

## Introduction

- The Normandy region of France is experiencing an ongoing outbreak of *Neisseria meningitidis* serogroup B (MenB) disease, caused by a ST32, B:14:P1.7,16 strain.
- No licensed vaccine is currently available to combat this MenB outbreak.
- An investigational recombinant meningococcal vaccine (rMenB) containing three core proteins has been developed. These include the NadA protein, factor H binding protein (fHBP) variant 1.1, and genome derived Neisserial antigen (GNA) 2132 [Giuliani *et al.*, 2006].
- This investigational vaccine has been formulated and trialled with and without outer membrane vesicles (OMV) from the New Zealand outbreak strain NZ 98/254 (B:4:P1.7-2,4) [Giuliani *et al.*, 2006].
- This vaccine may be suitable to combat the outbreak of MenB disease currently occurring in Normandy.
- However, immunogenicity of this vaccine has not been determined against a strain representative of the Normandy outbreak.

## Aim

- The aim of this study was to assess the immunogenicity of the investigational recombinant vaccine (formulated with and without OMVs) against a target strain representative of the Normandy outbreak in the serum bactericidal antibody (SBA) assay.
- This was to enable the prediction of the vaccines likely ability to protect individuals against strains from the Normandy outbreak.

## Methods

### Strain selection

- Outbreak strains have been previously determined to express the fHBP variant 1.1 (homologous variant to the vaccine) and a miss matched NadA.
- Five outbreak strains were selected for use in the SBA assay and all confirmed as B:14:P1.7,16, expressing fHBP variant 1.1.
- All five strains were determined as expressing similar amounts of fHBP using a whole cell ELISA (data not presented).
- Following validation, strain LNP 20404 was chosen for use in the standardised MenB SBA assay.

### Serum samples

- Serum samples from a phase II study of recombinant vaccine (rMenB) and recombinant vaccine with OMVs (rMenB+OMV) were utilised [Miller *et al.*, 2008]. Briefly, vaccines were administered as a three dose schedule at 2, 4, and 6 months of age in UK infants.
- Twenty subjects which had residual volumes remaining from pre-vaccination and 4 weeks post-second and third dose were selected for analysis (60 samples total).
- This included eight subjects which had received rMenB and twelve subjects which had received rMenB+OMV.

### SBA assay

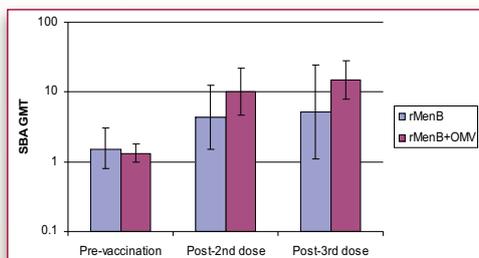
- The SBA assay was performed as previously described [Findlow *et al.*, 2005] incorporating human complement and a starting dilution of 1:2.
- SBA titres were expressed as the reciprocal of the final dilution giving  $\geq 50\%$  killing at 60 minutes.
- Sera with SBA titres  $<2$  were assigned as 1 for computational purposes.

### Data analysis

- Immunogenicity of vaccines was assessed by the calculation of the following;
  - SBA geometric mean titres (GMTs) with 95% confidence intervals (CI).
  - Proportions of subjects with putative protective SBA titres  $\geq 4$  [Borrow *et al.*, 2006].
  - Proportions of subjects achieving  $\geq 4$  fold rises in SBA titre from pre- to post-2nd and 3rd dose. This included subjects with pre-vaccination titres of  $<2$  and post-vaccination titres of 4.
- Due to small samples sizes, immunogenicity analysis did not include statistical tests.

## Results

**Figure 1. Serum bactericidal antibody geometric mean titres (95% confidence intervals), pre- and post-vaccination against LNP 20404.**



**Table 1. Proportions of subjects with serum bactericidal antibody titres  $\geq 4$  pre- and post-vaccination against LNP 20404.**

| Vaccination group | Pre-vaccination | Post-2 <sup>nd</sup> dose | Post-3 <sup>rd</sup> dose |
|-------------------|-----------------|---------------------------|---------------------------|
| rMenB             | 2/8 (25%)       | 5/8 (63%)                 | 5/8 (63%)                 |
| rMenB+OMV         | 1/12 (8%)       | 10/12 (83%)               | 12/12 (100%)              |

**Table 2. Proportions of subjects with  $\geq 4$  fold rise in serum bactericidal antibody titres from pre- to post-vaccination against LNP 20404.**

| Vaccination group | Post-2 <sup>nd</sup> dose | Post-3 <sup>rd</sup> dose |
|-------------------|---------------------------|---------------------------|
| rMenB             | 4/8 (50%)                 | 4/8 (50%)                 |
| rMenB+OMV         | 10/12 (83%)               | 11/12 (92%)               |

## Summary of results

- Pre-vaccination (2 months of age) infants had low GMTs (Figure 1) and low proportions with SBA titres  $\geq 4$  (Table 1).
- SBA GMTs rose post-two doses of vaccine to 4.4 and 10.1 for the rMenB and rMenB+OMV group, respectively (Figure 1). This was a significant rise for the rMenB+OMV group indicated by none overlapping 95% CI.
- Post-three doses, the rMenB group GMT rose to 5.2 whereas the rMenB+OMV group GMT rose to 15.1 (Figure 1).
- From pre- to post-three doses a 3.5 and 11.6 fold rise in SBA GMT was demonstrated for the rMenB and rMenB+OMV group, respectively (Figure 1).
- Following three doses, 63% and 100% of subjects had putative protective SBA titres of  $\geq 4$  in the rMenB and rMenB+OMV groups, respectively (Table 1).
- Following three doses, 50% and 92% of subjects had achieved a  $> 4$  fold rise in SBA titre from pre-vaccination with rMenB and rMenB+OMV, respectively (Table 2).
- The rMenB+OMV vaccine demonstrated greater immunogenicity than the rMenB vaccine following both the second and third dose. This is indicated by greater GMTs (Figure 1), proportions with SBA titres  $> 4$  (Table 1) and proportions with  $> 4$  fold rises in SBA titre (Table 2).

## Discussion

- Results from this study must be treated as exploratory due to the small sample size in each vaccination group.
- Interpretation of the study is limited in that it did not include a group receiving the OMV component of the vaccine alone.
- Both vaccines were immunogenic and induced SBA against LNP 20404.
- The rMenB+OMV vaccine demonstrated a greater immunogenicity than the rMenB vaccine alone. This is despite both vaccines containing the same variant and amount of fHBP.
- Reasons for the greater immunogenicity of the rMenB+OMV vaccine could be due to;
  - The OMV component inducing SBA against minor proteins which are expressed by LNP 20404.
  - The OMV component inducing antibodies which induce

SBA activity synergistically with those induced by the recombinant antigens.

- The OMV component of the vaccine acting as an "adjuvant" to the recombinant proteins.
- Similar differences have been demonstrated with other MenB target strains [Borrow *et al.*, 2008] however, the exact reason remains to be determined.
- The strain utilised in this investigation was "representative" of the Normandy outbreak, in terms of the phenotype, variant of fHBP + NadA and expression levels of fHBP.
- Immunogenicity was demonstrated in infants and vaccines may be more immunogenic in older age groups.
- Expression of fHBP has been previously demonstrated to correlate with SBA activity (lower expression can result in lower/no SBA activity) [Mascioni *et al.*, 2008].
- We only investigated the expression of fHBP in five strains from the Normandy outbreak. Expression among other strains may be different and could effect SBA activity.
- The vaccine has produced promising results when administered in schedules of 2, 4, 6 months and 6, 8, 12 months of age against other target strains [Su *et al.*, 2009].
- The vaccine is currently being evaluated in ongoing phase III trials.

## Conclusions

- The rMenB+OMV vaccine was demonstrated to elicit SBA against LNP 20404 which is representative of the Normandy outbreak. This was achieved in infants following a 2, 4 and 6 month of age schedule.
- The rMenB+OMV vaccine may be suitable to provide protection against the Normandy outbreak.

## Further work

- Investigate expression of fHBP + NadA among other strains from the Normandy outbreak.
- Confirm immunogenicity in a study incorporating a larger number of subjects.
- Confirm immunogenicity in older age groups which may be targeted in a vaccination "catch-up" campaign.
- Investigate and determine reason for greater immunogenicity of the rMenB+OMV vaccine.
- Investigate the potential benefit of a booster dose of vaccine.

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