APPLICATION FOR CONSENT TO RELEASE A GMO

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].

BPZE1 GMO has been manufactured according to GMP specifically as a biological medicinal product (attenuated bacteria) for investigational use humans. The GMO has been released previously in multiple clinical and non-clinical studies (please refer to Application Form Part A1 section 32).

PART A3: DETAILS OF PREVIOUS APPLICATIONS FOR RELEASE

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

Applications with multiple EU countries have been approved for classifying BPZE1 as a Biosafety Level 1 organism, including France, Germany, Belgium, Spain, and Sweden for the purpose of manufacturing and clinical studies.

PART A4: RISK ASSESSMENT AND A STATEMENT ON RISK EVALUATION

Risk Assessment: environmental impact of the release of the GMOs

To avoid accidental exposure actions are taken to minimise generation of aerosols, since the bacterium is strictly a respiratory tract organism. Persons handling the BPZE1 bacteria should wear gloves and PEP and must wash their hands with a suitable disinfecting soap before touching their skin and eyes. Effective antibiotic treatment with azithromycin (or an appropriate antibiotic if the subject is allergic to azithromycin) should be given in case of accidental transmission to other humans.

Due to the robust preclinical safety data, BPZE1 has been classified as a Biosafety Level 1 organism by French authorities Republique Francaise Ministere De L'enseignement Superieur Et De La Recherche (French Ministry of Higher Education and Research). Germany, Belgium, Spain, and Sweden have accepted the French Authority's Biosafety Level 1 rating for the purpose of manufacturing and clinical studies.

Risk assessment: factors affecting dissemination

Wild-type *B. pertussis* is spread mainly by aerosol formed by coughing of infected persons. The coughing is potentially induced by the TCT, which is more than 99% reduced in BPZE1. The BPZE1 strain is not expected to induce coughing, therefore transmission is highly unlikely. Furthermore, neither baboons nor human volunteers infected with doses of BPZE1 up to 10^9 CFU experienced any significant or

prolonged BPZE1-related cough, as described in Locht et al. J. Infect. Dis. 2017;216:117-124, Thorstensson et al. PLoS One 2014;9:e83449, Jahnmatz et al. Lancet Infect Dis 2020;20:1290-1301and Keech, et al. World Vaccine Congress 2020). The Clinical Study Report of the phase 2b study (n=300 subjects) has been submitted to the FDA and is under review. Data from the CSR is available in the IB for reference. *B. pertussis* has fastidious growth requirements and has limited survival time outside the human body.

Chronic carriage of wild-type *B. pertussis* has not been reported and is therefore not expected and BPZE1 has not been found to be chronically carried in any study to date. No cross-contamination between the subjects was noted when assessing the immunologic outcomes in the previous Phase 1 and 2 clinical trials of BPZE1, nor was any risk to the family members of study subjects reported. In case of transmission to other humans, accidentally exposed, an efficient treatment against *B. pertussis* is commercially available and is based on administering erythromycin or other macrolides. BPZE1 has been shown to be sensitive to erythromycin and other macrolides.

The subjects will stay at the study center for at least 30 minutes after administration to observe for any immediate reactogenicity or safety concerns. In addition, subjects with frequent contact with children less than 1 year of age (parent, childcare worker, nurse, etc.) or subjects who live in the same household as individuals with known immunodeficiency or individuals on immunosuppressant therapy will be excluded from participation in the study as a safety precaution due to the current development stage of the product.

Risk assessment: human health impact

The risks of BPZE1 administration are expected to be minimal and clinically manageable. *B. pertussis* colonization is strictly limited to respiratory epithelium without dissemination of the bacteria outside the respiratory tract, which also excludes systemic bacteremia of the BPZE1 strain, even in immune-compromised subjects.

B. pertussis has not been shown to be allergenic in any preclinical or clinical studies to date, nor to have any of the excipients in the lyophilised formulation. BPZE1 has been shown to protect against airway inflammation induced by allergens or viral infections in a murine model (Li et al Allergy 2012;67:1250-1258, Li et al. J. Virol. 2020;84:7105-7113). BPZE1 has also been shown to protect against wild type *B. pertussis* infection 3 hours after immunization in a murine model (Mielcarek et al PLoS Pathog 2006;2:e65) and in baboons (Locht et al. J. Infect. Dis. 2017;216:117-124). However, there remains a theoretical risk of allergic reaction, as is present with any vaccine product.

The attenuated BPZE1 bacteria colonises the upper respiratory tract slightly less well than wild-type *B. pertussis*. In the most recent (and largest) study to date (Phase 2b) BPZE1 was noted to be cleared from all individuals on Day 78 after vaccination using microbiological culture. Furthermore, attenuated challenge with BPZE1 at Day 85 demonstrated protection against re-colonization if BPZE1 was utilized as the

vaccine on Day 1 but not if Boostrix was utilized. In the current school age study, colonization of BPZE1 will be assessed using PCR from samples obtained from the mid-turbinate/nasopharyngeal on Days 7 (safety lead in) and documented clearance on Days 28 and 85 (approximately half of the subjects at each time point). In the sub -study (n~120 subjects) revaccination/attenuated challenge of BPZE1 on Day 85 (open label) with subsequent sampling on days 92 and 99 is designed to demonstrate that vaccination with BPZE1 (but not Boostrix) on Day 1 can avert subsequent colonization using an attenuated challenge model approach 3 months later. This sub-study design is similar to the design in the adult Phase 2b study.

In summary, the risk assessment for this study shows a very low potential risk for the study subjects and impact associated with administering BPZE1.

Risk assessment: environmental impact

The preliminary risk assessment for this study suggests there is an extremely low risk for potential environmental impact associated with administering the BPZE1 to study subjects.

Risk assessment: monitoring the GMO

Mid-turbinate/nasopharyngeal sampling followed by PCR detection of BPZE1 will be conducted at key time points to ensure that the GMO has a limited survival and clears as expected. In all study subjects to date, the GMO has cleared with 45 days, with most subjects having evidence of no colonization 28 days post-vaccination. The sub study is designed to demonstrate the difference in mode of action of BPZE1 versus current acellular pertussis vaccines (e.g. Boostrix).

In summary, the GMO is readily sampled and identified, and the colonization and clearance behavior has been consistent and controlled over a typically < 28-day duration.

Risk assessment: emergency response

Efficient antibiotics (erythromycin or other macrolide) treatment can be administered. The GMO has no resistance to macrolides. BPZE1 is resistant to streptomycin and nalidixic acid, which are not used to treat *B. pertussis* infections. Furthermore, due to the lack of horizontal gene transfer systems in BPZE1, resistance to streptomycin and nalidixic acid cannot be transferred to other organisms. As such, the risk assessment shows a clear and effective emergency response in the unlikely situation that the bacteria are disseminated to a non-study participant or the bacteria has prolonged colonization.

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.

N/A

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.

Peer-reviewed publications and issued patents have described the GMO's structure, safety, and genetic stability and use as a vaccine against pertussis.

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