The serogroup B meningococcal vaccine Bexsero elicits antibodies to \textit{Neisseria gonorrhoeae}

Evgeny A. Semchenko$^1$, Aimee Tan$^1$, Ray Borrow$^2$, Kate L. Seib$^{1,\#}$

$^1$ Institute for Glycomics, Griffith University, Gold Coast, QLD, 4222, Australia

$^2$ Vaccine Evaluation Unit, Public Health England, Manchester Royal Infirmary, Manchester M13 9WZ, United Kingdom

$\#$ Address correspondence to Kate L. Seib: k.seib@griffith.edu.au, +61 7 55527453.

Summary:

This study demonstrates that \textit{Neisseria gonorrhoeae} shares a high level of sequence identity with OMV antigens in serogroup B meningococcal vaccines, MeNZB and Bexsero. The Bexsero NHBA recombinant antigen is also conserved in \textit{N. gonorrhoeae}. Furthermore, serum from humans vaccinated with Bexsero is able to recognize several gonococcal proteins, including the gonococcal NHBA homologue.
Abstract

Background Neisseria gonorrhoeae and Neisseria meningitidis are closely related bacteria that cause a significant global burden of disease. Control of gonorrhoea is becoming increasingly difficult due to widespread antibiotic resistance. While vaccines are routinely used for *N. meningitidis*, no vaccine is available for *N. gonorrhoeae*. Recently, the outer membrane vesicle (OMV) meningococcal B vaccine, MeNZB, was reported to be associated with reduced rates of gonorrhoea following a mass vaccination campaign in New Zealand. To probe the basis for this protection we assessed cross reactivity to *N. gonorrhoeae* of serum raised to the meningococcal vaccine Bexsero, which contains the MeNZB OMV component plus three recombinant antigens (NadA, fHBP-GNA2091, and NHBA-GNA1030).

Methods Bioinformatic analysis was performed to assess the similarity of MeNZB OMV and Bexsero antigens to gonococcal proteins. Rabbits were immunised with the OMV component or the three recombinant antigens of Bexsero, and Western blot and ELISA were used to assess generation of antibodies recognising *N. gonorrhoeae*. Serum from humans immunised with Bexsero was investigated assess the nature of the anti-gonococcal response.

Results There is a high level of sequence identity between MeNZB OMV and Bexsero OMV antigens, and gonococcal proteins. NHBA is the only Bexsero recombinant antigen that is conserved and surfaced exposed in *N. gonorrhoeae*. Bexsero induces antibodies in humans that recognise gonococcal proteins.

Conclusions The anti-gonococcal antibodies induced by MeNZB-like OMV proteins could explain the previously seen decrease in gonococcal cases following MeNZB vaccination. The high level of anti-gonococcal-NHBA antibodies generated by Bexsero vaccination in humans may result in additional cross-protection against gonorrhoea.

Keywords: STI, gonorrhea, *Neisseria gonorrhoeae*, immune response, meningococcal vaccine
Introduction

The sexually transmitted infection gonorrhoea is a global public health concern [1]. It is estimated that there are approximately 100 million cases of gonorrhoea worldwide each year [2], with the number of cases rising in recent years [3, 4]. Symptomatic gonococcal infections most commonly present as urethritis in males and cervicitis in females, although mucosal infections of the rectum, pharynx and eye frequently occur. Furthermore, asymptomatic infections are common and if undiagnosed or untreated, gonorrhoea can lead to severe sequelae including pelvic inflammatory disease, adverse pregnancy outcomes, neonatal complications, and infertility. Gonococcal infection also increases the risk of HIV [1, 5]. The effectiveness of antibiotics has been significantly compromised, and strains with high level resistance to the last line of antibiotics, the expanded-spectrum cephalosporins, have been isolated from around the world [6]. As such, *N. gonorrhoeae* has been prioritized as an urgent public health threat for which immediate action is needed [7, 8], including development of a gonococcal vaccine [9].

Vaccine development has been challenging for *N. gonorrhoeae*, and none of the vaccine candidates tested in clinical trials have afforded protection against gonorrhoea. This is largely due to its various mechanisms of immune evasion, the lack of an animal model that mimics natural disease, and our limited understanding of what is required to induce a protective immune response [1, 10]. However, several different approaches have identified promising gonococcal vaccine candidates [11-17]. In addition, recently a vaccine to a closely-related pathogen, the *Neisseria meningitidis* serogroup B outer membrane vesicle (OMV) vaccine MeNZB, was associated with decreased rates of gonorrhoea [18, 19]. MeNZB was developed in response to a meningococcal epidemic in New Zealand, and over 1 million people were vaccinated between 2004-2008 [20]. A retrospective case-control study showed that individuals vaccinated with MeNZB were significantly less likely to contract gonorrhoea compared with unvaccinated controls, with an predicted vaccine efficacy of 31% [18].
OMVs are spherical bi-layered membrane structures that are naturally released from the outer membrane of Gram-negative bacteria, and contain phospholipids, lipopolysaccharides (LPS) and a mix of outer membrane proteins [21, 22]. The most abundant proteins in meningococcal OMVs include PorA, PorB, and OpcA, with the antigenically diverse PorA being immunodominant and the main target of serum bactericidal antibodies [23]. However, functional antibodies are raised against other OMV components and some cross-protection against heterologous strains with mismatched PorA types has been reported [24]. Despite causing distinct diseases, *N. meningitidis* and *N. gonorrhoeae* are genetically and antigenically very similar with 80-90% nucleotide identity across the genome, and many protein sharing high levels of identity (e.g., PorB share 60-70% amino acid homology) [14, 25]. As such, meningococcal OMV vaccines may induce functional antibodies against gonococcal strains. Several other observational studies have also reported reduced rates of gonorrhoea following the use of OMV-based meningococcal vaccines [26-30].

MeNZB is no longer available. However, the broad-spectrum serogroup B vaccine, Bexsero, contains the MeNZB OMV antigen plus three recombinant antigens (NadA, fHbp-GNA2091, and NHBA-GNA1030) [31]. Neisseria adhesin A (NadA), factor H binding protein (fHbp) and Neisserial Heparin Binding Antigen (NHBA) induce serum bactericidal antibodies against diverse strains [32, 33]. The accessory proteins GNA2091 [34] and GNA1030 [35] are fused with fHbp and NHBA, respectively, and increase their immunogenicity and serum bactericidal titres [33]. In *N. gonorrhoeae*, the gene encoding NadA is absent [36, 37], fHbp is present but not surface exposed [38], and genes encoding NHBA, GNA2091, and GNA1030 are present [36, 37] but have not been characterized in detail. These studies found NHBA present in 17/17 *N. gonorrhoeae* strains studies with an average identity of 81.2% to NHBA-2 peptide in Bexsero [37], and in 97/111 *N. gonorrhoeae* with 65.6% identity to the non-vaccine NHBA-3 peptide from *N. meningitidis* strain MC58 [36]. Here we investigate the similarity of antigens present in MeNZB and Bexsero to gonococcal proteins, the capacity of MeNZB-like OMVs and Bexsero recombinant antigens to induce anti-gonococcal antibodies, and the specificity of antibodies induced by Bexsero vaccinated humans to recognise gonococcal surface antigens.
Methods

Bacterial strains

*N. gonorrhoeae* strains 1291, FA1090 and WHO K were grown at 37°C with 5% CO₂ on GC agar or broth (Oxoid) supplemented with IsoVitalex (Becton Dickinson).

Sequence analysis

Allele and protein sequences of vaccine antigens are shown in Table 1. Sequences were aligned with CLUSTAL in MEGA7 and % amino acid identity and similarity calculated (BLOSUM90, threshold 0). Protein identity between gonococcal strains was determined by BLASTp of sequences from *N. gonorrhoeae* 1291 against 438 gonococcal genomes in GenBank.

NHBA distribution was investigated in >3,000 *N. gonorrhoeae* genomes (PubMLST [39]) using BLASTx with NHBA from *N. gonorrhoeae* 1291 (GenBank Accession EEH61857-1). Amino acid sequence alignments, phylogeny tree construction and annotation were performed using Clustal Omega at EMBL-EBI, MEGA (v7.0.26) and iTOL (v3.5.4), respectively.

OMV preparation

Naturally secreted gonococcal OMVs were isolated as described previously [40]. Briefly, OMVs were harvested from a 6-hour culture (OD₆₀₀ ~0.8) by brief centrifugation (5,000 x g), the supernatant filtered (0.22µm filter), filtrate centrifuged (100,000 x g, 1 hour, 4°C), pellet washed with PBS, then OMVs solubilized in PBS-0.2% SDS.
Expression of recombinant NHBA (rNHBA)

*E. coli* BL21(DE3) was transformed with pET19b carrying the mature NHBA (no signal sequence) from *N. gonorrhoeae* 1291 (amplified using 5'-ATTActcgagTCGCCGATGTCAAGTC-3' and 5'-TGAAgatccCGGCATCAACATCAATC-3' primers containing Xhol and BamHI sites shown in lower case, respectively). Expression of rNHBA was induced (100 mM IPTC, 16 hours, 25˚C) and protein purified using TALON affinity resin (Clontech), as described previously [40].

Polyclonal rabbit serum

The rabbit sera to Bexsero vaccine antigens were generated as per Giuliani et al [33], and provided by Novartis Vaccines. New Zealand White rabbits were immunized with 10 µg NZ98/254 OMV (α-OMV) or a combination of 25 µg each of the recombinant antigens NadA, fHbp-GNA2091, NHBA-GNA1030 (α-rMenB) with aluminum hydroxide on days 0, 21, and 35, and blood taken on day 49.

Human serum

Pre- and post-vaccination human serum was obtained from a previous phase II trial, in which adult laboratory staff were vaccinated with three doses of Bexsero at zero, three and six months [41]. Pre-vaccination (month 0) and one-month post third dose of Bexsero (month 7) serum samples from ten individuals were tested. In addition, samples from healthy adults, with no history of meningococcal disease, vaccinated with two doses of Bexsero at zero and two months (as per Australian recommendations [42]) was collected in accordance with Griffith University Human Ethics Committee (HREC 2012/798).
Western blot

Western blot analysis [40] was performed with whole cell lysates, OMV or rNHBA separated using 12% Bis-Tris NuPAGE polyacrylamide gels. Rabbit (1:2,000) or human (1:4,000) primary antibody, and horseradish peroxidase (HRP)-conjugated anti-immunoglobulin secondary antibody (Sigma-Aldrich) were used for protein detection. Duplicate gels were Coomassie stained to confirm equal sample loading.

ELISA

ELISAs [40] were performed with 96-well MaxiSorp (NUNC) plates coated with *N. gonorrhoeae* or *N. meningitidis* (50 µL/well of OD<sub>600</sub> 0.2), OMVs (2 µg/mL), rNHBA (1 µg/mL) or LPS (1 µg/mL [43]). Binding by rabbit or human antibodies was detected using Goat Anti-Rabbit HRP (1:2,000; Dako) or Goat Anti-Human IgG Fc HRP (1:20,000; ThermoFisher), respectively. The ELISA titre is the highest serum dilution with absorbance at 450 nm > mean negative (all reagents excluding primary sera) + 3 standard deviations.

Results

Gonococcal proteins share a high level of identity with serogroup B meningococcal vaccine antigens

To investigate the sequence conservation between serogroup B meningococcal vaccine antigens and *N. gonorrhoeae*, the major OMV protein antigens present in MeNZB and Bexsero, and the recombinant protein antigens present in Bexsero, were compared to gonococcal proteins from available *N. gonorrhoeae* genomes. OMVs contain a heterogeneous mix of numerous proteins, however proteomic analysis of OMVs from Bexsero vaccine preparations identified a core set of 22 proteins that comprise >90% of OMV content [44]. Several of these major OMV proteins induce an antibody response to meningococcal strains post MeNZB vaccination [45]. Our bioinformatic analysis identified homologues of 20 of the 22 core OMV proteins in *N. gonorrhoeae* strain FA1090 (table 1). Of these 20 homologues, 16 proteins have >90% identity, two proteins have >80%, and two proteins are poorly conserved in FA1090 (PorB [24] and OpcA...
For the two proteins absent in FA1090, porA is a pseudogene in *N. gonorrhoeae* [47] and lbpA is a pseudogene in *N. gonorrhoeae* strain FA1090 but is expressed by the majority of gonococcal strains. Of the major OMV proteins that have a homologue in *N. gonorrhoeae*, 14 of these also have a high level of sequence identity (94-100%) in the 438 gonococcal genome strains available in GenBank (table 1).

Analysis of the recombinant Bexsero antigens confirmed previous findings from smaller strain panels [36, 37], that the gene encoding NadA is absent in *N. gonorrhoeae*, while homologues of fHbp, NHBA, GNA2091 and GNA1030 are conserved *N. gonorrhoeae* strains (table 1). Since fHbp [38], GNA2091 [34], and GNA1030 [35] are not believed to be surface exposed in *N. gonorrhoeae*, further investigation focused on NHBA. Bexsero contains NHBA-2, which shares 68.8% identity to the NHBA variant from strain FA0190 (NHBA-527; table 1). Investigation of NHBA in *N. gonorrhoeae* genome strains in GenBank revealed that *nhba* is present in 100% of strains. An expanded search in the PubMLST database indicated that 72% of gonococcal strains (3,068/4,953) have an annotated *nhba* gene (NEIS2109). This is likely an underestimate of the presence of *nhba*, due to duplicate and incompletely annotated genomes. There are 41 unique NHBA variants reported for *N. gonorrhoeae* and 393 for *N. meningitidis* in PubMLST. Three NHBA variants, represented by *N. gonorrhoeae* strains WHO K (NHBA-475), PID332 (NHBA-481), and 1291 (NHBA-542), account for 82% gonococcal strains, and these variants each share 67% identity to the NHBA-2 in Bexsero (figure 1A). The phylogenetic relatedness of the most common NHBA variants (i.e., variants present in ≥1% of *N. meningitidis* or *N. gonorrhoeae* strains in the database) is shown in figure 1B. This tree highlights the relatively high conservation of NHBA in *N. gonorrhoeae*, with 93·7-100% amino acid identity between strains (figure 1, table 1). Epitope-mapping indicates that the human monoclonal antibodies 12E1 and 10C3 bind to the N-terminal region [48], while 5H2 interacts with a large cross-reactive conformational epitope in the C-terminal of NHBA-2 [49]. The regions bound by 12E1, 10C3 and 5H2 are conserved in the main gonococcal NHBA variants (figure 1).
Bexsero OMVs and recombinant antigens can elicit antibodies in rabbits that recognise *N. gonorrhoeae* antigens

To investigate the ability of antibodies raised to serogroup B meningococcal vaccine antigens to recognize gonococcal proteins, Western blot and ELISA analysis was performed using serum from rabbits immunised with either the OMV present in MenZB and Bexsero (anti-OMV) or a combination of the recombinant antigens of Bexsero (anti-rMenB). Several bands in whole cell lysates of *N. gonorrhoeae* strains WHO K, FA1090 and 1291 are recognized by the anti-OMV sera, and these proteins are consistent between the three gonococcal strains and similar, but not identical, to proteins recognized in *N. meningitidis* (figure 2A&B, supplementary figure S1 in the supplementary appendix). The anti-OMV sera had an ELISA titre of 128,000 to OMVs from *N. gonorrhoeae* 1291 (supplementary figure S1).

The anti-rMenB sera recognized all Bexsero protein antigens in *N. meningitidis* strain MC58 (NadA, fHbp, NHBA, GNA2091, and GNA1030), while only NHBA, GNA2091, and GNA1030 were recognised in *N. gonorrhoeae* (figure 2A&C). NHBA was not recognized in trypsin treated *N. gonorrhoeae*, confirming that NHBA is surface exposed (figure 2A&C). Detection of GNA2091 and GNA1030 were unchanged by trypsin treatment of *N. gonorrhoeae* (figure 2A&C) indicating that they are located inside the cell, as previously described for *N. meningitidis* [34, 35]. The anti-rMenB sera recognised rNHBA from *N. meningitidis* MC58 and *N. gonorrhoeae* 1291 equally (figure 2D), with an ELISA titre of 2,048,000 to both proteins (supplementary figure S1).

Bexsero vaccination in humans generates antibodies that recognise *N. gonorrhoeae* whole cells, OMVs and NHBA

To investigate the ability of human, Bexsero-induced antibodies to recognize gonococcal proteins, Western blot and ELISA analysis was performed using serum from humans vaccinated with either three (0, 1, 3 months) or two (0, 1 month) doses of Bexsero. For the ten individuals given three doses of Bexsero, there
was no significant increase in the geometric mean ELISA titre (GMT) of the samples from pre-vaccination to one-month post-vaccination (dose three) for gonococcal OMVs, which is likely due to the high pre-vaccine titres of some individuals. However, titres were significantly increased from pre- to post-vaccination for whole-cell \textit{N. gonorrhoeae} (1.8-fold increased GMT, compared to 5.7-fold increase against whole-cell \textit{N. meningitidis}) and gonococcal NHBA (34-fold increase) (table 2, figure 3 & supplementary figure S2-S5).

Western blot analysis of whole cell lysates supports the ELISA data, and shows reactivity to several gonococcal and meningococcal antigens with vaccinated but not pre-immune serum (figure 3). There is a minimal amount of LPS present in detergent extracted OMVs, that can induce a weak increase in antibodies to meningococcal LPS [45]. We see a minor increase in antibodies to meningococcal LPS, but no response to gonococcal LPS in sera from Bexsero vaccinated individuals (supplementary figure S6).

Analysis of serum from individuals who received two doses of Bexsero was also performed as this is the current recommended adolescent schedule in Australia, UK, Canada, and USA. Antibodies recognising gonococcal OMV proteins were induced above pre-vaccination base-line to similar levels at one-month post first and second vaccine dose (ELISA titre of 8,000) (figure 4A(i)). Western blot analysis indicated that pre-vaccination serum did not cross react with gonococcal OMV proteins, while post second sera dose reacted with several proteins (figure 4A(ii)). Antibodies recognising the gonococcal NHBA were induced after dose one (titre of 64,000) and to a very high-level one-month post dose two (titre of 512,000; figure 4B(i)). Western blot analysis indicated that pre-vaccination serum did not cross react with gonococcal or meningococcal rNHBA (figure 4B(ii)), while post second dose reacted equally well with these rNHBA proteins (figure 4B(ii)).

Discussion
This study provides both bioinformatic and serological data on the potential of meningococcal vaccine antigens to generate an immune response that recognizes gonococcal proteins. These data provide experimental evidence for the concept that cross-reactive antibodies may be the mechanism that underlies the recent observation that the meningococcal serogroup B OMV vaccine MeNZB was associated with reduced rates of gonorrhoea [18]. The broad-spectrum serogroup B vaccine Bexsero, which contains the MeNZB OMV antigen plus three recombinant antigens (NadA, fHbp-GNA2091, and NHBA-GNA1030) is now licensed worldwide. In this study, we determined that there is a high level of amino acid identity between most of the major MeNZB/Bexsero OMV proteins and \emph{N. gonorrhoeae} homologues, and that OMV-induced antibodies recognise gonococcal proteins. Furthermore, we have shown that NHBA is the only Bexsero recombinant protein antigen with a homologue in \emph{N. gonorrhoeae} that is exposed on the surface of the bacteria, and therefore accessible to vaccine induced antibodies. We have also identified a high level of homology and cross-reactivity between the meningococcal and gonococcal NHBA proteins, which suggests that Bexsero may result in additional cross-protection against gonorrhoea, above that predicted for MeNZB.

The highly variable PorA protein is the main antigen in meningococcal OMV vaccines that induces bactericidal antibodies, and it has long been considered that OMV vaccines do not protect against \emph{N. meningitidis} strains expressing heterologous PorA types [23]. However, there is increasing evidence that some level of protection is provided against heterologous meningococcal strains, potentially due to minor OMV antigens, synergy between antigens, and/or a general immunomodulatory effect of OMVs induced by bacterial components such as LPS [24, 45, 50-52]. It is important to note that although serum bactericidal activity is the established correlate of immune protection for \emph{N. meningitidis}, the mechanisms of immune protection against \emph{N. gonorrhoeae} are unknown and may involve bactericidal, opsonophagocytic, and/or functional blocking activity of antibodies, or cell-mediated killing. Since \emph{N. gonorrhoeae} rarely causes the invasive, life-threatening sepsis that is typical of meningococcal infection, then sterilising immunity conferred by meningococcal vaccines may not be required or appropriate to prevent gonococcal
transmission and disease. Rather, a vaccine that is able to reduce mucosal colonisation and transmission (i.e., if antibodies that target a gonococcal adhesin are able to block bacterial adherence) or reduce pathology (i.e., if antibodies that target a gonococcal virulence factor are able to reduce bacterial ascension to the upper genital tract) may be sufficient to reduce prevalence and disease burden [53]. Other studies have shown various levels of cross-reactivity or functional activity to *N. gonorrhoeae* of antibodies raised to meningococcal vaccines. For example, mouse sera raised to an intranasal serogroup B Proteoliposome vaccine recognizes *N. gonorrhoeae* by ELISA [54] and mouse sera raised to the meningococcal NHBA or NHBA-GNA1030 fusion protein can cross-react with *N. gonorrhoeae* F62, and induce complement deposition as detected by flow cytometry [55]. Mouse sera raised to Bexsero or OMVs was able to reduced gonococcal adherence to epithelial cells, and serum bactericidal activity against *N. gonorrhoeae* FA1090 has been shown for mouse sera raised to either Bexsero, the Bexsero OMV or recombinant protein component, or NHBA, GNA1030 or GNA2091 alone, although similar SBA titres were seen for all these antigens despite whether they are surface localised on *N. gonorrhoeae* or not [55]. Mice immunized Bexsero have been reported to have a significant reduction in percentage of mice colonized and bacterial burden through 7 days post-infection [56]. Mouse sera raised to native meningococcal OMVs has also been shown to induce SBA activity against *N. gonorrhoeae* FA1090, but no SBA activity was seen with serum from humans vaccinated with Bexsero in this study [57].

Irrespective of the functional immune response required for protection against gonorrhoea, there was a high level of immune reactivity of human Bexsero induced antibodies to *N. gonorrhoeae*. There was a significant increase in ELISA GMT between pre- and post-Bexsero vaccine serum against gonococcal whole cells, and although the GMT to OMVs was not significantly increased, >50% of the samples did have an increased response to OMVs post vaccination. Several individuals had high pre-immune titres against whole cells and OMVs, which was consistent with findings from the original study where high baseline immunity against serogroup B meningococcal strains were seen (71% of individuals had pre-vaccination bactericidal antibody titres above the cut-off for PorA) [41], potentially due to prior meningococcal carriage or exposure.
in this at-risk laboratory cohort. The use of sera from laboratory workers is a potential limitation, and a larger study in the general population is needed. However it is important to note that carriage rates of up to 35% are seen in young adults [58]. Of the recombinant antigens present in Bexsero that are not components of MeNZB, NHBA is potentially the only protein that may be able to provide an additive, protective effect towards \(N.\ gonorrhoeae\). The Bexsero NHBA-2 shares 69% identity to the NHBA-527 variant from \(N.\ gonorrhoeae\) strain FA1090, and as previously reported, the main difference between NHBA-2 and gonococcal NHBA peptides is due to a 189 nucleotide deletion the N-terminal half of the gonococcal nhba gene [36]. Despite the presence of different NHBA variants, Western blot and ELISA data presented here show strong immune reactivity of anti-NHBA antibodies to \(N.\ gonorrhoeae\), with a 34-fold increase in the ELISA geometric mean titre between pre- and post-Bexsero vaccine serum against the gonococcal NHBA antigen. This cross-reactivity between NHBA variants is supported by recent analysis of meningococcal strains circulating in the USA, which demonstrated immune reactivity of anti-NHBA antibodies with 99.5% of isolates, irrespective of the NHBA genotype [59]. This MATS analysis predicted that NHBA provided high (84-100%) coverage of strains that expressed one of the eight major NHBA variants present in the USA isolates [59], including NHBA-10, 20, 21 and 29 (shown in figure 1) that share a similar level of identity to the NHBA-2 as the three-major gonococcal NHBA variants (72-79% identity for meningococcal vs 67% identity for gonococcal variants). NHBA induces antibodies that have serum bactericidal activity against a diverse collection of meningococcal strains [32, 33], are opsonophagocytic [60, 61], and that are able to block adherence of \(N.\ meningitidis\) to epithelial cells [62]. NHBA antibodies may have similar functional activities against \(N.\ gonorrhoeae\).

Mathematical modelling of hypothetical gonococcal vaccines indicated that a vaccine efficacy of 31%, as predicted for MeNZB [18], could decrease gonorrhoea prevalence by >30% in the 20 years after vaccine implementation, if vaccine-induced protection could be maintained for longer than 10 years [53]. A higher level of vaccine efficacy would be optimal and may potentially be afforded by Bexsero due to the additional NHBA component. Given that antibiotic resistant gonococcal infections are a rapidly emerging health
problem worldwide, vaccine development is an increasing priority [9] and if untreatable gonorrhoea [63] becomes widespread, then a modestly effective vaccine would be better than no vaccine. Ideally a gonococcal-specific vaccine consisting of a combination of promising candidate gonococcal antigens (as recently reviewed [1, 10]) should enter human clinical trials as soon as possible to determine whether a higher vaccine efficacy can be achieved. However, until now there have been limited tools to enable discovery of what is required to induce a protective immune response and there has been little to no clinical progress towards a gonococcal vaccine in the last three decades [1, 10]. The landmark finding that individuals vaccinated with MenB were significantly less likely to contract gonorrhoea compared with unvaccinated controls [18] represents the first time that any vaccine has been associated with protection against gonorrhoea in humans. This observation, and the data on cross-reactivity outlined here, provides a new opportunity to progress gonococcal vaccine development, guided by the human immune response to the vaccine-mediated presentation of antigens that are common between *N. gonorrhoeae* and the closely related *N. meningitidis*. Further work is needed to identify the full set of gonococcal targets recognized by Bexsero-induced antibodies, and the mechanism(s) of protection against gonorrhoea that are mediated by these antibodies or by other components of the immune system. However, this study provides a new framework to advance gonococcal vaccine development and firm evidence to justify new human trials to investigate the potential level of Bexsero-induced protection against gonorrhoea.

**Supplementary Data**

Figures S1-S6 are shown in the Supplementary Appendix.

**Acknowledgments**

We would like to acknowledge Michael P. Jennings for valuable discussions and critical review of the manuscript, and Novartis Vaccines, Siena, Italy for provision of rabbit sera.
This publication made use of the Neisseria Multi Locus Sequence Typing website (https://pubmlst.org/neisseria/) developed by Keith Jolley and sited at the University of Oxford; the development of this site has been funded by the Wellcome Trust and European Union.

**Funding**

This work was supported by the Australian National Health and Medical Research Council (NHMRC) (Career Development Fellowship and Project Grants 1028326 and 1099278 to KLS).

**Conflict of interest**

RB performs contract research on behalf of Public Health England for GlaxoSmithKline (GSK), Pfizer, and Sanofi Pasteur. KLS worked for Novartis Vaccines on the development of Bexsero, from 2006 to 2012.
References


23. Martin DR, Ruijne N, McCallum L, O'Hallahan J, Oster P. The VR2 epitope on the PorA P1.7-2,4 protein is the major target for the immune response elicited by the strain-specific group B meningococcal vaccine MeNZB. Clin Vaccine Immunol 2006; 13(4): 486-91.


Table 1. Bexsero vaccine components and their homology to gonococcal proteins

<table>
<thead>
<tr>
<th>Protein Description</th>
<th>Abundance in OMVs</th>
<th>% ID to Ng FA1090</th>
<th>% ID between Ng strains</th>
</tr>
</thead>
<tbody>
<tr>
<td>NMB2039PorB (porin, major OMP PIB)</td>
<td>42-54</td>
<td>67.3</td>
<td>88.6-100</td>
</tr>
<tr>
<td>NMB1429PorA (porin, serosubtype P1-4)</td>
<td>28-63</td>
<td>n/a</td>
<td>n/a</td>
</tr>
<tr>
<td>NMB1497TonB-dependent receptor</td>
<td>4-60</td>
<td>96.1</td>
<td>98-100</td>
</tr>
<tr>
<td>NMB0382RmpM (OMP class 4)</td>
<td>3.08</td>
<td>93.4</td>
<td>99.6-100</td>
</tr>
<tr>
<td>NMB0964TonB-dependent receptor</td>
<td>2.87</td>
<td>96.9</td>
<td>96.2-100</td>
</tr>
<tr>
<td>NMB1812PilQ (Tfp pilus assembly protein)</td>
<td>1.44</td>
<td>91.4</td>
<td>79.1-100</td>
</tr>
<tr>
<td>NMB0634FbpA (iron ABC transporter substrate-binding protein)</td>
<td>1.29</td>
<td>99.1</td>
<td>99.1-100</td>
</tr>
<tr>
<td>NMB1126/1164Putative lipoprotein NMB1126/1164</td>
<td>1.06</td>
<td>94.2</td>
<td>99.1-100</td>
</tr>
<tr>
<td>NMB1988FrpB (FetA, iron-regulated OMP)</td>
<td>0.96</td>
<td>94.3</td>
<td>94.6-100</td>
</tr>
<tr>
<td>NMB0461Tbp1 (transferrin binding protein)</td>
<td>0.92</td>
<td>93.7</td>
<td>38.3-100</td>
</tr>
<tr>
<td>NMB0182OMP85</td>
<td>0.87</td>
<td>95</td>
<td>99.2-100</td>
</tr>
<tr>
<td>NMB1053OpcA (class 5 OMP)</td>
<td>0.75</td>
<td>43.8</td>
<td>98.9-100</td>
</tr>
<tr>
<td>NMB0088OMP P1</td>
<td>0.54</td>
<td>94</td>
<td>98.9-100</td>
</tr>
<tr>
<td>NMB1540LbpA (lactoferrin binding protein A)</td>
<td>0.46</td>
<td>n/a</td>
<td>41.0-100</td>
</tr>
<tr>
<td>NMB Locus</td>
<td>Protein Description</td>
<td>Variant Name</td>
<td>%ID to Ng</td>
</tr>
<tr>
<td>-----------</td>
<td>---------------------</td>
<td>--------------</td>
<td>-----------</td>
</tr>
<tr>
<td>NMB0280</td>
<td>LptD (LPS assembly protein/organic solvent tolerance protein OstA)</td>
<td>0-44</td>
<td>89.8</td>
</tr>
<tr>
<td>NMB1714</td>
<td>MtrE (outer membrane efflux protein)</td>
<td>0-29</td>
<td>96.4</td>
</tr>
<tr>
<td>NMB0109</td>
<td>LysM peptidoglycan-binding domain containing protein</td>
<td>0-26</td>
<td>88.7</td>
</tr>
<tr>
<td>NMB1333</td>
<td>hypothetical protein</td>
<td>0-24</td>
<td>96.3</td>
</tr>
<tr>
<td>NMB1567</td>
<td>FkpA (macrophage infectivity protein)</td>
<td>0-23</td>
<td>97.8</td>
</tr>
<tr>
<td>NMB0946</td>
<td>antioxidation AhpC TSA family glutaredoxin</td>
<td>0-20</td>
<td>98.5</td>
</tr>
<tr>
<td>NMB0375</td>
<td>MafA adhesin (mafA-1)</td>
<td>0-18</td>
<td>98.8</td>
</tr>
<tr>
<td>NMB0633</td>
<td>NspA (OMP)</td>
<td>n/a</td>
<td>93.7</td>
</tr>
</tbody>
</table>

**Recombinant protein antigens**

<table>
<thead>
<tr>
<th>NMB Locus</th>
<th>Protein Description</th>
<th>Variant Name</th>
<th>%ID</th>
<th>%ID between Ng strains</th>
</tr>
</thead>
<tbody>
<tr>
<td>NMB2132</td>
<td>Neisseria heparin binding antigen (NHBA)</td>
<td>peptide 2 (NZ98/254)</td>
<td>68.8</td>
<td>93.7-100</td>
</tr>
<tr>
<td>NMB1870</td>
<td>Factor H binding protein (fHbp)</td>
<td>peptide 8, variant 1-1 (MC58)</td>
<td>62.6</td>
<td>98.9-100</td>
</tr>
<tr>
<td>NMB1994</td>
<td>Neisseria Adhesin A (NadA)</td>
<td>peptide 8, variant 2/3 (2996)</td>
<td>n/a</td>
<td>n/a</td>
</tr>
<tr>
<td>NMB1030</td>
<td>GNA1030 (Nubp)</td>
<td>n/a (2996)</td>
<td>92.6</td>
<td>98.8-100</td>
</tr>
</tbody>
</table>
For distribution and homology analysis of OMV proteins, allele and protein sequences were obtained from strains *N. meningitidis* NZ98/254 (isolate id 34532) from the PubMLST database (https://pubmlst.org/neisseria) [39]. When sequence for NZ98/254 was not available, sequence from the *N. meningitidis* NZ05/33 (isolate id 19263) was used. NMB locus tags corresponding to *N. meningitidis* strain MC58 (accession NC_003112) are used as this genome is fully annotated. * An antibody response is induced to this protein post MeNZB vaccination [45].

Average abundance calculated from average of six lots of Bexsero from Table 2 in Tani et al [44]. NB. NspA protein is detected poorly by the proteomic approached used, compared with its abundance on SDS-PAGE [44].

Sequence from *N. meningitidis* strain NZ05/33 (NZ98/254 genome is not available) was compared to *N. gonorrhoeae* (Ng) strain FA1090. 

Conservation of antigen in the 438 *N. gonorrhoeae* genomes in GenBank. 

Previously established nomenclature for Bexsero NHBA, fHBP and NadA was used, where every unique peptide sequence is assigned a unique identification number (e.g. NHBA peptide 2 (NHBA-2) is in Bexsero). OMP, outer membrane protein; n/a, not available.

Grey shading indicates level of identity: dark > 90%; medium > 80%, light >60%. ^The gene encoding LbpA is a pseudogene in FA1090, but is expressed by the majority of gonococcal strains. 

^The gonococcal fHbp is not expressed on the surface of the gonococcus due to the absence of a signal sequence for export [38]. It has previously been shown that the gonococcal fHbp signal sequence differs from that of *N. meningitidis*, and is identical in 111 gonococcal isolates examined [36]. We confirm that the N-terminal 33 amino acids are identical in all annotated fHbp sequences in the gonococcal genome strains available in GenBank.
Table 2. Enzyme linked immunosorbent assay (ELISA) geometric mean titres (GMTs) against Bexsero vaccine components in serum from Bexsero-vaccinated humans

<table>
<thead>
<tr>
<th>Antigen</th>
<th>Pre-vaccination GMT (95% CI)</th>
<th>1-month post dose GMT (95% CI)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ng OMV</td>
<td>34,297 (20,946 - 56,156)</td>
<td>42,224 (29,853 - 59,722)</td>
<td>0.596</td>
</tr>
<tr>
<td>Ng whole cell</td>
<td>48,503 (25,906 – 90,811)</td>
<td>78,793 (49,228 – 126,115)</td>
<td>0.035</td>
</tr>
<tr>
<td>Nm whole cell</td>
<td>97,006 (42,879 – 219,456)</td>
<td>388,023 (183,938 – 818,550)</td>
<td>0.0091</td>
</tr>
<tr>
<td>Ng rNHBA</td>
<td>34,297 (20,946-56,156)</td>
<td>1,176,267 (669,930-2,065,300)</td>
<td>0.0051</td>
</tr>
</tbody>
</table>

1 Outer membrane vesicles (OMV), intact, whole cells from *N. gonorrhoeae* (Ng), whole cells from *N. meningitidis* (Nm) or recombinant Neisseria heparin binding antigen (rNHBA) from *N. gonorrhoeae* strain 1291. 2 Three dose vaccine schedule (0, 1, 3 months), ten donors. CI, confidence interval. GMT is the arithmetic mean of the logarithms of individuals’ serum titres. P-value calculated using Wilcoxon signed-rank test.
Figure Legends

Figure 1. Conservation of NHBA in *N. meningitidis* and *N. gonorrhoeae*.

(A) Alignment of the amino acid sequence of the three main NHBA peptide variants in *N. meningitidis* (black) and *N. gonorrhoeae* (red). Bexsero contains NHBA peptide 2 from *N. meningitidis* strain NZ98/254. The percent identity (ID) and similarity (Sim) of each NHBA peptide to the Bexsero NHBA peptide 2 is shown on the left. Amino acids identified in epitopes bound by the human monoclonal antibodies 12E1 (gray bar; 9/10 amino acids identical to gonococcal NHBA) and 10C3 (open bar; 24/32 amino acids identical to gonococcal NHBA) in the N-terminal region [48], and 5H2 (black bar, 32/35 amino acids identical to gonococcal NHBA) interacts with in the C-terminal of NHBA-2 [49] are indicated above the sequence). (B) Phylogenetic tree of the most common NHBA peptides present in *N. meningitidis* (black) and *N. gonorrhoeae* (red) and the percent of strains that express this peptide are shown (all peptides present in ≥1% of strains are shown). The Bexsero NHBA peptide 2 is boxed, and the peptides included in the alignment in panel (A) are indicated with an asterisk (*).

Figure 2. Reactivity of rabbit serum raised against Bexsero antigens to *N. gonorrhoeae* antigens.

(A) Coomassie strained SDS-PAGE, (B) Western blot with rabbit serum immunised with the NZ98/254 outer membrane vesicle component of Bexsero (α-OMV), and (C) Western blot with rabbit serum immunised to the recombinant protein component of Bexsero (α-rMenB). Samples shown are whole cell lysates (equivalent to a final OD600 of 5) from *N. meningitidis* (strain MC58) and *N. gonorrhoeae* (strains WHO K, FA1090, 1291), and *N. gonorrhoeae* strain 1291 treated with trypsin for 60 min to remove surface proteins (1291+TRYPSIN). The protein ladder is shown on the left of each panel, with the protein sizes (kDa) on the far left. On the right of panel C, the recombinant proteins are indicated. For MC58 NHBA, the upper band is the full length NHBA protein and the lower band is the fragment generated by NalP cleavage. For GNA1030, the protein is weakly expressed, and a digitally over exposed blot is shown in supplementary figure S1A.
where GNA1030 is more evident. (D) Western blot with α-rMenB rabbit serum against recombinant NHBA (rNHBA) from N. meningitidis strain MC58 and N. gonorrhoeae strain 1291.

Figure 3. Reactivity of Bexsero-vaccinated human serum to whole cell N. gonorrhoeae and N. meningitidis.

Reactivity of pooled Bexsero-vaccinated human serum from ten donors vaccinated with three doses of Bexsero at zero, three and six months. (A) Enzyme linked immunosorbent assay (ELISA) titration curves of pre-vaccination (dashed line) and one-month post dose three (black line) against intact, whole cell N. gonorrhoeae 1291 and N. meningitidis MC58 are shown as the average absorbance (+/- standard deviation) at 450 nm versus reciprocal serum dilutions. (B) Western blot analysis of in whole cell lysates shows recognition of several gonococcal and meningococcal antigens from post-vaccination, but not pre-vaccination serum. Proteins recognised include those running at a molecular weight consistent with recombinant Bexsero antigens (NHBA, GNA2091 and GNA1030 in N. gonorrhoeae and NadA, NHBA, fHbp, GNA2091 and GNA1030 in N. meningitidis).

Figure 4. Reactivity of Bexsero-vaccinated human serum to N. gonorrhoeae OMV antigens and NHBA.

Reactivity of Bexsero-vaccinated human serum from one donor vaccinated with two doses of Bexsero at zero and two months to (A) N. gonorrhoeae strain 1291 outer membrane vesicles (Ng OMVs) and (B) recombinant NHBA (rNHBA) from N. gonorrhoeae strain 1291 (Ng) or N. meningitidis strain MC58 (Nm). (i) Enzyme linked immunosorbent assay (ELISA) titration curves of pre-vaccination (month 0, dashed line), one-month post dose one (month one, grey line) and one-month post dose two (month three, black line) are shown as the average absorbance (+/- standard deviation) at 450 nm versus reciprocal serum dilutions. (ii) Western blot analysis shows recognition of (A) several gonococcal OMV proteins and (B) rNHBA in post-vaccination, but not pre-vaccination serum.
Figure 2

A. whole cell lysate
B. whole cell lysate
C. whole cell lysate
D. rNHBA

Coomassie stained SDS-PAGE

α- OMV rabbit serum
α-rMenB rabbit serum
α-rMenB rabbit serum

MC58
WHOK
FA1090
1291
+TRYPsin
MC58
WHOK
FA1090
1291
+TRYPsin
MC58
WHOK
FA1090
1291
+TRYPsin
MC58
1291

NadA
NHBA
fHBP
GNA2091
GNA1030
**A i.**

Ng OMV

- 1-month post dose 2
- 1-month post dose 1
- pre-vaccination

**A ii.**

OMV

pre-vaccination 1-month post dose 2

**B i.**

Ng rNHBA

- 1-month post dose 2
- 1-month post dose 1
- pre-vaccination

**B ii.**

rNHBA

pre-vaccination 1-month post dose 2