TITLE: NEISSERIA GONORRHOEAE VACCINES – A CONTEMPORARY OVERVIEW

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SUMMARY

Neisseria gonorrhoeae infection is an important public health issue, with an annual global incidence of 87 million. *N. gonorrhoeae* infection causes significant morbidity and can have serious long-term impacts on reproductive and neonatal health and may rarely cause life-threatening disease. Global rates of *N. gonorrhoeae* infection have increased over the past 20 years. Importantly, rates of antimicrobial resistance to key antimicrobials also continue to increase, with the United States Centers for Disease Control and Prevention identifying drug-resistant *N. gonorrhoeae* as an urgent threat to public health. This review summarises the current evidence for *N. gonorrhoeae* vaccines, including historical clinical trials, key *N. gonorrhoeae* vaccine preclinical studies and studies of the impact of *Neisseria meningitidis* vaccines on *N. gonorrhoeae* infection. A comprehensive survey of potential vaccine antigens, including those identified through traditional vaccine immunogenicity approaches, as well as those identified using more contemporary reverse vaccinology approaches are

also described. Finally, the potential epidemiological impacts of a *N. gonorrhoeae* vaccine and research priorities for further vaccine development are described.

INTRODUCTION

Epidemiology and Clinical Manifestations of *Neisseria gonorrhoeae* Infection

Infection with *Neisseria gonorrhoeae* is an important public health issue, with an estimated annual global incidence of 87 million (1). Reported global rates of *N. gonorrhoeae* infection have significantly increased over the past 20 years (1, 2). In the United States (US), rates of *N. gonorrhoeae* infection increased 111% between 2009 and 2020 (3); in Europe, rates increased by 218% between 2009 and 2018 (4); while in Australia, rates increased 127% between 2012 and 2019 (5). *N. gonorrhoeae* infection disproportionately affects vulnerable populations, with over 90% of cases occurring in low- and middle-income (LMIC) settings (1). Within high-income countries, *N. gonorrhoeae* infection is more prevalent in certain populations, including men who have sex with men (MSM) (6, 7), transgender persons, sex workers, racial/ethnic minorities and indigenous populations (8).

N. gonorrhoeae infection causes a wide range of disease, including symptomatic urogenital disease, asymptomatic mucosal infection and infrequently, disseminated gonococcal infection (9). Urogenital infection most commonly manifests as lower genital tract infection, usually presenting as purulent anterior urethritis in men, and as cervicitis in women (10). Up to 40% of cases of urogenital *N. gonorrhoeae* infections in women are asymptomatic (11, 12). If urogenital infection is not diagnosed and treated early, severe sequelae can ensue. In women, infection can ascend to the upper genital tract to cause salpingitis and pelvic inflammatory disease. Tubal infection can result in ectopic pregnancy and infertility, and

97 infection during pregnancy is associated with preterm birth and low birthweight (9, 10, 13).
98 Neonatal infection most commonly presents as opthalmia neonaturm, a purulent
99 conjunctivitis that may result in blindness (14). In men, ascending infection can cause
100 epididymitis, and untreated infection may result in male infertility and urethral strictures
101 (15, 16). Extragenital mucosal infections in the oropharynx, rectum and conjunctiva also
102 occur. Oropharyngeal and rectal *N. gonorrhoeae* infections are more prevalent than urethral
103 infections in certain high-risk populations, such as MSM in high-income settings, where
104 regular asymptomatic screening with nucleic acid amplification testing (NAAT) at multiple
105 anatomical sites is recommended (17). While infections of the oropharynx and rectum are
106 often asymptomatic (18), they may represent a significant reservoir for *N. gonorrhoeae*
107 transmission (19). Manifestations of disseminated gonococcal infection include purulent
108 arthritis, tenosynovitis, dermatitis, polyarthritis and osteomyelitis. Rare life-threatening
109 complications of *N. gonorrhoeae* infection include meningitis and endocarditis (20). *N.*
110 *gonorrhoeae* infection also promotes the transmission and susceptibility to human
111 immunodeficiency virus (HIV) by causing local inflammation (21).
112
113 Importantly, resistance to all prior and currently recommended antimicrobials for treatment
114 of *N. gonorrhoeae* has been described (22). *N. gonorrhoeae* has the ability to develop
115 antimicrobial resistance (AMR) through numerous mechanisms (22). Consequently, the
116 World Health Organization (WHO) and the US Centers for Disease Control and Prevention
117 (CDC) have identified antimicrobial-resistant *N. gonorrhoeae* as an urgent threat to public
118 health (23, 24). *N. gonorrhoeae* has therefore been classified as a high priority pathogen on
119 the WHO *Global Priority List of Antibiotic-Resistant Bacteria to Guide Research, Discovery*
120 *and Development of New Antibiotics* (23). The first case of treatment failure due to an

extensively-drug-resistant (XDR) *N. gonorrhoeae* strain (resistant to both current first-line antimicrobials, ceftriaxone and azithromycin) was reported in the United Kingdom (UK) in 2016 (25); XDR *N. gonorrhoeae* with high-level resistance to both ceftriaxone and azithromycin has now been reported in the UK and Australia (26, 27). These cases demonstrate the growing global threat of untreatable *N. gonorrhoeae* infection. A number of novel gonococcal antimicrobial therapies have recently been tested in phase two and three trials, including solithromycin, zoliflodacin and gepotidacin. These studies have demonstrated several limitations of these new anti-gonococcal antimicrobials (28); in brief, a randomised trial found solithromycin to be inferior to standard-of-care dual ceftriaxone and azithromycin therapy (29); the efficacy of zoliflodacin was suboptimal for pharyngeal infection (30); and current data on the performance of gepotidacin for extragenital infections is sparse (31). As the spectre of untreatable *N. gonorrhoeae* infection looms, preventative strategies that overcome the extraordinary ability of *N. gonorrhoeae* to evade killing by antimicrobial therapy are therefore urgently required.

The Need for a *Neisseria gonorrhoeae* Vaccine

An effective and accessible *N. gonorrhoeae* vaccine could have a wide range of benefits, including: i) reduction of the individual and healthcare impact of urogenital infection; ii) improvement in reproductive and neonatal health; iii) reduction of individual and population antimicrobial usage and the unintended consequences arising from this, including the potential to drive further *N. gonorrhoeae* antimicrobial resistance; and iv) reduction in the healthcare costs associated with frequent screening for *N. gonorrhoeae* infection in asymptomatic individuals. However, there are multiple significant barriers to the development of a *N. gonorrhoeae* vaccine, including i) antigenic and phase variation of

potential vaccine targets; ii) the absence of protective immunity following natural infection; iii) the lack of a known immune correlate of protection; and iv) exclusive human host restriction, with limited appropriate animal models of infection (32). Encouragingly, the successes of vaccines for other sexually-transmitted infections (STIs) such as human papillomavirus (HPV), hepatitis A virus (HAV) and hepatitis B virus (HBV) (33), as well as closely-related pathogens, such as *Neisseria meningitidis,* have paved the way for further progress in *N. gonorrhoeae* vaccine development (34).

The development and implementation of safe and efficacious vaccines for HPV, HAV and HBV has had a significant impact on the incidence and resulting complications of these diseases (33). These successes have provided additional motivation for the development of new STI vaccines. In 2014, the WHO and National Institutes of Health (NIH) announced a comprehensive roadmap to accelerate the STI vaccine development (35). This roadmap comprised of nine areas of focus, including obtaining improved epidemiological data, modelling vaccine impact, accelerating basic science research, outlining preferred product characteristics and encouraging investment (35, 36). The WHO subsequently assembled a panel of international experts to define the potential public health value of and preferred product characteristics of a *N. gonorrhoeae* vaccine to inform vaccine development (37, 38). The WHO Global Health Sector Strategy on STIs has set a target of a 90% reduction in worldwide *N. gonorrhoeae* infection incidence by 2030. Given the rising incidence of *N. gonorrhoeae* infection worldwide and the limitations of current preventative interventions, this WHO strategy highlights *N. gonorrhoeae* vaccine development as a priority innovation to support this ambitious aim (39).

In this review, we examine the evidence for a *N. gonorrhoeae* vaccine, including i) historical clinical trials; ii) key *N. gonorrhoeae* vaccine preclinical studies; iii) observational and randomised studies of the impact of *N. meningitidis* vaccines on *N. gonorrhoeae* infection and iv) clinical trials currently underway. In addition, we present a comprehensive survey of potential vaccine antigens, including those identified through traditional vaccine immunogenicity approaches, as well as those identified using more contemporary approaches, such as bioinformatics, transcriptomics and proteomics. Finally, we review the potential epidemiological impacts of a *N. gonorrhoeae* vaccine, and outline research priorities for *N. gonorrhoeae* vaccine development.

References for this review were identified on the basis of the topics described above, with literature search conducted through PubMed and ClinicalTrials.gov. The websites of the WHO and US CDC were also reviewed and an online search engine was used to access press releases, conference abstracts and commercial information. Search terms included, “gonorrhoea*”, "gonococcal”, “Neisseria”, “vaccine”, “antigen”, “meningococcal”, “outer membrane vesicle”, “OMV”, “model*”, “impact”, “cost” and “economic”. In addition, a search was undertaken for each vaccine antigen listed in column 1 of Table 2. Relevant articles published between Jan 1, 1900 and March 1, 2023 were included. Articles published in English resulting from these searches and their relevant references were reviewed.

NEISSERIA GONORRHOEAE VACCINE CHALLENGES

A number of obstacles have impeded progress towards the development of an effective vaccine against *N. gonorrhoeae* (32). First, *N. gonorrhoeae* demonstrates significant surface antigen variability, such that key surface antigens have variable genomic sequences and

193 protein composition (antigenic variation) and/or change their protein expression (through
194 phase variation). Second, there is no epidemiologic evidence that *N. gonorrhoeae* infection
195 results in protective immunity against recurrent infections; indeed, repeated infections are
196 relatively common in high-risk populations. Third, given the lack of protective immunity
197 against reinfection, it has not been possible to define correlates of immunity that can be
198 measured using immunologic methods (32).
199
200 As an exclusive human pathogen, establishment of an appropriate animal infection model to
201 study the pathogenesis and preclinical immune responses to *N. gonorrhoeae* infection and
202 vaccines has been difficult. A 17-β-estradiol-treated mouse model (40, 41), using inbred
203 mice and recently modified by the use of transgenic mice with additional human host-cell
204 receptors such as human carcinoembryonic antigen cellular adhesion molecules (42, 43) and
205 human transferrin (44) or supplementation of inbred mice with human transferrin (45, 46)
206 have partially overcome this host-specific barrier. Although chimpanzees were also used in
207 early infection models (47), they are no longer available or ethically appropriate for this
208 work. A number of experimental systems have been used to assist drug development,
209 however these models are not appropriate for vaccine development. These include a hollow
210 fiber infection model that is well suited to characterize the pharmacodynamic and
211 pharmacokinetic responses of novel antimicrobials for treatment of *N. gonorrhoeae* (48,
212 49), and an invertebrate *Galleria mellonella* greater wax moth of gonococcal infection (50).
213 These latter models, however, lack the essential host immunity components required to test
214 gonococcal vaccines.
215

A *N. gonorrhoeae* male urethritis controlled human infection model (CHIM) was developed by investigators at Walter Reed Army Institute of Research and the University of North Carolina at Chapel Hill in the US in the 1980s (51). Over 200 individuals have participated in *N. gonorrhoeae* urethritis CHIM studies. These studies have been reviewed for safety and compliance with modern ethical standards, and have been undertaken without serious or unexpected adverse events (51). There are a number of advantages to a *N. gonorrhoeae* CHIM, compared to alternative study designs. In particular, compared to animal studies, the model not only assesses microbiological outcomes, but also clinical disease and immune responses. In addition, CHIM studies provide a model that has the power to test for statistically significant vaccine efficacy in a much smaller study population (<100 participants) compared to an efficacy trial conducted in a population with high risk for gonorrhoea infection (>1000 participants)(52). Notably, the only *N. gonorrhoeae* CHIM that is currently available is a male urethritis model, which could limit the generalizability of vaccine efficacy findings to oropharyngeal, rectal and cervical *N. gonorrhoeae* infections (52). *N. gonorrhoeae* male urethritis CHIM studies have already advanced the understanding of the complex pathogenesis and immune responses to *N. gonorrhoeae* infection (51). As promising vaccine candidates become available, *N. gonorrhoeae* CHIM studies may offer a safe and effective model for testing these novel vaccines, particularly if models of extragenital infection, such as an oropharyngeal *N. gonorrhoeae* CHIM become available (52).

Although these obstacles may have slowed the progress of *N. gonorrhoeae* vaccines, evidence suggesting partial effectiveness of the *N. meningitidis* serogroup B outer membrane vesicle (OMV) vaccines against *N. gonorrhoeae* infection (34, 53-62) has

reinvigorated the field, with an increased international focus on the development an effective *N. gonorrhoeae* vaccine.

HISTORICAL NEISSERIA GONORRHOEAE VACCINE STUDIES

The aim of developing a gonococcal vaccine has been pursued since the turn of the twentieth century. Initially, these vaccines were designed as a therapeutic strategy for persistent *N. gonorrhoeae* infection, rather than as a preventative measure. At this time there were numerous attempts made by different groups to immunize patients with symptomatic gonorrhoea with various whole cell vaccines to promote opsonophagocytosis (63). With the development of effective antimicrobial therapy, therapeutic vaccine discovery stalled. Further efforts were made in the 1970s and 1980s, when three different preventative *N. gonorrhoeae* vaccines were developed and trialled in humans, all of which were unsuccessful (64-66). These vaccine studies are described in Table 1. The first vaccine, a partially inactivated whole-cell vaccine prepared from two pooled *N. gonorrhoeae* strains elicited specific antibody responses among the majority of the 54 participants included in the initial phase I study (67). A subsequent placebo-controlled double-blind field trial of this vaccine was undertaken in 1972-1973, this time using whole cell preparations from three pooled *N. gonorrhoeae* strains. This study involved 62 participants from an Aboriginal Inuit population in the northern Canada village of Inuvik, with the immunization schedule comprising three 1ml intramuscular injections of vaccine or placebo at weekly intervals. No significant difference between the groups in the cumulative incidence of laboratory-confirmed *N. gonorrhoeae* infection was observed over the 12-month period following vaccination (30% incidence in the vaccinated group versus 24% in the placebo group; p=0.78)(64).

The second vaccine, a *N. gonorrhoeae* pilus vaccine, elicited serum and genital anti-pilus antibody responses against heterologous strains and demonstrated efficacy in an initial *N. gonorrhoeae* urethral CHIM study after challenge with a homologous strain (68, 69).This was followed by a placebo-controlled, double-blind trial in Korea in the 1980s, involving 3,250 high-risk US military personnel, using an immunization schedule comprising two 0.1ml intradermal injections of vaccine or placebo, two weeks apart. There was no significant difference in cumulative incidence of laboratory-confirmed *N. gonorrhoeae* infection in the 8-week period following vaccination between the two groups, with a cumulative incidence of 6.9% observed in the vaccinated group, compared with 6.5% in the placebo group (65). In a subsequent *N. gonorrhoeae* urethral CHIM study, no protection was observed against a heterologous *N. gonorrhoeae* strain expressing antigenically different pili (70), suggesting that despite the production of anti-pilus antibody responses against heterologous pili, these responses were insufficient to prevent infection with *N. gonorrhoeae* strains expressing antigenically different pili. Pilus antigen heterogeneity, a characteristic of circulating strains of *N. gonorrhoeae,* was the most likely explanation for the unsuccessful field trial.

The most recent *N. gonorrhoeae* vaccine to be trialled in humans was a *N. gonorrhoeae* outer membrane vaccine prepared from a single strain. In a randomized placebo-controlled *N. gonorrhoeae* urethral CHIM undertaken in 1985, 63 male participants received a single dose of intramuscular vaccine or placebo and underwent intraurethral challenge with a homologous *N. gonorrhoeae* strain two to four weeks later. No significant difference in infection was observed between the two groups. Infection rates were unexpectedly low in this study, with 46% of those vaccinated and 36% of placebo recipients remaining

uninfected (66). Although designed to enrich for the Porin (Por) outer membrane protein, this vaccine was contaminated with other membrane antigens, including lipooligosaccharide (LOS) and reduction modifiable protein (Rmp). Later studies demonstrated that anti-Rmp antibodies downregulate the bactericidal activity of antibodies against other antigens (71). The contamination of this vaccine by Rmp therefore likely resulted in anti-Rmp antibodies that may have antagonized the bactericidal effect of anti-Por and anti-LOS antibodies. This hypothesis was supported by a retrospective analysis of the vaccine trial data which incorporated data on risk for pre-existing immunity. This analysis demonstrated that the ratio of Por and LOS antibody concentration to Rmp antibody concentration correlated with protection from *N. gonorrhoeae* infection in both vaccine and placebo recipients (66).

These early studies demonstrate three key considerations for future gonococcal vaccine trials. Firstly, CHIM trials that are appropriately designed to test investigational vaccines may serve as go-no-go measure using a relatively small number of participants before more resource-intensive, larger-scale efficacy trials are undertaken. Secondly, pre-existing immunity should be incorporated into the design and analysis of future gonococcal vaccine trials by documenting baseline antibody levels and previous exposure. Finally, the heterogeneity of circulating *N. gonorrhoeae* strains must be considered both in the selection of potential vaccine antigens and the selection of challenge strains for future gonococcal CHIM vaccine trials.

POTENTIAL VACCINE TARGETS FOR NEISSERIA GONORRHOEAE VACCINES

Neisseria gonorrhoeae Vaccine Antigens

A number of potential *N. gonorrhoeae* vaccine candidates have been evaluated in pre-clinical testing including *in vitro*, in animal models and occasionally, early phase human studies. Key features of an ideal *N. gonorrhoeae* vaccine antigen include i) surface exposure; ii) conservation (lack of phase or antigenic variation); iii) high prevalence among globally-circulating strains; iv) immunogenicity; and v) evidence that the antigen plays an important role in virulence or survival. In the absence of known immune correlates of protection against *N. gonorrhoeae* infection, a widely used approach has been to identify surface antigens that elicit an antibody response that confers complement-dependent bactericidal activity, and/or mediates opsonophagocytosis (72), hypothesising that these may be surrogate predictors of prevention. However, antibody responses to natural uncomplicated *N. gonorrhoeae* infection are typically described as weak and short-lived (32). In addition, in early vaccine studies where the pilus, Por and LOS antigens (described above) were evaluated, no correlates of protective immunity were apparent. The bactericidal and opsonophagocytic activity of antibodies induced by natural reinfection is influenced by a number of factors, including downregulation by blocking antibodies (e.g. anti-Rmp antibodies)(71) and soluble complement regulators (e.g. Factor H and C4b-binding protein)(66), as well as poor cross-protection of antibodies to polymorphic antigens (e.g. pilus and Por)(65, 71). Given the complex humoral immune responses to *N. gonorrhoeae* infection and the lack of protective immunity induced by natural gonococcal infection (32), an optimal *N. gonorrhoeae* vaccine will need to induce immune responses that are qualitatively and quantitatively different to that induced by natural immunity.

A number of novel strategies have informed the contemporary approach to gonococcal vaccine antigen discovery. Reverse vaccinology is a process of vaccine antigen discovery that harnesses genomics, proteomics, immunoproteomics, transcriptomics and bioinformatics to identify highly conserved, widely distributed and surface exposed antigens that may represent promising vaccine antigens. A reverse vaccinology approach has been used to develop highly successful vaccines for other pathogens, such as *N. meningitidis* serogroup B (4CMenB; GlaxoSmithKline)(73). Identification of novel surface-exposed antigens of *N. gonorrhoeae* have used proteomic techniques to characterise *N. gonorrhoeae* membrane vesicle and cell envelope proteins (74). Such an approach can be coupled with a range of bioinformatic tools to predict function, subcellular localization, post-translational modification and immunogenicity (74).

Using a proteomic-based approach, Zielke et al identified 305 cell envelope and 46 membrane vesicle proteins that were uniformly present among four well-characterized *N. gonorrhoeae* strains, many of which were newly discovered proteins or proteins that had not previously been characterized in *N. gonorrhoeae* (75). Using such proteomic approaches it has been possible to identify candidate vaccine antigens with a range of attractive characteristics, such as expression in physiologically-relevant environmental conditions, including both aerobic and anaerobic, iron deprivation, exposure to normal human serum and exposure to extended spectrum cephalosporins (76-78). Analysis of the genes expressed during natural human mucosal infection, coupled with immune characterisation, has also led to the discovery of a number of novel putative vaccine antigen targets (79). The availability of public genomic databases, such as Neisseria PubMLST, has also enabled

assessment of the presence and conservation of putative vaccine antigens across globally diverse strains (80).

Another strategy to improve vaccine discovery efforts for *N. gonorrhoeae* has been to target antigens that not only elicit an antibody response with bactericidal and opsonophagocytic activity, but also those that elicit a functional antibody response that inhibits important physiological functions in the pathogenesis of gonococcal infection (81-83). These physiological functions include i) adherence to and invasion of mucosal epithelial cells; ii) nutrient acquisition and metabolism; iii) immune evasion; iv) intracellular survival and iv) protection from oxidative stress or antimicrobial substances. Promising vaccine antigens from each of these categories will be briefly highlighted below, and a comprehensive summary of potential *N. gonorrhoeae* vaccine antigens is presented in Table 2.

Adherence and invasion of mucosal epithelial cells

The potential of targeting with a vaccine a number of key mediators of attachment and invasion, such as type IV pili, LOS and the opacity-associated outer membrane proteins (Opa), has been confounded by the high levels of antigenic variation and/or phase variation in these antigens. For example, although the gonococcal porin protein, PorB is the most highly abundant outer membrane protein and constitutively expressed, targeting it with a vaccine is confounded by a high level of antigenic variation within the eight surface-exposed loops in different gonococcal strains (32, 84). However, PorB is an essential protein that plays a key role in host cellular attachment, invasion, nutrient acquisition, apoptosis and serum complement resistance (85) and has immune enhancing activity, making it a promising vaccine adjuvant (86). Preclinical studies of putative PorB vaccines are described

below. Alternative targets include mediators of host cell adherence such as the type IV pilus-associated outer membrane proteins PilC (87, 88), involved in pilus biogenesis and attachment; and PilQ, the secretin through which pili are extruded (89-91). Phospholipase D, which participates in host cell invasion and survival, is another potential outer membrane protein vaccine target (92, 93). In addition, the *Neisseria* heparin binding antigen (NHBA), which is also involved in host cell adherence and survival, has recently been demonstrated to be a promising vaccine antigen candidate, as it is widely distributed, highly conserved and induces bactericidal and opsonophagocytic antibodies (94-96).

Nutrient acquisition and metabolism

A number of antigens involved in nutrient acquisition through iron and zinc uptake have shown promise as potential vaccine antigen targets. The transferrin receptor proteins transferrin binding protein A (TbpA) and transferrin binding protein B (TbpB) facilitate iron acquisition, and are essential for experimental urethral infection of male volunteers when alternative iron acquisition mechanisms are not available (97). The transferrin receptor proteins are immunogenic, with the intranasal immunization of mice with TbpA and TbpB proteins fused to cholera toxin subunit B inducing serum and vaginal mucosal anti-TbpA and anti-TbpB bactericidal antibodies (98). However preliminary evidence suggests that antibodies to gonococcal TbpA have only a modest inhibitory effect on ligand binding (81). Nitrate reductase (AniA) is required for anaerobic growth and biofilm formation of *N. gonorrhoeae* (99, 100). Antibodies against AniA protein inhibit nitrite reductase activity (101, 102), suggesting this may be another promising function-blocking vaccine target.

Immune evasion and intracellular survival

Potential vaccine antigens involved in immune evasion and intracellular survival of *N. gonorrhoeae* include alpha-2,3-sialyltransferase (Lst) and Neisserial surface protein A (NspA). Lst expressed by gonococci scavenge sialic acid from the host and sialylate the gonococcal LOS, thereby inhibiting complement-mediated and polymorphonuclear leukocyte-mediated killing (103, 104). However recent evidence suggests that Lst is a cytoplasmic rather than surface-exposed protein (105). NspA plays an important role in complement evasion by binding to complement regulator human factor H and factor H-like protein 1 (106). Immunization of mice with plasmid DNA containing the NspA gene followed by boosting with recombinant NspA protein induced serum and mucosal antibodies with bactericidal and opsonophagocytic activity (107).

The conserved LOS epitope 2C7, defined by lactose substitutions at HepI and HepII in the LOS core promotes gonococcal colonization and survival, and is another important *N. gonorrhoeae* vaccine target. Although this epitope is phase variable, the *lgtG* glycosyltransferase gene that controls this phase variation is expressed in 95% of gonococci in human infection (108, 109). Monoclonal antibodies against this epitope are bactericidal and opsonophagocytic (108). In an intraperitoneal mouse immunization study of a multi-antigenic 27C peptide mimic (MAP1) with a T helper type 1 (Th1)-inducing adjuvant (Monophosphoryl lipid A; MPL), immunization induced Th-1 biased anti-LOS antibodies that were also bactericidal. Immunization also reduced the length of gonococcal carriage and bacterial burden in experimentally infected mice (110). Further studies of a LOS 2C7 vaccine candidate with greater potential for scalability and economic production comprising a

426 stable, homogenous tetrapeptide 2C7 mimitope (TMCP2), administered with a
427 glucopyranosyl lipid A adjuvant in a stable oil-in-water nanoemulision replicated these
428 findings (111). Anti-Rmp antibodies have been demonstrated to inhibit the efficacy of 2C7
429 monoclonal antibody in mice in a dose-dependent fashion. Therefore, an effective LOS 2C7
430 vaccine would likely need to produce concentrations of protective antibody sufficient to
431 overcome this inhibitory effect in individuals with pre-existing anti-Rmp antibodies (112).
432
433 **Protection from oxidative stress and antimicrobial substances**
434 Proteins that protect *N. gonorrhoeae* from the threats of oxidative stress and antimicrobial
435 substances play an important role in the pathogenesis of *N. gonorrhoeae*. A number of
436 these proteins have recently been identified as promising vaccine targets. Gonococcal
437 methionine sulfoxide reductase protein (MsrA/B) reduces methionine sulfoxide to
438 methionine to protect the organism from oxidative stress (83). MsrA/B is surface-exposed
439 and the gene encoding MsrA/B is highly conserved. Immunisation of mice with an
440 adjuvanted recombinant MsrA/B vaccine results in the production of function-blocking
441 antibodies with bactericidal and opsonophagocytic activity (83). Another promising vaccine
442 antigen is multiple transferable resistance protein E (MtrE), an outer membrane channel of
443 a multidrug transporter system (MtrCDE) which mediates export of hydrophobic
444 antimicrobial substances (fatty acids, long-chain lipids, bile salts and antimicrobials) from
445 the cell and survival after neutrophil exposure (113). It also plays a key role in the FarA-FarB-
446 MtrE active efflux pump, an additional efflux pump system that mediates resistance to
447 hydrophobic agents (114). Mice immunized with an adjuvanted recombinant MtrE vaccine
448 produce anti-MtrE antibodies that are bactericidal and reduce the activity of the MtrCDE
449 efflux pump in the presence of hydrophobic compounds (82).

Key reverse vaccinology antigen discoveries

Finally, a number of promising vaccine antigens have also been discovered using reverse vaccinology approaches described above. These include several antigens involved in cell envelope homeostasis and translocation, including beta-barrel assembly machinery protein A (BamA), lipopolysaccharide assembly protein D (LptD) and translocation and assembly module A (TamA) as well as two human lysozyme inhibitors, adhesin complex protein (ACP) and surface-exposed lysozyme inhibitor of c-type lysozyme (SliC). BamA, LptD and TamA are surface-exposed, highly conserved, stably expressed and immunisation with them elicits antibodies with bactericidal activity (76). Both lysozyme inhibitor antigens ACP and SliC are highly conserved and stably expressed (115-117); antibodies to ACP are both bactericidal and inhibit binding to human lysozyme (116).

Another promising vaccine candidate discovered by reverse vaccinology is MetQ, the methionine binding component of an ATP-binding cassette transporter system (118). MetQ plays a role in epithelial cell adherence and survival. It is a highly conserved surface-exposed protein that is constitutively expressed (76, 118). Anti-MetQ antibodies are bactericidal and reduce adherence of *N. gonorrhoeae* to cervical epithelial cells (118). Mice immunized with a recombinant MetQ lipoprotein formulated with a Th1-stimulating adjuvant (cytosine phosphoguanine; CpG) developed robust Th1-biased serum and vaginal antibodies. After vaginal challenge, the immunized mice demonstrated accelerated clearance of gonococcal infection and a lower bacterial burden (119).

Novel Vaccine Delivery Systems

In addition to novel vaccine targets, there has been significant development in vaccine adjuvants that augment vaccine antigen immune responses. These include nanoparticle technologies such as liposome-based adjuvants which contain immunogens such as toll-like receptor ligands; and oil-in-water emulsions which activate myeloid cells to stimulate innate and adaptive immune responses (120). Novel adjuvants that have been assessed in preclinical *N. gonorrhoeae* vaccine studies include the Th1-stimulating adjuvants, microencapsulated interleukin-12 (IL-12) (121) and CpG oligodextronucleotides (119, 122). In recent mouse model studies, *N. gonorrhoeae* has been shown to be able to suppress the development of Th1 and T helper type 2 (Th2) T cell response, and to induce a T helper type 17- (Th17) driven immune response that facilitates immune evasion (123-125). Elevated levels of the Th17 cytokine, interleukin-17 (IL-17), have also been demonstrated in serum and genital secretions of patients with *N. gonorrhoeae* infection compared to healthy subjects or those with non-bacterial STIs, suggesting that the experimental observations in mice of a Th17-driven immune response may also apply in human *N. gonorrhoeae* infection (126, 127). Rational vaccine design using Th-1 stimulating adjuvants harnesses this key discovery. Th1-stimulating adjuvants have been shown to induce a Th1-driven response, generate anti-gonococcal antibodies and gamma-interferon secreting CD4+ T cells and accelerated clearance of *N. gonorrhoeae* infection in preclinical mouse model studies (119, 121).

The past decade has seen a number of newly licensed vaccines for important infectious diseases which use novel vaccine delivery systems, including nucleic acid vaccines (such as those used in messenger ribonucleic acid (mRNA) severe acute respiratory syndrome

497 coronavirus 2 (SARS-CoV-2) vaccines), virus-like particles (as used in HPV vaccines) and OMV
498 vaccines (used in serogroup B meningococcal vaccines) (128). A number of these novel
499 vaccine delivery systems have been studied in preclinical mouse models of *N. gonorrhoeae*
500 vaccines. Nucleic acid vaccines, viral replicon particles and recombinant vaccines are
501 particularly attractive for putative PorB gonococcal vaccines as these techniques avoid
502 potential problems of contamination by Rmp and inadvertent stimulation of anti-Rmp
503 blocking antibodies by the putative vaccine. Zhu et al have undertaken a number of studies
504 investigating various vaccine delivery techniques and prime-boost schedules for putative
505 PorB vaccines, including PorB deoxyribonucleic acid (DNA), renatured recombinant PorB
506 (rrPorB), PorB expressed from Venezuelan equine encephalitis virus replicon particles (PorB
507 VRPs) and OMV vaccines (in which the major constituent antigen is PorB)(129, 130). These
508 studies have demonstrated that different immune responses are triggered by various
509 vaccine antigen delivery systems and sites of inoculation. For example, mice immunized
510 subcutaneously with a rrPorB vaccine developed high levels of PorB-specific IgG antibodies,
511 with immunization administered in the hind footpad inducing a Th1 response and
512 immunization administered in the dorsal area inducing a Th2 response. In this study,
513 immunization with PorB VRPs induced a Th1 response while an intranasal OMV vaccine was
514 the only vaccine that generated serum bactericidal antibodies (129). Antibodies induced by
515 a PorB DNA vaccine alone were modest, however boosting by either rrPorB or PorB VRPs
516 significantly increased PorB-specific serum antibody levels (130).
517
518 Other novel vaccine delivery technologies such as bacterial ghosts have also been explored
519 in preclinical *N. gonorrhoeae* vaccines. Bacterial ghosts are empty Gram-negative bacterial
520 cell envelopes that retain the cellular morphology and antigenic determinants of the cell

envelope and provide a promising system for the delivery of nucleic acid (DNA or RNA) vaccines. This delivery system provides intrinsic adjuvant activity due to the enhanced immune responses produced against cell envelope antigens, including T cell activation and mucosal immunity (131). Jiao et al have demonstrated that *N. gonorrhoeae* PorB and NspA DNA vaccines delivered using *Salmonella enteritidis* ghosts induce serum IgG antibodies that are bactericidal in an experimental mouse model (132, 133).

Meningococcal Outer Membrane Vesicle Vaccines

Meningococcal OMV vaccines have been a key focus of both observational and preclinical *N. gonorrhoeae* vaccine studies. OMVs are spherical lipid bi-layer membrane structures that are released spontaneously from the outer membrane of Gram-negative bacteria and contain surface-exposed phospholipids, lipopolysaccharide/LOS and membrane proteins as well as RNA, DNA, proteins and peptidoglycans within the lumen of the vesicle (134-136). The role of OMVs in bacterial pathogenesis includes modulation of host immune response, nutrient acquisition, and biofilm formation (134-136). OMVs present a number of advantages as a novel vaccine platform, including the ability to enter lymphatic vessels for uptake by antigen-presenting cells and presentation of membrane surface antigens in their native configuration, thereby evoking humoral and cell-mediated responses (134-136). The association between immunization with currently-available meningococcal B vaccines and *N. gonorrhoeae* infection and preclinical studies of these vaccines will be discussed in further detail below. The focus in preclinical studies of novel OMV vaccines has recently shifted to optimizing meningococcal OMV design based on known features of gonococcal pathogenesis, such as the use of meningococcal isolates lacking Rmp proteins and avoiding detergent-based preparation of outer membranes (137, 138). Although detergent-based

preparation of outer membrane vesicles extracts LOS and decreases endotoxin activity, it also removes key meningococcal antigens, including factor H binding protein (fHbp) (137).A detoxified meningococcal OMV vaccine (lacking PorA, PorB and Rmp) has been shown to improve gonococcal clearance in a murine model (137). In addition, a meningococcal native OMV vaccine with attenuated endotoxin and overexpressed fHbp has been shown to induce high levels of serum immunoglobulin G (IgG) anti-FHbp as well as serum bactericidal antibodies against heterologous gonococcal strains (138). Furthermore, the next generation of OMV vaccines developed from *N. gonorrhoeae* strains, and designed specifically to induce protection against *N. gonorrhoeae* infection are under study, with a number of preclinical studies demonstrating promising results, including production of serum and vaginal antibodies and accelerated clearance of gonococcal infection in the estradiol-treated female mouse model (139-141). These include the dmGC_0817560 (140) and NGoXIM (141) native OMV vaccines described in further detail below.

Route of Immunization

The route of immunization may also play a significant role in determining the immunogenicity of a *N. gonorrhoeae* vaccine. It has been observed that the ability of parenteral immunization to induce mucosal immunoglobulin A (IgA) antibodies for other sexually transmitted pathogens is limited (142). By contrast, mucosal administration of vaccines via intranasal immunization has demonstrated relatively higher mucosal IgA and IgG antibodies compared with parenteral vaccines (98, 142). This has been shown in mouse model studies of a number of different experimental *N. gonorrhoeae* vaccines (98, 119, 129, 139, 141, 143). For a number of these vaccines, accelerated clearance of gonococcal infection has been observed in intranasally immunized mice, including a gonococcal OMV

569 preparation (139) and a recombinant MetQ-CpG adjuvant vaccine (119). In addition,
570 intravaginal and intranasal immunization using a native gonococcal OMV plus
571 microencapsulated IL-12 vaccine (NGoXIM) in a female mouse model induced serum and
572 vaginal IgG and IgA antibodies and accelerated clearance of gonococcal infection (121, 141).
573 Other novel routes of *N. gonorrhoeae* vaccine delivery studied in preclinical settings include
574 a transdermal microneedle skin patch that enables slow release of antigens using a
575 formalin-inactivated whole-cell gonococcal microparticle vaccine formulation. Mouse model
576 studies of this vaccine demonstrated that the transdermal skin patch vaccine induced
577 increased IgG antibody titres compared with the comparator subcutaneously administered
578 vaccine (144).
579
580 Table 3 provides a summary of *N. gonorrhoeae* vaccines that have proceeded to
581 contemporary preclinical studies in the experimental mouse model, many of which have
582 included novel vaccine antigens, vaccine delivery systems or routes of immunization.
583
584 **THE IMPACT OF NEISSERIA MENINGITIDIS OUTER MEMBRANE VESICLE VACCINES ON**
585 **GONORRHOEA INFECTION**
586 The most significant step in *N. gonorrhoeae* vaccine progress in the past decade was a
587 landmark study that demonstrated 31% vaccine efficacy of a *N. meningitis* serogroup B
588 outer membrane vesicle (OMV) vaccine (MeNZB) against *N. gonorrhoeae* infection in a
589 retrospective observational case-control study of 15-30-year-olds attending sexual health
590 clinics in New Zealand (34). This finding demonstrated the biological plausibility of vaccine-
591 mediated protective immunity against *N. gonorrhoeae,* and provided a proof-of-concept
592 that an effective *N. gonorrhoeae* vaccine may be possible (145). This observation was

supported by evidence from ecological studies in Cuba, Norway and Canada where an association between the introduction of *N. meningitidis* serogroup B OMV-containing vaccines and reduced rates of gonorrhoea infection were apparent (58-62).The impact of 4CMenB (Bexsero; GSK) has been assessed in further retrospective observational case-control and cohort studies. 4CMenB is a *N. meningitidis* serogroup B OMV-containing vaccine which incorporates the OMV included in MeNZB, as well as three recombinant antigens, Neisseria adhesin A (NadA), fHbp and NHBA, as well as two accessory proteins (GNA2091 fused with fHbp and GNA1030 fused with NHBA) that increase the immunogenicity of the target recombinant antigens (146). These studies have also demonstrated a protective effect of 4CMenB on *N. gonorrhoeae* infection, with estimated vaccine effectiveness for a two-dose schedule ranging between 33-46% in various settings across the world, including in the US, Australia and Italy (53-56). These studies are summarised in Table 4.

Several studies are currently recruiting participants into randomized placebo-controlled trials of the 4CMenB vaccine to assess efficacy against *N. gonorrhoeae* infection (https://clinicaltrials.gov/study/NCT04415424; https://clinicaltrials.gov/study/NCT04350138; https://clinicaltrials.gov/study/NCT05766904; https://clinicaltrials.gov/study/NCT05766904; https://clinicaltrials.gov/study/NCT05294588; 147). Furthermore, in recently reported interim analysis of a randomised, open-label factorial study of the 4CMenB vaccine coupled with doxycycline post-exposure prophylaxis in MSM on HIV pre-exposure prophylaxis (PrEP) (DOXYVAC), a reduced incidence of first-episode *N. gonorrhoeae* infection in the 4CMenB group was observed compared to the no vaccine group (adjusted hazard ratio 0.49; 95% CI 0.27-0.88)(148). However, the final study

report is awaited, as review of the study data indicates that a number of *N. gonorrhoeae* infections were not included in the interim analysis (149). The randomised studies of 4CMenB will provide further high-level evidence of the protective efficacy of *N. meningitidis* serogroup B OMV vaccines against *N. gonorrhoeae* infection. Here we describe the clinical and basic science studies of meningococcal serogroup B OMV vaccines in further detail.

Observational studies

The first studies to suggest an association between various meningococcal serogroup B OMV vaccines and *N. gonorrhoeae* incidence were ecological analyses of the impact of mass serogroup B meningococcal OMV vaccination programs on *N. gonorrhoeae* infection rates in Cuba, Norway and Canada (58-62). In Cuba, a *N. meningitidis* serogroup B OMV-containing meningococcal vaccine, VA-MENGOC-BC, was used in a national mass vaccination program of individuals aged 3 months to 24 years between 1989 and 1990, and subsequently incorporated into the national infant immunization schedule (150). Reported vaccine coverage of the mass vaccination program in the target population was 95% (150). In the years immediately after the program (1989-1993), the incidence of gonorrhoea decreased from 381.9 to 190.3 cases per 100,000 ($r=0.9607$, $p=0.001$), despite an increase in other sexually transmitted infections such as syphilis (58-60).

In a second ecological study undertaken in Norway using trial registry data of a *N. meningitidis* serogroup B OMV-containing vaccine, MenBvac, delivered to 63% of 13-15-year-olds between 1988-1992, a reduced incidence rate ratio (IRR) of gonorrhoea was observed in the subsequent years 1993-2008 among 20 to 24-year-olds in the vaccinated cohort compared to the pre-vaccination cohort (IRR 0.58, 95% CI 0.42-0.8 for women and

adjusted IRR 0.68, 95% CI 0.51-0.93 for men)(62). In a third an ecological study the incidence of *N. gonorrhoeae* infection was studied in the context of a mass vaccination campaign undertaken in Canada in 2014 among individuals aged 6 months to 20 years vaccinated with the *N. meningitidis* serogroup B OMV-containing 4CMenB vaccine in the Sanguenay-Lac-Saint-Jean region of Quebec. Although an association between vaccination and reduced gonorrhoea incidence of 59% (95% CI -22% to 84%; p=0.1) was observed, this finding was not statistically significant.(61)

A landmark retrospective observational case-control study of the MeNZB *N. meningitidis* serogroup B OMV vaccine was the first to describe vaccine efficacy against *N. gonorrhoeae* infection among 14,730 sexual health clinic patients aged 15-30 years who were eligible to receive MeNZB vaccination through a mass vaccination program of individuals aged 6 weeks to 20 years implemented in New Zealand between 2004-2006 (34). This study demonstrated that vaccinated individuals were significantly less likely to be cases (*N. gonorrhoeae* mono-infection) than controls (*Chlamydia trachomatis* mono-infection); 41% vs 51%; adjusted odds ratio (OR) 0.69 (95% CI 0.61-0.79; p<0.0001). After adjustment for ethnicity, deprivation status, geographical area and sex, the estimated vaccine effectiveness of MeNZB against *N. gonorrhoeae* infection was 31% (95% CI 21-39; p<0.0001)(34). Further study of individuals vaccinated with MeNZB during New Zealand's mass vaccination program demonstrated that vaccinated individuals were also significantly less likely to be hospitalized due to *N. gonorrhoeae* infection, with an estimated vaccine effectiveness against *N. gonorrhoeae*-related hospitalization of 24% (95% CI 1-42%)(57).

664 A similar association between the *N. meningitidis* serogroup B OMV-containing vaccine
665 4CMenB and reduced risk of *N. gonorrhoeae* infection has been reported in subsequent
666 retrospective observational case-control studies using jurisdictional health registry and
667 immunization data in various populations in the US, Australia and Italy, with vaccine
668 effectiveness of a two-dose schedule ranging between 33% and 46% (53-56). In a
669 retrospective matched cohort study of 15-30 year-olds resident in Southern California, the
670 incidence of *N. gonorrhoeae* infection among individuals who received 4CMenB (with or
671 without a MenACWY *N. meningitidis* serogroup A, C, W, Y polysaccharide conjugate vaccine)
672 was compared with the incidence of *N. gonorrhoeae* infection among individuals who
673 received the MenACWY vaccine alone; the hazard ratio (HR) of incident *N. gonorrhoeae*
674 infection was 0.54 (95% CI 0.34-0.86)(55).
675
676 In contrast, no association has been noted between receipt of an alternative *N. meningitidis*
677 serogroup B vaccine, MenB-fHbp (Trumenba; Pfizer) and *N. gonorrhoeae* infection. The
678 MenB-fHbp vaccine contains recombinant fHbp, but does not contain OMVs. Importantly,
679 the homologue of fHbp in *N. gonorrhoeae* is not surface exposed, does not bind factor H
680 and is therefore not predicted to be protective against *N. gonorrhoeae* infection (151). In a
681 retrospective, observational case-control study of 96,235 persons aged 16-23 years of age
682 with a diagnosis of *N. gonorrhoeae* or *C. trachomatis* infection between 2016 and 2018 in
683 New York City and Philadelphia, no significant association between MenB-fHbp vaccination
684 and *N. gonorrhoeae* mono-infection was observed after adjustment for ethnicity, gender
685 and jurisdiction (adjusted prevalence ratio 0.97, 95% CI=0.79-1.19)(151). This suggests that
686 healthy vaccinee bias (where persons who adopt preventive vaccinations may be more likely
687 to adopt other protective behaviours and therefore have reduced risk of disease acquisition)

688 has not played a significant role in the association between meningococcal serogroup B
689 OMV vaccine and *N. gonorrhoeae* protection.
690
691 Collectively, these retrospective, observational studies are limited by potential biases
692 resulting from possible missing data associated with the use of health and immunization
693 registry data. In addition, this non-randomised data may be confounded by differences in
694 risk behaviour between vaccinated and non-vaccinated persons, such that those who adopt
695 a preventative meningococcal vaccine may also be more likely to adopt preventative
696 behaviours that reduce the risk of *N. gonorrhoeae* infection. Further data are also required
697 to determine vaccine effectiveness against *N. gonorrhoeae* infection in subpopulations at
698 high risk of *N. gonorrhoeae* infection, such as people living with HIV (PLHIV) and men who
699 have sex with men (MSM). Encouragingly, the first study investigating the impact of
700 4CMenB in PLHIV demonstrated promising results. This retrospective case-control study
701 comprised of 1,051 MSM living with HIV in Milan, Italy and demonstrated vaccine
702 effectiveness of 4CMenB against *N. gonorrhoeae* infection of 42% (95% CI 6-64, p=0.027), a
703 figure that remained significant after adjustment in multivariable analysis (56). A further
704 uncertainty remains regarding whether there are any differences in the protective efficacy
705 of meningococcal serogroup B OMV-containing vaccines against *N. gonorrhoeae* infection at
706 specific anatomical sites (ie. genital, anorectal or oropharyngeal infections) as well as the
707 duration of vaccine-induced protection against *N. gonorrhoeae* infection.
708
709 Finally, the evidence regarding vaccine effectiveness against *N. gonorrhoeae* and *C.*
710 *trachomatis* co-infection in published studies is mixed. In the initial New Zealand
711 retrospective case-control study of MeNZB, vaccine effectiveness was observed against *N.*

gonorrhoeae/*C. trachomatis* co-infection, albeit with a lower effect size. The estimated vaccine effectiveness against *N. gonorrhoeae*/*C. trachomatis* co-infection compared to *C. trachomatis*-only controls was 14% (95% CI, 1-26%) in this study, while estimated vaccine effectiveness against *N. gonorrhoeae*-only infection was 31% (95% CI, 21-39%)(34). In contrast, in the large retrospective case-control study described above of 109,737 individuals aged 16-23 years with *C. trachomatis or N. gonorrhoeae* infection in New York City and Philadelphia between 2016 and 2018, vaccination with 4CMenB was not protective against *N. gonorrhoeae*/*C. trachomatis* co-infection, despite an estimated two-dose vaccine effectiveness against *N. gonorrhoeae*-only infection of 40% (95% CI 23-53%)(53).

Randomised studies

The interim findings of the first randomised study of a meningococcal serogroup B OMV vaccine with 4CMenB were made available in February 2023. The French National Agency for AIDS Research (ANRS) DOXYVAC trial was a phase III randomised open-label factorial design trial of MSM on HIV PrEP with a history of STI in the previous 12 months (148). In this study participants were randomised to two interventions, i) two doses of 4CMenB or no vaccine (randomised 1:1); and ii) doxycycline post-exposure prophylaxis (PEP) (200mg within 72 hours of condomless sex) or no PEP (randomised 2:1). Participants underwent testing for *N. gonorrhoeae* infection at baseline, every three months and whenever they had symptoms of STIs. Testing for *N. gonorrhoeae* infection comprised NAAT (nucleic acid amplification test) of urine, oropharyngeal and anorectal swabs every three months. The primary endpoint of the study was the incidence of a first episode of *N. gonorrhoeae* infection one month after the second dose using an intention-to-treat analysis. Of 546 MSM enrolled, 502 were included in the intention-to-treat analysis. The interim findings reported a significant

reduction in incident *N. gonorrhoeae* infection between the two-dose 4CMenB recipients and unimmunized participants followed for 9 months, with the incidence of first episode *N. gonorrhoeae* infection 9.8 and 19.7 per 100 person-years in the 4CMenB arm and no vaccine arms, respectively (adjusted hazard ratio 0.49; 95% CI 0.27-0.88). There was no interaction for the primary endpoints between the doxycycline PEP and 4CMenB vaccination. No vaccine-related serious adverse events were reported. However, the results of this study are now under independent review due to a discrepancy between the results of the reported interim and final results, explained by the omission of a number of *N. gonorrhoeae* infections from the interim analysis (149). As the first reported randomised trial of a meningococcal B OMV vaccine, the final results of this trial and independent review are highly anticipated.

Further randomised studies of a two-dose schedule of 4CMenB are currently underway and are described in Table 5. Notably, four double-blind randomised-controlled trials are actively enrolling participants, including two large placebo-controlled multi-centre clinical trials and a CHIM study (https://clinicaltrials.gov/study/NCT04415424; https://clinicaltrials.gov/study/NCT04350138; https://clinicaltrials.gov/study/NCT05766904; https://clinicaltrials.gov/study/NCT05294588). In addition, a randomised, open-label, single-site trial of 18-50 year-old gay and bisexual men on HIV pre-exposure prophylaxis or recent *N. gonorrhoeae* infection is planned (147). The three double-blind, randomised, placebo-controlled trials evaluating the impact of 4CMenB on natural infection will recruit from different populations, including i) a multi-site Australian study of 18-50 year-old men (cis and trans), transexual women and non-binary people who have sex with men(https://clinicaltrials.gov/study/NCT04415424); ii) a multi-site American study of 18-50

760 year-old healthy men and women (https://clinicaltrials.gov/study/NCT04350138); and iii) a
761 single-site Hong Kong study of MSM aged 18 or above with risk factors for gonorrhoea
762 infection (https://clinicaltrials.gov/study/NCT05766904). The randomised-controlled CHIM
763 study is a single-site, double-blind randomised controlled trial where two doses of 4CMenB
764 are compared to quadrivalent influenza and tetanus/diphtheria vaccination. The aim is to
765 recruit up to 140 male participants who will undergo urethral challenge with *N. gonorrhoeae*
766 strain FA1090 after immunization with 4CMenB or the comparator vaccine. Participants will
767 be randomised 1:1 to the 4CMenB or control vaccine arm, and receive two immunizations
768 prior to the anterior urethral bacterial challenge with 10^6 colony-forming units of *N.*
769 *gonorrhoeae* strain FA1090 in suspension. The primary outcome measured will be
770 microbiological confirmation of urethral infection via detection of *N. gonorrhoeae* by culture
771 or NAAT of urine or urethral swab (https://clinicaltrials.gov/study/NCT05294588). In
772 addition, this CHIM will measure the proportion of participants that develop symptomatic
773 disease and also presents an opportunity for intensive biological sampling and
774 immunological characterisation of responses in those who have received 4CMenB compared
775 to the control group. Furthermore, subgroup analysis of this data regarding anatomical site-
776 specific risk of *N. gonorrhoeae* infection (eg. genital, anorectal and oropharyngeal) between
777 the vaccinated and non-vaccinated groups in these randomised studies will be important to
778 inform the potential impact of the 4CMenB vaccine on *N. gonorrhoeae* transmission at a
779 population level. Increasing evidence suggests that oropharyngeal *N. gonorrhoeae* infection
780 may play a significant role in *N. gonorrhoeae* transmission (19) and modelling studies
781 suggest that the impact of a *N. gonorrhoeae* vaccine will be significantly reduced if the
782 vaccine is not effective at the oropharynx (152).
783

Biological plausibility

Biological plausibility for the association between meningococcal serogroup B OMV vaccines and protection against *N. gonorrhoeae* infection has been strengthened by basic science studies demonstrating a high level of genomic sequence identity between *N. gonorrhoeae* and the serogroup B *N. meningitidis* OMV protein antigens present in the MeNZB and 4CMenB vaccines (94, 153). Bioinformatic analysis of twenty-two proteins that comprise >90% of 4CMenB OMV content resulted in the identification of twenty orthologues of these proteins in *N. gonorrhoeae* strain FA1090, including sixteen with >90% identity and two with >80% identity (153). Of the OMV proteins that have an orthologue in *N. gonorrhoeae*, fourteen of these also have a high level of sequence identity with >400 *N. gonorrhoeae* strains available on GenBank (153). A further study comprising bioinformatic analysis of abundant 4CMenB OMV vaccine antigens among 940 *N. gonorrhoeae* strains from the US, found that of all the predicted outer membrane proteins, OpcA (45%) and PorB (70%) had the lowest mean sequence similarity between the NZ98/254 *N. meningitidis* strain from which the 4CMenB OMV is derived and *N. gonorrhoeae*. In addition, although the *porA* gene was identified in 99.5% of *N. gonorrhoeae* isolates in this study, inactivating mutations render PorA a pseudogene in *N. gonorrhoeae* (94). Analysis of the additional recombinant antigens present in 4CMenB indicates that NadA is absent in *N. gonorrhoeae* (94) and although orthologues of NHBA, fHbp, GNA2091 and GNA1030 are present in *N. gonorrhoeae* strains, fHbp (154), GNA2091 (155) and GNA1030 (156) are not thought to be surface exposed. Importantly, the 4CMenB NZ98/254 *N. meningitidis* strain NHBA antigen shares 67% mean amino acid sequence similarity to *N. gonorrhoeae* (153), suggesting the presence of this antigen in the 4CMenB vaccine may provide an additive protective effect against *N. gonorrhoeae* infection.

In addition, analysis of the antibody response of rabbits, mice and humans after immunization with 4CMenB, or the OMV present in 4CMenB has demonstrated the induction of cross-reacting gonorrhoea-specific antibodies (153, 157). For example, in rabbits immunized with the OMV present in 4CMenB, several cross reactive proteins were detected by Western blot analysis of whole-cell lysates comprising three different *N. gonorrhoeae* strains, and an elevated ELISA titre to *N. gonorrhoeae* strain 1291 OMVs was observed (153). Similar findings were observed in a serology study of humans who had received three doses of 4CMenB, with a significant rise in the enzyme-linked immunosorbent assay (ELISA) titre against *N. gonorrhoeae* whole-cell lysates between pre- and post-vaccination. Western blot analysis of human post-vaccination sera also demonstrated reactivity to several gonococcal and meningococcal proteins (153). Further investigation in the estradiol-treated female mouse model demonstrated that subcutaneous and intraperitoneal immunization of mice with 4CMenB induced serum and vaginal antibodies to whole-cell lysates of six different *N. gonorrhoeae* strains, as well as serum and vaginal antibodies that cross-react with several OMV proteins, including promising *N. gonorrhoeae* vaccine targets such as MtrE and PilQ. Furthermore, vaccination with 4CMenB significantly reduced *N. gonorrhoeae* bacterial load and accelerated clearance of infection after *N. gonorrhoeae* vaginal inoculation in the estradiol-treated mouse model (157).

A number of investigators are currently undertaking studies to further characterise the immunological responses to a two-dose schedule of 4CMenB vaccine. These include a study comprising up to 15 male and female participants conducted at the University of North Carolina, Chapel Hill in which the change in anti-gonococcal OMV- specific IgG, IgA and

immunoglobulin M (IgM) concentrations and the mean change in the proportion of CD4+ T lymphocytes expressing at least two different activation markers (interferon-gamma, tumour necrosis factor-alpha and interleukin-2) will be measured after *in vitro* stimulation with *N. gonorrhoeae* strain FA1090 OMVs in participants after vaccination with two doses of 4CMenB (https://clinicaltrials.gov/study/NCT04094883). In another study, investigators at the University of Oxford and KEMRI-Wellcome Trust Collaborative Research Program aim to recruit approximately 50 male and female participants, including HIV-uninfected and HIV-infected individuals from existing follow-up cohorts in Mtwapa, Kenya. These investigators will also measure serum humoral and T cell responses to *N. gonorrhoeae* before and after two-doses of 4CMenB (https://clinicaltrials.gov/study/NCT04297436). Furthermore, in a study at the National Institute of Allergy and Infectious Diseases, 50 male and female participants will be recruited and serum and mucosal antibody responses at oropharyngeal, rectal and vaginal sites will be measured before and after vaccination with two-dose of 4CMenB (https://clinicaltrials.gov/study/NCT04722003). In addition, a number of the randomised two-dose 4CMen B vaccine efficacy studies described above will investigate serum (https://clinicaltrials.gov/study/NCT04350138) or serum and mucosal immune responses (https://clinicaltrials.gov/study/NCT04415424, 147).

In summary, there is substantial evidence of an association between meningococcal serogroup B OMV vaccines and reduced *N. gonorrhoeae* infection. This includes human ecological and observational trial data, evidence of overlap in important vaccine targets in meningococcal serogroup B OMV vaccines and *N. gonorrhoeae* and induction of cross-reactive antibody responses. Lacking are data defining the impact of meningococcal serogroup B OMV vaccines on *N. gonorrhoeae* infection at various anatomical sites and in

different population groups. With a number of randomised-controlled studies assessing the vaccine efficacy of 4CMenB currently underway, further information will become available. Given the promising findings of meningococcal serogroup B OMV vaccines against *N. gonorrhoeae* infection to date, as well as the widespread availability and demonstrated safety data of vaccines such as 4CMenB (158), implementation of this vaccine in settings with particularly high *N. gonorrhoeae* prevalence should be considered.

IN THE PIPELINE: NEISSERIA GONORRHOEAE OUTER MEMBRANE VESICLE VACCINES

Importantly, several *N. gonorrhoeae*-specific OMV vaccines are in preclinical or clinical development (141, 159, https://clinicaltrials.gov/study/NCT05630859). These include the NGoXIM (141) and dmGC_0817560 (140, 159) native OMV vaccine candidates which are in the late stages of preclinical development (160), and a generalized modules for membrane antigens (GMMA) vaccine, which is currently recruiting participants into a phase 1/2 study (https://clinicaltrials.gov/study/NCT05630859). The NGoXIM vaccine is being developed by Intravacc and TherapyX in the Netherlands and the United States, with funding from the US National Institute of Allergy and Infectious Diseases (161). This vaccine is a *N. gonorrhoeae* native OMV vaccine formulated for intranasal mucosal delivery combined with a sustained-release microsphere encapsulated IL-12 adjuvant (160). Studies have demonstrated that intravaginal and intranasal administration of this vaccine induced Th1-driven responses that accelerated clearance of *N. gonorrhoeae* genital tract infection in mice (121, 141). Intranasal administration of this experimental vaccine generated antigonococcal serum IgG, salivary IgA and vaginal IgG and IgA antibodies in female mice and antigonococcal serum IgG and salivary IgA antibodies in male mice. In addition, female mice that received intranasal immunization with this experimental vaccine demonstrated accelerated clearance of

880 homologous and heterologous strains of *N. gonorrhoeae* infection. Further, intranasal
881 immunization with vaccines comprising various adaptations to this vaccine include
882 detergent-extracted OMVs to reduce LOS content, and OMVs from *N. gonorrhoeae* strains
883 with deleted *rmp* and *lpxl1* genes to eliminate anti-Rmp blocking antibodies and reduce LOS
884 endotoxicity. These have shown accelerated clearance of vaginal gonococcal infection in the
885 female mouse model (141).
886
887 The dmGC_0817560 vaccine is being developed by the Jenner Institute and Oxford Vaccine
888 Group in the United Kingdom, with funding from CARB-X (160). This vaccine is also a native
889 OMV vaccine formulated from a Chilean *N. gonorrhoeae* strain in which genes for Rmp and
890 LpxL1 have deleted, combined with an aluminium hydroxide adjuvant. Preclinical studies
891 demonstrate that parenteral delivery of this experimental vaccine induced anti-gonococcal
892 serum and vaginal mucosal IgG and IgA antibodies and gonococcal-specific Th1/Th17 CD4+
893 T-cell responses in the female mouse model. In addition, female mice immunized with the
894 candidate vaccine demonstrated accelerated clearance of genital *N. gonorrhoeae* infection
895 with a heterologous strain and cleared infection significantly faster than mice immunized
896 with 4CMenB (140).
897
898 The intramuscular NgG generalized modules for membrane antigens (GMMA) vaccine is
899 being developed by GlaxoSmithKline in the US
900 (https://clinicaltrials.gov/study/NCT05630859). GMMA vaccines are OMV vaccines that have
901 been produced from bacterial strains that have been genetically modified to increase
902 production of OMVs and reduce endotoxin levels (162).To our knowledge, preclinical studies
903 of this experimental vaccine have not been published, however a phase 1/2 study of this

experimental vaccine has commenced recruitment, aiming to evaluate the safety, reactogenicity, immunogenicity and efficacy of this experimental vaccine in a randomised, observer-blind, placebo-controlled multicentre study in an estimated 774 participants aged 18-50 years of age (https://clinicaltrials.gov/study/NCT05630859). The phase 1 dose-escalation safety study for this vaccine is now complete and the study has entered phase 2; furthermore, the US Food and Drug Administration (FDA) has granted a Fast Track designation to accelerate its path to US FDA submission (163).

These vaccines represent the next generation of anti-gonococcal OMV vaccines that have been specifically engineered to build on the scientific advances in understanding of *N. gonorrhoeae* pathogenesis and host immune response, as well as the significant progress made in the past decade to explore the association between serogroup B meningococcal OMV vaccines and reduced *N. gonorrhoeae* infection. These include i) use of a *N. gonorrhoeae* strain to produce OMVs for use in next-generation vaccines, potentially increasing the specificity of the immune responses induced by this multi-antigen vaccine technology; ii) inclusion of adjuvants that stimulate a Th1 response; iii) genetically modifying selected gonococcal strains to reduce the endotoxicity associated with LOS and blocking antibodies induced by Rmp; and iv) evaluation of mucosal administration to increase the immune response at the mucosal sites of gonorrhoea infection.

POTENTIAL PUBLIC HEALTH IMPACT OF A NEISSERIA GONORRHOEAE VACCINE

Determining the potential public health impact of a *N. gonorrhoeae* vaccine requires consideration of the health, economic and societal value of future *N. gonorrhoeae* vaccines. The WHO convened an international panel of experts in 2019 to define the public health

928 value and preferred product characteristics of *N. gonorrhoeae* vaccines (37, 38). At this
929 meeting, prevention of poor sexual and reproductive health outcomes and addressing the
930 threat of AMR were identified as the key goals of future *N. gonorrhoeae* vaccines. Important
931 considerations to define the target product profile of a *N. gonorrhoeae* vaccine include i)
932 defining the target endpoint for assessment of vaccine efficacy (eg. prevention of infection,
933 versus prevention of symptomatic disease, versus prevention of AMR); ii) the target
934 population for the vaccine (eg. all individuals prior to sexual activity or high risk populations;
935 whether to include both females and males) and iii) the target programmatic delivery
936 program (eg. schools or sexual health clinics). Notably, the preferred product characteristics
937 of a potential vaccine may also vary according to the epidemiology of *N. gonorrhoeae*
938 infection and AMR in the target population. The promotion of a vaccine against a sexually
939 transmitted infection may also require adaptation to the specific socio-cultural context in
940 order to maximise acceptability.
941
942 Modelling studies are important to understanding the potential impact of *N. gonorrhoeae*
943 vaccines on gonococcal infection and AMR, and to aid policy development and programme
944 delivery. The public health impact and cost-effectiveness of potential *N. gonorrhoeae*
945 vaccines have been modelled in several studies, including various target population groups,
946 vaccine program strategies and levels of vaccine coverage. In addition, the effects of various
947 levels of vaccine efficacy and duration of protection have been investigated.
948
949 **Modelling The Impact of *Neisseria gonorrhoeae* Vaccines in Heterosexual Populations**
950 The impact of gonococcal vaccines delivered prior to commencement of sexual activity has
951 been estimated in a number of heterosexual population model studies. Craig et al used an

individual-based, epidemiological simulation model of a *N. gonorrhoeae* vaccine delivered prior to commencement of sexual activity in a heterosexual population of 100,000 individuals using theoretical vaccines of 10-100% efficacy and 2.5-20 year duration of protection (164). The model output predicted that *N. gonorrhoeae* prevalence could be reduced by at least 90% after 20 years by a non-waning vaccine with 50% efficacy and universal vaccination coverage. The duration of protection of a theoretical vaccine had a significant effect on the prevalence of *N. gonorrhoeae* in the model; a vaccine with 100% efficacy that waned after 7.5 years was predicted to reduce *N. gonorrhoeae* prevalence by at least 90% after 20 years, one whose protection waned after 5 years by 50% and one with 2.5 years protection having minimal impact on prevalence. Similarly, vaccine coverage played a key role in predicted vaccine impact, with 50% vaccine coverage of a *N. gonorrhoeae* vaccine with 50% efficacy predicted to reduce *N. gonorrhoeae* prevalence by 50% after 20 years, compared to at least 90% reduction if the same vaccine had universal vaccine coverage (164).

The impact of a 4CMenB adolescent vaccine on *N. gonorrhoeae* prevalence has been estimated in a number of studies using transmission models of *N. gonorrhoeae* infection among heterosexual populations. Carey et al developed a heterosexual transmission model of 15-24 year-olds in the US using Approximate Bayesian Computation analysis to account for uncertainty in key transmission factors (rates of natural clearance, rates of screening, proportion of symptomatic infections and annual number of sexual contacts). The results of this analysis estimated that a vaccine with 30% efficacy and 2-year duration of protection would result in a 12.2-39.4% reduction in *N. gonorrhoeae* prevalence if 50% vaccine coverage was achieved in this population, and 4.8-14.3% reduction in prevalence if 20%

vaccine coverage was achieved (165). Looker et al developed a developed a deterministic transmission-dynamic model of heterosexual 13-64 year-olds in England and estimated the impact of a vaccinating 14-year-olds with a vaccine with 31% efficacy, 6-year duration of protection and 85% vaccine uptake (166). The results of this analysis indicated that 10% (95%CrI 8-13%), 18% (95%CrI 13-23%) and 25% (95%CrI 17-33%) of cases of *N. gonorrhoeae* infections would be prevented in this population over a 10-, 20- and 70-year period, respectively (166). Regnier et al modelled the potential health and economic impact of a 4CMenB adolescent vaccination on *N. gonorrhoeae* infection with an estimated 20% vaccine efficacy, 10-year duration of protection and 70.5% vaccination rate using a decision-analysis model developed using published US healthcare utilization and cost data (167). This model predicted that vaccination could prevent 83,167 lifetime *N. gonorrhoeae* infections and 55 lifetime HIV infections per vaccinated birth cohort in the US. This was predicted to reduce the direct medical costs of *N. gonorrhoeae* infection by US$28.7 million and reduce income and productivity losses by US$40.0 million (167).

Modelling the Impact of *Neisseria gonorrhoeae* Vaccines in Men Who Have Sex With Men Populations

The impact of gonococcal vaccines within a male population of MSM has been modelled in four studies. Using a stochastic transmission-dynamic model that incorporated heterogenous sexual behaviour and symptomatic and asymptomatic infection in an MSM population based on surveillance data from England, Whittles et al assessed potential *N. gonorrhoeae* vaccination impact and the feasibility of achieving the WHO target of reducing *N. gonorrhoeae* incidence by 90% by 2030 (168). This study estimated that the WHO target is achievable even if the worst-case scenario where untreatable AMR infection emerges, if

all MSM attending sexual health clinics receive a vaccine with ≥52% efficacy and ≥6 years or vaccination; or ≥70% efficacy and ≥3 years protection (168). Heinje et al developed a compartmental model of *N. gonorrhoeae* transmission among a population of MSM with heterogenous sexual behaviour and symptomatic and asymptomatic infection. This model also incorporated AMR as a stepwise increase in minimum inhibitory concentration (MIC) and eventual resistance to ceftriaxone. The impact of a partially protective vaccine with 30% efficacy that provided 2 years of protection delivered to high risk MSM (with baseline gonorrhoea prevalence of 12.5%) on *N. gonorrhoeae* prevalence and AMR was assessed. The modelling output indicated that a vaccine with 30% vaccine effectiveness could not prevent AMR despite high uptake or long-term protection, but would increase time to development of AMR by several years (169).

More recent modelling studies of *N. gonorrhoeae* vaccines within male populations of MSM have added increasing layers of complexity to their models. Hui et al simulated anatomical site-specific data into their individual-based mathematical model of *N. gonorrhoeae* transmission in an urban population of 10,000 MSM with heterogenous sexual behaviour and symptomatic and asymptomatic infection (152). Three types of vaccine efficacy were investigated, including i) 'protective efficacy', the protection of a vaccinated individual against acquiring *N. gonorrhoeae* infection; ii) 'transmission suppression efficacy', the reduction of *N. gonorrhoeae* transmission from a vaccinated individual; and iii) 'symptom suppression efficacy', the reduction of symptoms of *N. gonorrhoeae* infection in the setting of infection in a vaccinated individual. It was estimated that *N. gonorrhoeae* elimination may be possible within the population in this model in eight years with vaccines with ≥50% efficacy and two years of protection if 30% of MSM presenting for sexually transmitted

infection testing were vaccinated and underwent a booster vaccination every three years. Importantly, it was estimated that vaccine impact may be substantially reduced if a *N. gonorrhoeae* vaccine is not effective at the oropharynx and that prevalence may actually increase if a vaccine prevents symptoms but does not prevent infection or transmission. In addition, this study estimated that *N. gonorrhoeae* vaccines that reduced transmission without conferring protection from *N. gonorrhoeae* infection would have a similar impact on *N. gonorrhoeae* prevalence as vaccines with protective efficacy, and that the impact of vaccines with both transmission suppression and protective efficacy would be additive (152).

Whittles et al's most recent study incorporated a cost-effectiveness analysis into their transmission-dynamic model that incorporated heterogenous sexual behaviour and symptomatic and asymptomatic infection in an MSM population based on surveillance data from England (170). The impact and cost-effectiveness of four different vaccination strategies were assessed in this study. It was estimated that vaccination of adolescents in schools would have little impact on *N. gonorrhoeae* prevalence, whereas vaccination of individuals on attendance for STI testing at sexual health clinics would have the largest impact. Vaccination on diagnosis of *N. gonorrhoeae* infection at sexual health clinics would have a moderate impact but require fewer doses than a vaccination on attendance approach, while vaccination of sexual health clinic attendees according to risk (defined as individuals diagnosed with *N. gonorrhoeae* infection in the past 12 months or with >5 sexual partners per year) was estimated to have a similar impact as vaccination of all STI clinic attendees, however required administration of fewer vaccine doses. The most cost-effective strategy for vaccines with moderate efficacy or duration of protection was vaccination

according to risk, whereas vaccination on diagnosis of *N. gonorrhoeae* infection was most cost-effective for highly efficacious and long-lasting vaccines. The impact of 4CMenB vaccination against *N. gonorrhoeae* infection, assuming a vaccine efficacy of 31% and protection lasting 18 months after two-dose primary vaccination and 36 months after single-dose booster vaccination, was also evaluated. A strategy comprising 4CMenB vaccination administered according to risk was estimated to prevent 110,200 cases, gaining a mean of 100.3 QALYs and save a mean £7.9 million over 10 years (170).

Modelling The Impact of *Neisseria gonorrhoeae* Vaccines In Low- and Middle-Income Settings

The use of modelling to assess the impact of *N. gonorrhoeae* vaccines in a high prevalence LMIC setting was reported in a recent study (171). Using a compartmental model of *N. gonorrhoeae* transmission among a 15-49-year-old heterosexual population in a high prevalence LMIC setting similar to South Africa, Padeniya et al modelled the impact of vaccines with varying levels of protective and transmission suppression efficacy on the prevalence *N. gonorrhoeae* infection. In addition, the impact of vaccination programs delivered to various age- and sexual-activity groups was assessed. Vaccination of 15-49-year-olds with a vaccine with protective efficacy of 25%, a 5 year duration of protection and 10% annual vaccine uptake would have the greatest impact on *N. gonorrhoeae* prevalence, with the model predicting that a 50% reduction in prevalence would be achieved, compared to 25% reduction in prevalence if only 15-24-year-olds were vaccinated. Vaccination of only individuals with high sexual-activity was predicted to achieve an almost equivalent reduction in *N. gonorrhoeae* prevalence to vaccinating the entire 15-49-year-old population using theoretical vaccines with same efficacy, duration of protection and uptake

characteristics over the same time period, but was able to achieve this more efficiently, requiring approximately 3 times fewer vaccinations. Similar to the findings of the modelling study by Hui et al's of an urban MSM population, this study estimated that a vaccine with both protective and transmission suppression efficacy would have an additive impact on reducing *N. gonorrhoeae* prevalence (171).

In summary, modelling studies undertaken in both heterosexual and MSM populations using data from various international settings have demonstrated that delivery of vaccines with efficacy and duration of protection derived from estimates of the currently-available 4CMenB vaccine could have a significant impact on *N. gonorrhoeae* prevalence, and even be cost-saving when implemented in select high-risk populations (167, 170). In addition, such vaccines could delay the development of AMR, providing time for more efficacious vaccines and novel antimicrobials to be developed (169). Furthermore, even moderate improvements in *N. gonorrhoeae* vaccine efficacy and duration of protection may have a significant impact on *N. gonorrhoeae* infection prevalence, with some studies estimating that *N. gonorrhoeae* infection may be eliminated or prevalence reduced by 90% through the implementation of vaccines with approximately 50% efficacy and 2-6 years duration of protection (152, 168). Given the prediction that vaccine impact may be reduced if a vaccine is not effective at the oropharynx (152), further data regarding vaccine efficacy at different anatomical sites is pivotal in informing current and future vaccine implementation strategies. In addition, modelling studies simulating the epidemiological characteristics of *N. gonorrhoeae* infection in LMIC settings, where the burden of gonorrhoea infection is greatest, should be prioritised.

QUESTIONS REMAINING: RESEARCH PRIORITIES FOR GONOCOCCAL VACCINES

This is an exciting time for *N. gonorrhoeae* vaccine development, with evidence from observational studies suggesting that meningococcal B OMV vaccines may have efficacy against *N. gonorrhoeae* infection and multiple randomised trials underway. However, there are several key questions that remain unanswered about the currently available serogroup B meningococcal vaccines. These include i) the major effector antigen/s responsible for the efficacy of OMV vaccines; ii) the efficacy of vaccination on infection at various anatomical sites; iii) the duration of protective immunity; and iv) whether there is an immune correlate of protection that can be measured by laboratory tests. A number of these knowledge gaps, such as efficacy at various anatomical sites and further data on duration of protective immunity may be informed by currently recruiting clinical trials of the 4CMenB vaccine. In addition, a randomised trial of 4CMenB in a male urethritis gonorrhoea CHIM may provide more detailed data regarding immune responses to key serogroup B meningococcal OMV vaccine antigens.

The priority research areas outlined in the WHO Global STI Vaccine Roadmap and recently reviewed in the WHO stakeholder consultation regarding public health value and preferred product characteristics of gonococcal vaccines in 2019 remain pertinent today. These include i) improving access to quality epidemiological data regarding infection including AMR ii) advancing the understanding of the natural history of gonorrhoea infection; iii) modelling predicted gonorrhoea vaccine impact and cost-effectiveness; iv) accelerating basic science, translational, immunobiologic and clinical research; and v), advocating for investment and planning for policy and implementation decisions (35, 37). Although there is

much work to be done, there is significant momentum in *N. gonorrhoeae* vaccine development that is being fuelled by the bench to bedside research described in this review.

CONCLUSION

In this review, we have described the unique challenges involved in development of a *N. gonorrhoeae* vaccine. We have reviewed the breadth of data pertaining to *N. gonorrhoeae* vaccines, ranging from an overview of historical vaccines; to multi-omics vaccine antigen discovery and preclinical vaccine research; as well as contemporary clinical trials and modelling studies to inform potential vaccine implementation strategies. As we approach an important inflection point, with the imminent release of the results of six randomised trials of the efficacy of 4CMenB against *N. gonorrhoeae* infection, it is important to consider first how to best implement vaccination programs using currently available vaccines to protect against *N. gonorrhoeae* infection and secondly how to improve upon these technologies to develop the next generation of *N. gonorrhoeae* vaccines. The next generation of *N. gonorrhoeae*-specific OMV vaccines that include modifications of currently available vaccines may improve efficacy. However, alternative vaccines utilizing a range of gonococcal antigens that have shown promise in preclinical studies should also be pursued. Although these vaccine candidates are at a much earlier stage of development and their safety and efficacy in humans has not yet been demonstrated, there is good reason to hold optimism that they will confer improved protection over those currently available. As *N. gonorrhoeae* prevalence continues to increase and the threat of AMR to treatment of gonorrhoea becomes increasingly urgent, expediting the development of highly efficacious *N. gonorrhoeae* vaccines and implementing high-coverage vaccine programs is a key priority for sexual and reproductive health.

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Deborah A. Williamson is a member of the Royal College of Physicians and a Fellow of the Royal College of Pathologists. She has had a number of roles in clinical and public health microbiology in Australia, including as Deputy Director of Microbiological Diagnostics Unit Public Health Laboratory, Director of Microbiology at Royal Melbourne Hospital and Director of the Victorian Infectious Diseases Reference Laboratory. Her research has focused on public health microbiology, particularly sexually-transmitted infections, microbial genomics and antimicrobial resistance. Over the past decade, Deborah has undertaken numerous

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TABLES

Table 1. Historical *Neisseria gonorrhoeae* vaccine trials in humans

Clinical trial design	Vaccine	Immunization schedule	Study population	Result	Reference
Randomized double-blind placebo-controlled trial	Inactivated whole-cell vaccine prepared from three strains of *N. gonorrhoeae*	1ml dose of intramuscular immunization 3 times at 1-week intervals	62 participants recruited from an indigenous population of Inuit in northern Canada (background yearly *N. gonorrhoeae* infection incidence of 25%)	Cumulative infection rate of 30% in immunized participants compared to 24% in placebo in the 12 month follow-up period following immunization (ns)	Greenberg et al 1974 (64)

Randomized double-blind placebo-controlled trial	Single-antigen pilus protein vaccine prepared from single strain of *N. gonorrhoeae*	0.1ml dose of intradermal immunization 2 times at 2-week interval	3250 US military personnel stationed in Korea (96% men; 39% with self-reported history of prior *N. gonorrhoeae* infection)	Cumulative infection rate 6.9% in immunized participants compared to 6.5% in placebo in 8-week follow-up period following immunization (ns)	Boslego et al 1991 (65)
Placebo-controlled human challenge trial	Outer membranes vaccine prepared from single strain of *N. gonorrhoeae*	Participants vaccinated (dosing schedule not available) then inoculated with homologous *N.*	63 male participants	Post-challenge infection rate 54% in immunized participants compared to 64% in placebo (ns)	Rice et al (66)

		gonorrhoeae strain per urethra 2-4 weeks later			

ns, not significant; US, United States.

Table 2. Potential *Neisseria gonorrhoeae* vaccine antigens discovered by traditional or reverse vaccinology approaches

Gene	Protein/ Antigen name	Function	Location	Conservation*	Immunogenicity	Data	Reference
Adherence and invasion of epithelial cells							
Phospholipase							
pldA	Outer membrane phospho-lipase A (OMPLA)	Phospholipid hydrolysis of endogenous phospholipids. Autolysin	Outer membrane	Highly conserved	Murine antibodies elicited by *N. meningitidis* homologue are not bactericidal or protective against infection	Preclinical	Senff et al 1976 (172); Cacciapuoti et al 1978 (173); Bos et al 2005 (174)
PLD	*Neisseria gonorrhoea e* phospho-	Regulator of gonococcal invasion of and survival within	Outer membrane	Highly conserved	Antibodies decrease adherence to and invasion of primary cervical cells	Preclinical	Edwards et al 2003 (92); Edwards and Apicella 2006

	lipase D (NgPLD)	cervical epithelia					(93); Edwards et al 2016 (175)
Pilin							
pilE	Major subunit of the type 4 pilus	Type 4 pilus fiber. Channel for pilus extrusion. Mediates adherence to epithelial cells	Outer membrane	Antigenically variable. Conserved at C terminus	Antibodies to pili block cell attachment but are directed at variable epitopes	Historical vaccine trial	Tramont et al 1981 (176); Siegel et al 1982 (177); Brinton et al 1982 (178); Schoolnik et al 1983 (179); Virji and Heckels 1984 (180); Rothbard et al 1985 (181); Tramont et al

							1985 (70); Boslego et al 1991 (65)
pilC	PilC	Type 4 pilus tip-associated adhesin. Plays key role in pilus biogenesis and adhesion	Outer membrane	Antigenically variable. Phase variable	No data	Preclinical	Backman et al 1998 (87); Morand et al 2001 (88)
pilQ	PilQ	Outer membrane channel for pilus extrusion.	Outer membrane	Antigenically variable. Conserved at C terminus	Antibodies elicited by *N. meningitidis* homologs are bactericidal	Preclinical	Drake and Koomey 1995 (89); Helm et al 2007 (90); Haghi et al 2012 (91)

		Essential role in pilus biogenesis					
Porin							
porB	Porin	Major outer membrane protein. Nutrient channel. Binds complement factors C4bp and Factor H to down-regulate	Outer membrane	Antigenically variable surface loops and conserved membrane-spanning regions	Antibodies are bactericidal, opsonophagocytotic and block gonococcal entry into epithelial cells	Preclinical	Hook et al 1984 (182); Heckels et al 1992 (183); Christodoulides et al 1993 (184); Ram et al 1998 (185); Ram et al 1998 (186); Ram et al 2001 (187); Edwards et al 2002 (188);

		complement activation at gonococcal surface. Suppresses neutrophil oxidative burst and neutrophil apoptosis					Zhu et al 2004 (130); Zhu et al 2005 (129); Kulewein et al 2006 (189); Garvin et al 2008 (190); Faulstich et al 2013 (191); McKnew et al 2013 (84); Yuen et al 2019 (86)
Other outer membrane proteins							

opa	Opacity proteins	Adherence and invasion of host cells Influence innate and adaptive immune responses by binding CEACAM receptors on T and B lymphocytes	Outer membrane	Antigenically variable. Phase variable	Antibodies are bactericidal	Preclinical and controlled human challenge studies	Virji et al 1993 (192); Plummer et al 1994 (193); Chen et al 1997 (194); de Jonge et al 2004 (143); Cole et al 2009 (195); Callaghan et al 2011 (196); Sadarangani et al 2011 (197);

opcA	OpcA	Adhesion and invasion of host epithelial and endothelial cells	Outer membrane	Antigenically variable	Antibodies elicited by *N. meningitidis* homologues are bactericidal	Preclinical	Zhu et al 2003 (198); Moore et al 2005 (199); Keiser et al 2010 (200);
ompA	Outer membrane protein A (OmpA)	Adhesion and invasion of host epithelial and endothelial cells	Outer membrane	Highly conserved	No data	Preclinical	Serino et al 2007 (201); Starnino et al 2010 (202)
nhba	*Neisseria* heparin binding	Involved in adherence to epithelial cells	Outer membrane	Highly conserved	Antibodies are bactericidal, opsono-phagocytotic and block	Preclinical	Marjuki et al 2019 (94); Semchenko et al

	antigen (NHBA)	and serum survival			gonococcal adherence to epithelial cells		2019 (153); Semchenko et al 2020 (96); Semchenko et al 2020 (203)
Nutrient acquisition and metabolism							
Iron metabolism							
tbpA	Transferrin-binding protein A (TbpA)	Essential receptor for iron uptake from transferrin	Outer membrane	Highly conserved	Antibodies are bactericidal	Preclinical and controlled human challenge studies	Cornelissen et al 1998 (97); Masri and Cornelissen 2002 (204); Price et al 2004 (205); Price et al 2005 (98); Price et al 2007 (206);

							Hobbs et al 2011 (51); Cash et al 2015 (81)
tbpB	Transferrin-binding protein B (TbpB)	Increases efficiency of iron uptake from transferrin	Outer membrane	Antigenically variable with conserved segments	Antibodies are bactericidal	Preclinical and controlled human challenge studies	Cornelissen et al 1998 (97); Price et al 2004 (205); Price et al 2005 (98); Price et al 2007 (206); de Rocco and Cornelissen 2007 (207); Hobbs et al 2011 (51); Ostberg et al

							2013 (208); Cash et al 2015 (81)
lbpA	Lactoferrin-binding protein A (LbpA)	Essential receptor for iron uptake from lactoferrin	Outer membrane	Highly conserved. Present in approximately half of isolates.	Antibodies elicited by *N. meningitidis* homologues are bactericidal but cross reactivity (in *N. meningitidis*) is limited	Preclinical and controlled human challenge studies	Mickelsen et al 1982 (209); Biswas et al 1999 (210); Anderson et al 2003 (211); Pettersson et al 2006 (212); Adamiak et al 2012 (213); Noinaj et al 2013 (214)

lbpB	Lactoferrin-binding protein B (LbpB)	Increases the efficiency of iron transport from lactoferrin	Outer membrane	Antigenically variable with conserved segments. Phase variable. Present in approximately half of isolates	Antibodies elicited by *N. meningitidis* homologues are bactericidal but cross reactivity (in *N. meningitidis*) is limited	Preclinical and controlled human challenge studies	Mickelsen et al 1982 (209); Biswas et al 1999 (210); Anderson et al 2003 (211); Pettersson et al 2006 (212); Adamiak et al 2012 (213); Noinaj et al 2013 (214)
fetA	Ferric entero-bactin	Involved in iron uptake through	Outer membrane	Antigenically variable. Phase variable	Antibodies elicited by *N. meningitidis* homologues are bactericidal but cross	Preclinical	Black et al 1986 (215); Dyer et al 1988 (216);

	transporter A (FetA)	scavenging siderophores from other bacteria via binding and transport of ferric enterobactin			reactivity (in *N. meningitidis*) is limited		Pettersson et al 1990 (217); Van der Ley et al 1996 (218); Carson et al 1999 (219); Carson et al 2000 (220)
fetB	Ferric entero-bactin transporter B (FetB)	Involved in iron uptake through scavenging siderophores from other bacteria via	Outer membrane	Antigenically variable	No data	Preclinical	Baarda et al (80)

		binding and transport of ferric enterobactin					
Zinc metabolism							
tdfJ	TonB-dependent family J (TdfJ)	Facilitates uptake of zinc via human protein S100A7	Outer membrane	Highly conserved	Antibodies elicited by *N. meningitidis* homologue are bactericidal	Preclinical	Stork et al 2010 (221); Cornelissen and Hollander 2011 (222); Maurakis 2019 (223)
tdfH	TonB-dependent family H (TdfH)	Facilitates uptake of zinc via human calprotectin	Outer membrane	Highly conserved	No data	Preclinical	Turner et al 2001 (224); Jean et al 2016 (225);

							Kammerman et al 2020 (226)
Anaerobic metabolism							
aniA	Anaerobically induced protein A (AniA)	Inducible nitrite reductase, required for anaerobic growth and biofilm formation	Outer membrane	Highly conserved	Antibodies block nitrite reductase activity	Preclinical	Clark et al 1988 (227); Boulanger et al 2002 (228); Ku et al 2008 (229); Falsetta et al 2009 (99); Falsetta et al 2011 (230); Shewell et al 2013 (102); Shewell et al 2017 (101)

Immune evasion							
lst	Alpha-2,3-sialy-transferase (Lst)	Sialylates the surface lipooligo-saccharide to protect gonococci from complement-mediated killing and phagocytic killing by neutrophils. Incorporates	Cytoplasm (previously thought to be outer membrane)	Highly conserved	Antibodies partially inhibit sialyltransferase activity of *N. gonorrhoeae* however this is inhibited in the presence of exogenous 5'-cytidinemonophospho-N-acetylneuraminic acid (CMP-NANA) present in *N. gonorrhoeae* strains. KDO-specific monoclonal antibody 6E4 is opsonophagocytic.	Preclinical	Smith 1995 (231); Shell 2002 (232); Packiam 2006 (233); Wu & Jerse 2006 (104); Lewis et al 2015 (103); Jen et al 2021 (105)

		keto-deoxyoctanoate (KDO) as the terminal glycan on the LOS					
nspA	Neisserial surface protein A (NspA)	Subverts complement pathway activation by binding to complement inhibitor factor H	Outer membrane	Highly conserved	Antibodies are bactericidal and opsonophagocytic	Preclinical	Martin et al 1997 (234); Li et al 2011 (107); Lewis et al 2019 (106)
Intracellular survival							

lgtG	Lipooligo-saccharide (LOS) epitope 2C7	Inner glycose core of LOS. Promotes colonization and survival	Outer membrane	High antigenic conservation. Phase variable.	Antibodies are bactericidal and opsonophagocytotic	Preclinical	Gulati et al 1996 (108, 235); Banerjee et al 1998 (236); Ngampasutadol 2006 (237); Gulati et al 2012 (238), Gulati et al 2013 (110); Chakraborti et al 2016 (239) Gulati et al 2019 (109, 111)
iga	IgA1-specific	Promotes intracellular	Outer membrane	Highly conserved.	No data	Preclinical	Mulks and Knapp 1987

	protease (IgA1)	survival and release of inflammatory cytokines		Present in approximately 50% of isolates			(240); Simpson et al 1988 (241); Lomholt et al 1995 (242); Lin et al 1997 (243); Lorenzen et al 1999 (244); Karlinsky et al 2022 (245)
mip	Macro-phage Infectivity Potentiator (MIP) lipoprotein	Bacterial persistence within macrophages and protects *Neisseria*	Outer membrane	Highly conserved	Antibodies are bactericidal	Preclinical	Leuzzi et al 2005 (246) Humbert & Christodoulides 2018 (247);

		gonorrhoeae from bactericidal activity of immune effector cells					Christodoulides 2022 (248)
Oxidative stress and antimicrobial substance protection							
msrA/B	Methionine sulfoxide reductase (MsrA/B)	Protects from oxidative stress by reducing methionine sulfoxide to methionine	Outer membrane	Highly conserved	Antibodies are bactericidal, opsonophagocytic and functionally block the activity of MsrA/B by binding to its substrate, methionine sulfoxide	Preclinical	Jen et al 2019 (83)

mtrE	Multiple transferable resistance protein E (MtrE)	Surface-exposed channel of the MtrCDE and FarAB-MtrE efflux pumps that export antimicrobial substances	Outer membrane	Highly conserved. Expression upregulated in multi-drug resistant strains	Antibodies are bactericidal	Preclinical	Delahay et al 1997 (249); Lee & Schafer 1999 (250); Veal et al 2002 (251); Jerse & Deal 2013 (114); Wang et al 2018 (82); Handing et al 2018 (113); Baarda et al 2018 (252)
Other							

NgoΦ6	Filamentous bacterio-phage proteins	Encodes proteins needed for progeny phage production	Outer membrane	Highly conserved	Antibodies are bactericidal and block adherence to cervical epithelial cells	Preclinical	Piekarowiczet al 2016 (253); Klyz & Piekarowicz 2018 (254)
Proteomic and bioinformatic vaccine antigen discovery							
acp	Adhesin complex protein (ACP)	Inhibition of host lysozyme activity, promotes host cell colonization	Outer membrane	Highly conserved	Antibodies are bactericidal and inhibit human lysozyme	Preclinical	Humbert et al 2017 (115); Almonacid-Mendoza et al 2018 (116)
iga2	IgA2 protease (AidA)	Putative adhesion and	Cell envelope	Antigenically variable	No data	Preclinical	El Rami et al 2019 (77); Huang et al

		penetration protein					2020 (255); Baarda et al 2021 (80)
bamA	Beta-barrel assembly machinery protein A (BamA)	Folds and inserts beta-barrel proteins into the outer membrane	Outer membrane	Highly conserved	Antibodies are bactericidal	Preclinical	Zielke et al 2016 (76); Baarda et al 2018 (252)
bamE	Beta-barrel assembly machinery protein E (BamE)	Contributes to outer membrane assembly and integrity	Outer membrane	Highly conserved	No data	Preclinical	El-Rami et al 2019 (77); Baarda et al 2021 (80)

csgG	Curl-specific gene G (CsgG)	Membrane protein	Outer membrane	Moderately conserved	No data	Preclinical	El Rami et al 2019(77); Baarda et al 2021 (80)
lolB	Lipoprotein outer membrane localization lipoprotein B (LolB)	Putative role in lipoprotein trafficking to the outer membrane	Outer membrane	Moderately conserved	No data	Preclinical	El Rami et al 2019 (77); Baarda et al 2021 (80)
lprI	Lipoprotein I (LprI)	Putative lysozyme resistance protein	Cell envelope	Moderately conserved	No data	Preclinical	El Rami et al 2019 (77); Baarda et al 2021 (80)

lptD	Lipopoly-saccharide assembly protein D (LptD)	Lipopoly-saccharide assembly	Outer membrane	Moderately conserved	Antibodies are bactericidal	Preclinical	Zielke et al 2014 (76); Zielke et al 2016 (76)
lptE	Lipopoly-saccharide assembly protein E (LptE)	Putative role in lipopoly-saccharide assembly	Outer membrane	No data	No data	Preclinical	El-Rami et al 2019 (77)
mafA	Multiple adhesin family A (MafA)	Adhesin	Cell envelope	Antigenically variable	No data	Preclinical	El Rami et al 2019 (77); Baarda et al 2021 (80)

metQ	Methionine binding lipoprotein Q (MetQ)	Methionine transport adhesin involved in epithelial cell adherence and survival	Outer membrane	Highly conserved	Antibodies are bactericidal and block gonococcal adherence to human cervical epithelial cells	Preclinical	Zielke et al 2016 (76); Semchenko et al 2017 (118); El-Rami et al 2019 (77); Sikora et al 2020 (119);
NGO0416	-	Hypothetical protein with conserved domain similarity to N-terminal domain of LamB	Periplasm	Moderately conserved	Limited bactericidal antibodies	Preclinical	Zhu et al 2019 (79)

		carbohydrate-specific outer membrane porin					
NGO0425	-	Hypothetical protein	Cell envelope	Moderately conserved	No data	Preclinical	El Rami et al 2019 (77); Baarda et al 2021 (80)
NGO0690	-	Putative lipoprotein possibly involved in threonine biosynthesis	Periplasm/ outer membrane	Moderately conserved	Antibodies are bactericidal	Preclinical	Zhu et al 2019 (79)

		and pilin antigenicity					
NGO0778	-	Membrane protein	Cell envelope	Highly conserved	No data	Preclinical	El Rami et al 2019 (77); Baarda et al 2021 (80)
NGO0948	-	Lipoprotein member of NlpB/DapX family	Periplasm/ outer membrane	Moderately conserved	Antibodies are bactericidal	Preclinical	Zhu et al 2019 (79)
NGO1043	-	Putative lipoprotein, possibly glycosylated and a	Periplasm/ outer membrane	Moderately conserved	Antibodies are bactericidal	Preclinical	Zhu et al 2019 (79)

		substrate for phosoph-ethanolamine addition					
NGO1215	-	Putative protein with homology to a copper chaperone superfamily	Periplasm	Moderately conserved	Antibodies are bactericidal	Preclinical	Zhu et al 2019 (79)
NGO1251	-	Lipoprotein	Cell envelope	Highly conserved	No data	Preclinical	El Rami et al 2019 (77); Baarda et al 2021 (80)

NGO1701	-	Putative with homology to copper - binding protein of the DUF326 super family	Periplasm	Moderately conserved	Antibodies are bactericidal	Preclinical	Zhu et al 2019 (79)
NGO2054	-	Unknown	Outer membrane	Highly conserved	Antibodies are bactericidal	Preclinical	Zielke et al 2016 (76)
ompU	Outer membrane porin protein U (OmpU)	Putative iron uptake protein	Outer membrane	Moderately conserved	No data	Preclinical	El Rami et al 2019 (77); Baarda et al 2021 (80)

sliC	Surface-exposed lysozyme inhibitor of c-type lysozyme (SliC)	Inhibition of host lysozyme activity, promotes host colonization	Outer membrane	Highly conserved	No data	Preclinical	Zielke et al 2018 (117); Baarda et al 2021 (80)
tamA	Trans-location and assembly module A (TamA)	Translocation assembly	Outer membrane	Moderately conserved	Antibodies are bactericidal	Preclinical	Zielke et al 2016 (76)

*Conservation: Amino acid sequence conservation between *N. gonorrhoeae* strains: highly conserved ≥ 80%, moderately conserved ≥50%, antigenically variable <50%.

Table 3. Contemporary *Neisseria gonorrhoeae* vaccines that have proceeded to preclinical studies in the experimental mouse model

Trial design	Vaccine	Immunization schedule	Immunogenicity	Attenuation of gonococcal infection	Reference
Estradiol-treated BALB/c mouse model inoculated vaginally with *N. gonorrhoeae* MS11 approximately 3 weeks after immunization	Gonococcal outer membrane preparation from *N. gonorrhoeae* strain MS11	IN or SC administration 3 times at 3 week intervals	Serum and vaginal antibodies induced by both IN and SC immunization. SBA to heterologous gonococcal strain induced by IN immunization.	Clearance of gonococcal colonization significantly faster in IN immunized compared to control mice.	Plante et al 2000 (139)

BALB/c mouse model immunized with putative vaccine	Gonococcal recombinant plasmid encoding PorB DNA (PorB DNA) from *N. gonorrhoeae* strain FA1090 prime vaccine followed by either PorB DNA, renatured recombinant PorB protein (rrPorB) plus Ribi R-700 adjuvant or PorB expressed from Venezuelan equine encephalitis virus	IM or epidermal gene gun bombardment administration with prime PorB DNA followed by boost with PorB DNA, rrPorB or PorB-VRPs 4-weeks later	Serum antibodies induced by both IM and epidermal gene gun bombardment, with Th1 response induced by IM administration and Th2 response induced by gene gun bombardment. Boosting with rrPorB and PorB VRPs significantly	No data	Zhu et al 2004 (130)

	replicon particles (PorB-VRPs)		increased PorB IgG and IgA antibodies. Serum OPA to homologous gonococcal strain, SBA not produced.		
BALB/c mouse model immunized with various combinations of putative vaccines	*N. gonorrhoeae* vaccines produced from strain FA1090, including renatured recombinant PorB protein (rrPorB) plus Ribi R-700 adjuvant or PorB expressed from Venezuelan equine	SC administration into dorsal area or hind footpad (rrPorB), SC administration into hind footpad (PorB VRP) or IN	Serum anti-PorB antibodies induced by all vaccines tested, with Th1 bias for PorB-VRP and rrPorB in footpad and Th2 bias when	No data.	Zhu et al 2005 (129)

	encephalitis virus replicon particles (PorB-VRPs) or outer membrane vesicle (OMV) vaccine	(OMV) 3-4 times, 3 weeks apart.	rrPorB given in dorsal area. IN OMV induced SBA whilst other vaccines did not.		
BALB/c mouse model immunized with various combinations of vaccine components	Various combinations of *N. gonorrhoeae* strain FA19 1) recombinant transferrin binding protein A (rTbpA) plus Ribi R-700 adjuvant, 2) recombinant transferrin binding protein B (rTbpB) and 3) cholera	IN or SC administration 3 times at 10 day intervals	Serum and vaginal antibodies induced by IN immunization for each Tbp antigen combined with Ctb. SBA induced by IN immunization.	No data	Price et al 2005 (98)

	toxin B subunit (Ctb), either as conjugates or admixed.				
BALB/c mouse model immunized with putative vaccine	*N. gonorrhoeae* vaccines produced from strain FA1090, including renatured recombinant TbpB (rrTbpB) and TbpB expressed from Venezeulan equine encephalitis virus replicon particles (TbpB-VRPs)	SC immunization at 0, 4, 7 and 10 weeks	Serum antibodies induced by both TbpB vaccines, with highest titers in mice immunized with rrTbpB. TbpB-VRP responses Th1-biased. Mucosal antibodies produced by both vaccines with	No data	Thomas et al 2006 (256)

			highest titers in mice immunized or boosted with rrTbpB. Bactericidal antibodies not produced		
Estradiol-treated BALB/c female mouse model inoculated with *N. gonorrhoeae* strain FA1090 approximately 2 weeks after immunization	OMV preparation from *N. gonorrhoeae* strain FA1090 combined with IL-12 microspheres	Intravaginal immunization 3 times at 1 week intervals	Intravaginal OMV/IL-12 microsphere vaccination induced serum and vaginal IgG and IgA antibodies against	Clearance of *N. gonorrhoeae* colonization significantly faster in mice immunized with OMV/IL-12 microsphere vaccine candidate compared to those	Liu et al 2017 (121)

			homologous and heterologous strains.	immunized with OMV or IL-12 microspheres alone	
Smith Webster (CFW) mouse model immunized with putative vaccine	Whole-cell formalin-inactivated microparticle vaccine from *N. gonorrhoeae* strain CDC-F62 loaded in dissolvable microneedles	Transdermal immunization applied for 20 minutes, 3 times, 2 weeks apart	Transdermal microparticle vaccination induced greater serum IgG antibodies than SC vaccination and induced elevated CD4+ and CD8+ responses comparable to SC vaccination.	No data	Gala et al 2018 (144)

BALB/c mouse model immunized with putative vaccine	*porB* gene DNA from *N. gonorrhoeae* strain WHO-A inserted into eukaryotic expression vector pVAX1 (pVAX1-porB) loaded in *S. enteritidis* ghosts (SE ghosts (pVAX1-porB))	PO immunization 3 times at 2 week intervals	SE bacterial ghosts (pVAX1-porB) vaccination induced greater serum IgG antibodies, CD4+ and CD8+ T cell responses than pVAX1-porB DNA vaccine alone. SBA induced.	No data	Jiao et al 2018 (132)
BALB/cAnNCr mouse model inoculated with *N. gonorrhoeae* F62 3 weeks after immunization	Meningococcal detoxified outer membrane vesicle (dOMV) vaccine	IP immunization 3 times at 3 week intervals	dOMV vaccine produced induced serum and vaginal	A significantly higher proportion of mice immunized with meningococcal dOMV	Beernik et al 2019 (138)

	prepared from meningococcal strains deleted for major outer membrane proteins (including PorA, PorB and RmpM) plus Alhydrogel adjuvant		antibodies. SBA not detected.	vaccines prepared from strains deleted for major outer membrane proteins were cleared of Ng compared to control.	
BALB/c mouse model immunized with putative vaccine	*nspA* gene DNA from *N. gonorrhoeae* strain WHO-A inserted into eukaryotic expression vector pVAX1 (pVAX1-nspA) either alone (SE ghosts pVAX1-nspA) or in combination with SE	PO immunization 3 times at 2 week intervals	Co-administered SE ghosts (pVAX1-nspA) and SE ghosts (pVAX1-porB) vaccination induced the highest level of anti-nspA and	No data	Jiao et al 2020 (133)

	ghosts (pVAX1-porB) vaccine described above		anti-porB serum IgG and the highest SBA titres		
BALB/c mouse model immunized inoculated with *N. gonorrhoeae* FA1090 approximately 3 weeks after immunization	Gonococcal recombinant MetQ protein combined with Titermax gold oil-in-water immersion adjuvant subcutaneous vaccine, then subsequently combined CpG 1826 adjuvant intranasal vaccine (rMetQ-CpG)	SC immunization, followed by 3 IN boosts on days 14, 24 and 35	Immunization with rMetQ-CpG induced the highest level of anti-MetQ IgG and IgA serum and vaginal antibodies, with a serum IgG1/IgG2a ratio suggestive of a Th1 response.	Clearance of *N. gonorrhoeae* colonization significantly faster and with a lower burden of infection in mice immunized with rMetQ-CpG vaccine candidate compared to those immunized with PBS or adjuvant alone.	Sikora et al 2020 (119)

CD-1 mouse model immunized with putative vaccine	Meningococcal native outer membrane vesicle (NOMV) vaccine prepared from meningococcal strain with genetically attenuated endotoxin and overexpressed factor H binding protein (FHbp) or inactivated gene encoding FHbp (NOMV-KO) or recombinant FHbp	IP immunization 2 times at 3-week intervals	Immunization with NOMV-FHbp and NOMV-KO induced gonococcal SBA	No data.	Matthias et al 2022 (137)
Estradiol-treated BALB/c mouse model inoculated	Gonococcal native outer membrane	IN or intravaginal immunization 2	IN and intravaginal	Female mice immunized with IN or	Liu et al 2023 (141)

with *N. gonorrhoeae* strain FA1090,FA19 or WHO strain F, L or W approximately 2 weeks after immunization; plus BALB/c male mouse model.	vesicle vaccine (NOMV) from strains FA1090; Gonococcal detergent-extracted OMV (dMV) from strain FA19 and double deletion mutant OMV (dm OMV) prepared from mutant *N. gonorrhoeae* strain MS11 in which genes for Rmp and LpxL1 were deleted to eliminate induction of blocking antibodies against Rmp and to	times at 2-week interval.	immunization of female mice with NOMV plus IL-12 ms induced comparable serum IgG, salivary IgA and vaginal IgG and IgA antigonococcal antibodies. IN immunization of male mice with NOMV plus IL-12 ms induced comparable serum	intravaginal NOMV plus IL-12 ms cleared gonococcal infection faster than mice immunized with control immunization. In addition, female mice cleared gonococcal infection with heterologous strains faster than mice immunized with control immunization. Gonococcal clearance was also accelerated in	

	decrease LOS endotoxicity; all vaccines combined with IL-12 microspheres (ms)		IgG and saliva IgA antigonococcal antibodies to female mice. IFN-gamma production by CD4+ T cells from iliac lymph nodes was elevated after IN or intravaginal immunization with NOMV plus IL-12 ms.	mice immunized with deOMVs comparable to that seen for NOMV immunized mice; Gonococcal clearance was accelerated in mice immunized with OMV plus IL-12 ms vaccine produced from mutant *N. gonorrhoeae* in which genes for Rmp and LpxL1 were deleted to eliminate induction of blocking antibodies against Rmp and to	

				decrease LOS endotoxicity comparable to that seen for NOMV immunized mice	
Estradiol-treated BALB/c mouse model inoculated with *N. gonorrhoeae* strain FA1090	Gonococcal native outer membrane vesicle vaccine (NOMV) from Chilean gonococcal strain GC_08175680 (dmGC0817560 NOMV) and FA1090 (dmFA1090 NOMV) with *lpxL1* and *rmp* genes deleted to	Parenteral administration.	Immunization with dmGC_08175680 NOMV and dmFA1090 NOMV induced gonococcal specific serum and vaginal mucosal IgG and IgA	Immunization of mice with dmGC_08175680 OMV and dmFA1090 NOMV accelerated clearance of FA1090 from mice significantly faster than 4CMenB	MacLennan et al 2022 (140)

	reduce reactogenicity, minimise production of potentially unprotective antibodies and increase NOMV yield; both vaccines formulated with aluminium hydroxide		antibodies and gonococcal-specific Th1/Th17 CD4+ T cell responses		

IM, intramuscular; IN, intranasal; IN, intraperitoneal; OPA: opsonophagocytic antibodies; PO, per oral; SBA: serum bactericidal antibodies; SC, subcutaneous

Table 4. Completed observational trials assessing vaccine effectiveness of meningococcal vaccines on gonorrhoea infection.

Clinical trial design	Vaccine	Immunization schedule	Study population	Result	Reference
Retrospective ecological study of *N. gonorrhoeae* infection incidence in Cuba before and after introduction of VA-MENGOC-BC vaccine	VA-MENGOC-BC *N. meningitidis* vaccine (OMV from *N. meningitidis* serogroup B strain CU385 plus serogroup C capsular polysaccharide from N. meningitidis	Intramuscular; single-dose given in mass vaccination program; 2-dose schedule, 2 months apart given in routine schedule.	Cuban national health registry data comprising annual incidence rates of *N. gonorrhoeae* infection, meningococcal disease and syphilis between 1970-2018, including period of vaccine efficacy trial of VA-MENGOC-BC, mass vaccination campaign	Decreased incidence of *N. gonorrhoeae* infection compared to other STIs observed between 1990-1993 after mass vaccination campaign, and between 2010-2018 compatible with possible impact of routine infant vaccination program.	Sotolongo et al 2007 (150); Perez et al 2009 (60); Azze et al 2019 (58); Reyes Diaz et al 2021 (59)

	serogroup C strain C11)		in age 3 months to 20 years from 1989-1990; and incorporation in routine vaccination schedule from 1991-2018.		
Retrospective ecological study of *N. gonorrhoeae* infection incidence in Norway before and after introduction of MenBvac vaccine.	MenBvac *N. meningitidis* serogroup B vaccine (OMV from *N. meningitidis*	Intramuscular 2-dose schedule.	Norwegian national health registry data comprising incidence rates of gonorrhoea from 1993 onwards and data of vaccine efficacy trial in 13-15	Incidence rate ratio (IRRs) analysis (defined as number of new diagnoses of *N. gonorrhoeae* infection per 100,000 population for vaccinated cohort	Whelan et al 2016 (62)

	serogroup B strain H44/76)		year-old students enrolled in secondary schools between 1988-1992. 93,611 (63%) of the 148,589 children resident in Norway and born during 1973-1976 received MenBvac. Total 2,601 cases of *N. gonorrhoeae* infection reported during 1993-2008.	compared to pre-vaccination and post-vaccination cohorts) demonstrated reduced crude IRR for women aged 20-24 years in the vaccinated cohort (IRR 0.58, 95% CI 0.42-0.8) and reduced adjusted IRR for men aged 20-24 years in the vaccinated cohort (0.68, 95% CI 0.51-0.93) and post-vaccination cohort	

				(0.51, 95% CI 0.33-0.78) between 1993-2008.	
Retrospective case-control study of 15–30-year-old sexual health clinic patients eligible to receive MeNZB vaccine in New Zealand. Cases are defined as confirmed laboratory detection of *N. gonorrhoeae* only from clinical specimen; and controls defined as confirmed laboratory	MeNZB *N. meningitidis* vaccine (OMV from *N. meningitidis* serogroup B strain NZ98/254)	Intramuscular, 3-dose schedule. Infants: age 6 weeks, 3 months and 5 months Children >6 months: 3 doses, 6 weeks apart	Sexual health clinic patients aged 15-30 years and eligible to receive MeNZB (mass vaccination program 2004-2006 age 6 weeks to 20 years and available in schools and primary care until 2008) and diagnosed with *N. gonorrhoeae* and/or *C. trachomatis* infection between Jan	Vaccinated individuals significantly less likely to be cases than controls (511 (41%) vs 6,424 (51%); adjusted OR 0.69 (95% CI 0.61-0.79; p<0.0001). Estimated vaccine effectiveness of MeNZB against *N. gonorrhoeae* infection adjusted for ethnicity, deprivation,	Petoussis-Harris et al 2017 (34)

detection of *C. trachomatis* only from clinical specimen			1, 2005 and Dec 31, 2016. 14,730 cases and controls for analysis: 1,241 incidences of *N. gonorrhoeae* infection; 12,487 incidences of C. trachomatis infection; and 1002 incidences of co-infection.	geographical area and sex 31% (95% CI 21-39; p<0.0001)	
Retrospective ecological study of *N. gonorrhoeae* infection incidence in Sanguenay-Lac-Saint-Jean	4CMenB *N. meningitidis* vaccine (OMV from	Intramuscular, 2-dose schedule.	Public health registry data comprising cases of *N. gonorrhoeae* infection	Decrease in the number of *N. gonorrhoeae* infections and incidence rate of among the	Longtin et al 2017 (61)

region of Quebec, Canada before and after introduction of 4CMenB vaccination program.	*N. meningitidis* serogroup B strain NZ98/254 plus three recombinant protein antigens)		notified between January 2006 and June, 2017 and vaccination uptake data for mass vaccination campaign (mass vaccination campaign of individuals aged 6 months to 20 years conducted May to December, 2014). Overall vaccine coverage was 82%. A total of 231	vaccinated cohort (age 14-20 years) observed during post-vaccination period, whereas it increased in unvaccinated cohort (age 21 years and older). Estimated vaccine impact: *N. gonorrhoeae* infection risk reduction of 59% (95% CI 22-84; p=0.1).	

			gonorrhoea cases were reported among persons aged 14 years and older between January, 2006 and June, 2017.		
Retrospective cohort study of individuals born 1984-1999 eligible for MeNZB vaccination 2004-2008 in New Zealand with primary outcome hospitalization for primary diagnosis of *N. gonorrhoeae* infection.	MeNZB *N. meningitidis* vaccine (OMV from *N. meningitidis* serogroup B strain NZ98/254)	Intramuscular vaccine, 3-dose schedule. Infants: age 6 weeks, 3 months and 5 months Children >6 months: 3 doses, 6 weeks apart.	Individuals born 1984-1999 and residing in New Zealand from 2004 until 2015 (mass vaccination program 2004-2006 age 6 weeks to 20 years and available in schools and primary care until	Vaccinated individuals were significantly less likely to be hospitalized due to *N. gonorrhoeae* infection after adjusting for gender, ethnicity and deprivation (HR 0.76, 95% CI 0.58-0.99) with estimated vaccine	Paynter et al 2019 (57)

			2008) with data available through national registry on vaccination status, sex, ethnicity and deprivation. 935,496 individuals included in the analysis. Overall vaccination coverage 59.2%. 261 cases of hospitalization attributable to *N. gonorrhoeae*.	effectiveness of 24% (95% CI 1-42%).	

Retrospective case-control study of 16-23 year-old individuals with *N. gonorrhoeae* or *Chlamydia trachoomatis* infection in New York City and Philadelphia. Cases defined as confirmed laboratory detection of *N. gonorrhoeae* (NAAT or culture) but not *C. trachomatis*; and controls defined as confirmed laboratory detection of *C.*	4CMenB *N. meningitidis* vaccine (OMV from *N. meningitidis* serogroup B strain NZ98/254)	Intramuscular vaccine, 2-dose schedule minimum 30 days and maximum 180 days apart (single dose categorized as partial vaccination).	Individuals aged 16-23 years old with *C. trachomatis* and/or *N. gonorrhoeae* reported to STI surveillance systems of the New York City Department of Health and Mental Hygiene and the Philadelphia Department of Public Health, with data matched to vaccine registry data system to obtain number and	Vaccinated individuals were significantly less likely to be diagnosed with *N. gonorrhoeae i*nfection. Complete vaccination series unadjusted prevalence ratio (UPR) 0.64, 95% CI 0.51-0.79; p<0.0001 in bivariate analyses and adjusted prevalence ratio (APR) 0.60, 95% CI 0.47-0.77; p<0.0001 in multivariate analyses.	Abara et al 2022 (53)

trachomatis only (NAAT or culture) but not *N. gonorrhoeae*			dates of MenB-4C vaccine doses between Jan 1, 2016 and Dec 31, 2018. 109,737 individuals with 167,706 reported STIs for analysis. 124,876 *C. trachomatis* infections, 18,099 *N. gonorrhoeae* infections and 24,731 were gonococcal and chlamydia co-infections.	Partial vaccination series UPR 0.83, 95% CI 0.72-0.96, p=0.0204 in bivariate analyses and APR 0.74, 95% CI 0.63-0.88; 0=0.0012. Estimated vaccine effectiveness for complete vaccination series 40% (95% CI 23-53) and partial vaccination series 26% (95% CI 12-37%).	

			3,058 STIs occurred after complete vaccination series, 6,519 after partial vaccination series and 155,330 among vaccine-naïve individuals.		
Retrospective case-control study of adolescents and young adults with gonorrhoea or chlamydia infection in the state of South Australia, Australia.	4CMenB *N. meningitidis* vaccine (OMV from *N. meningitidis* serogroup B strain NZ98/254)	Intramuscular vaccine, 2-dose schedule, 8 weeks apart.	Individuals born between Feb 1, 1998 and Feb 1, 2005 that had *N. gonorrhoea* or *C. trachomatis* disease notification between Feb 1, 2019 and Jan	Estimated vaccine effectiveness using *C. trachomatis* infection as controls was 32.6% (95% CI 10.6-49.1) for individuals who received at least one	Wang et al 2022 (54)

Cases defined as all gonorrhoea-positive cases who did or did not have *C. trachomoatis* co-infection at the time of first episode *N. gonorrhoeae* infection. Controls defined *as C. trachomatis*-positive infections only.			31, 2021 (in 2019, a 2-dose vaccination schedule for 15-17 year-old school-based immunization programme was implemented and between 2019-2020, a catch-up programme was available for those aged 17-20 years). 53,356 individuals received at least 1 dose of 4CMenB and	dose; and 32.7% (95% CI 8.3-50.6) for people who received two doses compared to those who were unvaccinated.	

			46,083 received 2 doses. 512 patients with total 575 episodes of gonorrhoea and 3140 patients with 3847 episodes of chlamydia included in analysis.		
Retrospective matched cohort study of 15-30 year-olds who received 4CMenB (plus/minus MenACWY) or MenACWY only in Southern California, United States.	4CMenB *N. meningitidis* vaccine (OMV from *N. meningitidis* serogroup B strain NZ98/254);	4CMenB intramuscular vaccine, 2-dose schedule. Minimum 1 dose included in analysis.	Individuals aged 15-30 years old in Kaiser Permanente Southern California health records noted to be vaccinated with 4CMenB, matched in a	Incident gonorrhoea rates 2.0 (95% CI 1.3-2.8) per 1000-person years for 4CMenB recipients; 5.2 (95% CI 4.6-5.8) per 1000-person years for	Bruxvoort et al 2022 (55)

The exposed group comprising recipients of 4CMenB were matched in a ratio of 1:4 to the unexposed group comprising recipients of MenACWY only by age, sex and year of index vaccination with study outcome positive gonorrhoea NAAT or culture or chlamydia NAAT (negative control).	MenACWY *N. meningitidis* vaccine (serogroup A, C, W, Y polysaccharide conjugate vaccine)	MenACWY intramuscular vaccine, 2-dose schedule. Minimum 1 dose included in analysis	ratio of 1:4 to recipients of MenACWY only by age, sex and year of index vaccination between Jan 1, 2016 and Dec 12, 2019. 6,641 4CMenB recipients; matched to 26,471 MenACWY only recipients.	MenACWY only recipients. Incident chlamydia rates 12.4 (95% CI 10.7-14.4) per 1000-person years for 4CMenB recipients; 15.2 (95% CI 14.2-16.2) per 1000-person years for MenACWY only recipients. Hazard ratio (HR) for incident gonorrhoea in 4CMenB recipients compared to MenACWY	

				only recipients 0.54 (95% CI 0.34-0.86) in multivariable analyses.	
Retrospective case-control study of ≥18 year old MSM living with HIV with gonorrhoea or syphilis, chlamydia or anal HPV in Milan, Italy. Cases defined as all gonorrhoea-positive cases by NAAT or culture; Controls were defined as	4CMenB *N. meningitidis* vaccine (OMV from *N. meningitidis* serogroup B strain NZ98/254)	Intramuscular vaccine, 2-dose schedule, 8 weeks apart.	≥18 year old MSM living with HIV diagnosed with gonorrhoea or syphilis, chlamydia or anal HPV included in the database of the Infectious Diseases Unit at the San Raffaele Scientific Institute, Milan, Italy	Estimated vaccine effectiveness was 42% (95% CI 6-64, p=0.027) and remained significant at 44% (95% CI 9-65, p=0.020) after adjustment in multivariable analysis.	Raccagni et al 2023 (56)

chlamydia positive by NAAT, syphilis positive by serology and HPV positive by anal NAAT to 28 HPV genotypes or following a diagnosis of condylomatosis.			between July, 2016 and February 2021. 349/1051 (33%) received 4CMenB vaccination. 103 cases and 948 controls analysed. Median follow up 3.8 years (2.1-4.3)		

HIV, human immunodeficiency virus; HPV, human papillomavirus; MSM, men who have sex with men; NAAT, nucleic acid amplification test; OMV, outer membrane vesicle

Table 5. Randomised trials of vaccine effectiveness of meningococcal vaccines on gonorrhoea infection currently in design, recruitment or pre-publication phases.

Clinical trial design	Vaccine and immunization schedule	Study population	Recruitment strategy	Primary outcome	Reference
Phase III, double-blinded, randomised,	4CMenB; Intramuscular administration, 2	18-50 year-old men (cis and trans), transexual	730 participants enrolled and randomised	1. To measure whether 4CMenB changes the incidence of first	Seib et al (https://clinicaltrials.gov/study/NCT04415424)

placebo-controlled, multi-centred trial evaluating the efficacy of 4CMenB in prevention of gonorrhoea infection (GoGoVax).	doses, 3 months apart OR placebo.	women and non-binary people who have sex with men; either HIV-negative and on PrEP or HIV-positive with HIV viral load <200 copies/ml and CD4	1:1. Recruitment for 12 months. After vaccination, all participants followed-up 3-monthly for 24 months.	episode *N. gonorrhoeae* infection. 2. To compare overall incidence of all episodes of *N. gonorrhoeae* infection diagnosed during the study period between vaccine and placebo arms.	

		count >350 cells/cmm.			
Phase II, randomised, observer-blind, placebo-controlled, multi-centre trial evaluating the efficacy of 4CMenB in prevention of urogenital/and	4CMenB; Intramuscular administration, 2 doses, 2 months apart OR placebo.	18-50 year-old healthy men and women	Approximately 2,200 participants and randomised 1:1. After vaccination, participants followed 3-monthly for 16 months.	To measure efficacy of 4CMenB in prevention of urogenital and/or anorectal infection.	Marazzo et al (https://clinicaltrials.gov/study/NCT04350138)

or anorectal gonorrhoea infection					
Single-site double-randomised controlled trial evaluating the efficacy of 4CMenB in prevention of gonorrhoea using a controlled	Initial vaccination phase: 2 doses of intramuscular 4CMenB OR quadrivalent influenza and tetanus/diphtheria vaccine; Post-challenge vaccination: crossover arm	18-35 year-old men without a history of 4CMenB vaccination	Approximately 120-140 participants enrolled and randomized 1:1.	Infectivity of *N. gonorrhoeae* inoculum defined as the proportion of participants with microbiological evidence of *N. gonorrhoeae* by culture or NAAT in urine or urethral swab culture on the	Duncan et al (https://clinicaltrials.gov/study/NCT05294588)

human experimental infection with *N. gonorrhoeae* strain FA1090	with receipt of either quadrivalent influenza and tetanus/diphtheria vaccines or 2 doses of 4Cmen B.			post-inoculation antibiotic treatment day in each study group.	
Single-site, parallel, double-blind, randomised, placebo-controlled trial evaluating the efficacy of	4CMenB; intramuscular administration, 2 doses, 1 month apart OR placebo	MSM aged 18 or above at risk of gonorrhoea infection (condomless sex with >1 man in last 6	150 participants.	Incidence of *N. gonorrhoeae* infection between control and intervention groups	Kwan T et al (https://clinicaltrials.gov/study/NCT05766904)

4CMenB in prevention of gonorrhoea infection		moths, history of STI, inclination to have condomless sex and other HIV PrEP-eligible criteria)			
Randomised, open-label, single-site trial evaluating the efficacy of	4CMenB; intramuscular administration, 2 doses, 3 months apart	18-50 year old gay and bisexual men that are currently	130 participants enrolled and randomised 1:1. Followed	Number of *N. gonorrhoeae* infections in participants over 2	Thng et al (147)

4CMenB in prevention of gonorrhoea infection (MenGo)		taking HIV PrEP or have been diagnosed with gonorrhoea in the past 3 months	3-monthly for 24 months.	years measured by NAAT	

Footnotes: HIV, human immunodeficiency virus; MSM, men who have sex with men; NAAT, nucleic acid amplification test; PrEP, pre-exposure prophylaxis