genogroups at 12 months (yes/no) will be compared between groups using logistic regression, with generalized estimating equations (GEE) used to account for clustering at the school level. The difference in carriage between groups will be expressed as an odds ratio with 95% confidence interval. Adjustment will be made for baseline carriage, randomisation strata (school size, ICSEA) and other baseline variables pre-specified for adjustment. Missing data on the primary outcome will be addressed using multiple imputation. All secondary outcomes will be compared between groups using logistic GEEs. In planned sub-group analyses of the primary and secondary outcomes, the effect of the 4CMenB vaccine will also be examined separately for metropolitan and rural schools and year 10 and year 11 students. Effect modification by these factors will be assessed separately by including an interaction term involving randomised group within each statistical model.

Laboratory Procedures

The protocol, informed consent forms, recruitment materials, social media and all participant materials have been reviewed and approved by the Women's and Children's Health Network Human Research Ethics Committee.

DISCUSSION

This study is being conducted in SA which has (i) the highest IMD notification rate in Australia with a predominance of serogroup B, and (2) IMD notifications that are uniquely higher in adolescents than children. It is estimated by the MATS assay that vaccine coverage of invasive strains in SA will be high (~ 90%).(28) The predominant genotype over the past decade in SA is the B P1.7-2,4, which is the New Zealand epidemic strain and the PorA type contained in 4CMenB. Whilst 4CMenB is available and recommended in Australia, uptake on the private market has been low and should not impact on baseline carriage rates.

It is feasible to conduct a large population study of this kind in SA due to the infrastructure and partnerships between the University of Adelaide, SA Health, the Women's and Children's Health Network, the NHMRC SA Academic Health Science and Translation Research Centre and Education sectors (Department of Education, Independent and Catholic Schools). The school immunisation program which successfully delivers vaccines to adolescents supports the feasibility and potential high engagement in this study. We are cognisant of the risk of potential bias in having a control group with vaccination delayed and potential for disproportionate withdrawal from this group, however we will encourage continual involvement in the study and document any privately accessed vaccines in these individuals. We are also aware of the risk of inter-operator variability in oropharyngeal swab collection in a study of this size. To mitigate this risk all immunisation providers have been trained in a standardised technique for posterior oropharyngeal swab collection which includes face to face training and unlimited access to a video outlining the swab collection technique.

As IMD is rare, the impact of the vaccine on carriage is an important component of cost-effectiveness analyses. This study will allow assessment of any association between the intervention and changes in carriage prevalence, to predict the likelihood of indirect effects of 4CMenB in reduction in disease in a national immunisation program which includes adolescents.

The question of the ability of any vaccine to provide indirect effects on the unvaccinated population (i.e. herd protection) has important implications for vaccine policy. This is a particularly important question for meningococcal vaccines due to the unique epidemiology of asymptomatic pharyngeal carriage and more critically important for protein-based MenB vaccines, where no such information exists. High rates of serogroup B meningococcal disease, despite very low rates of carriage in infants, are likely explained by transmission from older age groups where carriage rates are relatively high. Understanding the potential impact of this vaccine on carriage in older age groups has important public health implications with the potential to inform worldwide policy on the implementation of adolescent MenB vaccination programs.

This will be the first study to assess the impact of a large population 4CMenB program on *N. meningitidis* carriage. Understanding any effects on carriage will assist Australian regulatory authorities and authorities in other countries in assessing the potential indirect effects to assist in the cost-effectiveness estimates of a MenB vaccine for inclusion in a national immunisation program. Carriage data will also inform the vaccine type and age group for implementation.(8) In particular it will be of interest to establish whether the remarkable herd protection effect seen with introduction of the conjugate meningococcal C vaccines is also replicated for meningococcal B vaccine, 4CMenB.(12) In addition, the data gathered in

this study will be invaluable for the development of mathematical models to predict the outcome of a national 4CMenB immunisation program.

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Council immunisation providers: Berri Barmera Council, Booleroo Medical Centre, Broughton Clinic, City of Charles Sturt, Coorong District Council, Country Health SA Local Health Network, Eastern Health Authority, Health and Immunisation Management Services, Kadina Medical Associates, District Council of Karoonda East Murray, District Council of Lower Eyre Peninsula, District Council of Loxton Waikerie, Mallee Medical Practices, Mid Murray Council, City of Mitcham, Mount Barker District Council, Nganampa Health Council Inc, City of Onkaparinga, District Council of Peterborough, City of Playford, Pop Up Medics, City of Port Lincoln, Renmark Paringa Council, Royal Flying Doctors Service, Streaky Bay Medical Clinic, Tatiara District Council, City of Tea Tree Gully, District Council of Tumby Bay, Wakefield Plains Medical Clinic, City of West Torrens, Whyalla City Council, Watto Purrunna Aboriginal Primary Health Care Service, Wudinna District Council, District Council of Yankalilla

Reference Group: Don Roberton, Ann Koehler, Maureen Watson, Noel Lally, Paddy Philips, Monica Conway, Carolyn Grantskalns, Ann-Marie Hayes, Naomi Dwyer, Andrew Lawrence, Amo Fioravanti, Lyn Olsen, Alistair Burt, Sarah Robertson, Steve Wesselingh, David Johnson, Debra Petrys, Larissa Biggs, Tahlia Riessen

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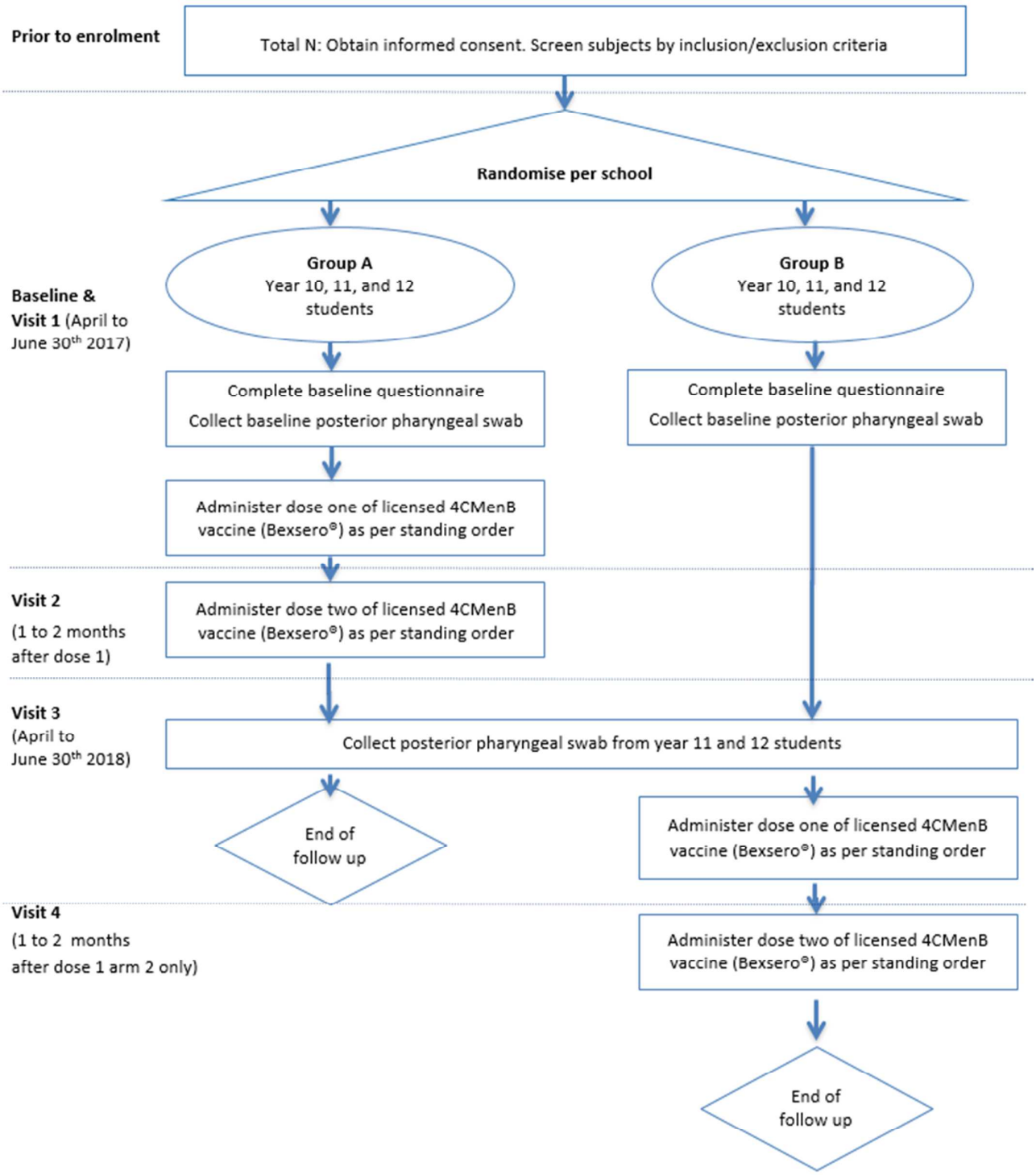
Competing Interests:

HM is an investigator on vaccine trials sponsored by Industry (GSK, Novavax, Pfizer). HM's and MM's institution receives funding for investigator led studies from Industry (Pfizer, GSK). HM and MM receive no personal payments from Industry. CT has received a consulting payment from GSK and an honorarium from Sanofi Pasteur. RB performs contract

research on behalf of Public Health England for GSK, Pfizer and Sanofi Pasteur. PR is an investigator on vaccine trials sponsored by Industry (GSK, Novavax, Pfizer). PR's institution receives funding for investigator led studies from Industry (Pfizer, GSK, CSL). PR has been a member of scientific vaccine advisory boards for industry (Pfizer, GSK, Sanofi) but has not received any personal payments from Industry. AF's institution is in receipt of research funding from GlaxoSmithKline, Pfizer and consultancy fees from Alios BioPharma/Johnson & Johnson, BioNet-Asia and VBI Vaccines. AF is a member of the UK Department of Health's Joint Committee on Vaccination, Chair of the WHO European Technical Advisory Group of Experts and President of the European Society for Paediatric Infectious Diseases which receives sponsorship for its annual meeting from vaccine manufacturers. KV and JW are employees of the GSK group of companies and hold shares in the GSK group of companies as part of their employee remuneration.

Figure 1: Schematic of parallel cluster randomised study design



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B Part of It Protocol: A cluster randomised controlled trial to assess the impact of 4CMenB vaccine on nasopharyngeal carriage of Neisseria meningitidis in adolescents

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B Part of It Protocol: A cluster randomised controlled trial to assess the impact of 4CMenB vaccine on pharyngeal carriage of *Neisseria meningitidis* in adolescents

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ABSTRACT

Introduction:

South Australia (SA) has the highest notification rate of invasive meningococcal disease in Australia with the majority of cases due to serogroup B. *Neisseria meningitidis* is carried in the pharynx, with adolescents having the highest rates of carriage in the population. A vaccine designed to offer protection against serogroup B (4CMenB) was licensed in Australia in 2013. The SA MenB vaccine carriage study, aims to assess the impact of 4CMenB on carriage of *N. meningitidis* in adolescents.

Methods and Analysis:

This is a parallel cluster randomised controlled trial enrolling year 10, 11 and 12 school students (approximately 16-18 years of age) throughout SA, in metropolitan and rural/remote areas. Schools will be randomised to intervention (4CMenB vaccination at baseline) or control (4CMenB vaccination at study completion) with randomisation stratified by school size and socio-economic status, as measured by the Index of Community Socio-Educational Advantage (Australian Curriculum, Assessment and Reporting Authority). Oropharyngeal swabs will be taken from all students at the first visit and then 12 months later from year 11 and 12 students. Students unvaccinated in 2017 will receive vaccine at the 12 month follow-up. Carriage prevalence of *N. meningitidis* will be determined by PCR at baseline and 12 months following 4CMenB vaccination and compared to carriage prevalence at 12 months in unvaccinated students. A questionnaire will be completed at baseline and 12 months to assess risk factors associated with carriage.

The primary outcome of carriage prevalence of disease causing *N. meningitidis* at 12 months will be compared between groups using logistic regression, with generalised estimating

equations used to account for clustering at the school level. The difference in carriage prevalence between groups will be expressed as an odds ratio with 95% confidence interval.

Trial registration number: The study is registered with the Australian and New Zealand Clinical Trials Registry ACTRN12617000079347 and clinicaltrials.gov NCT03089086 registries.

Strengths and limitations of this study

- A parallel cluster randomised controlled trial will allow a causal determination of the impact of meningococcal B vaccine on oropharyngeal carriage of *N. meningitidis*.
- The primary outcome is an objective measure, laboratory confirmed PCR positivity, which is measured by one centralised laboratory.
- This clinical trial will be the largest interventional population study of its kind.
- Attrition of participants over the 12 month follow-up may compromise group comparisons.
- Control and intervention students are independent but limited school mixing between schools may occur reducing the estimation of impact of 4CMenB on carriage.
- Acquisition rates of N. meningitidis are unknown in this population and may be lower than expected, limiting the potential to show an impact of 4CMenB on carriage.
- It is not known what percentage reduction in pharyngeal carriage will be sufficient to provide herd immunity.

INTRODUCTION

Neisseria meningitidis infection is an important cause of morbidity (~500,000 – 1,200,000 cases/year) and mortality (50,000 – 135,000 deaths/year) worldwide.(1, 2) Clinically the most important serogroups are A, B, C, W, X and Y. The global serogroup distribution is dynamic over time and there are regional variations in disease epidemiology.(3)

Carriage of N. meningitidis

Exposure to *N. meningitidis* is common in the general population, leading to asymptomatic pharyngeal carriage which may be transient, temporary, or long term. Age influences carriage, with a rapid rise from 15 years of age to a peak in carriage at around 19 years, likely due to increases in the number and closeness of social contacts. (4, 5) Other factors that influence carriage are male gender, concomitant or predisposing respiratory infections, active and passive smoking, and low socioeconomic status.(6) Disease is a rare outcome of infection and the relationship between carriage and disease incidence is not fully understood.(4, 7) Given that carriage and transmission rates are significantly higher in adolescents than other members of the population and very low in infants, a reduction of carriage in adolescents has the potential to provide indirect protection to unvaccinated individuals, including infants.(8)

Epidemiology in Australia and South Australia

As in many countries, the incidence of invasive meningococcal disease (IMD) in Australia is highest in children under 1 year of age (3.7/100,000), followed by adolescents between the

ages of 15 to 19 years (2.6/100,000).(9) In 2016, 262 cases of IMD were notified nationally (1.1/100,000), with 28 notifications in South Australia (SA) including one death.(10) SA has a population of 1.7 million and has the highest notification rate of IMD in Australia (1.65/100,000), with serogroup B predominating (n=23/28, 82%; 2016).(10) The most common serogroup causing IMD nationally between 1999 and 2015 was serogroup B. In 2016, serogroup W notifications exceeded serogroup B notifications nationally (110 versus 93 cases, respectively).(10)

Meningococcal vaccines and herd protection

Since the early 2000s, countries that offer universal vaccination against meningococcal serogroup C (MenC) have seen a dramatic decrease in the incidence of serogroup C disease.(11-13) Aligned to this, where adolescents have been targeted for vaccination, carriage of serogroup C in adolescents has reduced, resulting in indirect protection through reduced transmission and herd protection, with disease rates reduced across all age groups as a consequence.(12, 13) The ability of a meningococcal vaccine to impact colonisation and transmission of meningococci and, in turn, provide indirect effects through herd protection, has important implications for evaluating the population impact and risk/benefit of the vaccine and for determining vaccine policy. As a result, there is high interest in assessing meningococcal B vaccines in relation to their impact on carriage, ideally in a large post-licensure population study.(14)

In Australia, 4CMenB is registered for use in persons ≥2 months of age for the prevention of invasive disease caused by serogroup B meningococci and is recommended by the Australian Technical Advisory Group on Immunisation for children <2years of age and adolescents 15-19 years of age.(15) However, 4CMenB is only available through purchase

on the private market in Australia as it has not been included on the National Immunisation Program. The Pharmaceutical Benefits Advisory Committee, Commonwealth Government, which reviewed the cost-effectiveness of a meningococcal B vaccine program in 2013 identified lack of data on effectiveness in a population program (prior to implementation of the infant program in the UK) and herd protection to inform cost-effectiveness estimates.(16)

In contrast to serogroups A, C, W and Y, the poor immunogenicity of the meningococcal serogroup B polysaccharide capsule, coupled with the marked genetic variability of the immunodominant serogroup B surface proteins, has prevented the development of a universal serogroup B vaccine. As the meningococcal B vaccines have been developed with novel technologies, their ability to induce herd protection is unknown.(14) In Australia, based on the Meningococcal Antigen Typing System (MATS) data, approximately 76% of 373 MenB isolates from invasive disease collected from 2007-2011 were predicted to be covered by this vaccine with the predicted coverage for SA at that time being 90%. A recent longitudinal study covering the past 15 year (2000-2014) history of meningococcal disease in Western Australia, a neighbouring state, indicates that although there was fluctuation over time in MenB vaccine coverage, the overall 15 year average remained high (60% with an annual range of 40% to 82%).(17)

Vaccine effectiveness in an infant 4CMenB population program in the UK has been reported as 82.9% (95%CI 24.1, 95.2).(18)

In the UK, a randomised, multi-centre controlled study was conducted to examine carriage in 18-24 year old university students pre-vaccination and at serial follow-up points post-vaccination with 4CMenB.(19) From 3 months after dose 2, 4CMenB vaccination resulted in

significantly lower carriage of any meningococcal genogroup (18.2% (95% CI 3.4-30.8) carriage reduction), and 26.6% (95%CI 10.5, 39.9) reduction in genogroups BCWY. A significant carriage reduction for disease-associated sequence types of capsular B meningococci compared to controls was not observed (12.6% (95%CI -15.9-34.1). This non-significant finding may in part be attributable to low acquisition of meningococcal strains, a low level of expression of vaccine antigens in carriage isolates, a slower than expected enrolment, and limited vaccination prior to or during the period of maximal carriage acquisition.(19)

The SA MenB vaccine carriage study "B Part of It" aims to assess the impact of 4CMenB on carriage of disease causing *N. meningitidis* by comparing carriage prevalence at 12 months post implementation of a MenB vaccine program in schools, with participating schools randomised to intervention or control.

METHODS AND ANALYSIS

Study Design

This parallel cluster randomised controlled trial (RCT) will measure the impact of 4CMenB on carriage prevalence in adolescents in SA. All 260 schools in metropolitan and rural/remote SA are invited to participate with immunisation provided through the school immunisation program, managed by the Immunisation Branch, SA Health, in SA. For the purposes of the study, a school is defined as an educational institution at which students in years 10, 11 and 12 physically attend school during the week. Each school year level in SA has a cohort of 19,000-20,000 students aged approximately 16-18 years of age, with year 12 being the final year of school.

As carriage of the meningococcus is temporary and fluctuates over time and the adolescent years, a control group is essential to assess a causal relationship between the intervention, MenB vaccination, and any change in carriage prevalence during this study. Two doses of 4CMenB will be given with a 2 month interval to all students attending school in years 10, 11, and 12. Individuals eligible to be enrolled into this study are South Australian secondary school students in years 10, 11, and 12 in 2017, who provide informed consent, are available at school for at least the first oropharyngeal swab and willing to comply with study procedures. Students are ineligible if they have previously received any doses of Bexsero® (4CMenB) or had an anaphylactic reaction to any component of the vaccine or are known to be pregnant.

All students will undergo baseline oropharyngeal swab sampling, with schools randomised for students to receive either 4CMenB in 2017 (Group A) or 4CMenB in 2018 (Group B)(Figure 1). The latter will receive 4CMenB at the 12 month follow-up swab visit. As follow-up swabs will only be available for year 10 and 11 students, the primary outcome is PCR positivity in year 10 and 11 students enrolled in the study. Year 12 students will undergo baseline posterior oropharyngeal swabs only. Year 12 students in Group B will be offered 4CMenB vaccine in 2018 at designated immunisation clinics as the majority will have completed school in 2017. The advantages of conducting a study in school rather than university students include the opportunity to vaccinate prior to rapid carriage acquisition and the relatively closed accessible environment with an existing vaccination program infrastructure. Year 12 students are included as they are likely to have the highest carriage rates and mixing of unimmunised year 12 students with immunised year 10 and 11 students could potentially reduce any vaccine impact on carriage.

Primary Objective

- Estimate the difference in overall carriage prevalence of disease causing genogroups of *N. meningitidis* (A, B, C, W, X, Y) following the 12 month pharyngeal swab in year 10 and 11 students who received two doses of Bexsero®, compared to unvaccinated students.

Secondary objectives

- Estimate the difference in carriage prevalence of each disease causing genogroup of *N. meningitidis* (A, B, C, W, X, Y) following the 12 month pharyngeal swab in year 10 and 11 students who received two doses of Bexsero®, compared to unvaccinated students.
- Estimate the difference in carriage prevalence of all genogroups of *N. meningitidis* following the 12 month pharyngeal swab in year 10 and 11 students who received two doses of Bexsero ®, compared to unvaccinated students.
- Estimate the difference in acquisition (negative at baseline, positive at 12 month followup) of carriage of disease causing genogroups of *N. meningitidis* (A, B, C, W, X, Y) over a 12 month period in students who received two doses of Bexsero ®, compared to unvaccinated students.
- Estimate the difference in acquisition (negative at baseline, positive at 12 month followup) of carriage of all genogroups of *N. meningitidis* over a 12 month period in students who received two doses of Bexsero ®, compared to unvaccinated students.
- Identify characteristics associated with carriage prevalence of all genogroups *N. meningitidis* in South Australian school students at baseline and 12 months.

- Identify characteristics associated with carriage prevalence of disease causing genogroups of *N. meningitidis* (A, B, C, W, X, Y) in South Australian school students at baseline and 12 months.

Randomisation

Randomisation will take place at the school level and will be stratified by school size (<60, 60 to 119, and ≥120 students per year level) and school socio-economic status, as measured by the Index of Community Socio-Educational Advantage (ICSEA); (ICSEA <970, 970 to 1020, >1020).(20) All schools agreeing to participate will be randomised to intervention (4CMenB vaccine) in 2017 or control (vaccination at the follow-up visit in 2018) (Figure 1). The randomisation schedule will be generated by an independent statistician not otherwise involved in the study using Stata version 14. Schools and students will be unaware of their allocation to intervention or control until the day of the study immunisation provider visit. Laboratory personnel are blinded to assignment of intervention or control for the duration of the study.

Study Processes

Immunisation providers will be trained in all aspects of the study processes, including collection of a posterior oropharyngeal swab, using a standardised technique. A flocculated swab will be wiped across the posterior oropharynx from one tonsillar area to the other and the swab placed immediately in STGG (skim milk, tryptone, glucose, glycerine; Thermo-Fisher Scientific Australia) transport medium.(21) Swab vials will be labelled and placed in a portable cooler and delivered to the nearest SA Pathology collection centre.

School immunisation providers and the study team will approach all schools in SA to confirm their involvement in the study. Consent forms and information sheets will be sent home to

parents and both parental consent and student assent will be obtained. Consent forms will be collected from the schools by the immunisation nurses, checked for completeness and data entered into the designated "B Part of It" study web based database established by Adelaide Health Technology Assessment (AHTA), The University of Adelaide.

Immunisation providers will explain the process of swab collection and immunisation to each student prior to any procedures being performed. All students will have an oropharyngeal swab taken and complete the risk factor questionnaire from 01 April – 30 June 2017. All Group A students will be administered the first dose of 4CMenB (Figure 1).

Participants will be asked to complete a one page de-identified questionnaire to collect information on characteristics that may be relevant to carriage of *N. meningitidis* (e.g. smoking history, household size, recent antibiotic use) at each swab visit (Figure 2). The questionnaire will be re-identified by subject number to link questionnaire data with carriage data.

Participants will be offered a A$20 iTunes card for completion of the questionnaire and oropharyngeal swabs to compensate them for their time. A SMS reminder will be sent 2 days prior to the school visits to notify parent/participants of the first and follow up school visits

All collected data (student consent forms, questionnaires and swab analysis results) will be securely stored on a database held by AHTA, The University of Adelaide, with access to the database controlled by password protection. Range and logic checks will be performed on all collected data. Any data presented will be de-identified prior to presentation.

Patient and Public Involvement

The research question was developed in response to policy advisors recommendations. Study materials were reviewed by a Youth Advisory Group at several stages during study design. Feedback was also sought through social media including twitter, Instagram, Facebook and enquires/feedback on the study website, early in development of the website. Student, parent and immunisation ambassadors will support awareness of the study and recruitment through schools.

The three Education Sectors (public, independent and Catholic schools) in SA will provide information to schools and support the study within schools. A communications officer will work with stakeholders on establishing appropriate and accessible avenues of communication. Involving students in the planning and delivery of communication strategies is expected to facilitate communication and provide opportunities for students to engage in research. A multi-media strategy will be overseen by the University of Adelaide, with the support of a public relations/communications company and SA Health. Key activities include website development *www.bpartofit.com.au*,(22) brand identity "B Part of It", advertising and creation of supporting materials, ambassador engagement, public relations management and media training, social media strategy and amplification and bespoke content development. Study results will be provided to students through communication to schools and presentations at public forums. Results will also be reported in the media including television, radio and print media.

Study Safety Monitoring and Surveillance

Vaccine safety will be monitored through the South Australian Vaccine Safety Surveillance (SAVSS), an enhanced passive surveillance system used for timely detection of signals

suggestive of an increase in adverse events following immunisation. Serious adverse events (SAE) considered possibly or probably related to administration of 4CMenB vaccine will be reported to the Research Ethics Committee (REC), The study Sponsor, The Therapeutic Goods Administration, (Australian Government) and the vaccine manufacturer within 72 hours of the site becoming aware of the SAE. A Study vaccine safety committee including independent vaccine safety experts has been established and will review all participant reported safety data in accordance with a vaccine safety surveillance protocol.

Monthly summaries of all adverse events reported will be provided to the International Scientific Advisory Committee (ISAC), and the vaccine manufacturer. The ISAC has oversight of the study and has decision making capacity over the scientific, technical and logistical aspects of study conduct.

Training of immunisation providers

Training for the study has been conducted in metropolitan Adelaide and major rural locations. A detailed training manual and standard medication order has been provided to all immunisation providers. Nurses are trained in and practice swab collection at the scheduled training days to ensure standardized and adequate posterior oropharyngeal swab collection technique. Schools will be randomly selected for monitoring of protocol related study processes including throat swab technique.

Laboratory Processes

On receipt of samples, DNA will be extracted using an automated extraction on the Roche MagnaPure system and subjected to PCR screening for the presence of specific meningococcal DNA (using PorA gene detection). Any samples yielding a positive PCR will be

identified and cultured for Neisseria species on selective and non-selective agar and incubated overnight in CO2 at 35°C. Plates will be examined daily for isolates for up to 72 hours. *N. meningitidis* will be identified by standard diagnostic laboratory bacteriological methods using oxidase reaction and MALDI ToF with further PCR testing to determine the capsular group (A, B, C, W, X, Y).

Quantitative PCR will be applied to the positive screen samples for estimation of the density of carriage of the Neisseria species.(23) A standard curve will be generated allowing comparison of crossing point values from the specimen analysis with the standard curve allowing the estimation of Neisseria density in the specimen. Samples will be stored long term in STGG broth at -80◦C for future whole genome sequencing.(24)

Sample size and analysis plan

Students attending school have been chosen as the study population, as carriage of *N. meningitidis* increases from around 15 years of age (4) and a funded program for adolescents would likely be introduced in this age group. Study results will then predict the likelihood of indirect effects of 4CMenB in a national immunisation program which includes adolescents.

Consistent with previous published carriage rates in school students,(25, 26) we estimate the overall carriage prevalence in unvaccinated South Australian adolescents will be 6-8 %.

With around 80% uptake and 20% attrition, we anticipate 12160 vaccinated and 12160 unvaccinated year 10 and 11 students with a 12 month oropharyngeal swab. Assuming the carriage rate among the unvaccinated cohort is 8%, this sample size will provide 90% power to detect a 20% relative reduction in carriage to 6.4% in vaccinated participants (two tailed alpha = 0.05). These calculations incorporate a design effect of 2.19, based on an average of

120 students per school providing 12 month swab data and an intra-class correlation coefficient estimate of 0.01 as reported in other studies involving students in schools.(27) Should uptake or study completion be suboptimal, the study will still have 80% power provided that at least 8,970 participants per arm contribute 12 month swab results.

All analyses will be undertaken according to a pre-specified statistical analysis plan. Available outcome data for students will be analysed according to the randomised group of their school (intention to treat principle). A sensitivity per-protocol analysis of the primary outcome will also be conducted in vaccine group students that followed a 2 dose schedule of 4CMenB and control group students that did not receive 4CMenB before the 12 month follow-up.

The primary outcome of carriage of disease causing *N. meningitidis* genogroups detected by PCR at 12 months (yes/no) will be compared between groups using logistic regression, with generalized estimating equations (GEE) used to account for clustering at the school level. The difference in carriage between groups will be expressed as an odds ratio with 95% confidence interval. Adjustment will be made for baseline carriage, randomisation strata (school size, ICSEA) and other baseline variables pre-specified for adjustment. Missing data on the primary outcome will be addressed using multiple imputation. All secondary outcomes will be compared between groups using logistic GEEs. In planned sub-group analyses of the primary and secondary outcomes, the effect of the 4CMenB vaccine will also be examined separately for metropolitan and rural schools and year 10 and year 11 students. Effect modification by these factors will be assessed separately by including an interaction term involving randomised group within each statistical model.

DISCUSSION

This study is being conducted in SA which has (i) the highest IMD notification rate in Australia with a predominance of serogroup B, and (ii) IMD notifications that are uniquely higher in adolescents than children. The predominant genotype over the past decade in SA is the B P1.7-2,4, which is the New Zealand epidemic strain and the PorA type contained in 4CMenB. Whilst 4CMenB is available and recommended in Australia, uptake on the private market has been low and should not impact on baseline carriage rates.

It is feasible to conduct a large population study of this kind in SA due to the infrastructure and partnerships between the University of Adelaide, SA Health, the Women's and Children's Health Network, the NHMRC SA Academic Health Science and Translation Research Centre and Education sectors (Department of Education, Independent and Catholic Schools). The school immunisation program which successfully delivers vaccines to adolescents supports the feasibility and potential high engagement in this study. We are cognisant of the risk of potential bias in having a control group with vaccination at study completion and potential for disproportionate withdrawal from this group, however we will encourage continual involvement in the study and document any privately accessed vaccines in these individuals. We are also aware of the risk of inter-operator variability in oropharyngeal swab collection in a study of this size. To mitigate this risk all immunisation providers have been trained in a standardised technique for posterior oropharyngeal swab collection which includes face to face training and unlimited access to a video outlining the swab collection technique.

As IMD is rare, the impact of the vaccine on carriage is an important component of cost-effectiveness analyses. This study will allow assessment of any association between the

intervention and changes in carriage prevalence, to predict the likelihood of indirect effects of 4CMenB in reduction in disease in a national immunisation program which includes adolescents. A single 12 month time-point for repeat oropharyngeal swabs has been chosen for a number of reasons including to void any seasonal variation in carriage prevalence and to ensure enough time to measure a vaccine effect but also to ensure such an effect is sustained in order to be confident about a herd immunity impact at a population level. This time point is approximately 10 months after the second dose of vaccine (12 months post first dose), with a previous vaccine effect shown 3 months after the second dose in the Read et al study.(19) A single time-point was chosen for feasibility reasons as 6 months post dose 2 would occur during the exam period and following holidays and there would likely be large numbers of students lost to follow-up. The timing of the swabs took into account the calendar year and avoided the busy periods where there would be competing priorities such as school commencement and other school immunisation programs and enough time for parents and students to learn about the study and return consent forms and eligibility checklists for careful review by the immunisation nurses.

The question of the ability of any vaccine to provide indirect effects on the unvaccinated population (i.e. herd protection) has important implications for vaccine policy. This is a particularly important question for meningococcal vaccines due to the unique epidemiology of asymptomatic pharyngeal carriage and more critically important for protein-based MenB vaccines, where limited information exists. High rates of serogroup B meningococcal disease, despite very low rates of carriage in infants, are likely explained by transmission from older age groups where carriage rates are relatively high. Understanding the potential impact of this vaccine on carriage in older age groups has important public

health implications with the potential to inform worldwide policy on the implementation of adolescent MenB vaccination programs.

This will be the first study to assess the impact of a large population 4CMenB program on *N. meningitidis* carriage. Understanding any effects on carriage will assist Australian regulatory authorities and authorities in other countries in assessing the potential indirect effects to assist in the cost-effectiveness estimates of a MenB vaccine for inclusion in a national immunisation program. A study to examine the impact of 4CMenB and MenB:fHBp (Pfizer) on carriage is planned for commencement in the UK in 2018 (personal communication Dr Matthew Snape, Oxford University). Carriage data will also inform the vaccine type and age group for implementation.(8) In particular it will be of interest to establish whether the remarkable herd protection effect seen with introduction of the conjugate meningococcal C vaccines is replicated for meningococcal B vaccine, 4CMenB.(12) In addition, the data gathered in this study will be invaluable for the development of mathematical models to predict the outcome of a national 4CMenB immunisation program.

Ethics and dissemination:

The study was approved by the Women's and Children's Health Network Human Research Ethics Committee (WCHN HREC). The protocol, informed consent forms, recruitment materials, social media and all participant materials have been reviewed and approved by the WCHN HREC and updated on clinicaltrials.gov.

Results will be published in international peer review journals and presented at national and international conferences. The study findings will be provided in public forums and to study participants and participating schools.

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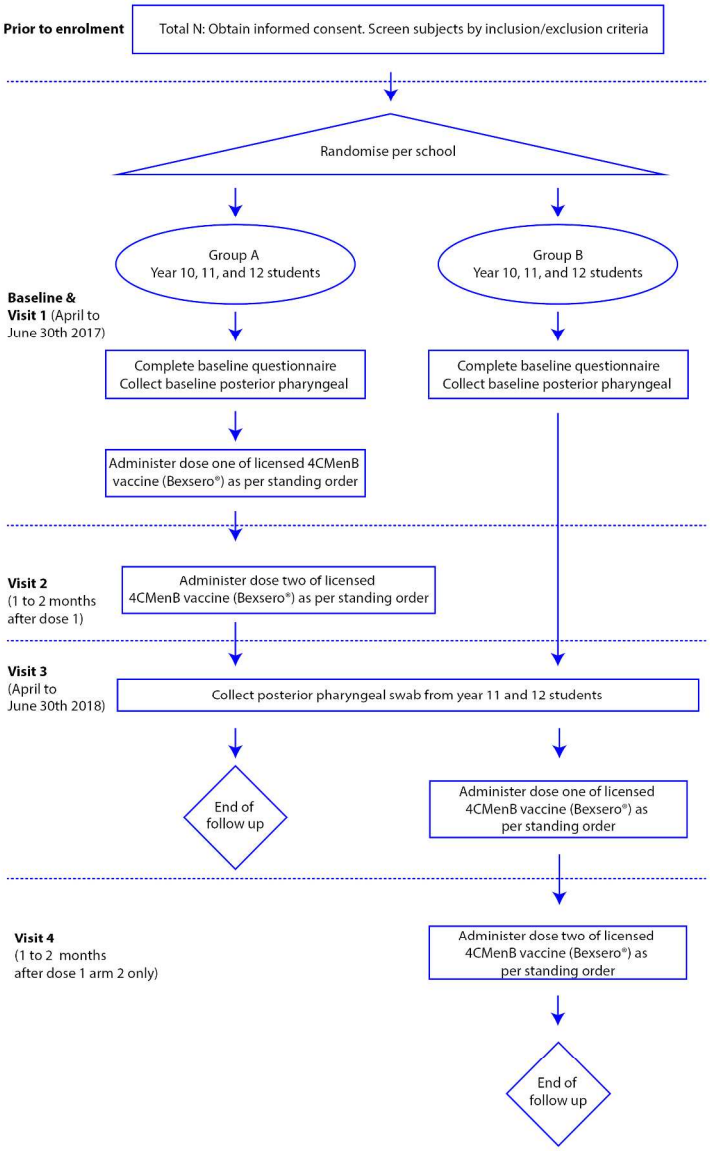
Competing Interests:

HM is supported by a NHMRC CDF APP1084951 and is a member of the Australian Technical Advisory Group on Immunisation, Australian Government. HM is an investigator on vaccine trials sponsored by Industry (GSK, Novavax, Pfizer). HM's and MM's institution receives funding for investigator led studies from Industry (Pfizer, GSK). HM and MM receive no personal payments from Industry. CT has received a consulting payment from GSK and an honorarium from Sanofi Pasteur. RB performs contract research on behalf of Public Health England for GSK, Pfizer and Sanofi Pasteur. PR is an investigator on vaccine trials sponsored by Industry (GSK, Novavax, Pfizer). PR's institution receives funding for investigator led studies from Industry (Pfizer, GSK, CSL). PR has been a member of scientific vaccine advisory boards for industry (Pfizer, GSK, Sanofi) but has not received any personal payments from Industry. AF's institution is in receipt of research funding from GlaxoSmithKline, Pfizer and consultancy fees from Alios BioPharma/Johnson & Johnson, BioNet-Asia and VBI Vaccines. AF is a member of the UK Department of Health's Joint Committee on Vaccination, Chair of the WHO European Technical Advisory Group of Experts and President of the European Society for Paediatric Infectious Diseases which receives sponsorship for its annual meeting from vaccine manufacturers. KV and JW are employees of the GSK group of companies and hold shares in the GSK group of companies as part of their employee remuneration.

Figure Legends:

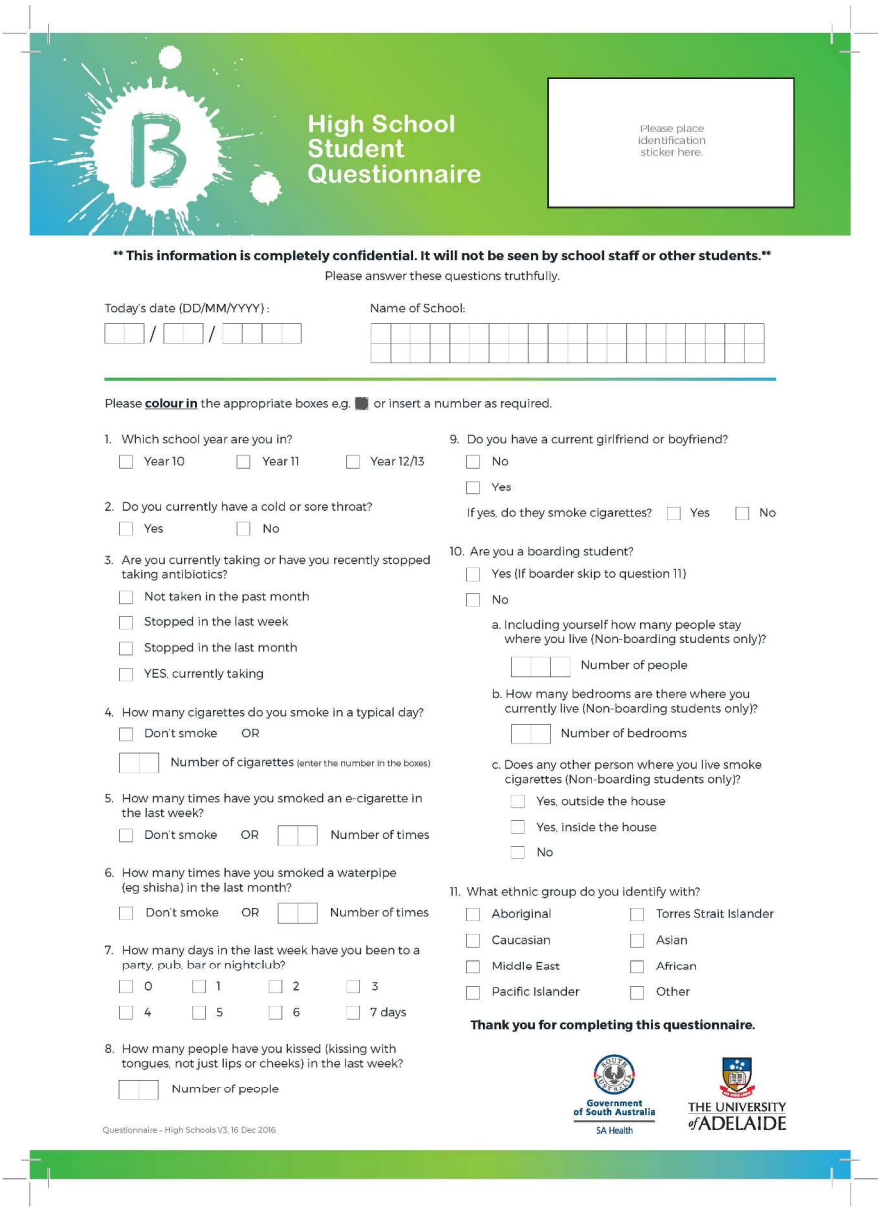
Figure 1: Study Design

Figure 2: High School Questionnaire



Study Design

209x297mm (300 x 300 DPI)



High School Student Questionnaire

Please place identification sticker here.

** This information is completely confidential. It will not be seen by school staff or other students.**

Please answer these questions truthfully.

Today's date (DD/MM/YYYY) : / /

Name of School:

Please **colour in** the appropriate boxes e.g. ■ or insert a number as required.

1. Which school year are you in?
   - ☐ Year 10
   - ☐ Year 11
   - ☐ Year 12/13

2. Do you currently have a cold or sore throat?
   - ☐ Yes
   - ☐ No

3. Are you currently taking or have you recently stopped taking antibiotics?
   - ☐ Not taken in the past month
   - ☐ Stopped in the last week
   - ☐ Stopped in the last month
   - ☐ YES, currently taking

4. How many cigarettes do you smoke in a typical day?
   - ☐ Don't smoke OR
   - ☐☐ Number of cigarettes (enter the number in the boxes)

5. How many times have you smoked an e-cigarette in the last week?
   - ☐ Don't smoke OR ☐☐ Number of times

6. How many times have you smoked a waterpipe (eg shisha) in the last month?
   - ☐ Don't smoke OR ☐☐ Number of times

7. How many days in the last week have you been to a party, pub, bar or nightclub?
   - ☐ 0 ☐ 1 ☐ 2 ☐ 3
   - ☐ 4 ☐ 5 ☐ 6 ☐ 7 days

8. How many people have you kissed (kissing with tongues, not just lips or cheeks) in the last week?
   - ☐☐ Number of people

9. Do you have a current girlfriend or boyfriend?
   - ☐ No
   - ☐ Yes

   If yes, do they smoke cigarettes? ☐ Yes ☐ No

10. Are you a boarding student?
    - ☐ Yes (If boarder skip to question 11)
    - ☐ No

    a. Including yourself how many people stay where you live (Non-boarding students only)?
    ☐☐☐ Number of people

    b. How many bedrooms are there where you currently live (Non-boarding students only)?
    ☐☐ Number of bedrooms

    c. Does any other person where you live smoke cigarettes (Non-boarding students only)?
    - ☐ Yes, outside the house
    - ☐ Yes, inside the house
    - ☐ No

11. What ethnic group do you identify with?
    - ☐ Aboriginal
    - ☐ Torres Strait Islander
    - ☐ Caucasian
    - ☐ Asian
    - ☐ Middle East
    - ☐ African
    - ☐ Pacific Islander
    - ☐ Other

Thank you for completing this questionnaire.

Government of South Australia SA Health

THE UNIVERSITY of ADELAIDE

Questionnaire – High Schools V3, 16 Dec 2016

High School Questionnaire

234x321mm (300 x 300 DPI)

SPIRIT 2013 Checklist: Recommended items to address in a clinical trial protocol and related documents*

Section/item	Item No	Description	Addressed on page number
Administrative information			
Title	1	Descriptive title identifying the study design, population, interventions, and, if applicable, trial acronym	1
Trial registration	2a	Trial identifier and registry name. If not yet registered, name of intended registry	3
	2b	All items from the World Health Organization Trial Registration Data Set	N/A
Protocol version	3	Date and version identifier	3
Funding	4	Sources and types of financial, material, and other support	21
Roles and responsibilities	5a	Names, affiliations, and roles of protocol contributors	2 and 21
	5b	Name and contact information for the trial sponsor	3
	5c	Role of study sponsor and funders, if any, in study design; collection, management, analysis, and interpretation of data; writing of the report; and the decision to submit the report for publication, including whether they will have ultimate authority over any of these activities	21
	5d	Composition, roles, and responsibilities of the coordinating centre, steering committee, endpoint adjudication committee, data management team, and other individuals or groups overseeing the trial, if applicable (see Item 21a for data monitoring committee)	12,13

Introduction			
Background and rationale	6a	Description of research question and justification for undertaking the trial, including summary of relevant studies (published and unpublished) examining benefits and harms for each intervention	4-7
	6b	Explanation for choice of comparators	8
Objectives	7	Specific objectives or hypotheses	9
Trial design	8	Description of trial design including type of trial (eg, parallel group, crossover, factorial, single group), allocation ratio, and framework (eg, superiority, equivalence, noninferiority, exploratory)	7-8
Methods: Participants, interventions, and outcomes			
Study setting	9	Description of study settings (eg, community clinic, academic hospital) and list of countries where data will be collected. Reference to where list of study sites can be obtained	7-8
Eligibility criteria	10	Inclusion and exclusion criteria for participants. If applicable, eligibility criteria for study centres and individuals who will perform the interventions (eg, surgeons, psychotherapists)	8 and 13
Interventions	11a	Interventions for each group with sufficient detail to allow replication, including how and when they will be administered	10-11
	11b	Criteria for discontinuing or modifying allocated interventions for a given trial participant (eg, drug dose change in response to harms, participant request, or improving/worsening disease)	12-13
	11c	Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return, laboratory tests)	13
	11d	Relevant concomitant care and interventions that are permitted or prohibited during the trial	N/A
Outcomes	12	Primary, secondary, and other outcomes, including the specific measurement variable (eg, systolic blood pressure), analysis metric (eg, change from baseline, final value, time to event), method of aggregation (eg, median, proportion), and time point for each outcome. Explanation of the clinical relevance of chosen efficacy and harm outcomes is strongly recommended	9
Participant timeline	13	Time schedule of enrolment, interventions (including any run-ins and washouts), assessments, and visits for participants. A schematic diagram is highly recommended (see Figure)	Figure 1

Sample size	14	Estimated number of participants needed to achieve study objectives and how it was determined, including clinical and statistical assumptions supporting any sample size calculations	14
Recruitment	15	Strategies for achieving adequate participant enrolment to reach target sample size	11-12
Methods: Assignment of interventions (for controlled trials)			
Allocation:			
Sequence generation	16a	Method of generating the allocation sequence (eg, computer-generated random numbers), and list of any factors for stratification. To reduce predictability of a random sequence, details of any planned restriction (eg, blocking) should be provided in a separate document that is unavailable to those who enrol participants or assign interventions	10
Allocation concealment mechanism	16b	Mechanism of implementing the allocation sequence (eg, central telephone; sequentially numbered, opaque, sealed envelopes), describing any steps to conceal the sequence until interventions are assigned	10
Implementation	16c	Who will generate the allocation sequence, who will enrol participants, and who will assign participants to interventions	10
Blinding (masking)	17a	Who will be blinded after assignment to interventions (eg, trial participants, care providers, outcome assessors, data analysts), and how	10
	17b	If blinded, circumstances under which unblinding is permissible, and procedure for revealing a participant's allocated intervention during the trial	N/A
Methods: Data collection, management, and analysis			
Data collection methods	18a	Plans for assessment and collection of outcome, baseline, and other trial data, including any related processes to promote data quality (eg, duplicate measurements, training of assessors) and a description of study instruments (eg, questionnaires, laboratory tests) along with their reliability and validity, if known. Reference to where data collection forms can be found, if not in the protocol	11, 13, fig. 2
	18b	Plans to promote participant retention and complete follow-up, including list of any outcome data to be collected for participants who discontinue or deviate from intervention protocols	11-12, 15

Data management	19	Plans for data entry, coding, security, and storage, including any related processes to promote data quality (eg, double data entry; range checks for data values). Reference to where details of data management procedures can be found, if not in the protocol	11
Statistical methods	20a	Statistical methods for analysing primary and secondary outcomes. Reference to where other details of the statistical analysis plan can be found, if not in the protocol	14,15
	20b	Methods for any additional analyses (eg, subgroup and adjusted analyses)	14,15
	20c	Definition of analysis population relating to protocol non-adherence (eg, as randomised analysis), and any statistical methods to handle missing data (eg, multiple imputation)	15
Methods: Monitoring			
Data monitoring	21a	Composition of data monitoring committee (DMC); summary of its role and reporting structure; statement of whether it is independent from the sponsor and competing interests; and reference to where further details about its charter can be found, if not in the protocol. Alternatively, an explanation of why a DMC is not needed	11
	21b	Description of any interim analyses and stopping guidelines, including who will have access to these interim results and make the final decision to terminate the trial	N/A
Harms	22	Plans for collecting, assessing, reporting, and managing solicited and spontaneously reported adverse events and other unintended effects of trial interventions or trial conduct	12-13
Auditing	23	Frequency and procedures for auditing trial conduct, if any, and whether the process will be independent from investigators and the sponsor	12
Ethics and dissemination			
Research ethics approval	24	Plans for seeking research ethics committee/institutional review board (REC/IRB) approval	18
Protocol amendments	25	Plans for communicating important protocol modifications (eg, changes to eligibility criteria, outcomes, analyses) to relevant parties (eg, investigators, REC/IRBs, trial participants, trial registries, journals, regulators)	18

Consent or assent	26a	Who will obtain informed consent or assent from potential trial participants or authorised surrogates, and how (see Item 32)	10
	26b	Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies, if applicable	N/A
Confidentiality	27	How personal information about potential and enrolled participants will be collected, shared, and maintained in order to protect confidentiality before, during, and after the trial	11
Declaration of interests	28	Financial and other competing interests for principal investigators for the overall trial and each study site	21-22
Access to data	29	Statement of who will have access to the final trial dataset, and disclosure of contractual agreements that limit such access for investigators	11
Ancillary and post-trial care	30	Provisions, if any, for ancillary and post-trial care, and for compensation to those who suffer harm from trial participation	N/A
Dissemination policy	31a	Plans for investigators and sponsor to communicate trial results to participants, healthcare professionals, the public, and other relevant groups (eg, via publication, reporting in results databases, or other data sharing arrangements), including any publication restrictions	18
	31b	Authorship eligibility guidelines and any intended use of professional writers	N/A
	31c	Plans, if any, for granting public access to the full protocol, participant-level dataset, and statistical code	N/A
Appendices			
Informed consent materials	32	Model consent form and other related documentation given to participants and authorised surrogates	Not included
Biological specimens	33	Plans for collection, laboratory evaluation, and storage of biological specimens for genetic or molecular analysis in the current trial and for future use in ancillary studies, if applicable	12-13

*It is strongly recommended that this checklist be read in conjunction with the SPIRIT 2013 Explanation & Elaboration for important clarification on the items. Amendments to the protocol should be tracked and dated. The SPIRIT checklist is copyrighted by the SPIRIT Group under the Creative Commons "Attribution-NonCommercial-NoDerivs 3.0 Unported" license.

BMJ Open

B Part of It Protocol: A cluster randomised controlled trial to assess the impact of 4CMenB vaccine on pharyngeal carriage of Neisseria meningitidis in adolescents

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B Part of It Protocol: A cluster randomised controlled trial to assess the impact of 4CMenB vaccine on pharyngeal carriage of *Neisseria meningitidis* in adolescents

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ABSTRACT

Introduction:

South Australia (SA) has the highest notification rate of invasive meningococcal disease in Australia with the majority of cases due to serogroup B. *Neisseria meningitidis* is carried in the pharynx, with adolescents having the highest rates of carriage. A vaccine designed to offer protection against serogroup B (4CMenB) is licensed in Australia. The SA MenB vaccine carriage study, aims to assess the impact of 4CMenB on carriage of *N. meningitidis* in adolescents.

Methods and Analysis:

This is a parallel cluster randomised controlled trial enrolling year 10, 11 and 12 school students (approximately 16-18 years of age) throughout SA, in metropolitan and rural/remote areas. Schools are randomised to intervention (4CMenB vaccination at baseline) or control (4CMenB vaccination at study completion) with randomisation stratified by school size and socio-economic status, as measured by the Index of Community Socio-Educational Advantage (Australian Curriculum). Oropharyngeal swabs will be taken from all students at visit one and 12 months later from year 11 and 12 students. Students unvaccinated in 2017 will receive vaccine at the 12 month follow-up. Carriage prevalence of *N. meningitidis* will be determined by PCR at baseline and 12 months following 4CMenB vaccination and compared to carriage prevalence at 12 months in unvaccinated students. A questionnaire will be completed at baseline and 12 months to assess risk factors associated with carriage.

The primary outcome of carriage prevalence of disease causing *N. meningitidis* at 12 months will be compared between groups using logistic regression, with generalised estimating

equations used to account for clustering at the school level. The difference in carriage prevalence between groups will be expressed as an odds ratio with 95% confidence interval.

Ethics and dissemination:

The study was approved by the Women's and Children's Health Network Human Research Ethics Committee. Results will be published in international peer review journals.

Trial registration number: The study is registered with the Australian and New Zealand Clinical Trials Registry ACTRN12617000079347 and clinicaltrials.gov NCT03089086 registries.

Strengths and limitations of this study

- A parallel cluster randomised controlled trial will allow a causal determination of the impact of meningococcal B vaccine on oropharyngeal carriage of *N. meningitidis*.
- The primary outcome is an objective measure, laboratory confirmed PCR positivity, which is measured by one centralised laboratory.
- This clinical trial will be the largest interventional population study of its kind.
- Attrition of participants over the 12 month follow-up may compromise group comparisons.
- Control and intervention students are independent but limited school mixing between schools may occur reducing the estimation of impact of 4CMenB on carriage.

INTRODUCTION

Neisseria meningitidis infection is an important cause of morbidity (~500,000 – 1,200,000 cases/year) and mortality (50,000 – 135,000 deaths/year) worldwide.(1, 2) Clinically the most important serogroups are A, B, C, W, X and Y. The global serogroup distribution is dynamic over time and there are regional variations in disease epidemiology.(3)

Carriage of N. meningitidis

Exposure to *N. meningitidis* is common in the general population, leading to asymptomatic pharyngeal carriage which may be transient, temporary, or long term. Age influences carriage, with a rapid rise from 15 years of age to a peak in carriage at around 19 years, likely due to increases in the number and closeness of social contacts. (4, 5) Other factors that influence carriage are male gender, concomitant or predisposing respiratory infections, active and passive smoking, and low socioeconomic status.(6) Disease is a rare outcome of infection and the relationship between carriage and disease incidence is not fully understood.(4, 7) Given that carriage and transmission rates are significantly higher in adolescents than other members of the population and very low in infants, a reduction of carriage in adolescents has the potential to provide indirect protection to unvaccinated individuals, including infants.(8)

Epidemiology in Australia and South Australia

As in many countries, the incidence of invasive meningococcal disease (IMD) in Australia is highest in children under 1 year of age (3.7/100,000), followed by adolescents between the ages of 15 to 19 years (2.6/100,000).(9) In 2016, 262 cases of IMD were notified nationally (1.1/100,000), with 28 notifications in South Australia (SA) including one death.(10) SA has a

population of 1.7 million and has the highest notification rate of IMD in Australia (1.65/100,000), with serogroup B predominating (n=23/28, 82%; 2016).(10) The most common serogroup causing IMD nationally between 1999 and 2015 was serogroup B. In 2016, serogroup W notifications exceeded serogroup B notifications nationally (110 versus 93 cases, respectively).(10)

Meningococcal vaccines and herd protection

Since the early 2000s, countries that offer universal vaccination against meningococcal serogroup C (MenC) have seen a dramatic decrease in the incidence of serogroup C disease.(11-13) Aligned to this, where adolescents have been targeted for vaccination, carriage of serogroup C in adolescents has reduced, resulting in indirect protection through reduced transmission and herd protection, with disease rates reduced across all age groups as a consequence.(12, 13) The ability of a meningococcal vaccine to impact colonisation and transmission of meningococci and, in turn, provide indirect effects through herd protection, has important implications for evaluating the population impact and risk/benefit of the vaccine and for determining vaccine policy. As a result, there is high interest in assessing meningococcal B vaccines in relation to their impact on carriage, ideally in a large post-licensure population study.(14)

In Australia, 4CMenB is registered for use in persons ≥2 months of age for the prevention of invasive disease caused by serogroup B meningococci and is recommended by the Australian Technical Advisory Group on Immunisation for children <2years of age and adolescents 15-19 years of age.(15) However, 4CMenB is only available through purchase on the private market in Australia as it has not been included on the National Immunisation Program. The Pharmaceutical Benefits Advisory Committee, Commonwealth Government,

which reviewed the cost-effectiveness of a meningococcal B vaccine program in 2013 identified lack of data on effectiveness in a population program (prior to implementation of the infant program in the UK) and herd protection to inform cost-effectiveness estimates.(16)

In contrast to serogroups A, C, W and Y, the poor immunogenicity of the meningococcal serogroup B polysaccharide capsule, coupled with the marked genetic variability of the immunodominant serogroup B surface proteins, has prevented the development of a universal serogroup B vaccine. As the meningococcal B vaccines have been developed with novel technologies, their ability to induce herd protection is unknown.(14) In Australia, based on the Meningococcal Antigen Typing System (MATS) data, approximately 76% of 373 MenB isolates from invasive disease collected from 2007-2011 were predicted to be covered by this vaccine with the predicted coverage for SA at that time being 90%. A recent longitudinal study covering the past 15 year (2000-2014) history of meningococcal disease in Western Australia, a neighbouring state, indicates that although there was fluctuation over time in MenB vaccine coverage, the overall 15 year average remained high (60% with an annual range of 40% to 82%).(17)

Vaccine effectiveness in an infant 4CMenB population program in the UK has been reported as 82.9% (95%CI 24.1, 95.2).(18)

In the UK, a randomised, multi-centre controlled study was conducted to examine carriage in 18-24 year old university students pre-vaccination and at serial follow-up points post-vaccination with 4CMenB.(19) From 3 months after dose 2, 4CMenB vaccination resulted in significantly lower carriage of any meningococcal genogroup (18.2% (95% CI 3.4-30.8) carriage reduction), and 26.6% (95%CI 10.5, 39.9) reduction in genogroups BCWY. A

significant carriage reduction for disease-associated sequence types of capsular B meningococci compared to controls was not observed (12.6% (95%CI -15.9-34.1). This non-significant finding may in part be attributable to low acquisition of meningococcal strains, a low level of expression of vaccine antigens in carriage isolates, a slower than expected enrolment, and limited vaccination prior to or during the period of maximal carriage acquisition.(19)

The SA MenB vaccine carriage study "B Part of It" aims to assess the impact of 4CMenB on carriage of disease causing *N. meningitidis* by comparing carriage prevalence at 12 months post implementation of a MenB vaccine program in schools, with participating schools randomised to intervention or control.

METHODS AND ANALYSIS

Study Design

This parallel cluster randomised controlled trial (RCT) will measure the impact of 4CMenB on carriage prevalence in adolescents in SA. All 260 schools in metropolitan and rural/remote SA are invited to participate with immunisation provided through the school immunisation program, managed by the Immunisation Branch, SA Health, in SA. For the purposes of the study, a school is defined as an educational institution at which students in years 10, 11 and 12 physically attend school during the week. Each school year level in SA has a cohort of 19,000-20,000 students aged approximately 16-18 years of age, with year 12 being the final year of school.

As carriage of the meningococcus is temporary and fluctuates over time and the adolescent years, a control group is essential to assess a causal relationship between the intervention, MenB vaccination, and any change in carriage prevalence during this study. Two doses of

4CMenB will be given with a 2 month interval to all students attending school in years 10, 11, and 12. Individuals eligible to be enrolled into this study are South Australian secondary school students in years 10, 11, and 12 in 2017, who provide informed consent, are available at school for at least the first oropharyngeal swab and willing to comply with study procedures. Students are ineligible if they have previously received any doses of Bexsero® (4CMenB) or had an anaphylactic reaction to any component of the vaccine or are known to be pregnant.

All students will undergo baseline oropharyngeal swab sampling, with schools randomised for students to receive either 4CMenB in 2017 (Group A) or 4CMenB in 2018 (Group B)(Figure 1). The latter will receive 4CMenB at the 12 month follow-up swab visit. As follow-up swabs will only be available for year 10 and 11 students, the primary outcome is PCR positivity in year 10 and 11 students enrolled in the study. Year 12 students will undergo baseline posterior oropharyngeal swabs only. Year 12 students in Group B will be offered 4CMenB vaccine in 2018 at designated immunisation clinics as the majority will have completed school in 2017. The advantages of conducting a study in school rather than university students include the opportunity to vaccinate prior to rapid carriage acquisition and the relatively closed accessible environment with an existing vaccination program infrastructure. Year 12 students are included as they are likely to have the highest carriage rates and mixing of unimmunised year 12 students with immunised year 10 and 11 students could potentially reduce any vaccine impact on carriage.

Primary Objective

- Estimate the difference in overall carriage prevalence of disease causing genogroups of *N. meningitidis* (A, B, C, W, X, Y) following the 12 month pharyngeal swab in year 10 and 11 students who received two doses of Bexsero®, compared to unvaccinated students.

Secondary objectives

- Estimate the difference in carriage prevalence of each disease causing genogroup of *N. meningitidis* (A, B, C, W, X, Y) following the 12 month pharyngeal swab in year 10 and 11 students who received two doses of Bexsero®, compared to unvaccinated students.
- Estimate the difference in carriage prevalence of all genogroups of *N. meningitidis* following the 12 month pharyngeal swab in year 10 and 11 students who received two doses of Bexsero ®, compared to unvaccinated students.
- Estimate the difference in acquisition (negative at baseline, positive at 12 month followup) of carriage of disease causing genogroups of *N. meningitidis* (A, B, C, W, X, Y) over a 12 month period in students who received two doses of Bexsero ®, compared to unvaccinated students.
- Estimate the difference in acquisition (negative at baseline, positive at 12 month followup) of carriage of all genogroups of *N. meningitidis* over a 12 month period in students who received two doses of Bexsero ®, compared to unvaccinated students.
- Identify characteristics associated with carriage prevalence of all genogroups *N. meningitidis* in South Australian school students at baseline and 12 months.
- Identify characteristics associated with carriage prevalence of disease causing genogroups of *N. meningitidis* (A, B, C, W, X, Y) in South Australian school students at baseline and 12 months.

Randomisation

Randomisation will take place at the school level and will be stratified by school size (<60, 60 to 119, and ≥120 students per year level) and school socio-economic status, as measured by the Index of Community Socio-Educational Advantage (ICSEA); (ICSEA <970, 970 to 1020, >1020).(20) All schools agreeing to participate will be randomised to intervention (4CMenB vaccine) in 2017 or control (vaccination at the follow-up visit in 2018) (Figure 1). The randomisation schedule will be generated by an independent statistician not otherwise involved in the study using Stata version 14. Schools and students will be unaware of their allocation to intervention or control until the day of the study immunisation provider visit. Laboratory personnel are blinded to assignment of intervention or control for the duration of the study.

Study Processes

Immunisation providers will be trained in all aspects of the study processes, including collection of a posterior oropharyngeal swab, using a standardised technique. A flocculated swab will be wiped across the posterior oropharynx from one tonsillar area to the other and the swab placed immediately in STGG (skim milk, tryptone, glucose, glycerine; Thermo-Fisher Scientific Australia) transport medium.(21) Swab vials will be labelled and placed in a portable cooler and delivered to the nearest SA Pathology collection centre.

School immunisation providers and the study team will approach all schools in SA to confirm their involvement in the study. Consent forms and information sheets will be sent home to parents and both parental consent and student assent will be obtained. Consent forms will be collected from the schools by the immunisation nurses, checked for completeness and

data entered into the designated "B Part of It" study web based database established by Adelaide Health Technology Assessment (AHTA), The University of Adelaide.

Immunisation providers will explain the process of swab collection and immunisation to each student prior to any procedures being performed. All students will have an oropharyngeal swab taken and complete the risk factor questionnaire from 01 April – 30 June 2017. All Group A students will be administered the first dose of 4CMenB (Figure 1).

Participants will be asked to complete a one page de-identified questionnaire to collect information on characteristics that may be relevant to carriage of *N. meningitidis* (e.g. smoking history, household size, recent antibiotic use) at each swab visit (Figure 2). The questionnaire will be re-identified by subject number to link questionnaire data with carriage data.

Participants will be offered a A$20 iTunes card for completion of the questionnaire and oropharyngeal swabs to compensate them for their time. A SMS reminder will be sent 2 days prior to the school visits to notify parent/participants of the first and follow up school visits

All collected data (student consent forms, questionnaires and swab analysis results) will be securely stored on a database held by AHTA, The University of Adelaide, with access to the database controlled by password protection. Range and logic checks will be performed on all collected data. Any data presented will be de-identified prior to presentation.

Patient and Public Involvement

The research question was developed in response to policy advisors recommendations. Study materials were reviewed by a Youth Advisory Group at several stages during study design. Feedback was also sought through social media including twitter, Instagram, Facebook and enquires/feedback on the study website, early in development of the website. Student, parent and immunisation ambassadors will support awareness of the study and recruitment through schools.

The three Education Sectors (public, independent and Catholic schools) in SA will provide information to schools and support the study within schools. A communications officer will work with stakeholders on establishing appropriate and accessible avenues of communication. Involving students in the planning and delivery of communication strategies is expected to facilitate communication and provide opportunities for students to engage in research. A multi-media strategy will be overseen by the University of Adelaide, with the support of a public relations/communications company and SA Health. Key activities include website development *www.bpartofit.com.au*,(22) brand identity "B Part of It", advertising and creation of supporting materials, ambassador engagement, public relations management and media training, social media strategy and amplification and bespoke content development. Study results will be provided to students through communication to schools and presentations at public forums. Results will also be reported in the media including television, radio and print media.

Study Safety Monitoring and Surveillance

Vaccine safety will be monitored through the South Australian Vaccine Safety Surveillance (SAVSS), an enhanced passive surveillance system used for timely detection of signals suggestive of an increase in adverse events following immunisation. Serious adverse events

(SAE) considered possibly or probably related to administration of 4CMenB vaccine will be reported to the Research Ethics Committee (REC), The study Sponsor, The Therapeutic Goods Administration, (Australian Government) and the vaccine manufacturer within 72 hours of the site becoming aware of the SAE. A Study vaccine safety committee including independent vaccine safety experts has been established and will review all participant reported safety data in accordance with a vaccine safety surveillance protocol.

Monthly summaries of all adverse events reported will be provided to the International Scientific Advisory Committee (ISAC), and the vaccine manufacturer. The ISAC has oversight of the study and has decision making capacity over the scientific, technical and logistical aspects of study conduct.

Training of immunisation providers

Training for the study has been conducted in metropolitan Adelaide and major rural locations. A detailed training manual and standard medication order has been provided to all immunisation providers. Nurses are trained in and practice swab collection at the scheduled training days to ensure standardized and adequate posterior oropharyngeal swab collection technique. Schools will be randomly selected for monitoring of protocol related study processes including throat swab technique.

Laboratory Processes

On receipt of samples, DNA will be extracted using an automated extraction on the Roche MagnaPure system and subjected to PCR screening for the presence of specific meningococcal DNA (using PorA gene detection). Any samples yielding a positive PCR will be identified and cultured for Neisseria species on selective and non-selective agar and

incubated overnight in CO2 at 35°C. Plates will be examined daily for isolates for up to 72 hours. *N. meningitidis* will be identified by standard diagnostic laboratory bacteriological methods using oxidase reaction and MALDI ToF with further PCR testing to determine the capsular group (A, B, C, W, X, Y).

Quantitative PCR will be applied to the positive screen samples for estimation of the density of carriage of the Neisseria species.(23) A standard curve will be generated allowing comparison of crossing point values from the specimen analysis with the standard curve allowing the estimation of Neisseria density in the specimen. Samples will be stored long term in STGG broth at -80◦C for future whole genome sequencing.(24)

Sample size and analysis plan

Students attending school have been chosen as the study population, as carriage of *N. meningitidis* increases from around 15 years of age (4) and a funded program for adolescents would likely be introduced in this age group. Study results will then predict the likelihood of indirect effects of 4CMenB in a national immunisation program which includes adolescents.

Consistent with previous published carriage rates in school students,(25, 26) we estimate the overall carriage prevalence in unvaccinated South Australian adolescents will be 6-8 %.

With around 80% uptake and 20% attrition, we anticipate 12160 vaccinated and 12160 unvaccinated year 10 and 11 students with a 12 month oropharyngeal swab. Assuming the carriage rate among the unvaccinated cohort is 8%, this sample size will provide 90% power to detect a 20% relative reduction in carriage to 6.4% in vaccinated participants (two tailed alpha = 0.05). These calculations incorporate a design effect of 2.19, based on an average of 120 students per school providing 12 month swab data and an intra-class correlation

coefficient estimate of 0.01 as reported in other studies involving students in schools.(27) Should uptake or study completion be suboptimal, the study will still have 80% power provided that at least 8,970 participants per arm contribute 12 month swab results.

All analyses will be undertaken according to a pre-specified statistical analysis plan. Available outcome data for students will be analysed according to the randomised group of their school (intention to treat principle). A sensitivity per-protocol analysis of the primary outcome will also be conducted in vaccine group students that followed a 2 dose schedule of 4CMenB and control group students that did not receive 4CMenB before the 12 month follow-up.

The primary outcome of carriage of disease causing *N. meningitidis* genogroups detected by PCR at 12 months (yes/no) will be compared between groups using logistic regression, with generalized estimating equations (GEE) used to account for clustering at the school level. The difference in carriage between groups will be expressed as an odds ratio with 95% confidence interval. Adjustment will be made for baseline carriage, randomisation strata (school size, ICSEA) and other baseline variables pre-specified for adjustment. Missing data on the primary outcome will be addressed using multiple imputation. All secondary outcomes will be compared between groups using logistic GEEs. In planned sub-group analyses of the primary and secondary outcomes, the effect of the 4CMenB vaccine will also be examined separately for metropolitan and rural schools and year 10 and year 11 students. Effect modification by these factors will be assessed separately by including an interaction term involving randomised group within each statistical model.

DISCUSSION

This study is being conducted in SA which has (i) the highest IMD notification rate in Australia with a predominance of serogroup B, and (ii) IMD notifications that are uniquely higher in adolescents than children. The predominant genotype over the past decade in SA is the B P1.7-2,4, which is the New Zealand epidemic strain and the PorA type contained in 4CMenB. Whilst 4CMenB is available and recommended in Australia, uptake on the private market has been low and should not impact on baseline carriage rates.

It is feasible to conduct a large population study of this kind in SA due to the infrastructure and partnerships between the University of Adelaide, SA Health, the Women's and Children's Health Network, the NHMRC SA Academic Health Science and Translation Research Centre and Education sectors (Department of Education, Independent and Catholic Schools). The school immunisation program which successfully delivers vaccines to adolescents supports the feasibility and potential high engagement in this study. We are cognisant of the risk of potential bias in having a control group with vaccination at study completion and potential for disproportionate withdrawal from this group, however we will encourage continual involvement in the study and document any privately accessed vaccines in these individuals. We are also aware of the risk of inter-operator variability in oropharyngeal swab collection in a study of this size. To mitigate this risk all immunisation providers have been trained in a standardised technique for posterior oropharyngeal swab collection which includes face to face training and unlimited access to a video outlining the swab collection technique.

As IMD is rare, the impact of the vaccine on carriage is an important component of cost-effectiveness analyses. This study will allow assessment of any association between the

intervention and changes in carriage prevalence, to predict the likelihood of indirect effects of 4CMenB in reduction in disease in a national immunisation program which includes adolescents. A single 12 month time-point for repeat oropharyngeal swabs has been chosen for a number of reasons including to void any seasonal variation in carriage prevalence and to ensure enough time to measure a vaccine effect but also to ensure such an effect is sustained in order to be confident about a herd immunity impact at a population level. This time point is approximately 10 months after the second dose of vaccine (12 months post first dose), with a previous vaccine effect shown 3 months after the second dose in the Read et al study.(19) A single time-point was chosen for feasibility reasons as 6 months post dose 2 would occur during the exam period and following holidays and there would likely be large numbers of students lost to follow-up. The timing of the swabs took into account the calendar year and avoided the busy periods where there would be competing priorities such as school commencement and other school immunisation programs and enough time for parents and students to learn about the study and return consent forms and eligibility checklists for careful review by the immunisation nurses.

The question of the ability of any vaccine to provide indirect effects on the unvaccinated population (i.e. herd protection) has important implications for vaccine policy. This is a particularly important question for meningococcal vaccines due to the unique epidemiology of asymptomatic pharyngeal carriage and more critically important for protein-based MenB vaccines, where limited information exists. High rates of serogroup B meningococcal disease, despite very low rates of carriage in infants, are likely explained by transmission from older age groups where carriage rates are relatively high. Understanding the potential impact of this vaccine on carriage in older age groups has important public

health implications with the potential to inform worldwide policy on the implementation of adolescent MenB vaccination programs.

This will be the first study to assess the impact of a large population 4CMenB program on *N. meningitidis* carriage. Understanding any effects on carriage will assist Australian regulatory authorities and authorities in other countries in assessing the potential indirect effects to assist in the cost-effectiveness estimates of a MenB vaccine for inclusion in a national immunisation program. A study to examine the impact of 4CMenB and MenB:fHBp (Pfizer) on carriage is planned for commencement in the UK in 2018 (personal communication Dr Matthew Snape, Oxford University). Carriage data will also inform the vaccine type and age group for implementation.(8) In particular it will be of interest to establish whether the remarkable herd protection effect seen with introduction of the conjugate meningococcal C vaccines is replicated for meningococcal B vaccine, 4CMenB.(12) In addition, the data gathered in this study will be invaluable for the development of mathematical models to predict the outcome of a national 4CMenB immunisation program.

Ethics and dissemination:

The study was approved by the Women's and Children's Health Network Human Research Ethics Committee (WCHN HREC). The protocol, informed consent forms, recruitment materials, social media and all participant materials have been reviewed and approved by the WCHN HREC and updated on clinicaltrials.gov.

Results will be published in international peer review journals and presented at national and international conferences. The study findings will be provided in public forums and to study participants and participating schools.

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Trademarks: Bexsero is a trademark owned by GSK Group of companies.

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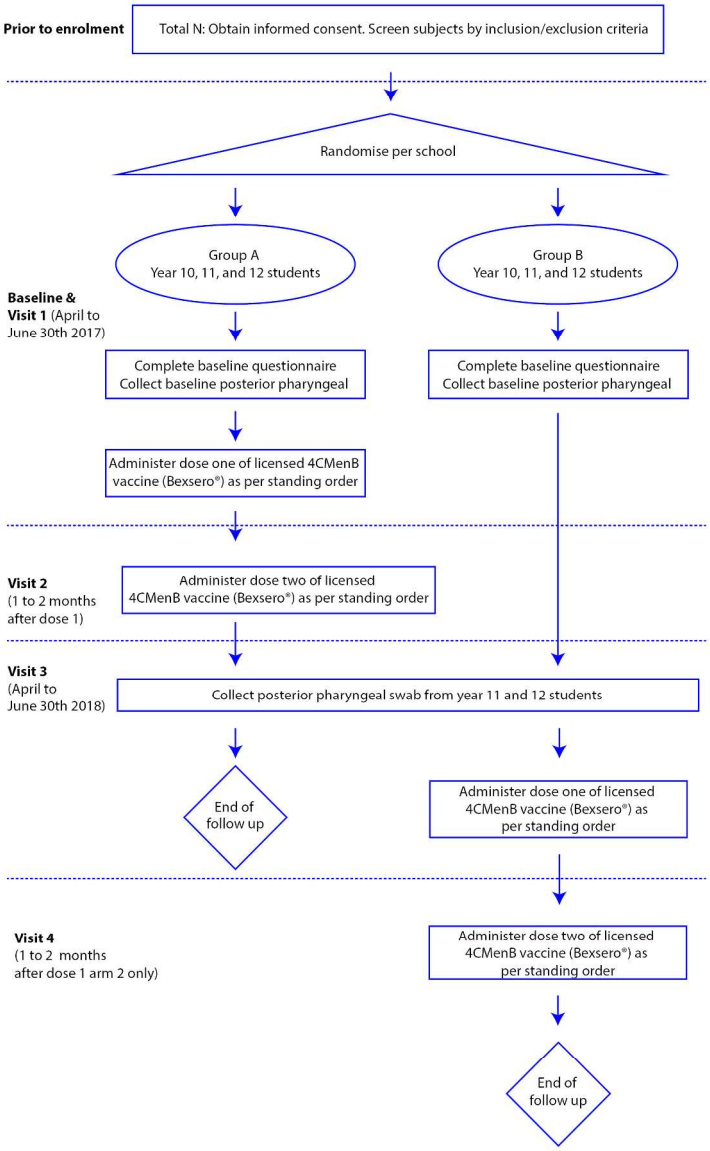
Competing Interests:

HM is supported by a NHMRC CDF APP1084951 and is a member of the Australian Technical Advisory Group on Immunisation, Australian Government. HM is an investigator on vaccine trials sponsored by Industry (GSK, Novavax, Pfizer). HM's and MM's institution receives funding for investigator led studies from Industry (Pfizer, GSK). HM and MM receive no personal payments from Industry. CT has received a consulting payment from GSK and an honorarium from Sanofi Pasteur. RB performs contract research on behalf of Public Health England for GSK, Pfizer and Sanofi Pasteur. PR is an investigator on vaccine trials sponsored by Industry (GSK, Novavax, Pfizer). PR's institution receives funding for investigator led studies from Industry (Pfizer, GSK, CSL). PR has been a member of scientific vaccine advisory boards for industry (Pfizer, GSK, Sanofi) but has not received any personal payments from Industry. AF's institution is in receipt of research funding from GlaxoSmithKline, Pfizer and consultancy fees from Alios BioPharma/Johnson & Johnson, BioNet-Asia and VBI Vaccines. AF is a member of the UK Department of Health's Joint Committee on Vaccination, Chair of the WHO European Technical Advisory Group of Experts and President of the European Society for Paediatric Infectious Diseases which receives sponsorship for its annual meeting from vaccine manufacturers. KV and JW are employees of the GSK group of companies and hold shares in the GSK group of companies as part of their employee remuneration.

Figure Legends:

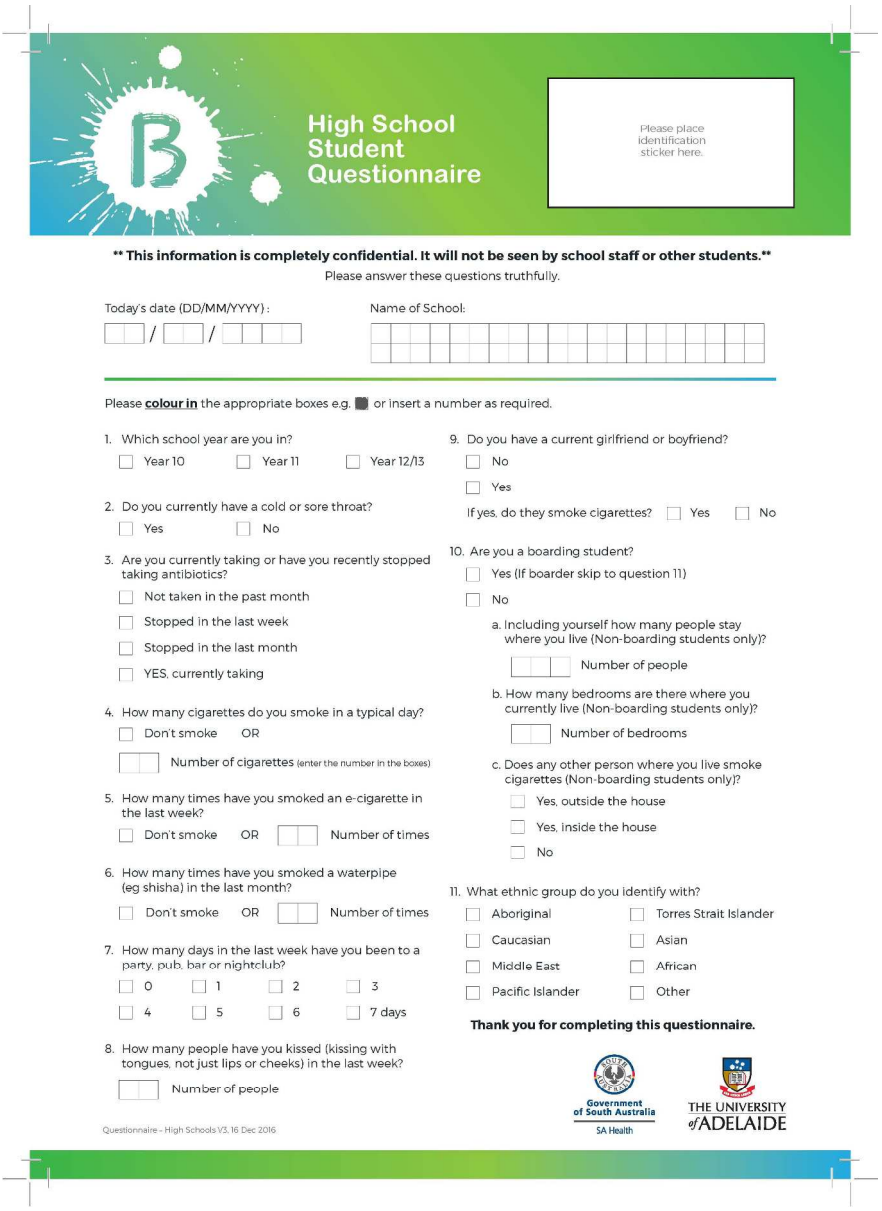
Figure 1: Study Design

Figure 2: High School Questionnaire



Study Design

209x297mm (300 x 300 DPI)



High School Student Questionnaire

Please place identification sticker here.

** This information is completely confidential. It will not be seen by school staff or other students.**

Please answer these questions truthfully.

Today's date (DD/MM/YYYY) : / /

Name of School:

Please **colour in** the appropriate boxes e.g. ■ or insert a number as required.

1. Which school year are you in?
   - ☐ Year 10
   - ☐ Year 11
   - ☐ Year 12/13

2. Do you currently have a cold or sore throat?
   - ☐ Yes
   - ☐ No

3. Are you currently taking or have you recently stopped taking antibiotics?
   - ☐ Not taken in the past month
   - ☐ Stopped in the last week
   - ☐ Stopped in the last month
   - ☐ YES, currently taking

4. How many cigarettes do you smoke in a typical day?
   - ☐ Don't smoke OR
   - ☐☐ Number of cigarettes (enter the number in the boxes)

5. How many times have you smoked an e-cigarette in the last week?
   - ☐ Don't smoke OR ☐☐ Number of times

6. How many times have you smoked a waterpipe (eg shisha) in the last month?
   - ☐ Don't smoke OR ☐☐ Number of times

7. How many days in the last week have you been to a party, pub, bar or nightclub?
   - ☐ 0 ☐ 1 ☐ 2 ☐ 3
   - ☐ 4 ☐ 5 ☐ 6 ☐ 7 days

8. How many people have you kissed (kissing with tongues, not just lips or cheeks) in the last week?
   - ☐☐ Number of people

9. Do you have a current girlfriend or boyfriend?
   - ☐ No
   - ☐ Yes

   If yes, do they smoke cigarettes? ☐ Yes ☐ No

10. Are you a boarding student?
    - ☐ Yes (If boarder skip to question 11)
    - ☐ No

    a. Including yourself how many people stay where you live (Non-boarding students only)?
    ☐☐☐ Number of people

    b. How many bedrooms are there where you currently live (Non-boarding students only)?
    ☐☐ Number of bedrooms

    c. Does any other person where you live smoke cigarettes (Non-boarding students only)?
    - ☐ Yes, outside the house
    - ☐ Yes, inside the house
    - ☐ No

11. What ethnic group do you identify with?
    - ☐ Aboriginal
    - ☐ Torres Strait Islander
    - ☐ Caucasian
    - ☐ Asian
    - ☐ Middle East
    - ☐ African
    - ☐ Pacific Islander
    - ☐ Other

Thank you for completing this questionnaire.

Government of South Australia SA Health

THE UNIVERSITY of ADELAIDE

Questionnaire – High Schools V3, 16 Dec 2016

High School Questionnaire

234x321mm (300 x 300 DPI)

SPIRIT 2013 Checklist: Recommended items to address in a clinical trial protocol and related documents*

Section/item	Item No	Description	Addressed on page number
Administrative information			
Title	1	Descriptive title identifying the study design, population, interventions, and, if applicable, trial acronym	1
Trial registration	2a	Trial identifier and registry name. If not yet registered, name of intended registry	3
	2b	All items from the World Health Organization Trial Registration Data Set	N/A
Protocol version	3	Date and version identifier	3
Funding	4	Sources and types of financial, material, and other support	21
Roles and responsibilities	5a	Names, affiliations, and roles of protocol contributors	2 and 21
	5b	Name and contact information for the trial sponsor	3
	5c	Role of study sponsor and funders, if any, in study design; collection, management, analysis, and interpretation of data; writing of the report; and the decision to submit the report for publication, including whether they will have ultimate authority over any of these activities	21
	5d	Composition, roles, and responsibilities of the coordinating centre, steering committee, endpoint adjudication committee, data management team, and other individuals or groups overseeing the trial, if applicable (see Item 21a for data monitoring committee)	12,13

Introduction			
Background and rationale	6a	Description of research question and justification for undertaking the trial, including summary of relevant studies (published and unpublished) examining benefits and harms for each intervention	4-7
	6b	Explanation for choice of comparators	8
Objectives	7	Specific objectives or hypotheses	9
Trial design	8	Description of trial design including type of trial (eg, parallel group, crossover, factorial, single group), allocation ratio, and framework (eg, superiority, equivalence, noninferiority, exploratory)	7-8
Methods: Participants, interventions, and outcomes			
Study setting	9	Description of study settings (eg, community clinic, academic hospital) and list of countries where data will be collected. Reference to where list of study sites can be obtained	7-8
Eligibility criteria	10	Inclusion and exclusion criteria for participants. If applicable, eligibility criteria for study centres and individuals who will perform the interventions (eg, surgeons, psychotherapists)	8 and 13
Interventions	11a	Interventions for each group with sufficient detail to allow replication, including how and when they will be administered	10-11
	11b	Criteria for discontinuing or modifying allocated interventions for a given trial participant (eg, drug dose change in response to harms, participant request, or improving/worsening disease)	12-13
	11c	Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return, laboratory tests)	13
	11d	Relevant concomitant care and interventions that are permitted or prohibited during the trial	N/A
Outcomes	12	Primary, secondary, and other outcomes, including the specific measurement variable (eg, systolic blood pressure), analysis metric (eg, change from baseline, final value, time to event), method of aggregation (eg, median, proportion), and time point for each outcome. Explanation of the clinical relevance of chosen efficacy and harm outcomes is strongly recommended	9
Participant timeline	13	Time schedule of enrolment, interventions (including any run-ins and washouts), assessments, and visits for participants. A schematic diagram is highly recommended (see Figure)	Figure 1

Sample size	14	Estimated number of participants needed to achieve study objectives and how it was determined, including clinical and statistical assumptions supporting any sample size calculations	14
Recruitment	15	Strategies for achieving adequate participant enrolment to reach target sample size	11-12
Methods: Assignment of interventions (for controlled trials)			
Allocation:			
Sequence generation	16a	Method of generating the allocation sequence (eg, computer-generated random numbers), and list of any factors for stratification. To reduce predictability of a random sequence, details of any planned restriction (eg, blocking) should be provided in a separate document that is unavailable to those who enrol participants or assign interventions	10
Allocation concealment mechanism	16b	Mechanism of implementing the allocation sequence (eg, central telephone; sequentially numbered, opaque, sealed envelopes), describing any steps to conceal the sequence until interventions are assigned	10
Implementation	16c	Who will generate the allocation sequence, who will enrol participants, and who will assign participants to interventions	10
Blinding (masking)	17a	Who will be blinded after assignment to interventions (eg, trial participants, care providers, outcome assessors, data analysts), and how	10
	17b	If blinded, circumstances under which unblinding is permissible, and procedure for revealing a participant's allocated intervention during the trial	N/A
Methods: Data collection, management, and analysis			
Data collection methods	18a	Plans for assessment and collection of outcome, baseline, and other trial data, including any related processes to promote data quality (eg, duplicate measurements, training of assessors) and a description of study instruments (eg, questionnaires, laboratory tests) along with their reliability and validity, if known. Reference to where data collection forms can be found, if not in the protocol	11, 13, fig. 2
	18b	Plans to promote participant retention and complete follow-up, including list of any outcome data to be collected for participants who discontinue or deviate from intervention protocols	11-12, 15

Data management	19	Plans for data entry, coding, security, and storage, including any related processes to promote data quality (eg, double data entry; range checks for data values). Reference to where details of data management procedures can be found, if not in the protocol	11
Statistical methods	20a	Statistical methods for analysing primary and secondary outcomes. Reference to where other details of the statistical analysis plan can be found, if not in the protocol	14,15
	20b	Methods for any additional analyses (eg, subgroup and adjusted analyses)	14,15
	20c	Definition of analysis population relating to protocol non-adherence (eg, as randomised analysis), and any statistical methods to handle missing data (eg, multiple imputation)	15
Methods: Monitoring			
Data monitoring	21a	Composition of data monitoring committee (DMC); summary of its role and reporting structure; statement of whether it is independent from the sponsor and competing interests; and reference to where further details about its charter can be found, if not in the protocol. Alternatively, an explanation of why a DMC is not needed	11
	21b	Description of any interim analyses and stopping guidelines, including who will have access to these interim results and make the final decision to terminate the trial	N/A
Harms	22	Plans for collecting, assessing, reporting, and managing solicited and spontaneously reported adverse events and other unintended effects of trial interventions or trial conduct	12-13
Auditing	23	Frequency and procedures for auditing trial conduct, if any, and whether the process will be independent from investigators and the sponsor	12
Ethics and dissemination			
Research ethics approval	24	Plans for seeking research ethics committee/institutional review board (REC/IRB) approval	18
Protocol amendments	25	Plans for communicating important protocol modifications (eg, changes to eligibility criteria, outcomes, analyses) to relevant parties (eg, investigators, REC/IRBs, trial participants, trial registries, journals, regulators)	18

Consent or assent	26a	Who will obtain informed consent or assent from potential trial participants or authorised surrogates, and how (see Item 32)	10
	26b	Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies, if applicable	N/A
Confidentiality	27	How personal information about potential and enrolled participants will be collected, shared, and maintained in order to protect confidentiality before, during, and after the trial	11
Declaration of interests	28	Financial and other competing interests for principal investigators for the overall trial and each study site	21-22
Access to data	29	Statement of who will have access to the final trial dataset, and disclosure of contractual agreements that limit such access for investigators	11
Ancillary and post-trial care	30	Provisions, if any, for ancillary and post-trial care, and for compensation to those who suffer harm from trial participation	N/A
Dissemination policy	31a	Plans for investigators and sponsor to communicate trial results to participants, healthcare professionals, the public, and other relevant groups (eg, via publication, reporting in results databases, or other data sharing arrangements), including any publication restrictions	18
	31b	Authorship eligibility guidelines and any intended use of professional writers	N/A
	31c	Plans, if any, for granting public access to the full protocol, participant-level dataset, and statistical code	N/A
Appendices			
Informed consent materials	32	Model consent form and other related documentation given to participants and authorised surrogates	Not included
Biological specimens	33	Plans for collection, laboratory evaluation, and storage of biological specimens for genetic or molecular analysis in the current trial and for future use in ancillary studies, if applicable	12-13

*It is strongly recommended that this checklist be read in conjunction with the SPIRIT 2013 Explanation & Elaboration for important clarification on the items. Amendments to the protocol should be tracked and dated. The SPIRIT checklist is copyrighted by the SPIRIT Group under the Creative Commons "Attribution-NonCommercial-NoDerivs 3.0 Unported" license.