Department for Environment, Food and Rural Affairs

Application for consent to release a GMO – organisms other than higher plants

Part A2: Data or results from any previous releases of the GMO

Give information on data or results from any previous releases of this GMO by you either inside or outside the European Community [especially the results of monitoring and the effectiveness of any risk management procedures].

This is the first application for deliberate release of this GMO.

Part A3: Details of previous applications for release

Give details of any previous applications to release the GMO made to the Secretary of State under the 2002 Regulations or to another Member State under the Deliberate Release Directive 2001/18/EC.

This is the first application for deliberate release of this GMO.

Part A4: Risk assessment and a statement on risk evaluation

The GMO is a modified form of the human commensal organism *Neisseria lactamica*.

Wild type *Neisseria lactamica* (Nlac) is a non-pathogenic Gram-negative organism, frequently found in the nasopharynx, particularly in young children. Transmission occurs through close contact and only a few cases of clinical significance caused by the wild type have been reported, [1-3]. *Neisseria lactamica* is a member of the same genus as *Neisseria meningitidis* (Nmen), which is a pathogen and causes meningitis and severe sepsis. Although Nlac and Nmen, colonise the same location within the upper respiratory tract, previous studies suggest they engage with the human mucosal immune system in very different ways [4]. In contrast to Nmen, Nlac maintains a commensal relationship with the host in the absence of an adaptive immune response. Nlac lacks a polysaccharide capsule, so is unable to survive in circulating blood.

The first GMO (strain 4NB1) expresses the meningococcal gene *nadA*, which codes for an outer membrane adhesin called *Neisseria* adhesin A (NadA). NadA is a member of the type V autotransporter family of outer membrane proteins, and in Nmen is associated with an increased level of adhesion to and invasion of human epithelial cell lines, but is not known to confer increased virulence in animal models. Expression of NadA is observed both in hypervirulent and in carriage strains of Nmen. The NadA protein is one of the 4 major immunogenic proteins in the 4CMenB vaccine against serogroup B meningococcal disease (Bexsero), and is the

only component of this vaccine to induce sterilising (i.e. colonisation-inhibiting) immunity in animal models [5].

The second GMO is the control strain (strain 4YB2), which will be used in experimental medicine projects to normalise for effects observed with the NadA expressing GMO. The second GMO has been genetically modified in exactly the same ways as the NadA-expressing strain, except that it does not contain the coding sequence for the *nadA* gene.

Neither of the GMOs possesses capsular polysaccharide, which is the major virulence determinant of Nmen, which confers resistance to serum components enabling spread of Nmen in the bloodstream and subsequent disease.

1. Likelihood of the GMO to become persistent and invasive in natural habitats under the conditions of the proposed release(s).

The GMOs are likely to behave in the same ways the wild type Nlac. In previous projects we have performed experimental human challenge on almost 400 volunteers, and in those who become colonised with wild type Nlac, the organism has been carried harmlessly in the nose and throat in most of those colonised, for up to 6 months. No subject has ever experienced invasive disease; indeed this is likely to be the case with the GMO because neither the GMO nor the wild type Nlac expresses a polysaccharide capsule, which allows the related bacterial species, Nmen to survive in the blood stream and cause disease.

2. Any selective advantage or disadvantage conferred to the GMO and the likelihood of this becoming realised under the conditions of the proposed release(s).

The GMO expresses NadA on its surface; at least as much as wild type Nmen strain MC58, as measured by flow cytometry. Despite this, the *in vitro* growth of the bacterium in rich culture medium (TSB) appears to be unaffected. Expression of NadA by the GMO significantly increases the numbers of bacteria binding to the human epithelial cell line, HEp2. It is not known whether this will confer any selective advantage following inoculation into the human, but there is reason to postulate that it will confer a selective disadvantage through immune mechanisms over the longer term (i.e. that NadA-expressing bacteria will be selected against following seroconversion of the inoculated volunteer against the NadA protein). In a longitudinal study of nasopharyngeal meningococcal carriage, it was shown that NadA expression in serial Nmen isolates decreased over time, hypothesised to be a result of seroconversion against NadA and the development of an antibody-mediated selective pressure against NadA expression. This hypothesis is partially corroborated by the finding that immunisation with recombinant NadA, prior to attempted Nmen colonisation in a transgenic mouse model, leads to sterilizing immunity, whereby strains expressing a cognate NadA antigen were unable to colonise the murine nasopharynx. In the GMO, *nadA* expression is instead controlled by a hybrid, constitutively active promoter that drives expression of the gene to a high level.

3. Potential for gene transfer to other species under conditions of the proposed release of the GMO and any selective advantage or disadvantage conferred to those species.

There is the possibility of transfer of the gene expression cassette into other bacteria resident in the human nasopharynx, but the risk of this occurring is considered

negligible. This is because the gene expression cassette containing the *nadA* and/or *lacZ* genes is incorporated into the chromosome of the GMOs at the NHCIS1 locus, meaning only one copy of each gene is present in any one bacterium. In addition, the cassette does not contain sequences that will support extrachromosomal replication, which necessitates its incorporation into a host genome in order to be maintained. Recombination of exogenous material into the genome is itself a relatively rare event. The most likely recipient of the cassette in the event of allele escape would be another member of the *Neisseriaceae*, due to the close proximity of the genes to *Neisseria* DNA Uptake Sequences (DUS). These sequences bias uptake of exogenous DNA by *Neisseria* in favour of DUS-containing nucleic acids, as a means of limiting the incorporation of potentially deleterious sequences. However, it is likely that any recipient of the gene expression cassette would be affected similarly to the original GMO, insofar as the constitutively active *nadA* gene is likely to become a selective disadvantage following seroconversion of the human host against the NadA protein. We predict this will result in a survival liability over the longer term.

4. Potential immediate and/or delayed environmental impact of the direct and indirect interactions between the GMO and target organisms (if applicable).

The GMO is likely to colonise the participants harmlessly for periods up to 6 months. In a large human challenge study we showed that the wild type organism (a commensal) inhibited colonisation of the nasopharynx by the related pathogen, Nmen (a pathobiont). We anticipate that the same effect will be observed with the GMO expressing NadA; in fact the effect may be enhanced because the GMO should induce immunity against NadA, which is expressed on the surface of many strains of Nmen. We cannot predict whether there will be a similar beneficial effect on the exclusion of other pathobionts, e.g. *Streptococcus pneumoniae* or *Haemophilus influenza*, but a preliminary analysis of microbiome data from a subset of experimentally colonised individuals showed that the displacement of Nmen by Nlac was an exquisite event and non-disruptive to other bacterial genera.

5. Possible immediate and/or delayed effects on human health resulting from potential direct and indirect interactions of the GMO and persons working with, coming into contact with or in the vicinity of the GMO release(s).

It is possible that the GMO may transmit to other humans (it is an exclusively human commensal) though we will take steps to minimise this possibility. It is anticipated that the effect of secondary transmission will be same as described in paragraph 4 above, i.e. harmless colonisation and possible displacement of pathiobionts.

6. Possible immediate and/or delayed effects on animal health and consequences for the feed/food chain resulting from consumption of the GMO and any product derived from it, if it is intended to be used as animal feed.

Not applicable. The GMO is an exclusively human commensal that is unable to colonise other animals. Neither the GMO nor any product derived from it is intended for use as animal feed.

7. Possible immediate and/or delayed effects on biogeochemical processes resulting from potential direct and indirect interactions of the GMO and target and non-target organisms in the vicinity of the GMO release(s).

The GMOs are nonpathogenic commensal bacteria found exclusively in humans. They are not involved in any biogeochemical processes and have limited survivability outside of their biological niche.

8. Possible immediate and/or delayed, direct and indirect environmental impacts of the specific techniques used for the management of the GMO where these are different from those used for non-GMOs.

The techniques used for the management of the GMO related waste in the hospital are all accepted standard practices for example, autoclaving, incineration or disinfection with Virkon. In the unexpected event that the GMO causes disease, the public health response could include similar strategies to those used for meningococcal outbreaks, such as administration of antibiotics to close contacts of inoculated volunteers. The GMO remains acutely sensitive to the frontline antibiotics used clinically to treat meningococcal disease (i.e. ceftriaxone and ciprofloxacin). NadA is also included as an immunogenic component of the 4CMenB, anti-meningococcal disease vaccine (Bexsero). Vaccination with Bexsero is also a possible public health response in the event of GMO pathogenesis.

Part A5: Assessment of commercial or confidentiality of information contained in this application.

Identify clearly any information that is considered to be commercially confidential. A clear justification for keeping information confidential must be given.

Not applicable.

Part A6: Statement on whether detailed information on the description of the GMO and the purpose of release has been published

Make a clear statement on whether a detailed description of the GMO and the purpose of the release have been published, and the bibliographic reference for any information so published.
This is intended to assist with the protection of the applicant's intellectual property rights, which may be affected by the prior publication of certain detailed information, e.g. by its inclusion on the public register.

A detailed description of the GMO and the purpose of this release have not been published. The technology used to derive the GMOs, and the GMOs themselves, are proprietary to the University of Southampton, comprising UK Patent Application number 1522153.4.

1. Bidmos, F.A., et al., *Persistence, replacement, and rapid clonal expansion of meningococcal carriage isolates in a 2008 university student cohort.* J Clin Microbiol, 2011. **49**(2): p. 506-12.
2. Denning, D.W. and S.S. Gill, *Neisseria lactamica meningitis following skull trauma.* Rev Infect Dis, 1991. **13**(2): p. 216-8.
3. Brown, N.M., N.K. Ragge, and D.C. Speller, *Septicaemia due to Neisseria lactamica--initial confusion with Neisseria meningitidis.* J Infect, 1987. **15**(3): p. 243-5.
4. Lauer, B.A. and C.E. Fisher, *Neisseria lactamica meningitis.* Am J Dis Child, 1976. **130**(2): p. 198-9.
5. Johswich, K.O., et al., *In vivo adaptation and persistence of Neisseria meningitidis within the nasopharyngeal mucosa.* PLoS Pathog, 2013. **9**(7): p. e1003509.