Identification of *Neisseria meningitidis* specific patient derived antibodies using reverse vaccinology 2.0

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### 1. Introduction

**Neisseria meningitidis**
- The most common cause of bacterial meningitis and septicaemia in the UK
- 10% of infections are fatal
- 25% of survivors have long term effects

**Current vaccines**
- Effective conjugate vaccines against *N. meningitidis* serogroups A, C, W and Y
- Effective recombinant protein vaccine (Bexsero) licensed against serogroup B in the UK in 2013
- Strains of serogroup B protected against by this vaccine vary worldwide:
  - 91% in USA, 70% in England and Wales, only 37% in Argentina

New vaccine antigens could be used to extend strain coverage and increase protection against meningitis

### 2. Reverse Vaccinology 2.0

**Antigen binding region**
- Cloning of antibody IgH and IgL variable regions into *E. coli*
- Transfection of HEK293 cells - production of IgG antibodies into supernatant
- hmAb characterisation and identification of epitope i.e. ELISA, flow cytometry, western blot, hSBA, LC-MS/MS

**Immunoassays**
- ELISA and flow cytometry
- Western blot

**Functional assays**
- CDA: C3c
- CDA: C5b-9

### 3. Results

- **35 anti-meningococcal human monoclonal antibodies (hmAbs) cloned from six patients**
- These antibodies had binding to *N. meningitidis* tested using ELISA and flow cytometry, before the size of their target protein was assessed using western blot
- A selection went on for testing of functional activity:
  - **Serum bactericidal assay (SBA)** tests if an antibody kills *N. meningitidis* when combined with human complement
  - **Complement deposition assay (CDA)** assesses whether the antibody (when bound to *N. meningitidis*) can recruit complement components C3c or C5b-9
    - C3c is an opsoniser which labels the bacteria for killing by phagocytes
    - C5b-9 is a complex which causes bacterial lysis and therefore bacterial killing

- Here we highlight the results from **five promising antibodies**

<table>
<thead>
<tr>
<th>Antibody</th>
<th>ELISA and flow cytometry</th>
<th>Western blot</th>
<th>Target size (kDa)</th>
<th>SBA</th>
<th>CDA: C3c</th>
<th>CDA: C5b-9</th>
</tr>
</thead>
<tbody>
<tr>
<td>P02-1A1</td>
<td></td>
<td></td>
<td>30 - 40</td>
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<tr>
<td>P02-5A2</td>
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<td>ND</td>
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<tr>
<td>P02-5E10</td>
<td></td>
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<td>30 - 40</td>
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<tr>
<td>P09-2F2</td>
<td></td>
<td></td>
<td>20-30</td>
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<tr>
<td>P09-2F7</td>
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<td>ND</td>
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</tbody>
</table>

Green boxes indicate a positive result, whilst red boxes indicate a negative result. ND = no data available

### 4. Conclusions

- To date we have cloned 35 hmAbs that bind to one or more strains of *N. meningitidis*
  - Some antibodies, including P02-1A1 and P09-2F2, bind to a wide range of *N. meningitidis* strains
  - So far, three antibodies have shown SBA activity, with nine hmAbs recruiting human complement C3c and/or C5b-9 in CDA
  - Five antibodies have had their target antigen size identified through western blot
- Future work will focus on further characterisation of all 35 hmAbs, and identifying the target antigens, before assessing these as vaccine candidates

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