Title: A Phase I/IIa Clinical Trial to Assess Safety and Immunogenicity of an Adenoviral-Vectored Capsular Group B Meningococcal Vaccine

Overline: VACCINES

One Sentence Summary: The group B meningococcus adenovirus-based vaccine ChAdOx1 MenB.1 is safe and immunogenic in healthy volunteers.

Authors: Christina Dold[1§#], Blanché Oguti[1,2§], Laura Silva-Reyes[1], Anna Stanzelova[1], Meriel Raymond[1], Catherine C. Smith[1], Maria Moore[1], Anna Barton[1†], Edward M Choi[1], Emma Plested[1], Kiarash Tanha[1], Jennifer Louth[3], Ann Holland[3], Robert Cook[3], Jessica King[3], Jay Lucidarme[4], Ray Borrow[3,4], Adrian V. S. Hill[5], Peter T. Beernink[6], Xinxue Liu[1], Andrew J. Pollard[1§], Christine S. Rollier[1,7*§]

Affiliations:

[1] Oxford Vaccine Group, Department of Paediatrics, University of Oxford and the NIHR Oxford Biomedical Research Centre, CCVTM, Churchill Lane, Oxford, OX37LE, United Kingdom

[2] Division of Infection and Immunity, Faculty of Medical Sciences, University College London; London, WC1E 6JF, United Kingdom

[3] Vaccine Evaluation Unit, UK Health Security Agency, Manchester, M13 9WL, United Kingdom

[4] Meningococcal Reference Unit, UK Health Security Agency, Manchester, M13 9WL, United Kingdom

[5] Jenner Institute, University of Oxford, Old Road Campus Research Building, Oxford, OX3 7DQ, United Kingdom

[6] Department of Paediatrics, University of California, San Francisco; 94143 San Francisco, USA

[7] School of Biosciences, Faculty of Health and Medical Sciences, University of Surrey, Guildford, GU2 7XH, United Kingdom

[§]**Contributed equally to the manuscript**

***Corresponding Author: c.rollier@surrey.ac.uk**

[†] Deceased

[#] Current affiliation: Moderna, Inc, Cambridge, Massachusetts, US

Abstract:

Capsular group B meningococcus (MenB) remains an important cause of disease in the globally and additional vaccines against MenB would aid in reducing the incidence of infection. Previous work has demonstrated that a MenB adenoviral-vectored vaccine, ChAdOx1 MenB.1, elicited high serum bactericidal responses in preclinical models after a single dose, supporting further clinical development of this vaccine. Here, we report the results of a trial designed to assess the safety and immunogenicity of ChAdOx1 MenB.1 in healthy adults aged 18 to 50. In this phase I/IIa, single-center trial, participants received one or two doses of ChAdOx1 MenB.1 at days 0 and 180. One dose of ChAdOx1 MenB.1 was also given at day 180 to some individuals primed with one dose of 4CMenB at day 0. Participants recorded their symptoms in an electronic diary after vaccination and safety blood readouts were monitored. Serum bactericidal antibody (SBA) assays were performed against a panel of MenB target strains. ChAdOx1 MenB.1 was well tolerated with no safety concerns and elicited protective SBA titers against a MenB strain expressing a homologous factor H-binding protein (fHbp) variant in 100% of participants after two doses. However, cross-reactivity analysis indicated a low SBA response to strains expressing heterologous fHbp, suggesting that a multivalent vaccine may be needed. In sum, ChAdOx1 MenB.1 is immunogenic in humans, and addition of another fHbp variant or of another antigen in this platform could provide an approach to extend protection against endemic MenB disease.

Editor's Summary: Mitigating MenB. There are currently only two approved vaccines for capsular group B meningococcal (MenB), but their cost effectiveness could be improved upon. To that end, Dold *et al.* developed an adenoviral-vectored MenB vaccine, ChAdOx1 MenB.1, and tested its safety and immunogenicity in a clinical trial enrolling healthy adults. The authors found that the vaccine was indeed safe and elicited immunity against the homologous bacteria, proving the proof of concept with this approach. However, cross-reactivity to other MenB strains was limited. These data support further development of ChAdOx1 MenB vaccines, particularly multivalent versions that could elicit broader immunity. –Courtney Malo

Main Text:

INTRODUCTION

Neisseria meningitidis remains an important cause of meningitis and septicemia, particularly in infant, toddler, and young adult populations*(1)*. It is a human-restricted pathogen that has an ecological niche in the nasopharynx; it can be found as a commensal in approximately 10% of healthy adults *(2)* and above 50% in certain populations, and can cause invasive meningococcal disease in a minority of individuals. Amongst the twelve *N. meningitidis* capsular serogroups, six (A, B, C, W, X and Y) cause most cases of invasive meningococcal disease *(3)*. Capsular group B meningococcus (MenB) remains the most common cause of invasive meningococcal disease in individuals under the age of 25 in high income regions *(4)*. Experimental vaccines based on serogroup B polysaccharides are poorly immunogenic *(5)* due to molecular mimicry with fetal neural cell adhesion molecules *(6)*.

Two vaccines, 4CMenB (Bexsero) and rLP2086 (Trumenba) are licensed in several countries. 4CMenB, licensed in the European Union (2013) and in the United States (2015), includes four non-polysaccharide components: non-lipidated factor H-binding protein (fHbp variant 1 peptide 1; alternative nomenclature B24), neisserial heparin binding antigen (NHBA peptide 2), each expressed as a recombinant fusion protein, recombinant *Neisseria* adhesin A (variant NadA-2/3 peptide 8) protein, and outer membrane vesicles from *N. meningitidis* group B strain NZ98/254 containing Porin A (PorA; subtype P1.4) and multiple minor antigens. The vaccine rLP2086 (Trumenba, or MenB-FHbp), licensed for adolescents and adults in the United States in 2014, and in the European Union in 2017, contains two recombinant lipidated fHbp peptides 45 and 55 (A05 and B01, respectively), and is also formulated in combination with meningococcal conjugate vaccines against other capsular groups (Penbraya, pentavalent ABCWY vaccine). Vaccine efficacy was inferred from serum bactericidal antibody (SBA) responses against MenB test strains. In clinical trials, SBA

responses elicited by the bivalent rLP2086 vaccine were assessed against four primary vaccine antigen-heterologous strains and ten secondary strains *(7)*, and those elicited by 4CMenB were assessed against four vaccine antigen-homologous strains. In the United Kingdom (UK), 4CMenB was included in the national immunization program for infants in 2015 and vaccine effectiveness reached 59.1% after a 2+1 schedule *(8–10)*.

We previously developed a ChAdOx1 MenB.1, a replication-deficient (E1 and E3 deleted) chimpanzee adenovirus (ChAd) vaccine *(11)*. The vaccine expresses fHbp variant 1 peptide 1 with a point mutation at the amino acid position 223 to abrogate its binding to human factor H (fH) while retaining immunogenicity *(12)*. In humanized mouse models, a single dose ChAdOx1 MenB.1 elicited robust bactericidal activity after a single immunization *(12)*. Whether this strong immunogenicity would occur in humans remained to be determined. Here, we report the results of an open-label, dose-escalation, non-randomized, single-site phase I/IIa clinical trial, designed to evaluate the safety and tolerability of ChAdOx1 MenB.1 as a vaccine against MenB in healthy adults aged 18 to 50 in Oxford (VAMBOX). The original trial design comprised three groups who received ChAdOx1 MenB.1, one group received a combination of ChAdOx1 MenB.1 and 4CMenB in a heterologous prime – boost regimen, and two groups immunized with 4CMenB at one or 6 months interval. The trial was then extended by adding two large groups of volunteers to enable the development of new reference serum standards against the two licensed meningococcal vaccines available in the UK, 4CMenB and bivalent rLP2086, led by the National Institute for Biological Standards and Controls (NIBSC). This trial therefore provided an opportunity to compare the safety profile of the study vaccine, 4CMenB, and bivalent rLP2086 in the same trial. The secondary and tertiary objectives of the VAMBOX trial were to characterize the immunogenicity of one or two doses of ChAdOx1 MenB.1 in healthy adults.

RESULTS

Participant demographics

Eighty-eight healthy volunteers were enrolled into the trial and sequentially assigned first to groups one to six (first-in-human part of the trial), and then to groups eight and nine (the part of the trial aiming at raising a serum standard) (Table 1), in a single center in Oxford, UK. The first vaccine date for the first dose was 2018-04-18, the last vaccine date for the first dose was 2019-12-16, and the last (visit 8) vaccine date for the booster dose was 2020-11-26. Recruitment was open to 18 to 50 years old healthy volunteers, and the enrolled participants were aged between 19 and 50 years old, median 28.15 years [interquartile range (IQR) 25.25-38.33], with 60.23% identifying as female. The participants were 90.9% White, 3.4% Mixed, and 5.7% of other ethnic origin (Table 2). All 88 participants adhered to the initial vaccination schedule and were therefore included in the primary safety analysis. However, eight participants missed their second vaccine dose across various groups (Fig. 1). Overall, 13 participants withdrew; four moved out of area, four cited heavy work or personal time commitments, three were lost to follow-up or unable to attend visits, and two were withdrawn by investigators because of low hemoglobin.

Safety and tolerability

ChAdOx1 MenB.1 was administered at $2.5x10^{10}$ (low dose) or $5x10^{10}$ (standard dose for ChadOx1-based vaccines, from here on referred to as standard dose) viral particles (vp). Only three participants received the low dose of $2.5x10^{10}$ vp, as this group was included in the trial design for safety purposes. An interim safety review was undertaken with the independent Data and Safety Monitoring Committee before proceeding with the target dose of $5x10^{10}$ vp.

ChAdOx1 menB.1 was well tolerated at doses up to $5x10^{10}$ viral particles when given as a single dose, in two doses, or as a booster following 4CMenB, with no serious adverse reactions (Fig. 2). Participants receiving a low dose of ChAdOx1 MenB.1 (ChAd low dose) of $2.5x10^{10}$ vp reported the fewest adverse reactions of all the trial arms: on day 1, 33.33% of participants had a mild headache, moderate fever, or experienced mild or moderate injection site pain, swelling, or redness that resolved by day 3 (Fig. 2A). Both a single standard dose of ChAdOx1 MenB.1 ($5x10^{10}$ vp) and a single dose of 4CMenB had the highest frequency of reactions. Up to 22.22% of participants receiving ChAdOx1 MenB.1 standard dose reported a mild or moderate fever, whereas none of the individuals who received a single dose 4CMenB reported a fever (and only 4.76% experienced a moderate fever in the rLP2086 group at Day 0). There was a high incidence of headaches among the ChAdOx1 MenB.1 standard dose recipients, which were resolved by day 2. Headaches occurred in the single dose 4CMenB and rLP2086 group participants and persisted to day 6. Apart from headache, "generally unwell" reaction was the only systemic severe reaction reported in the ChAdOx1 MenB.1 group. In contrast, muscle ache and joint pain reactions graded severe were reported in the 4CMenB and rLP2086 groups, respectively. Across all trial arms, the distribution of local reactions was similar, with the most frequently reported vaccine reaction being injection site pain, followed injection site redness. Severe-graded hardness and swelling were reported in all three groups.

The booster vaccinations elicited fewer adverse reactions than the standard dose prime vaccinations, particularly fewer systemic reactions, whether they were homologous or heterologous vaccine combinations (Fig. 2B). The ChAdOx1 MenB.1 prime at day 0 and second dose at day 180 (ChAd-ChAd(6)) vaccine schedule was well-tolerated; there were few reported adverse reactions that were predominantly mild, with no severe adverse reactions. The 4CMenB-4CMenB schedule was reactogenic, and the regimen with the shorter 1-month

interval (4CMenB-4CMenB(1)) appeared more reactogenic than the regimen with the 6-month interval (4CMenB-4CMenB(6)). The 4CMenB-4CMenB(1) participants also had a higher frequency of severe reactions than those in the 4CMenB-4CMenB(6) arm. The heterologous prime-boost combination of 4CMenB at day 0 followed by ChAdOx1 MenB.1 at day 180 (4CMenB-ChAd(6)) was well-tolerated and caused only mild or moderate adverse reactions. There were no clear differences in the reactogenicity profile between the prime dose of rLP2086 at day 0 and the booster dose at day 180 (rLP2086-rLP2086(6)). The summary of reported Adverse Events in the clinical trial is reported in Table 3. There were three serious adverse events (SAEs) reported during the trial: one participant had appendicitis requiring surgery, one participant had severe anemia, and one participant had cholecystitis related to known gallstones. None of these SAEs were considered vaccine-related.

ChAdOx1 MenB.1 induces SBA responses against a strain expressing a homologous fHbp in healthy adults

The ability of the study vaccines to induce SBA against homologous fHbp variant (v) following vaccination was explored using the target strain 44/76-SL, which expresses the fHbp variant 1 peptide1 included in the ChAdOx1 MenB.1 and 4CMenB vaccines (Table 4). Prior to vaccination, most individuals (range: 20% in ChAd standard dose to 55% in rLP2086-rLP2086) did not have SBA titers greater than 1:4 specific for the target strain 44/76-SL (Fig. 3A, day 0 and 3B, day 0; table S1). Both ChAdOx1 MenB.1 and 4CMenB prime vaccination elicited 44/76-SL -specific SBA responses (Fig. 3A). Immunization with ChAdOx1 MenB.1 induced SBA titers that continued to increase up to day 56 (geometric mean titers (GMT): 35.3, 95%CI [9.8-155.28]) (table S1). Individuals who received two doses of 4CMenB, one (GMT: 50.7, 95%CI [17.15-150.47]) or six months apart (GMT: 22.6, 95%CI [4.95-103.35]), had high 44/76-SL-specific SBA titers 28 days after the second dose

(table S1). Heterologous boosting did not generate similarly high 44/76-SL SBA titers. SBA waning was observed over the course of the trial observation period in all groups regardless of which vaccine was received (Fig. 3A, from day 180). However, the percentage of individuals with protective titers (≥1:4) following vaccination with a fHbp variant 1 peptide 1-containing vaccine (ChAdOx1 MenB.1 or 4CMenB) at standard dose remained at or above 78% at day 365 (Fig. 3B). The bivalent rLP2086 vaccine did not induce a high degree of cross-bactericidal activity against 44/76-SL (day 28 GMT: 4, 95%CI [1.74 – 9.22]) (table S1).

The proportion of baseline responders with SBA titers ≥ 1:4 by day 28 was 67% for ChAdOx1 MenB.1 low dose, 78% for ChAdOx1 MenB.1 standard dose, 75% for ChAd-ChAd(6), 75% for 4CMenB-ChAd(6), 89% for 4CMenB-4CMenB(1), 100% for 4CMenB-4CMenB(6), and 50% for rLP2086-rLP2086(6) (Fig. 3B). By day 56, this rose to 100% for ChAdOx1 MenB.1 low dose, 100% for ChAdOx1 MenB.1 standard dose, 86% for ChAd-ChAd(6), 86% for 4CMenB-ChAd(6), 100% for 4CMenB-4CMenB(1), and 100% for 4CMenB-4CMenB(6) (table S2). 67% of participants receiving a single dose of ChAdOx1 MenB.1 low dose and 63% of participants receiving a single dose of ChAdOx1 MenB.1 standard dose achieved a greater than 4-fold increase in SBA titers against the 44/76-SL strain at day 28 from baseline. This response peaked at day 56 before starting to wane (Fig. 3C). By day 208 (28 days post second dose), 100% of the participants in the ChAd-ChAd(6), 4CMenB-ChAd(6) and 4CMenB-4CMenB(6) arms had achieved at least a 4-fold increase in SBA activity against the 44/76-SL strain, as well as 86% of the participants in the 4CMenB-4CMenB(1) arm.

We also explored whether the two vaccines containing fHbp 1.1 induced B cell responses by characterizing IgG subclass profile by ELISA, and enumerating the fHbp 1.1 -specific antibody-secreting cell responses in participants who received ChadOx1 MenB.1 and

4CMenB. Serum IgG1 and IgG3 antibody titers were measured after a single injection of ChAdOx1 MenB.1 or 4CMenB at 3 time points during the first month (fig. S1A and S1B). Increases in the IgG1 and IgG3 responses were observed between day 0 and day 28 for both vaccines, and the IgG1 to IgG3 ratio remained stable at day 7 and day 28 for each vaccine type (fig. S1C). Additionally, antibody-secreting cell responses were increased after the 6 month boosts as measured by enzyme-linked immunosorbent spot (ELIspot) assay (day 208, fig. S1D).

ChAdOx1 MenB.1 induces only modest heterologous SBA responses in humans

The capability of the study vaccines to induce SBA against heterologous fHbp variants following vaccination was explored using the target strains M01 240355 (v3.31), M01 240101 (v1.15), M01 240185 (v1.10), M00 242922 (v1.4) and M15 240912 (v1.13) (Table 4, table S3 to S8 for GMT, and tables S9 to S13 for percentage of participants with SBA over 4). Variable SBA titers were present at day 0 prior to vaccination (Fig. 4A, day 0, tables S4 to S8). A single low dose of ChAdOx1 MenB.1 induced a transient increase in SBA titers to strain M01 240101 (expressing fHbp 1.15, v1.15) from a GMT of 4 [0.5-31.87] at day 0 to 20.1 [3.94-103.18] at day 28 (Fig. 4A, table S4). A single standard dose also induced a modest increase in SBA titers against this strain from 7.46 [2.69-20.73] at day 0 to 21.7 [8.77-54.03] at day 28 (Fig. 4A). A second dose of ChAdOx1 MenB.1 at day 180 did not further increase these SBA titers (Fig. 4A, day 208). ChAdOx1 MenB.1-immunized individuals maintained a low abundance of bactericidal antibodies against the variant 3 fHbp expressing strain (strain M01 240355, Fig. 4A, table S5) as well as low proportion of individuals with titers superior or equal to 1:4 (Fig. 4B). Negligible v1.10-specific SBA responses were observed in ChAdOx1 MenB.1- and 4CMenB-vaccinated individuals across

all schedules at all time-points (Fig. 4A and B, M01 240185 and table S6). The individual fold change in SBA activity against four heterologous MenB strains is shown in fig. S2 (Fig. S2), and the percentage of participants with SBA over the putative protective titer against each of the heterologous strains are shown in tables S9 to S13 (Tables S9 to S13).

ChAdOx1 MenB.1 vaccination induces a Th1-biased immune response

Adenoviral vaccines are expected to induce higher Th1-biased T cell responses than protein-based vaccines containing aluminum adjuvants. To investigate if this is the case when the same antigen is delivered with these two vaccine platforms, plasma cytokine concentrations were assessed in individuals 24 hours following vaccination with ChAdOx1 MenB.1 or 4CMenB, and fHbp 1.1-specific T cell responses were measured pre- and post-vaccination. Of the 10 plasma cytokines evaluated, five [interferon (IFN)-γ, interleukin (IL)-2, IL-6, IL-10, and tumor necrosis factor (TNF)-α, Fig. 5A] were noticeably increased at day one post-injection in those who received ChAdOx1 MenB.1. In contrast, IL-6 was the only cytokine with an observed increase in participants who received 4CMenB post-injection. A decrease in IL-8 was observed one day following immunization with 4CMenB. Homologous boosting with ChAdOx1 MenB.1 or 4CMenB did not elicit substantial increase in any plasma cytokine at day 181. 4CMenB prime followed by a heterologous boost with ChAdOx1 MenB.1 induced increases in IFN-γ, IL-10, IL-6, and TNF-α. Nearly all IL-2 concentrations were below the limit of detection (Fig. 5B). Neither vaccine, regardless of regimen, elicited an elevation in IL-1β or IL-13 in the plasma within 24 hours following immunization. Finally, we analyzed the fHbp 1.1-specific T cell response by IFN-γ ELIspot assay. These data indicated that, as expected, one or two injections with ChAdOx1 MenB.1 induced antigen-specific T cell responses (Fig. 5C).

DISCUSSION

ChAdOx1 MenB.1 was safe and well tolerated in this first-in-human trial in healthy adults. Both the low dose and SD elicited robust bactericidal activity against the homologous target *N. meningitidis* strain, 44/76-SL after a single immunization, which remained detectable for a year. This provides proof of concept that a Gram-negative outer membrane protein can be effectively expressed from an adenoviral vector and can induce bactericidal responses in humans. Additionally, the results show that fHbp with a point mutation abrogating binding to human factor H is immunogenic. However, the use of the adenoviral platform and the presence of the mutation induced only modest cross-reactive bactericidal responses.

ChAdOx1-based vaccine candidates and other viral-vectored vaccines in development, including those based on adenovirus (Ad) serotype 5, ChAdOx2, Adenovirus serotype 26, vesicular stomatitis virus, modified vaccinia Ankara, and yellow fever 17D, are mainly against viral or parasitic targets *(13)*. Adenoviral-vectored vaccines expressing bacterial proteins can produce a functional immune response in animal models, as demonstrated for *Bacillus anthracis (14)* and *Clostridium botulinum (15)*, although these were targeting the excreted toxin proteins, rather than the complex bacterial surface-exposed antigens. An experimental adenoviral-vectored vaccine against non-typeable *Haemophilus influenzae* tested in chinchillas elicited robust but non-protective immune responses *(16)*. Although we previously identified challenges in correctly expressing other meningococcus antigen targets *(17)*, our study contributes to the increasing evidence that viral vectors are suitable vehicles that can elicit functional antibody responses against complex conformational bacterial epitopes.

All adverse reactions reported by participants receiving the ChAdOx1 MenB.1 were mild or moderate and short-lived, and there were no vaccine-related SAEs. This is in keeping with results from both clinical trials and real-world effectiveness studies of ChAdOx1-based vaccines. Similar safety and reactogenicity profiles were observed in the trials for ChAdOx1-MERS against Middle East respiratory syndrome coronavirus *(18)*, ChAdOx1 NP+M1 against influenza A virus *(19)* , and ChAdOx1 Chik against chikungunya virus *(20)*. More than 3 billion doses of ChAdOx1 nCoV-19 (AZD1222), a vaccine against SARS-CoV-2, have been distributed to over 180 countries following the success of a multi-center, international, phase II/III trial, which reported it to be safe and efficacious *(21)*. Investigators have also been able to validate these findings in real world effectiveness studies *(22)*. There were also very few reported incidents of fever among the participants who received the 4CMenB vaccine in the trial described here, in agreement with current safety data in this age group.

As the incidence of capsular group B invasive meningococcal disease is low, vaccine efficacy trials are challenging to conduct and not likely to be feasible. However, the rise of SBA above the putative protective titer of 1:4 is accepted as an indication of efficacy and was used for licensure of both 4CMenB and rLP2086. Based on this metric, ChAdOx1 MenB.1 elicited a protective immune response against the 44/76-SL strain. It has been proposed that the percentage of participants with titers of ≥1:4 at 28 days post-immunization correlates with short-term protection against infection with a homologous strain, whereas the percentage of participants with ≥4-fold titer increases represent a more robust response *(23, 24)*. In our trial, 75% of the participants in the ChAd-ChAd(6) arm had a ≥ 4-fold increase in SBA titers against the 44/76-SL strain by day 28, after a single dose, rising to 100% after the second dose. Most adults will have carried *N. meningitidis* and subsequently developed SBA against multiple antigens in their lifetime, which is supported by the SBA detected in the trial

participants at baseline. Importantly, this phenomenon was present in participants in all arms of the trial. Of note, the SBA assays were validated, quality-controlled, and performed blinded, therefore differences at baseline were likely not a result of technical issues.

As a first-in-human trial, this trial was not powered to compare the immunogenicity between groups, therefore no comparison in immunogenicity between the different vaccines included were made, as they could have been affected by the small sample size and differences in baseline titers. Moreover, the last two larger groups were added at a later time point and for a different purpose (generation of reference standards), therefore not all analysis were performed for these groups. As an indication, the SBA responses observed in participants who received two doses of 4CMenB (100% participants with SBA ≥4 and hSBA GMT titers of 147 (94-229) against strain 44/76-SL) were in similar ranges to SBA responses observed in adults in other studies *(23, 25)*. In Toneatto *et al*., the percentage of participants with SBA titers ≥4 against strain 44/76-SL after two doses was 100% with 95% CI 87 to 100. The SBA titers were only reported after 3 doses in adults in that trial (GMT 77 with 95% CI of 57 to 106) *(25)*. In a trial including naïve young adults aged 18 to 24 years old, two doses of 4CMenB resulted in 100% participants with SBA ≥4, and GMTs of 57 (45–73) in the naïve participants from Canada and Australia or 64 (52–78) in the naïve participants from Chile *(26)*.

ChAdOx1 MenB.1 elicited a limited cross-reactive SBA response. SBA immune responses were generated against strains M00 242922 (expressing fHbp variant v1.4) and M01 240355 (fHbp v3.31) in a proportion of participants. The fHbp variant 1.1 is closer to variant 1.4 than variant 3.31 on the phylogenetic tree, and this is reflected in the degree of cross-reactivity of the SBA response against each strain. The poor cross-reactivity of immune responses against specific MenB fHbp variants has been well-described and is a universal challenge for all

protein-based MenB vaccines *(27)*, indicating the need for delivery of a multi-antigen vaccine for broad protection. This appears to be similar here when using the ChAdOx1 platform and the mutated fHbp. ChAdOx1 MenB.1 elicited poor SBA activity against MenB strain M01 240185, which contains a vaccine-heterologous fHbp subvariant (v1.10), and a modest effect against M01 240101 (v1.15). These data suggest that ChAdOx1 MenB.1 induced limited cross-reactivity in humans, contrary to the results observed in mice during pre-clinical development *(12)*. This may be linked with the dose of ChAdOx1 in human, as the dose used in mice was in the high range of the dose-response. Moreover, although we observed a drastic decrease of the mutant binding to human factor H during preclinical development *(12)*, this was performed in a cell lines, therefore the binding of factor H after immunization in human is not known.

The ChAdOx1-based vaccine induced a cellular mediated response, as expected with replication-deficient adenoviral vectors in humans *(18–20)*. T-cell responses can improve vaccine efficacy by supporting the B-cell response *(28)*, as shown for meningococcal polysaccharide conjugate vaccines *(29, 30)*. Therefore, the T-cell response induced by the ChAdOx1 may provide the conditions to increase the longevity of the memory B-cell response. T-cell responses against outer membrane proteins are induced after meningococcal disease *(31, 32)*. However, whereas the SBA is an established surrogate marker of efficacy against invasive meningococcal disease *(33)*, there is no evidence for the potential of such cellular responses to support direct protection in addition to, and independently of the SBA responses.

The main limitation of this trial is its small sample size, which was acceptable for a first-in-human trial aiming to assess safety. Although adequate expression of fHbp was assessed in vitro *(12)* and is assumed by the capacity to elicit an SBA response, we did not assess post-

translational changes and whether all fHbp B cell epitopes were presented in their natural conformation. Another limitation is that it is impossible to evaluate the overall dose of expressed fHbp from ChAdOx1 MenB.1 and how it compares with fHbp doses in protein-based vaccines. We have not attempted to measure the quantity of antigen expressed after injection in preclinical models as this would involve complex in vivo studies that cannot be reliably recapitulated in vitro. After in vivo injection, the amount of fHbp produced per infected cell includes cell-bound as well as secreted antigen, and varies depending on the cell type, its immediate environment (influenced by the innate immune response), anatomical location (muscle tissue, secondary lymphoid organs) *(34)*, and time since vaccination *(35)*. The amount of antigen expression can also be influenced by the vaccine-elicited T cell response itself, as demonstrated with DNA-based vaccines *(36)*. Geiben-Lynn *et al.* performed a comprehensive study using a luciferase-based real-time photon imaging and found that intramuscular adenovirus delivery resulted in transgene expression for a week, and that the T cell response contributed to the decrease of the antigen expression from day 4 *(37)*. There was no association between the peak amount of antigen expression in the first 2 days and T cell response, nor with the establishment of long-term immune response. However, the study did not explore the relation with the antibody response. Another limitation is that we did not assess the response to the vector itself. Anti-ChAdOx1 vector responses were analyzed in detail in a larger trial after one or two doses of the ChAdOx1 COVID-19 vaccine *(38)*, and the results showed no relationship between antibodies to ChAdOx1 on the day of vaccination and the antibody or T cell responses to the target antigen. It is unlikely that the anti-vector data generated from our trial with small sample size would support any further meaningful interpretation. We have also not measured the serum IgA titers in the participants. Restrictions on research activities, introduced in response to the COVID-19 pandemic, led to missed trial visits; however, very few of these were key immunological time points.

Furthermore, this first-in-human phase I/IIa trial was conducted in adults and may not be generalizable to other age groups or populations. Finally, although adenovirus vectors are considered an inexpensive vaccine platform owing to low production costs, without cost-effectiveness modelling, we cannot infer whether such vaccine would have a favorable cost-effectiveness profile for adolescent programs *(39, 40)*.

In conclusion, this phase I/IIa trial demonstrates that ChAdOx1 MenB.1 vaccine is well-tolerated and immunogenic. Our findings show that ChAdOx1 MenB.1 and 4CMenB elicited comparable SBA activity against 44/76-SL, and although the trial was not powered to assess differences in immunogenicity between the groups, the results suggest that ChAdOx1 MenB.1 did not induce strong cross-reactivity. The inclusion of another variant of fHbp or another antigen, similarly to other vaccine platform *(41)*, would potentially result in a MenB vaccine with broader cross-reactivity.

MATERIALS AND METHODS

Study design

This is a first-in-human trial to evaluate the use of a viral-vectored vaccine with a transgene expressing a Gram-negative bacterial surface protein. The trial was an open-label, dose-escalation, non-randomized, single-site Phase I/IIa clinical trial in healthy adults aged 18 to 50. Participants were enrolled following an informed consent process. Participants received one or two doses of either the vaccine under investigation, ChAdOx1 MenB.1, or one of the licensed MenB vaccines. The observations included safety and tolerability (primary endpoints) as well as immunogenicity, measured by SBA, ELISPOT and quantification of serum cytokines. The analyses for this study were descriptive and did not include any hypothesis testing or presentation of p-values for group comparisons. Consequently, there

were no power calculations used to determine the trial sample. Anyone who received at least one dose of study vaccine was included and all data obtained up to the point of withdrawal were used in the analysis and there were no imputations for missing data. Rules for stopping data collection and inclusion/exclusion criteria are in the protocol (data file S1).

Baseline assessments and information was collected by a study investigator as part of the assessment of inclusion/exclusion criteria (detailed in the study protocol, data file S1). Blood samples were taken for routine hematological and biochemical markers, as well as a blood-borne virus screen. Urinalysis was performed and a point-of-care pregnancy test was taken by participants with child-bearing potential.

In the first group, ChAdOx1 MenB.1 was administered at a dose of $2.5x10^{10}$ (low dose) for safety purpose (three volunteers, group 1), and then at $5x10^{10}$ (standard dose) viral particles. The 4CMenB (Bexsero, GlaxoSmithKline) and rLP2086 (Trumenba, Pfizer) vaccines were given as per summary of product characteristics at the standard dose of 0.5mL. All vaccines were administered as an intramuscular injection into the deltoid.

The trial contained eight groups (Table 1 and Fig. 1) with a sequential, non-randomized allocation: groups who received a single low or standard dose of ChAdOx1 MenB.1 (groups 1 and 2); then groups who received two doses of either standard dose of ChAdOx1 MenB.1 (group 3) or a heterologous immunization with 4CMenB followed by ChAdOx1 MenB.1 six months later (group 4); 4CMenB was administered at day 0 and either 1 month (group 5) or a 6 months later (group 6); the larger groups dedicated to raising a serum standard received two doses of 4CMenB or rLP2086 as per the respective vaccine licenses (groups 7 and 8). The latter two groups were supported by the NIBSC and were independent of the original first-in-human design. The sampling strategy was therefore different with a focus on large serum sampling supplied to the NIBSC. Therefore, not all samples and time points were available in

these groups. For group 7 (19 participants immunized with two doses of 4CMenB at 6 months interval), only the large blood collection were performed for the generation of a serum standard (no immunology analysis). For group 8 (21 participants immunized with two doses of rLP2086 at 6 months interval), only a subset of 10 participants were randomly used for the SBA assays. All SBA assays were performed blinded at the Vaccine Evaluation Unit (UKHSA) (samples were coded at the size of study). As a first-in-human trial, this trial was not powered to compare the immunogenicity between groups; therefore, no comparison in immunogenicity between the different vaccines included were made. Participants were recruited to each group sequentially. A staggered enrollment approach was used for the first three participants in group 1 and 2, receiving the low dose and standard dose of ChAdOx1 MenB.1, respectively. An interim safety review was undertaken with the independent Data and Safety Monitoring Committee before each dose escalation.

Ethics and approvals

Clinical Trial Authorization was granted by the United Kingdom Medicines and Healthcare products Regulatory Agency (MHRA reference 21584/0379/001-0001). Ethical approval and amendments were granted by South Central - Oxford A Research Ethics Committee (17/SC/0470). The trial was registered with EudraCT (2017-000965-61). The trial was conducted in accordance with the clinical trial protocol and the principles of the Declaration of Helsinki (2008) and the International Conference on Harmonization (ICH) Good Clinical Practice standards.

Vaccine construction and preparation

458 ChAdOx1 MenB.1 is a replication-deficient viral vector expressing the fHbp variant 1.1
459 transgene and was produced as described previously *(12)*. The vaccine was produced to
460 current Good Manufacturing Practice by the Clinical Biomanufacturing Facility (the
461 University of Oxford) in a Tet-repressed HEK-293 cell line and sterile-filtered to generate a
462 clinical lot at a concentration of $1.1x10^{11}$ viral particles per mL. Vaccine aliquots were stored
463 at -80°C until use.

464

465 **Safety monitoring**

466 Participants were observed in the clinic for 30-60 minutes following immunization and each
467 recorded any AEs using electronic diaries, from day 0 (day of vaccination) to day 6 for
468 solicited symptoms (induration, redness, swelling, headache, malaise, myalgia, nausea and
469 vomiting, anorexia, arthralgia, and fever) and up to 28 days post-vaccination for unsolicited
470 adverse events. The grading of AEs were predefined, where 0 indicates absence of symptoms,
471 1 indicates transient or mild discomfort, 2 indicates some intervention or medical therapy
472 required, 3 indicates significant interference with daily activity, and 4 indicates emergency
473 department visit or hospitalization. Laboratory AEs were graded using a table adapted from
474 the US Food and Drug Administration (US FDA) toxicity grading scale. All reported or
475 observed AEs were recorded in the OpenClinica electronic database and SAEs could be
476 reported throughout the trial. An independent local safety monitor provided trial oversight.

477 Blood samples were taken and vital signs were recorded to undertake safety clinical
478 assessments before vaccination at day 0 and at days 1, 2, 7, 14, 28, 56, 84, 180, 208 and 365
479 following the first vaccination. Hematological parameters were entered in a blood safety
480 database and graded mild, moderate, severe, or potentially life-threatening based on the US
481 FDA guidelines *(42)*.

Meso scale discovery (MSD) cytokine profiling

Cytokine responses were measured in plasma samples with the MSD V-PLEX Proinflammatory Cytokine (human) Panel 1 kit (Meso scale K15049D). The 10 spot plates were coated with capture monoclonal antibodies against the following cytokines: IFN-γ, IL-1β, IL-2, IL-4, IL-6, IL-8, IL-10, IL-12p70, IL-13, and TNF-α. Plasma samples were diluted at 1:2 in MSD diluent 2 and added in duplicates to the plates for two hours at room temperature with shaking. Each plate also included MSD standard diluted 1:4 and 3 MSD controls diluted 1:2. After incubation and a wash step, detection antibodies were added to the plates and incubated for 2 hours at room temperature. After adding the read buffer, plates were read on a MESO QuickPlex SQ 120 reader and analysis was carried out with the MSD Discovery Workbench 4.0. Samples with a coefficient of variation greater than 20% between replicates were repeated. Concentrations in pg/ml were determined by back fitting to the standard curve and multiplied by the dilution factor.

SBA assay

Vaccine-induced functional bactericidal antibodies were determined using a standardized SBA assay *(43)*. As described previously, a starting serum dilution of 1:2 was tested using human serum as an exogenous complement source *(44)*. A panel of MenB target strains were selected to represent key 4CMenB and ChAdOx1 MenB.1 vaccine antigen variants. Vaccine type strains were considered homologous if the serum sample was from a participant vaccinated with a vaccine based on that strain and other comparisons were considered heterologous. All strains utilized are shown in Table 4. The strains utilized include the homologous fHbp v1.1 as well as different V1 variants including v1.14, v1.10, v1.13 and

v1.15 as well as a fHbp v3.31. These selected strains have been utilized in other clinical trials of 4CMenB *(44–46)*. A vaccine response was defined as a ≥4-fold rise in SBA titer compared with pre-vaccination baseline titer; no response was defined as less than a 4-fold rise in titer.

Detection of serum IgG1 and IgG3 by enzyme-linked immunosorbent assay (ELISA)

Immulon 2HB plates (Thermo Fisher Scientific) were coated with recombinant fHbp 1.1 protein at 2.5 µg/ml in carbonate-bicarbonate buffer (Sigma Aldrich). Serum samples were serially diluted in phosphate-buffered saline containing 0.5% (v/v) Tween-20 and 1% (w/v) bovine serum albumin and tested in duplicates. Antibody binding was detected with horseradish peroxidase-conjugated anti-human IgG1 or IgG3 (Invitrogen A 10648 and 05-3620, Thermo-Fisher Scientific) and visualized with 3,3',5,5'-Tetramethylbensidine substrate (Sigma Aldrich). The reaction was stopped with 50 µl H_2SO_4, and optical densities (O.D.) were measured at 450 nm with a reduction at 600 nm.

Enumeration of antibody-secreting cell responses and IFN-γ T cell responses by ELISpot

Antigen-specific antibody-secreting cell enumeration was performed using cryopreserved peripheral blood mononuclear cells by human B cell FLUOROSpot as described previously *(47)* and following the manufacturer's instructions (Mabtech) using recombinant fHbp 1.1. Briefly, peripheral blood mononuclear cells were isolated from fresh heparinized blood samples by density gradient centrifugation and cryopreserved. After thawing, cells were stimulated with a cocktail of R848 and IL-2 (Mabtech) and incubated for 72 hours at 37°C. Cells were then washed and added to the 96-well ELISPOT plates coated with recombinant

fHbp 1.1 protein. Cells were incubated overnight at 37°C/5%CO2/95% humidity and detection of spots was carried out according to the manufacturer's instructions (Mabtech) and analyzed with the iSpot EliSpot reader (Autoimmun Diagnostika). The T cell responses were measured in peripheral blood mononuclear cells by human T cell FLUOROspot according to the manufacturer's instructions (Mabtech). Briefly, peripheral blood mononuclear cells were stimulated overnight with a peptide pool of 15mer peptides overlapping by 11 amino acids and covering the entire sequence of fHbp 1.1. Plates were coated overnight at 4 °C with anti-human IFN-γ antibody (clone 1-D1K, Mabtech) in carbonate buffer, washed, and blocked with media for 2 hours minimum. 2.5×10^5 cells were incubated with the peptide pool, medium alone or overnight at 37 °C with 5% CO2. Plates were washed and developed, and spots were counted using an AID automated ELISpot counter (AID Diagnostika, algorithm C). For both assays, identical settings were used for all plates. Responses were averaged across the replicate wells. For the T cell ELISPOT, the mean response of the unstimulated (negative control) wells was subtracted and results expressed as spot forming cells per million peripheral blood mononuclear cells.

Statistical analysis

The analyses for this study were descriptive and did not include any hypothesis testing or presentation of p-values for group comparisons. Consequently, there were no power calculations used to determine the trial sample. Eighty-eight participants were included in the analyses according to the group to which they were assigned. Anyone who received at least one dose of study vaccine was included and all data obtained up to the point of withdrawal were used in the analysis and there were no imputations for missing data. Safety data were summarized by each dose and in total.

The comparability of the groups was assessed using the demographic and baseline characteristics. Continuous variables that followed an approximately normal distribution were summarized using means and standard deviations. Skewed continuous variables were summarized using medians and inter-quartile ranges. Categorical variables were summarized using frequencies and percentages. All confidence intervals (CI) for descriptive analyses were set at 95%.

The geometric mean titer (GMT), with 95% CI, of the SBA were determined for each group at Day 0, 7, and 28 after each vaccination dose. The percentage of people with 4-fold increase compared to baseline and the percentage of participants with SBA titers greater or equal to 1:4, with 95% CI, are reported. Values less than 0.25, which are not detectable, were replaced by 0.125 in the analysis. As a first-in-human trial, this trial was not powered to compare the immunogenicity between groups, therefore no comparison in SBA between the different vaccines included were made. All available data were used in the analyses without imputations for missing data using R version 4.2.2, in agreement with the statistical analysis plan (data file S2). Individual-level data are presented in data file S3. For the plasma cytokine data, some data were missing at random, therefore a mixed-effects analysis with multiple comparisons (Šidák's comparisons test) was used, and the adjusted P value for significance indicated in the graphs.

List of Supplementary Materials

Fig. S1 and S2

Tables S1 to S13

MDAR Reproducibility Checklist

577 Data files S1 to S3

578

579

References and Notes

1. D. van de Beek, M. C. Brouwer, U. Koedel, E. C. Wall, Community-acquired bacterial meningitis. *The Lancet* 398, 1171–1183 (2021).

2. D. Pace, A. J. Pollard, Meningococcal A, C, Y and W-135 polysaccharide-protein conjugate vaccines. *Arch Dis Child* 92, 909–915 (2007).

3. L. H. Harrison, C. L. Trotter, M. E. Ramsay, Global epidemiology of meningococcal disease. *Vaccine* 27, B51–B63 (2009).

4. Health Protection Report, *Invasive meningococcal disease in England: epidemiological year 2019 to 2020 https://assets.publishing.service.gov.uk/government/uploads/system/uploads/attachment_data/file/951142/hpr0121_imd-ann.pdf* (2021).

5. F. A. Wyle, M. S. Artenstein, B. L. Brandt, E. C. Tramont, D. L. Kasper, P. L. Altieri, S. L. Berman, J. P. Lowenthal, Immunologic Response of Man to Group B Meningococcal Polysaccharide Vaccines. *Journal of Infectious Diseases* 126, 514–522 (1972).

6. J. Finne, M. Leinonen, P. H. Mäkelä, ANTIGENIC SIMILARITIES BETWEEN BRAIN COMPONENTS AND BACTERIA CAUSING MENINGITIS. Implications for Vaccine Development and Pathogenesis. *The Lancet* 322, 355–357 (1983).

7. T. Vesikari, L. Østergaard, J. Diez-Domingo, J. Wysocki, C.-E. Flodmark, J. Beeslaar, J. Eiden, Q. Jiang, K. U. Jansen, T. R. Jones, S. L. Harris, R. E. O'Neill, L. J. York, G. Crowther, J. L. Perez, Meningococcal Serogroup B Bivalent rLP2086 Vaccine Elicits Broad and Robust Serum Bactericidal Responses in Healthy Adolescents. *J Pediatric Infect Dis Soc* 5, 152–160 (2016).

8. S. N. Ladhani, N. Andrews, S. R. Parikh, H. Campbell, J. White, M. Edelstein, X. Bai, J. Lucidarme, R. Borrow, M. E. Ramsay, Vaccination of Infants with Meningococcal Group B Vaccine (4CMenB) in England. *New England Journal of Medicine* 382, 309–317 (2020).

9. Department of Health and Social Care and Public Health England, Meningococcal B vaccine: JCVI position statement: https://www.gov.uk/government/publications/meningococcal-b-vaccine-jcvi-position-statement*JCVI position statement on the use of meningococcal B (MenB) vaccine in the UK.* (2014).

10. R. M. Viner, R. Booy, H. Johnson, W. J. Edmunds, L. Hudson, H. Bedford, E. Kaczmarski, K. Rajput, M. Ramsay, D. Christie, Outcomes of invasive meningococcal serogroup B disease in children and adolescents (MOSAIC): a case-control study. *Lancet Neurol* 11, 774–783 (2012).

11. M. D. J. Dicks, A. J. Spencer, N. J. Edwards, G. Wadell, K. Bojang, S. C. Gilbert, A. V. S. Hill, M. G. Cottingham, A novel chimpanzee adenovirus vector with low human seroprevalence: Improved systems for vector derivation and comparative immunogenicity. *PLoS One* 7 (2012), doi:10.1371/journal.pone.0040385.

12. C. Dold, L. Marsay, N. Wang, L. Silva-Reyes, E. Clutterbuck, G. K. Paterson, K. Sharkey, D. Wyllie, P. T. Beernink, A. V. Hill, A. J. Pollard, C. S. Rollier, An adenoviral-vectored vaccine confers seroprotection against capsular group B meningococcal disease. *Sci Transl Med* 15 (2023), doi:10.1126/scitranslmed.ade3901.

13. N. McCann, D. O'Connor, T. Lambe, A. J. Pollard, Viral vector vaccines. *Curr Opin Immunol* 77, 102210 (2022).

14. M. J. McConnell, P. C. Hanna, M. J. Imperiale, Adenovirus-based Prime-boost Immunization for Rapid Vaccination Against Anthrax. *Molecular Therapy* 15, 203–210 (2007).

15. Q. Xu, M. E. Pichichero, L. L. Simpson, M. Elias, L. A. Smith, M. Zeng, An adenoviral vector-based mucosal vaccine is effective in protection against botulism. *Gene Ther* 16, 367–375 (2009).

16. L. E. Winter, S. J. Barenkamp, Construction and Immunogenicity of Recombinant Adenovirus Vaccines Expressing the HMW1, HMW2, or Hia Adhesion Protein of Nontypeable *Haemophilus influenzae*. *Clinical and Vaccine Immunology* 17, 1567–1575 (2010).

17. L. Marsay, C. Dold, G. K. Paterson, Y. Yamaguchi, J. P. Derrick, H. Chan, I. M. Feavers, M. C. J. Maiden, D. Wyllie, A. V. Hill, A. J. Pollard, C. S. Rollier, Viral vectors expressing group B meningococcal outer membrane proteins induce strong antibody responses but fail to induce functional bactericidal activity. *Journal of Infection* 84, 658–667 (2022).

18. P. M. Folegatti, M. Bittaye, A. Flaxman, F. R. Lopez, D. Bellamy, A. Kupke, C. Mair, R. Makinson, J. Sheridan, C. Rohde, S. Halwe, Y. Jeong, Y.-S. Park, J.-O. Kim, M. Song, A. Boyd, N. Tran, D. Silman, I. Poulton, M. Datoo, J. Marshall, Y. Themistocleous, A. Lawrie, R. Roberts, E. Berrie, S. Becker, T. Lambe, A. Hill, K. Ewer, S. Gilbert, Safety and immunogenicity of a candidate Middle East respiratory syndrome coronavirus viral-vectored vaccine: a dose-escalation, open-label, non-randomised, uncontrolled, phase 1 trial. *Lancet Infect Dis* 20, 816–826 (2020).

19. R. D. Antrobus, L. Coughlan, T. K. Berthoud, M. D. Dicks, A. V. Hill, T. Lambe, S. C. Gilbert, Clinical Assessment of a Novel Recombinant Simian Adenovirus ChAdOx1 as a Vectored Vaccine Expressing Conserved Influenza A Antigens. *Molecular Therapy* 22, 668–674 (2014).

20. P. M. Folegatti, K. Harrison, L. Preciado-Llanes, F. Ramos Lopez, M. Bittaye, Y. Chan Kim, A. Flaxman, D. Bellamy, R. Makinson, J. Sheridan, S. R. Azar, R. Kroon Campos, M. Tilley, N. Tran, D. Jenkin, I. Poulton, A. Lawrie, R. Roberts, E. Berrie, S. L. Rossi, A. Hill, K. J. Ewer, A. Reyes-Sandoval, A single dose of ChAdOx1 Chik vaccine induces neutralizing antibodies against four chikungunya virus lineages in a phase 1 clinical trial. *Nat Commun* Jul 3012, 4636 (2021).

21. M. Voysey, S. A. C. Clemens, S. A. Madhi, L. Y. Weckx, P. M. Folegatti, P. K. Aley, B. Angus, V. L. Baillie, S. L. Barnabas, Q. E. Bhorat, S. Bibi, C. Briner, P. Cicconi, A. M. Collins, R. Colin-Jones, C. L. Cutland, T. C. Darton, K. Dheda, C. J. A. Duncan, K. R. W. Emary, K. J. Ewer, L. Fairlie, S. N. Faust, S. Feng, D. M. Ferreira, A. Finn, A. L. Goodman, C. M. Green, C. A. Green, P. T. Heath, C. Hill, H. Hill, I. Hirsch, S. H. C. Hodgson, A. Izu, S. Jackson, D. Jenkin, C. C. D. Joe, S. Kerridge, A. Koen, G. Kwatra, R. Lazarus, A. M. Lawrie, A. Lelliott, V. Libri, P. J. Lillie, R. Mallory, A. V. A. Mendes, E. P. Milan, A. M. Minassian, A. McGregor, H. Morrison, Y. F. Mujadidi, A. Nana, P. J. O'Reilly, S. D. Padayachee, A. Pittella, E. Plested, K. M. Pollock, M. N. Ramasamy, S. Rhead, A. V Schwarzbold, N. Singh, A. Smith, R. Song, M. D. Snape, E. Sprinz, R. K. Sutherland, R. Tarrant, E. C. Thomson, M. E. Török, M. Toshner, D. P. J. Turner, J. Vekemans, T. L. Villafana, M. E. E. Watson, C. J. Williams, A. D. Douglas, A. V. S. Hill, T. Lambe, S. C. Gilbert, A. J. Pollard, M. Aban, F. Abayomi, K. Abeyskera, J. Aboagye, M. Adam, K. Adams, J. Adamson, Y. A. Adelaja, G. Adewetan, S. Adlou, K. Ahmed, Y. Akhalwaya, S. Akhalwaya, A. Alcock, A. Ali, E. R. Allen, L. Allen, T. C. D. S. C. Almeida, M. P. S. Alves, F. Amorim, F. Andritsou, R. Anslow, M. Appleby, E. H. Arbe-Barnes, M. P. Ariaans, B. Arns, L. Arruda, P. Azi, L. Azi, G. Babbage, C. Bailey, K. F. Baker, M. Baker, N. Baker, P. Baker, L. Baldwin, I. Baleanu, D. Bandeira, A. Bara, M. A. S. Barbosa, D. Barker, G. D. Barlow, E. Barnes, A. S. Barr, J. R. Barrett, J. Barrett, L. Bates, A. Batten, K.

Beadon, E. Beales, R. Beckley, S. Belij-Rammerstorfer, J. Bell, D. Bellamy, N. Bellei, S. Belton, A. Berg, L. Bermejo, E. Berrie, L. Berry, D. Berzenyi, A. Beveridge, K. R. Bewley, H. Bexhell, S. Bhikha, A. E. Bhorat, Z. E. Bhorat, E. Bijker, G. Birch, S. Birch, A. Bird, O. Bird, K. Bisnauthsing, M. Bittaye, K. Blackstone, L. Blackwell, H. Bletchly, C. L. Blundell, S. R. Blundell, P. Bodalia, B. C. Boettger, E. Bolam, E. Boland, D. Bormans, N. Borthwick, F. Bowring, A. Boyd, P. Bradley, T. Brenner, P. Brown, C. Brown, C. Brown-O'Sullivan, S. Bruce, E. Brunt, R. Buchan, W. Budd, Y. A. Bulbulia, M. Bull, J. Burbage, H. Burhan, A. Burn, K. R. Buttigieg, N. Byard, I. Cabera Puig, G. Calderon, A. Calvert, S. Camara, M. Cao, F. Cappuccini, J. R. Cardoso, M. Carr, M. W. Carroll, A. Carson-Stevens, Y. de M. Carvalho, J. A. M. Carvalho, H. R. Casey, P. Cashen, T. Castro, L. C. Castro, K. Cathie, A. Cavey, J. Cerbino-Neto, J. Chadwick, D. Chapman, S. Charlton, I. Chelysheva, O. Chester, S. Chita, J.-S. Cho, L. Cifuentes, E. Clark, M. Clark, A. Clarke, E. A. Clutterbuck, S. L. K. Collins, C. P. Conlon, S. Connarty, N. Coombes, C. Cooper, R. Cooper, L. Cornelissen, T. Corrah, C. Cosgrove, T. Cox, W. E. M. Crocker, S. Crosbie, L. Cullen, D. Cullen, D. R. M. F. Cunha, C. Cunningham, F. C. Cuthbertson, S. N. F. Da Guarda, L. P. da Silva, B. E. Damratoski, Z. Danos, M. T. D. C. Dantas, P. Darroch, M. S. Datoo, C. Datta, M. Davids, S. L. Davies, H. Davies, E. Davis, J. Davis, J. Davis, M. M. D. De Nobrega, L. M. De Oliveira Kalid, D. Dearlove, T. Demissie, A. Desai, S. Di Marco, C. Di Maso, M. I. S. Dinelli, T. Dinesh, C. Docksey, C. Dold, T. Dong, F. R. Donnellan, T. Dos Santos, T. G. dos Santos, E. P. Dos Santos, N. Douglas, C. Downing, J. Drake, R. Drake-Brockman, K. Driver, R. Drury, S. J. Dunachie, B. S. Durham, L. Dutra, N. J. W. Easom, S. van Eck, M. Edwards, N. J. Edwards, O. M. El Muhanna, S. C. Elias, M. Elmore, M. English, A. Esmail, Y. M. Essack, E. Farmer, M. Farooq, M. Farrar, L. Farrugia, B. Faulkner, S. Fedosyuk, S. Felle, S. Feng, C. Ferreira Da Silva, S. Field, R. Fisher, A. Flaxman, J. Fletcher, H. Fofie, H. Fok, K. J. Ford, J. Fowler, P. H. A. Fraiman, E. Francis, M. M. Franco, J. Frater, M. S. M. Freire, S. H. Fry, S. Fudge, J. Furze, M. Fuskova, P. Galian-Rubio, E. Galiza, H. Garlant, M. Gavrila, A. Geddes, K. A. Gibbons, C. Gilbride, H. Gill, S. Glynn, K. Godwin, K. Gokani, U. C. Goldoni, M. Goncalves, I. G. S. Gonzalez, J. Goodwin, A. Goondiwala, K. Gordon-Quayle, G. Gorini, J. Grab, L. Gracie, M. Greenland, N. Greenwood, J. Greffrath, M. M. Groenewald, L. Grossi, G. Gupta, M. Hackett, B. Hallis, M. Hamaluba, E. Hamilton, J. Hamlyn, D. Hammersley, A. T. Hanrath, B. Hanumunthadu, S. A. Harris, C. Harris, T. Harris, T. D. Harrison, D. Harrison, T. C. Hart, B. Hartnell, S. Hassan, J. Haughney, S. Hawkins, J. Hay, I. Head, J. Henry, M. Hermosin Herrera, D. B. Hettle, J. Hill, G. Hodges, E. Horne, M. M. Hou, C. Houlihan, E. Howe, N. Howell, J. Humphreys, H. E. Humphries, K. Hurley, C. Huson, A. Hyder-Wright, C. Hyams, S. Ikram, A. Ishwarbhai, M. Ivan, P. Iveson, V. Iyer, F. Jackson, J. De Jager, S. Jaumdally, H. Jeffers, N. Jesudason, B. Jones, K. Jones, E. Jones, C. Jones, M. R. Jorge, A. Jose, A. Joshi, E. A. M. S. Júnior, J. Kadziola, R. Kailath, F. Kana, K. Karampatsas, M. Kasanyinga, J. Keen, E. J. Kelly, D. M. Kelly, D. Kelly, S. Kelly, D. Kerr, R. de Á. Kfouri, L. Khan, B. Khozoee, S. Kidd, A. Killen, J. Kinch, P. Kinch, L. D. W. King, T. B. King, L. Kingham, P. Klenerman, F. Knapper, J. C. Knight, D. Knott, S. Koleva, M. Lang, G. Lang, C. W. Larkworthy, J. P. J. Larwood, R. Law, E. M. Lazarus, A. Leach, E. A. Lees, N.-M. Lemm, A. Lessa, S. Leung, Y. Li, A. M. Lias, K. Liatsikos, A. Linder, S. Lipworth, S. Liu, X. Liu, A. Lloyd, S. Lloyd, L. Loew, R. Lopez Ramon, L. Lora, V. Lowthorpe, K. Luz, J. C. MacDonald, G. MacGregor, M. Madhavan, D. O. Mainwaring, E. Makambwa, R. Makinson, M. Malahleha, R. Malamatsho, G. Mallett, K. Mansatta, T. Maoko, K. Mapetla, N. G. Marchevsky, S. Marinou, E. Marlow, G. N. Marques, P. Marriott, R. P. Marshall, J. L. Marshall, F. J. Martins, M. Masenya, M. Masilela, S. K. Masters, M. Mathew, H. Matlebjane, K. Matshidiso, O. Mazur, A. Mazzella, H. McCaughan, J. McEwan, J. McGlashan, L. McInroy, Z. McIntyre, D. McLenaghan, N. McRobert, S. McSwiggan, C. Megson, S. Mehdipour, W. Meijs, R. N. Á. Mendonça, A. J. Mentzer, N. Mirtorabi, C. Mitton, S. Mnyakeni, F. Moghaddas, K. Molapo, M. Moloi, M. Moore, M. I. Moraes-Pinto, M. Moran, E. Morey, R. Morgans, S. Morris, S. Morris, H. C. Morris, F. Morselli, G. Morshead, R. Morter, L. Mottal, A. Moultrie, N. Moya, M. Mpelembue, S. Msomi, Y. Mugodi, E. Mukhopadhyay, J. Muller, A. Munro, C. Munro, S. Murphy, P. Mweu, C. H. Myasaki, G. Naik, K. Naker, E. Nastouli, A. Nazir, B.

Ndlovu, F. Neffa, C. Njenga, H. Noal, A. Noé, G. Novaes, F. L. Nugent, G. Nunes, K. O'Brien, D. O'Connor, M. Odam, S. Oelofse, B. Oguti, V. Olchawski, N. J. Oldfield, M. G. Oliveira, C. Oliveira, A. Oosthuizen, P. O'Reilly, P. Osborne, D. R. J. Owen, L. Owen, D. Owens, N. Owino, M. Pacurar, B. V. B. Paiva, E. M. F. Palhares, S. Palmer, S. Parkinson, H. M. R. T. Parracho, K. Parsons, D. Patel, B. Patel, F. Patel, K. Patel, M. Patrick-Smith, R. O. Payne, Y. Peng, E. J. Penn, A. Pennington, M. P. Peralta Alvarez, J. Perring, N. Perry, R. Perumal, S. Petkar, T. Philip, D. J. Phillips, J. Phillips, M. K. Phohu, L. Pickup, S. Pieterse, J. Piper, D. Pipini, M. Plank, J. Du Plessis, S. Pollard, J. Pooley, A. Pooran, I. Poulton, C. Powers, F. B. Presa, D. A. Price, V. Price, M. Primeira, P. C. Proud, S. Provstgaard-Morys, S. Pueschel, D. Pulido, S. Quaid, R. Rabara, A. Radford, K. Radia, D. Rajapaska, T. Rajeswaran, A. S. F. Ramos, F. Ramos Lopez, T. Rampling, J. Rand, H. Ratcliffe, T. Rawlinson, D. Rea, B. Rees, J. Reiné, M. Resuello-Dauti, E. Reyes Pabon, C. M. Ribiero, M. Ricamara, A. Richter, N. Ritchie, A. J. Ritchie, A. J. Robbins, H. Roberts, R. E. Robinson, H. Robinson, T. T. Rocchetti, B. P. Rocha, S. Roche, C. Rollier, L. Rose, A. L. Ross Russell, L. Rossouw, S. Royal, I. Rudiansyah, S. Ruiz, S. Saich, C. Sala, J. Sale, A. M. Salman, N. Salvador, S. Salvador, M. Sampaio, A. D. Samson, A. Sanchez-Gonzalez, H. Sanders, K. Sanders, E. Santos, M. F. S. Santos Guerra, I. Satti, J. E. Saunders, C. Saunders, A. Sayed, I. Schim van der Loeff, A. B. Schmid, E. Schofield, G. Screaton, S. Seddiqi, R. R. Segireddy, R. Senger, S. Serrano, R. Shah, I. Shaik, H. E. Sharpe, K. Sharrocks, R. Shaw, A. Shea, A. Shepherd, J. G. Shepherd, F. Shiham, E. Sidhom, S. E. Silk, A. C. da Silva Moraes, G. Silva-Junior, L. Silva-Reyes, A. D. Silveira, M. B. V. Silveira, J. Sinha, D. T. Skelly, D. C. Smith, N. Smith, H. E. Smith, D. J. Smith, C. C. Smith, A. Soares, T. Soares, C. Solórzano, G. L. Sorio, K. Sorley, T. Sosa-Rodriguez, C. M. C. D. L. Souza, B. S. D. F. Souza, A. R. Souza, A. J. Spencer, F. Spina, L. Spoors, L. Stafford, I. Stamford, I. Starinskij, R. Stein, J. Steven, L. Stockdale, L. V. Stockwell, L. H. Strickland, A. C. Stuart, A. Sturdy, N. Sutton, A. Szigeti, A. Tahiri-Alaoui, R. Tanner, C. Taoushanis, A. W. Tarr, K. Taylor, U. Taylor, I. J. Taylor, J. Taylor, R. te Water Naude, Y. Themistocleous, A. Themistocleous, M. Thomas, K. Thomas, T. M. Thomas, A. Thombrayil, F. Thompson, A. Thompson, K. Thompson, A. Thompson, J. Thomson, V. Thornton-Jones, P. J. Tighe, L. A. Tinoco, G. Tiongson, B. Tladinyane, M. Tomasicchio, A. Tomic, S. Tonks, J. Towner, N. Tran, J. Tree, G. Trillana, C. Trinham, R. Trivett, A. Truby, B. L. Tsheko, A. Turabi, R. Turner, C. Turner, M. Ulaszewska, B. R. Underwood, R. Varughese, D. Verbart, M. Verheul, I. Vichos, T. Vieira, C. S. Waddington, L. Walker, E. Wallis, M. Wand, D. Warbick, T. Wardell, G. Warimwe, S. C. Warren, B. Watkins, E. Watson, S. Webb, A. Webb-Bridges, A. Webster, J. Welch, J. Wells, A. West, C. White, R. White, P. Williams, R. L. Williams, R. Winslow, M. Woodyer, A. T. Worth, D. Wright, M. Wroblewska, A. Yao, R. Zimmer, D. Zizi, P. Zuidewind, Safety and efficacy of the ChAdOx1 nCoV-19 vaccine (AZD1222) against SARS-CoV-2: an interim analysis of four randomised controlled trials in Brazil, South Africa, and the UK. *The Lancet* 397, 99–111 (2021).

22. R. Thiruvengadam, A. Awasthi, G. Medigeshi, S. Bhattacharya, S. Mani, S. Sivasubbu, T. Shrivastava, S. Samal, D. Rathna Murugesan, B. Koundinya Desiraju, P. Kshetrapal, R. Pandey, V. Scaria, P. Kumar Malik, J. Taneja, A. Binayke, T. Vohra, A. Zaheer, D. Rathore, N. Ahmad Khan, H. Shaman, S. Ahmed, R. Kumar, S. Deshpande, C. Subramani, N. Wadhwa, N. Gupta, A. K. Pandey, J. Bhattacharya, A. Agrawal, S. Vrati, S. Bhatnagar, P. K. Garg, Effectiveness of ChAdOx1 nCoV-19 vaccine against SARS-CoV-2 infection during the delta (B.1.617.2) variant surge in India: a test-negative, case-control study and a mechanistic study of post-vaccination immune responses. *Lancet Infect Dis* 22, 473–482 (2022).

23. S. Giuntini, E. Lujan, M. M. Gibani, C. Dold, C. S. Rollier, A. J. Pollard, D. M. Granoff, Serum bactericidal antibody responses of adults immunized with the MenB-4C vaccine against genetically diverse serogroup B meningococci. *Clinical and Vaccine Immunology* 24 (2017), doi:10.1128/CVI.00430-16.

24. R. Borrow, G. M. Carlone, N. Rosenstein, M. Blake, I. Feavers, D. Martin, W. Zollinger, J. Robbins, I. Aaberge, D. M. Granoff, E. Miller, B. Plikaytis, L. van Alphen, J. Poolman, R. Rappuoli, L. Danzig, J. Hackell, B. Danve, M. Caulfield, S. L. Lambert, D. Stephens, Neisseria meningitidis group B correlates of protection and assay standardization-International Meeting Report Emory University, Atlanta, Georgia, United States, 16-17 March 2005. *Vaccine* 24, 5093–5107 (2006).

25. D. Toneatto, S. Ismaili, E. Ypma, K. Vienken, P. Oster, P. Dull, The first use of an investigational multicomponent meningococcal serogroup B vaccine (4CMenB) in humans. *Hum Vaccin* 7, 646–653 (2011).

26. T. Nolan, M. E. Santolaya, F. de Looze, H. Marshall, P. Richmond, S. Henein, P. Rheault, K. Heaton, K. P. Perrett, H. Garfield, A. Gupta, M. Ferguson, D. D'Agostino, D. Toneatto, M. O'Ryan, Antibody persistence and booster response in adolescents and young adults 4 and 7.5 years after immunization with 4CMenB vaccine. *Vaccine* 37, 1209–1218 (2019).

27. R. Pajon, P. T. Beernink, L. H. Harrison, D. M. Granoff, Frequency of factor H-binding protein modular groups and susceptibility to cross-reactive bactericidal activity in invasive meningococcal isolates. *Vaccine* 28, 2122–2129 (2010).

28. A. J. Pollard, E. M. Bijker, A guide to vaccinology: from basic principles to new developments. *Nat Rev Immunol* 21, 83–100 (2021).

29. R. Lakshman, A. Finn, Meningococcal serogroup C conjugate vaccine. *Expert Opin Biol Ther* 2, 87–96 (2002).

30. D. F. Kelly, M. D. Snape, E. A. Clutterbuck, S. Green, C. Snowden, L. Diggle, L. Yu, A. Borkowski, E. R. Moxon, A. J. Pollard, CRM197-conjugated serogroup C meningococcal capsular polysaccharide, but not the native polysaccharide, induces persistent antigen-specific memory B cells. *Blood* 108, 2642–2647 (2006).

31. A. J. Pollard, R. Galassini, E. M. Rouppe van der Voort, M. Hibberd, R. Booy, P. Langford, S. Nadel, C. Ison, J. S. Kroll, J. Poolman, M. Levin, Cellular Immune Responses to *Neisseria meningitidis* in Children. *Infect Immun* 67, 2452–2463 (1999).

32. A. C. Cruz, B. S. Figueredo, S. L. Souza, M. C. Rebelo, A. G. A. Carvalho, L. G. Milagres, Antibody and memory CD4+ T-cell responses after meningococcal disease. *Vaccine* 32, 5145–5148 (2014).

33. I. Goldschneider, E. C. Gotschlich, M. S. Artenstein, Human immunity to the meningococcus. I. The role of humoral antibodies. *J Exp Med* 129, 1307–1326 (1969).

34. P. Gonin, C. Gaillard, Gene transfer vector biodistribution: pivotal safety studies in clinical gene therapy development. *Gene Ther* 11, S98–S108 (2004).

35. N. Tatsis, J. C. Fitzgerald, A. Reyes-Sandoval, K. C. Harris-McCoy, S. E. Hensley, D. Zhou, S.-W. Lin, A. Bian, Z. Q. Xiang, A. Iparraguirre, C. Lopez-Camacho, E. J. Wherry, H. C. J. Ertl, Adenoviral vectors persist in vivo and maintain activated CD8+ T cells: implications for their use as vaccines. *Blood* 110, 1916–1923 (2007).

36. J. R. Greenland, R. Geiben, S. Ghosh, W. A. Pastor, N. L. Letvin, Plasmid DNA Vaccine-Elicited Cellular Immune Responses Limit In Vivo Vaccine Antigen Expression through Fas-Mediated Apoptosis. *The Journal of Immunology* 178, 5652–5658 (2007).

37. R. Geiben-Lynn, J. R. Greenland, K. Frimpong-Boateng, N. L. Letvin, Kinetics of Recombinant Adenovirus Type 5, Vaccinia Virus, Modified Vaccinia Ankara Virus, and DNA Antigen Expression In

Vivo and the Induction of Memory T-Lymphocyte Responses. *Clinical and Vaccine Immunology* 15, 691–696 (2008).

38. J. R. Barrett, S. Belij-Rammerstorfer, C. Dold, K. J. Ewer, P. M. Folegatti, C. Gilbride, R. Halkerston, J. Hill, D. Jenkin, L. Stockdale, M. K. Verheul, P. K. Aley, B. Angus, D. Bellamy, E. Berrie, S. Bibi, M. Bittaye, M. W. Carroll, B. Cavell, E. A. Clutterbuck, N. Edwards, A. Flaxman, M. Fuskova, A. Gorringe, B. Hallis, S. Kerridge, A. M. Lawrie, A. Linder, X. Liu, M. Madhavan, R. Makinson, J. Mellors, A. Minassian, M. Moore, Y. Mujadidi, E. Plested, I. Poulton, M. N. Ramasamy, H. Robinson, C. S. Rollier, R. Song, M. D. Snape, R. Tarrant, S. Taylor, K. M. Thomas, M. Voysey, M. E. E. Watson, D. Wright, A. D. Douglas, C. M. Green, A. V. S. Hill, T. Lambe, S. Gilbert, A. J. Pollard, J. Aboagye, J. Alderson, A. Ali, E. Allen, L. Allen, R. Anslow, C. V. Arancibia-Cárcamo, E. H. Arbe-Barnes, M. Baker, P. Baker, N. Baker, I. Baleanu, E. Barnes, L. Bates, A. Batten, K. Beadon, R. Beckley, A. Beveridge, K. R. Bewley, E. M. Bijker, L. Blackwell, C. L. Blundell, E. Bolam, E. Boland, N. Borthwick, A. Boyd, T. Brenner, P. Brown, C. Brown-O'Sullivan, E. Brunt, J. Burbage, K. R. Buttigieg, N. Byard, I. Cabrera Puig, S. Camara, M. Cao, F. Cappuccini, M. Carr, M. W. Carroll, J. Chadwick, I. Chelysheva, J.-S. Cho, L. Cifuentes, E. Clark, R. Colin-Jones, C. P. Conlon, N. S. Coombes, R. Cooper, W. E. M. Crocker, C. J. Cunningham, B. E. Damratoski, M. S. Datoo, C. Datta, H. Davies, T. Demissie, C. Di Maso, D. DiTirro, T. Dong, F. R. Donnellan, N. Douglas, C. Downing, J. Drake, R. Drake-Brockman, R. E. Drury, S. J. Dunachie, O. El Muhanna, S. C. Elias, M. J. Elmore, K. R. W. Emary, M. R. English, S. Felle, S. Feng, C. F. Da Silva, S. Field, R. Fisher, K. J. Ford, J. Fowler, E. Francis, J. Frater, J. Furze, P. Galian-Rubio, H. Garlant, K. Godwin, G. Gorini, L. Gracie, G. Gupta, E. Hamilton, J. Hamlyn, B. Hanumunthadu, S. A. Harris, D. Harrison, T. C. Hart, S. Hawkins, J. A. Henry, G. Hodges, S. H. C. Hodgson, M. M. Hou, E. Howe, N. Howell, B. Huang, H. Humphries, P. Iveson, S. Jackson, F. Jackson, S. Jauregui, K. Jeffery, E. Jones, K. Jones, R. Kailath, J. Keen, S. Kelly, D. Kelly, E. Kelly, D. Kerr, L. Khan, B. Khozoee, A. Killen, J. Kinch, T. B. King, L. King, L. Kingham-Page, P. Klenerman, J. C. Knight, D. Knott, S. Koleva, C. W. Larkworthy, J. P. J. Larwood, E. A. Lees, A. Lelliott, S. Leung, Y. Li, A. M. Lias, S. Lipworth, S. Liu, L. Loew, R. Lopez Ramon, G. Mallett, K. Mansatta, N. G. Marchevsky, S. Marinou, E. Marlow, J. L. Marshall, P. Matthews, J. McEwan, J. McGlashan, L. McInroy, G. Meddaugh, A. J. Mentzer, N. Mirtorabi, E. Morey, R. Morgans, S. J. Morris, H. Morrison, G. Morshead, R. Morter, N. Moya, E. Mukhopadhyay, J. Muller, C. Munro, S. Murphy, P. Mweu, A. Noé, F. L. Nugent, E. Nuthall, K. O'Brien, D. O'Connor, D. O'Donnell, B. Oguti, V. Olchawski, C. Oliveria, P. J. O'Reilly, P. Osborne, N. Owino, K. Parker, H. Parracho, M. Patrick-Smith, Y. Peng, E. Penn, M. P. Peralta Alvarez, J. Perring, C. Petropoulos, K. Pfafferott, D. Pipini, D. Phillips, P. Proud, S. Provstgaard-Morys, D. Pulido, K. Radia, D. Rajapaksa, F. Ramos Lopez, H. Ratcliffe, T. Rawlinson, E. R. Pabon, S. Rhead, A. J. Ritchie, H. Roberts, S. Roche, I. Rudiansyah, S. Salvador, H. Sanders, K. Sanders, I. Satti, A. Schmid, E. Schofield, G. Screaton, C. Sedik, I. Shaik, H. R. Sharpe, A. Shea, S. Silk, L. Silva-Reyes, D. T. Skelly, C. C. Smith, D. J. Smith, A. J. Spencer, E. Stafford, A. Szigeti, A. Tahiri-Alaoui, R. Tanner, I. J. Taylor, K. Taylor, R. te Water Naude, Y. Themistocleous, A. Themistocleous, M. Thomas, T. M. Thomas, A. Thompson, L. Tinh, A. Tomic, S. Tonks, J. Towner, N. Tran, J. A. Tree, A. Truby, C. Turner, N. Turner, M. Ulaszewska, R. Varughese, I. Vichos, L. Walker, M. Wand, C. White, R. White, P. Williams, A. T. Worth, T. Wrin, X. L. Yao, D. Zizi, Phase 1/2 trial of SARS-CoV-2 vaccine ChAdOx1 nCoV-19 with a booster dose induces multifunctional antibody responses. *Nat Med* 27, 279–288 (2021).

39. I. B. Nwogu, M. Jones, T. Langley, Economic evaluation of meningococcal serogroup B (MenB) vaccines: A systematic review. *Vaccine* 39, 2201–2213 (2021).

40. I. L. Leeds, V. Namasivayam, A. Bamogo, P. Sankhla, W. M. Thayer, Cost Effectiveness of Meningococcal Serogroup B Vaccination in College-Aged Young Adults. *Am J Prev Med* 56, 196–204 (2019).

41. S. Bambini, A. Muzzi, P. Olcen, R. Rappuoli, M. Pizza, M. Comanducci, Distribution and genetic variability of three vaccine components in a panel of strains representative of the diversity of serogroup B meningococcus. *Vaccine* 27, 2794–2803 (2009).

42. Center for Biologics Evaluation and Research, *Toxicity Grading Scale for Healthy Adult and Adolescent Volunteers Enrolled in Preventive Vaccine Clinical Trials FDA-2005-D-0272* (Silver Spring, 2007).

43. J. Lucidarme, J. Louth, K. Townsend-Payne, R. Borrow, in (2019), pp. 169–179.

44. J. Findlow, R. Borrow, M. D. Snape, T. Dawson, A. Holland, T. M. John, A. Evans, K. L. Telford, E. Ypma, D. Toneatto, P. Oster, E. Miller, A. J. Pollard, Multicenter, Open-Label, Randomized Phase II Controlled Trial of an Investigational Recombinant Meningococcal Serogroup B Vaccine With and Without Outer Membrane Vesicles, Administered in Infancy. *Clinical Infectious Diseases* 51, 1127–1137 (2010).

45. M. D. Snape, T. Dawson, P. Oster, A. Evans, T. M. John, B. Ohene-Kena, J. Findlow, L.-M. Yu, R. Borrow, E. Ypma, D. Toneatto, A. J. Pollard, Immunogenicity of two investigational serogroup B meningococcal vaccines in the first year of life: a randomized comparative trial. *Pediatr Infect Dis J* 29, e71–e79 (2010).

46. J. Findlow, X. Bai, H. Findlow, E. Newton, E. Kaczmarski, E. Miller, R. Borrow, Safety and immunogenicity of a four-component meningococcal group B vaccine (4CMenB) and a quadrivalent meningococcal group ACWY conjugate vaccine administered concomitantly in healthy laboratory workers. *Vaccine* 33, 3322–3330 (2015).

47. C. S. Rollier, C. Dold, L. Blackwell, A. Linder, L. Silva-Reyes, E. Clutterbuck, K. Davis, K. Ford, X. Liu, A. Holland, H. Chan, H. Harbinson, D. O'Connor, R. Borrow, M. D. Snape, A. J. Pollard, Immunogenicity of a single 4CMenB vaccine booster in adolescents 11 years after childhood immunisation. *Vaccine* 40, 4453–4463 (2022).

Acknowledgements:

We would like to thank our active Data and Safety Monitoring Committee chaired by R. Heyderman and including A. Finn, A. Riordan, and H. Watt (statistician). The authors thank I. M. Feavers and M. C. J. Maiden for support and intellectual input including in funding applications. AVSH, AJP and CSR are Jenner Investigators. CSR is supported by the Equal Opportunities Foundation (Hong Kong), the Braithwaite Family Foundation, and the Bill and

Melinda Gates Foundation. BO is an Academic Clinical Fellow (NIHR unique award identifier: ACF-2020-18-018), funded in partnership by the NIHR and University College London. The views expressed in this publication are those of the authors and not necessarily those of the NIHR, contributing universities, NHS or the UK Department of Health and Social Care.

Funding:

This clinical trial was supported by Medical Research Council (MRC) grant DPFS-MR/M007693/1 (to CSR, AJP, and CD) and the National Institute of Healthcare Research (NIHR) / British Research Council (BRC). The serum standard arm of the trial was funded by the National Institute for Biological Standards and Control (NIBSC).

Author Contributions:

CSR, CD, AVH, and AJP conceptualized the study. AJP and CSR generated the funding. CD, CSR, and AJP led the investigations. CSR, CD, and BO developed the methodologies. The immunogenicity experiments, except for the SBAs, were performed by CD and LSR, as well as AS (ELISA). CD supervised all laboratory work (except for the SBAs). BO, MR, CCS, MM, and AB, led and delivered the clinical part of the work. Project management and administration was performed by EMC and EP. KT and XL led and performed the statistical analysis of the safety and reactogenicity (primary outcome). RB and his team, including J Louth, AH, RC, and JC led and performed the SBAs. RB and J Lucidarme advised and selected the panel of heterologous strains. Visualization of the results was performed by CD, BO, KT, LSR. CSR, and BO. CD wrote the paper. CD, BO, KS, PTB, AJP, and CSR edited the paper. All authors reviewed the paper.

Competing Interests:

AJP is Chair of the UK DHSC Joint Committee on Vaccination & Immunisation (JCVI). AJP waives his rights under any patent. PTB is a named inventor on patents related to meningococcal vaccines (Issued U.S. Patent No. 10,995,122 B2). AVSH is named as an inventor on a patent covering use of particular simian adenoviral-vectored vaccines (Simian adenovirus and hybrid adenoviral vectors US20150044766A1, WO2012172277A1). PTB has performed paid consultancy work for Pfizer. CSR has performed paid consultancy for Guidepoint. CD, CSR, AJP, AVSH, BO, XL, EP and MM are contributors to intellectual property licensed by Oxford University Innovation to AstraZeneca limited to COVID-19 applications. AJP waives his rights under this agreement. RB, AH, J Louth, and J Lucidarme perform contract research on behalf of UKHSA for GSK, PATH, Pfizer, and Sanofi. CD is currently employed by and holds stocks from Moderna, Inc. All other co-authors declare no conflict of interest.

Data and Materials Availability:

All data associated with this study are in the paper or supplementary materials.

Figure legends

Figure 1. CONSORT Diagram for the primary safety analysis. LD indicates low dose ($2.5x10^{10}$ vp); SD indicates standard dose ($5x10^{10}$ vp). 4CMenB and rLP2086 were administered as per product specifications. Gray boxes indicate the two groups added to generate serum standards. The numbers in parentheses in the allocation boxes indicate the interval between two doses in months. N/A, not applicable.

Figure 2. **Reported AEs from the day of vaccination until 6 days after vaccination**. AEs are reported for all trial arms. **(A)** Frequency of solicited local and systemic reactions on days 0 to 6 following the first dose of vaccination. **(B)** Frequency of solicited local and systemic reactions on days 0 to 6 following booster dose vaccination. ChAd, ChAdOx1 MenB.1; LD, low dose ($2.5x10^{10}$ vp); SD, standard dose ($5x10^{10}$ vp). Bars represent the percentage of participants with the reaction mentioned on the top, and the color indicates the severity of the reaction as indicated in the legend. *Rash was measured as a binary variable and color does not indicate severity.

Figure 2. ChAdOx1 MenB.1 induces SBA responses against the MenB strain 44/76-SL containing a homologous fHbp 1.1. (A) Shown are individual SBA titers in participants in each group of the trial as indicated at day 0 prior to vaccine (D0) and at days 28, 56, 180, 208 and 365 following the first vaccine dose. Each dot represents an individual. The number of individuals assessed at each time point is provided in table S3. Single tests were run for each sample, but multiple repeats were performed depending on the sample. The putative protective threshold titer of $\geq$ 1:4 is indicated by the horizontal red dotted line. Boxes and vertical lines indicate geometric means and 95% confidence intervals, respectively. **(B)** Proportion of participants with an SBA titer $\geq$1:4 at the indicated time points following first vaccination. Error bars show 95% confidence interval (CI). **(C)** Proportion of participants with a 4-fold increase in SBA titers at the indicated time points following first vaccination. Each dot represents an individual. The horizontal red dotted line indicates a value of 1 (no change). Boxes and vertical lines indicate geometric means and the 95% confidence intervals, respectively. The number of samples per group at day 0 are: ChAd LD, n = 3; ChAd SD, n = 10; ChAd-ChAd, n = 8; 4CMenB-ChAd, n = 8; 4CMenb-4CMenb(1), n = 10; 4CMenb-4CMenB (6), n = 8; and rLP2086-rLP2086, n = 10. Some of the samples at further time points were not collected, as some participants did not continue with the study.

Figure 4. ChAdOx1 MenB.1 did not elicit robust cross-reactive SBA responses following vaccination. (A) Individual SBA titers against each of the four strains indicated on the x-axis are shown for samples collected at the indicated time points from participants in each group of the trial. Each dot represents an individual. The number of individuals assessed at each time point is provided in table S3. Single tests were run for each sample, but multiple repeats were performed depending on the sample. The putative protective threshold titer of $\geq$ 1:4 is indicated by the horizontal red dotted lines. Boxes and vertical lines indicate **3**geometric means and 95% CIs, respectively. **(B)** Shown is the proportion of participants with an SBA titer $\geq$1:4 against the indicated strain at each time point. Error bars indicate 95% CIs.

Figure 5. ChAdOx1 MenB.1 elicits Th1-biased cytokine production and fHbp-specific IFN-γ secreting T cell responses in peripheral blood samples following vaccination. (A) Plasma cytokines indicated on top of the graphs were measured immediately prior to and 24 hours post the first vaccination with ChAdOx1 MenB.1 (blue, n = 18) or 4CMenB (gold, n = 25). **(B)** Plasma cytokines indicated on top of the graphs were measured immediately prior to and 24 hours after the second vaccination for participants that received two doses of ChAdOx1 MenB.1 (cyan, n = 6), two doses of 4CMenB (yellow, n = 5), or 4CMenB followed by ChAdOx1 MenB.1 (magenta, n = 7). Each dot represents an individual participant. The horizontal red line indicates the lower limit of detection and the horizontal black line indicates the lower limit of quantification. Data are presented as individual results, and the median ± interquartile range per group and time point. Significant differences between 24 hours post and prior to injection were measured using mixed-effects analysis with Sidak's multiple comparisons test. **(C)** IFN-γ secreting T cell responses were measured prior to and at the indicated time points after vaccination. Each dot represents an individual participant. The boxplots correspond to the median values with the first and third quartile, and whiskers show the highest and lowest values no more than 1.5 times the interquartile range from the corresponding edge of the box.

Tables

Table 1. Study Groups and Vaccination Schedules. LD indicates low dose ($2.5x10^{10}$ vp); SD indicates standard dose ($5x10^{10}$ vp). 4CMenB and rLP2086 were administered as per product specifications.

Study Group	Day 02	Day 28	Day 180
1	ChAdOx1 MenB.1 LD	-	-
2	ChAdOx1 MenB.1 SD	-	-
3	ChAdOx1 MenB.1 SD	-	ChAdOx1 MenB.1 SD
4	4CMenB	-	ChAdOx1 MenB.1 SD
5	4CMenB	4CMenB	-
6	4CMenB	-	4CMenB
7	4CMenB	-	4CMenB
8	rLP2086	-	rLP2086

Table 2. Summary of participant demographic characteristics. LD indicates low dose ($2.5x10^{10}$ vp); SD indicates standard dose ($5x10^{10}$ vp). 4CMenB and rLP2086 were administered as per product specifications. The numbers in parentheses in the top row indicate the interval in months between two doses.

		ChAd LD	ChAd SD	ChAd-ChAd(6)	4CMenB-ChAd(6)	4CMenB-4CMenB(1)	4CMenB-4CMenB(6)	4CMenB-4CMenB(6)	rLP2086-rLP2086(6)
		N=3	N=10	N=8	N=8	N=10	N=9	N=19	N=21
Age		21.0 (20.6-32.7)	29.0 (26.4-42.2)	29.2 (23.6-37.0)	28.8 (25.1-43.5)	23.8 (23.5-29.0)	26.1 (25.1-27.4)	30.4 (26.7-42.9)	28.7 (27.3-34.5)
Body mass index		25.5 (12.7-26.9)	22.8 (0.0-25.0)	22.5 (14.1-25.5)	24.3 (22.1-25.9)	22.9 (5.6-26.8)	19.8 (0.0-21.8)	23.5 (21.8-25.8)	23.4 (20.7-26.1)
Gender	Female	0 (0.0%)	4 (40.0%)	6 (75.0%)	5 (62.5%)	4 (40.0%)	7 (77.8%)	13 (68.4%)	14 (66.7%)
	Male	3 (100.0%)	6 (60.0%)	2 (25.0%)	3 (37.5%)	6 (60.0%)	2 (22.2%)	6 (31.6%)	7 (33.3%)
Ethnicity	Mixed	0 (0.0%)	0 (0.0%)	0 (0.0%)	1 (12.5%)	1 (10.0%)	0 (0.0%)	0 (0.0%)	1 (4.8%)
	Other	0 (0.0%)	0 (0.0%)	2 (25.0%)	1 (12.5%)	1 (10.0%)	0 (0.0%)	1 (5.3%)	0 (0.0%)
	White	3 (100.0%)	10 (100.0%)	6 (75.0%)	6 (75.0%)	8 (80.0%)	9 (100.0%)	18 (94.7%)	20 (95.2%)

Table 3. Summary of AEs and SAEs. LD indicates low dose ($2.5x10^{10}$ vp); SD indicates standard dose ($5x10^{10}$ vp). 4CMenB and rLP2086 were administered as per product specifications. The numbers in parentheses in the top row indicate the interval in months between two doses.

		ChAd LD	ChAd SD	ChAd-ChAd(6)	4CMenB-ChAd(6)	4CMenB-4CMenB(1)	4CMenB-4CMenB(6)	4CMenB-4CMenB(6)	rLP2086-rLP2086(6)
		N=3	N=10	N=8	N=8	N=10	N=9	N=19	N=21
No. of AEs		28	67	63	56	36	62	24	60
No. of participants		3	10	8	7	8	8	12	15
Severity	Mild	15	41	38	34	28	35	11	23
	Moderate	10	16	16	15	4	20	9	24
	Severe	2	6	5	4	3	5	1	11
	Emergency or Hospital visit	1	2	3	3	1	2	0	0
	Unknown	-	2	1	-	-	-	3	2
Relation to administered vaccine	No relationship	18	35	39	39	31	50	14	25
	Possibly related	6	23	20	10	2	9	3	12
	Probably related	0	7	3	5	2	2	3	12
	Definitely related	4	2	1	2	1	1	4	11
Duration of AE (days)	Median (Q1, Q3)	7 (1, 28)	7 (1, 34.75)	7 (4.5, 19.25)	7.5 (5.25, 27)	14 (5.5, 77)	13 (1.25, 28)	28 (14, 184.5)	6 (11, 14)
	No. of missing	-	5	1	2	5	8	13	11
Post-first dose		28	67	42	35	20	46	11	29
Post-second dose		0	0	21	21	16	16	13	31
SAE		0	0	1	1	1	0	0	0

Table 4. Characteristics of the MenB Strains used to assess SBA responses in relation with the fHbp variants contained in each study vaccine. VR, variable region; [a]Pfizer classification; [b]phase variable NadA allele in off state; [c]NadA gene inactivated by insertion sequence 1301. The ChAdOx1 MenB.1 vaccine contains fHbp variant 1 peptide 1 only (1.1); 4CMenB contains PorA-VR2 1.4, fHbp 1.1, NadA 3.8 and NHBA peptide 2; rLP2086 contains fHbp variants 1.55 and 3.45. n/a, not applicable.

Isolate	Country	Sequence type	Clonal Complex	PorA		fHbp			NadA		NHBA
				VR1	VR2	Variant	Peptide	Alternative Classification[a]	Variant	Peptide	Peptide
44/76-SL	Norway	32	ST-32	7	16	1	1	B24	n/a	0	3
M01 240355	UK	213	ST-213	22	14	3	31	A47	4/5	0[b]	18
M01 240101	UK	1049	ST-269	19-1	15-11	1	15	B44	n/a	0	21
M01 240185	UK	11	ST-11	5-1	10-8	1	10	B64	2/3	0[c]	20
M00 242922	UK	41	ST-41/44	7-2	4	1	4	B16	n/a	0	2
M15 240912	UK	1161	ST-269	22	9	1	13	B09	n/a	0	17

1