PART A4: RISK ASSESSMENT AND A STATEMENT ON RISK EVALUATION

Summary

The GMO is a severely attenuated strain of the human-specific pathogen *Salmonella typhi* expressing a Hepatitis B antigen and is intended for use as an immunotherapy for Hepatitis B infection. The immunotherapy will be given orally to volunteers who are likely to shed the organism in stools at low levels for no longer than 7 days. Shedding will constitute the release of the organism and, potentially, it could be released into the sewage system. Normal basic hygiene precautions, namely the use of toilets and hand washing, are considered sufficient to prevent person to person transmission.

The advantage of using attenuated *Salmonella typhi* as the vector is that there are no animal reservoirs and it does not persist in the environment. The GMO does not have a selective or survival advantage in the environment.

The potential for genetic exchange with any other organisms in the environment is extremely low, given that the GMO does not contain any plasmids (or antibiotic resistance markers), will not persist in the subjects and does not persist in the environment.

The GMO has previously been tested in healthy human volunteers and has been shown to be safe, well tolerated and not persistently shed. It is not likely that the GMO will become more persistent or invasive when administered to the subjects in the proposed study. The health of the volunteers will be carefully monitored as their safety is paramount in this study.

For the purpose of the release the immunotherapy will be given to a maximum of 30 subjects at two sites. The subjects will be patients who are chronic carriers of Hepatitis B. The GMO will be shed in stools for up to 7 days after administration and therefore the subjects will not be permitted to travel outside England for 14 days following each dosing day.

The information presented in this application demonstrates the safety of the GMO, in terms of its inability to cause harm and to persist in the environment. Clearly it is the key objective of the clinical trial to assess the safety of the GMO in the subjects. The risk assessment shows a low hazard associated with administering the GMO to these human volunteers, the risk to other humans is considered to be negligible and the risk to the wider environment is considered to be effectively zero.
RISK ASSESSMENT

Conclusions on the Potential Environmental Impact from the Release of GMOs

i Likelihood of the genetically modified organism (GMO) becoming more persistent and invasive in natural habitats under the conditions of the proposed release(s).

It not likely that the GMO will become more persistent or invasive under the conditions of this study.

Human hosts are the natural habitat of wild-type *Salmonella typhi* as there is no other animal, plant or insect vector.

The GMO is a severely attenuated *S. typhi* strain which has deletion mutations in two distantly located genes. The products of these genes are required for colonisation and replication in the host. Thus the GMO effectively has no natural habitat but the closest is the human host. The safety of the GMO has been assessed in healthy human subjects in a previous clinical study, under conditions similar to those of the proposed release. Safety data generated from this study demonstrated that the organism is well tolerated and it is not persistently shed. This shows that the strain is highly attenuated and can no longer survive or establish an infection in the human host.

The main purpose of the clinical trial is to assess safety of the GMO in the subjects. The subjects safety is paramount and they will be closely monitored for adverse effects of the GMO throughout.

It is not likely that the GMO will become more persistent or invasive under the conditions of the proposed study compared to the previous study.

In the proposed study, as in previous study the GMO will be administered orally in a bicarbonate solution. In the previous study the subjects were healthy volunteers whereas in the proposed study the subjects will be patients with a chronic Hepatitis B infection. The safety of the GMO is based on the 2 gene deletion mutations rendering the GMO incapable of colonisation and replication in the subjects and therefore the GMO will be no more persistent or invasive in patients who are chronic carriers of Hepatitis B than in healthy subjects.

In the proposed study it is intended that the subjects will receive higher dose levels of the GMO than were administered in the previous study. Additionally it is intended that a larger number of doses will be administered to each subject. Previously subjects received up to 2 doses of either $10^8$ or $10^9$ CFU of the GMO 56 days apart. In the proposed study it possible that the subjects will receive a maximum of one dose of $10^8$, one dose of $10^9$ and 4 doses of $10^{10}$ CFU, 28 days apart.
Receipt of the next dose and escalation of the dose level being dependant on tolerance of the previous dose. Administering higher numbers of the GMO to the subjects will not impact on the characteristics of the GMO itself; the GMO will still be unable to multiply within or colonise the subjects and so will not infect or persist in the subjects. Administering a larger number of doses to the subjects will also not impact on the characteristics of the GMO itself. It is not expected that there will be a cumulative effect of the multiple doses. Prior to each dose being administered the organisms from the previous dose will have cleared the GI tract. The only theoretical effect of the subjects receiving the $10^{10}$ CFU dose level compared the $10^8$ or $10^9$ CFU dose levels assessed in the previous study is that the GMO may be detected in subjects faecal stool samples more frequently over a greater number of days. This will be a reflection of the fact that more organisms will be passing through the GI tract. In addition, in this study where up to six doses will be given to each subject it is possible that an immune response may be generated by previous doses which will sequester the GMOs following administration of later doses of the GMO, thus reducing the number of GMOs shed in the faeces.

ii 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 does not have a selective advantage as it has been designed to have a strong selective disadvantage. The expressed Hepatitis B antigen will not confer a selective advantage or disadvantage, however the two gene deletion mutations result in a highly weakened form of *S. typhi*. This gives a selective disadvantage compared to wild type *S. typhi* which will be realised under the conditions of this release. First, as the GMO is highly weakened it will be unable to infect the subjects; it will not replicate in or colonise the subjects and consequently it will pass straight through the GI tract to be shed in the faeces.

Secondly, as the GMO is shed in stools it will be released into the sewage system; the GMO could possibly also enter the wider environment (e.g. soil and water bodies) either from the sewage system or if faecal stools are not disposed of via the mains sewage system. Wild type *S. typhi* is effectively contained and inactivated by normal sewage treatment processes and additionally does not persist in the wider environment. The highly weakened GMO does not have a selective advantage in these environments and indeed it may be expected to have selective disadvantage as it possesses a nutritional requirement not harboured by wild type *S. typhi*; this requirement results from one of the gene mutations introduced into the GMO. Emergent Europe has shown experimentally that the GMO does not have a competitive advantage over naturally occurring organisms in sewage and the GMO does not persist in this environment.
Higher numbers of the GMO may be released into sewage as a result of the proposed clinical study compared to the previous release but this will not influence the inherent weakness of the GMO and its inability to grow or persist in the environment. The larger numbers of organisms will not influence the ability of the sewage system to contain and inactivate the organisms. Typhoid fever patients shed wild type *S. typhi* in high numbers, up to $10^{11}$ CFU per gram of stool (this is only 20 fold less than the maximum total number of GMOs to be administered in the proposed study) and this is effectively contained and inactivated by normal sewage treatment processes.

It is clearly important to consider the possibility that the GMO may come into contact with aspects of the environment other than the sewage system (e.g. soil and water bodies). Emergent Europe has generated data showing that the GMO does not survive in soil for longer than 12 days and data to indicate that the GMO will be killed in tap water (chlorinated drinking water). Additionally published data indicate that the GMO will not persist in sea water or river water.

iii 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.

The potential for gene transfer from the GMO to other species is extremely low. The Hepatitis B antigen gene within the GMO is located on the chromosome and in addition, the GMO contains no plasmids or antibiotic resistance markers. Plasmids could potentially mediate gene transfer and antibiotic resistance markers could theoretically allow selection for organisms to which the markers and associated *Salmonella* DNA had been transferred.

The chromosomal location of the heterologous gene and the lack of plasmids reduce the likelihood of transfer of both the antigen gene and *Salmonella* genes to other organisms both in the gut flora, in sewage and in other aspects of the environment. In addition, the short transit time through the gut and the short survival time in the environment post-shedding from the volunteers reduces further the risk of gene transfer.

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

Following ingestion of the GMO by the target organism (subjects in the clinical trial) the GMO is predicted to interact with the immune system of the subjects to generate an immune response to the *Salmonella* antigens and the expressed Hepatitis B antigen. This is the direct interaction of the GMO with the subjects.
A large proportion of the GMOs will interact indirectly with the subjects as they will pass straight through the GI tract to be shed in faecal stools. From the faeces the GMO will enter the sewage system. This indirect interaction with the subjects therefore results in the GMO interacting directly with the wider environment. As a consequence of administration to the subjects the GMO will be released into the sewage system. Wild type \textit{S. typhi} is effectively contained and inactivated by normal sewage treatment processes. The GMO has a selective disadvantage in sewage compared to wild type \textit{S. typhi} and Emergent Europe has shown experimentally that the GMO does not have a competitive advantage over naturally occurring sewage organisms and that it does not persist in this environment. In a study to examine the persistence of the GMO in sewage results showed a steep drop in viable cell numbers between Day O when the GMO was added to the sewage and Day 1 (cell numbers dropped from 87% of the total population to less than 1%, representing a loss in 99.6% of the viable GMO cells). By Day 2 the GMO could no longer be detected amongst the native sewage organisms.

It is important to consider the possibility that the GMO may come into contact with aspects of the environment other than the sewage system (e.g. soil and water bodies). Wild-type \textit{S. typhi} can not persist in the environment. The GMO will not have a selective advantage over wild type, and indeed may be expected to have selective disadvantage due to the nutritional requirement resulting from one of the gene mutations present in the GMO. Emergent Europe has generated data showing that the GMO does not survive in soil for longer than 12 days and data indicating that the GMO will be killed in tap water (chlorinated drinking water). Additionally published data indicate that the GMO will not persist in sea water or river water.

The GMO may elicit an immune response in the subjects but it will not persist in the subjects or the wider environment. The potential impact to the environment of the direct and indirect interactions of the GMO with the subjects is therefore considered to be effectively zero.

Possible immediate and/or delayed environmental impact of the direct and indirect interactions between the GMO with non-target organisms, including impact on population levels of competitors, prey, hosts, symbionts, predators, parasites and pathogens.

The GMO will not interact with plants, animals or insects as humans are the only host for wild type \textit{S. typhi} and there are no animal vectors. The non-target organisms are therefore other humans and a consideration is faecal-oral transmission from the volunteers to non-target hosts. Strict exclusion criteria have been set for the trial to minimise the risk of transmission of the GMO. However, in the very unlikely event that the organism does come into contact with other humans the risk of causing harm to them and the impact on the environment is considered negligible. If the organisms are ingested they will not colonise or replicate in the host, they will pass straight through the GI tract and be shed in faeces.
The numbers shed will be very small and they will not persist in the sewage system or the wider environment. The environmental impact of the direct and indirect interactions between the GMO with non-target organisms is considered to be effectively zero.

vi 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 direct contact with or in the vicinity of the GMO release(s).

The potential for direct interactions to occur between the GMO and non-target hosts will be minimised during the clinical study. However, in the very unlikely event that the organism does come into contact with other humans the risk of causing harm is considered to be negligible.

Clinical study staff involved in administering the GMO to the trial subjects will receive appropriate training regarding their own safety (and that of other persons who might be affected by their actions) and regarding precautions to be taken to ensure no contamination will result from the activities associated with the dose administration and all materials are disposed of in a suitable manner. Staff in direct contact with trial subjects will wear appropriate protective clothing (aprons and gloves) which will be decontaminated after use. Following each administration of the GMO, hard surfaces in the room used for administration will be disinfected and disposable items will be incinerated or autoclaved.

Strict exclusion criteria have been set for the trial to minimise the risk of transmission of the GMO once the volunteers leave the clinical study site, and in particular to minimise transmission to potentially vulnerable groups. Women who are breastfeeding and individuals who work as commercial food handlers are excluded. Individuals who are health care workers with direct contact with high-risk patients, child care workers or other individuals who have routine contact with children less than 2 years of age, and individuals with household contacts with immuno-compromised individuals, pregnant women or the young and elderly will all be excluded from the trial. Volunteers will also be instructed to maintain strict personal hygiene and proper hand washing will be stressed to minimise the risk of faecal-oral transmission.

It is possible that not all of the subjects will shed the GMO and those that do are expected to shed the organisms in low numbers. However, in the very unlikely event that the organism does come into contact with any of these groups the risk of causing harm is considered negligible. It has already been demonstrated that 2 doses of $10^9$ CFU of the GMO can be safely given to healthy adult human volunteers whose stomach acid has been neutralised to facilitate survival of the vaccine strain.
Given that only a small number of organisms are likely to be shed by any subject over a short period of time following each dose it is considered unlikely that any of the perceived risk groups mentioned above could suffer any harm through transmission of the GMO through close/regular contact with the recipient of the GMO.

One of the main concerns about live vaccines is whether they are likely to be as attenuated in immuno-compromised subjects. A special focus of the pre-clinical programme for the recipient strain and the GMO has been to demonstrate safety in relevant immuno-compromised models. The recipient strain has been attenuated by the introduction of two independently attenuating deletion mutations in genes that are fundamental to the ability of S. typhi to colonise, persist and cause disease in humans. The GMO was constructed by inserting an inducible promoter-hepatitis B antigen fusion into the site of one of the gene deletions. The safety of the GMO results from the 2 gene deletions. The pre-clinical programme has demonstrated that the recipient strain is unable to replicate in a human macrophage like cell line which is a key indication of attenuation. Work with this strain has also demonstrated that the attenuating mutations have a particular safety advantage in the immuno-compromised. In the models utilised, the recipient strain was equally attenuated. This indicates that the attenuation of the GMO is not dependent on the ability of the innate or specific immune system to control the replication or spread of the organism and it should therefore be attenuated in immuno-compromised individuals.

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

There will be no effects on animal health as a result of this release. Wild type S. typhi is restricted in its host range to humans, and host range of GMO has not been increased.

The GMO is not intended to be used as an animal feed.

viii 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).

No immediate or delayed effects on biogeochemical processes are expected, as there is no known or predicted involvement of the GMO in biogeochemical processes.
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 will not differ from those used for non-GMO S. typhi. Wild type S. typhi is managed by disposal directly into the sewage system where it is effectively contained and inactivated by the normal sewage treatment processes. The GMO does not have a competitive advantage over naturally occurring sewage organisms and experimental data have been obtained to show it does not persist in sewage. The GMO may possibly enter the wider environment either from the sewage system or if faecal stools are not disposed of via the mains sewage system. Any GMO that reached the wider environment would not persist, or have a survival advantage over wild type S. typhi which does not persist in the environment.

Regular monitoring of public mains water by coliform counts by Public Water Supply Companies is in place to monitor for potential environmental contamination, and they will response as per standard operating procedures for any coliform bacteria.