PART 1 (COUNCIL DECISION 2002/813/EC)

SUMMARY NOTIFICATION INFORMATION FORMAT FOR THE RELEASE OF GENETICALLY MODIFIED ORGANISMS OTHER THAN HIGHER PLANTS IN ACCORDANCE WITH ARTICLE 11 OF DIRECTIVE 2001/18/EC

In order to tick one or several possibilities, please use crosses (meaning x or X) into the space provided as (.)

A. General information

1. Details of notification
   (a) Member State of notification: Sweden
   (b) Notification number: B/SE/19/2019-000680-24
   (c) Date of acknowledgement of notification: Submitted 2019-04-17
   (d) Title of the project: Lactobacillus reuteri expressing CXCL12 (activated ILP100-DP)

   Proposed period of release: Q3 2019 to Q1 2020

2. Notifier
   Name of institution or company: Ilya Pharma, AB

3. GMO characterization
   (a) Indicate whether the GMO is a:

   viroid (.)
   RNA virus (.)
   DNA virus (.)
   bacterium (X)
fungus

animal

- mammals
- insect
- fish
- other animal

specify phylum, class Firmicutes, Bacilli

other, specify ...

(b) Identity of the GMO (genus and species)

Order: Lactobacillales
Genus: Lactobacillus
Species: Lactobacillus reuteri

c) Genetic stability- according to Annex IIIa, II, A (10)

The evolutionary genomic history indicates a long, host-specific adaptation of most L. reuteri strains, including strains isolated from rodents. This long-term evolution has led to that different strains are highly adapted and not likely will undergo sudden genetic changes (Duar et al., 2017).

Therefore, chromosomal stability and content are expected to be equivalent to the wildtype L. reuteri R2LC. However, due to the energy burden of foreign plasmid maintenance, the GMO inserted plasmid is unstable and is steadily lost during growth and replication without the specific selection marker. Internal plasmid retention studies indicate GMO plasmid loss rate is estimated between 25%-50% in ≤10 generations (report no 20078 [cell banks], Karimi et al., 2016). This ultimately results in the GMO returning to its wildtype state. During production, wherein the selection pressure is provided during the entire process, plasmid retention is quite stable (>90%, Doc.no.20213).

4. Is the same GMO release planned elsewhere in the Community (in conformity with Article 6(1)), by the same notifier?

Yes (.) No (X)
If yes:

- Member State of notification
- Notification number

Please use the following country codes:

Austria AT; Belgium BE; Germany DE; Denmark DK; Spain ES; Finland FI; France FR; United Kingdom GB; Greece GR; Ireland IE; Iceland IS; Italy IT; Luxembourg LU; Netherlands NL; Norway NO; Portugal PT; Sweden SE

5. Has the same GMO been notified for release elsewhere in the Community by the same notifier?

Yes (.) No (X)

If yes:

- Member State of notification
- Notification number

Please use the following country codes:

Austria AT; Belgium BE; Germany DE; Denmark DK; Spain ES; Finland FI; France FR; United Kingdom GB; Greece GR; Ireland IE; Iceland IS; Italy IT; Luxembourg LU; Netherlands NL; Norway NO; Portugal PT; Sweden SE

6. Has the same GMO been notified for release or placing on the market outside the Community by the same or other notifier?

Yes (.) No (X)

If yes:

- Member State of notification
- Notification number

Please use the following country codes:

Austria AT; Belgium BE; Germany DE; Denmark DK; Spain ES; Finland FI; France FR; United Kingdom GB; Greece GR; Ireland IE; Iceland IS; Italy IT; Luxembourg LU; Netherlands NL; Norway NO; Portugal PT; Sweden SE

7. Summary of the potential environmental impact of the release of the GMOs.

Wild type *L. reuteri* R2LC is classified as a non-pathogenic, biosafety level-class I organism (BSL-1).

There is to date no indication that wild type *L. reuteri* R2LC is capable of causing disease or adverse reactions such as toxicity or allergenic responses in healthy adults.
There is to date no indication that wild type *L. reuteri* R2LC pose any known threat to the environment.

*L. reuteri* (the species) has a broad, natural dispersion throughout most members of the mammals and bird species. No special care or consideration is deemed necessary for use outside of normal good-hygiene practices (i.e. proper hand-washing). The species has been successfully marketed as a probiotic for decades.

Multiple strains of *L. reuteri* (food grade) have been considered safe for dosing of otherwise healthy infants (suffering only from colic and has been extensively documented). In relation to this, an *L. reuteri* rat isolate with very little chance of direct colonization of humans or wounds would naturally be acceptable for use in wound care treatment. The ILP100 modification carries no additional characteristics that would alter this assessment.

*L. reuteri* in general has never shown evidence (based on the data gathered by the Notifier [involving R2LC] and several relevant studies available in the literature) to change phenotype and/or lifestyle preference in order to colonize a non-native host and in a new environment (wounds/skin). Therefore, it is deemed highly unlikely that ILP100 would break the species barrier to become a new member of the human microbiota. However, if this were to occur, there is a risk, (as always associated with development of biologic drug candidates) of development of anti-drug-antibodies (ADA) and/or other immune reactions with regards to the secreted, recombinant CXCL12 protein. Therefore, the potential magnitude of colonization may be considered high, but the extreme unlikelihood of direct human colonization (together with monitoring efforts in place) renders this risk to be overall negligible.

In light of ILP100 containing the *ermB* gene (confers resistance to erythromycin with a cross-resistance clindamycin and possibly linezolid) a theoretical transfer of *ermB* cannot be entirely eliminated, however; based on what is known in the literature and on experimental results obtained by the Notifier, such an incidence remains highly unlikely. The *ermB* gene is found naturally among isolates of host-specific *Lactobacillus*, including human isolates. Therefore, even in the extremely unlikely event of horizontal gene transfer (HGT), no further environmental impact of this *ermB* gene is anticipated and in the receiving host they would be less fit for survival. The overall potential magnitude is low and the low likelihood of direct or even indirect transfer make this risk overall low-negligible. The magnitude of contribution to spread of antibiotic resistance from the overuse in clinical wound care cannot be compared to the hypothetical event of ILP100 transferring the synthetic pILP100_hCXCL12opt containing the *ermB* to other bacteria within the wounds wherein there is no immediate antibiotic selection pressure to drive the transfer and uptake of cost heavy foreign DNA.

With regards to the environment at large, the Notifier has identified a small risk for ILP100 to survive for a limited time in relevant dirty, stagnant water environments (e.g. drainage pipes, floor drains). Therefore, monitoring strategies will be conducted to
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Further analyze this potential risk with the aim of reducing this type of environmental exposure. However, even if ILP100 is shed into the drainage system, the most likely place for it to end up is in the sewage treatment plant, wherein it will not survive due to the three-step water purification strategy in Uppsala. Although, a remote risk for the indirect effect of the antibiotic resistance gene, \textit{ermB}, to remain intact within the sewage sludge cannot be entirely eliminated. The overall potential magnitude is low and the low likelihood of direct or even indirect genetic transfer make this risk overall negligible.

B. Information relating to the recipient or parental organism from which the GMO is derived

1. Recipient or parental organism characterization:

   (a) Indicate whether the recipient or parental organism is a:

   (select one only)

   - viroid
   - RNA virus
   - DNA virus
   - bacterium (X)
   - fungus
   - animal
     - mammals
     - insect
     - fish
     - other animal

2. Name

   (i) order and/or higher taxon (for animals) Lactobacillales
(ii) genus \( Lactobacillus \)

(iii) species \( Lactobacillus\) reuteri

(iv) subspecies N/A

(v) strain \( L.\) reuteri R2LC

(vi) pathovar (biotype, ecotype, race, etc.) N/A

(vii) common name \( L.\) reuteri R2LC

3. Geographical distribution of the organism

(a) Indigenous to, or otherwise established in, the country where the notification is made:

Yes (X) \hspace{1cm} No (.) \hspace{1cm} Not known (.)

(b) Indigenous to, or otherwise established in, other EC countries:

(i) Yes (.)

If yes, indicate the type of ecosystem in which it is found:

Atlantic (.)

Mediterranean (.)

Boreal (.)

Alpine (.)

Continental (.)

Macaronesian (.)

(ii) No (.)

(iii) Not known (X)
The wild type *L. reuteri* R2LC strain was isolated in Sweden, from the gastrointestinal tract of the rat. There is no information on prevalence of R2LC in the environment, but it is reasonable to assume prevalence is normal. It is also reasonable to assume, due to its strong ability to colonize the rat gut, that the strain, R2LC, is prevalently distributed among the rat populations of Europe (isolated in Sweden), and likely the rest of the world.

(c) Is it frequently used in the country where the notification is made?

Yes (.)  No (X)  Not known (.)

(d) Is it frequently kept in the country where the notification is made?

Yes (.)  No (X)  Not known (.)

4. Natural habitat of the organism

(a) If the organism is a microorganism

- water (.)
- soil, free-living (.)
- soil in association with plant-root systems (.)
- in association with plant leaf/stem systems (.)
- other, specify Specific hosts are rats and possibly other rodents

(b) If the organism is an animal: natural habitat or usual agroecosystem:

Not applicable

5. Detection techniques

- *L. reuteri* R2LC normally expresses an orange pigment (colony growth on specific agar medium)
- Most lactobacilli are generally and inherently resistant to vancomycin (colony growth on specific agar medium containing vancomycin)
- CXCL12 chemokine expression, measured by ELISA
- PCR with strain- and GMO specific primers
- 16S RNA gene sequencing
Identification techniques

- CXCL12 chemokine expression, measured by ELISA
- PCR with strain- and GMO specific primers
- 16S RNA sequencing

6. Is the recipient organism classified under existing Community rules relating to the protection of human health and/or the environment?

Yes (X) No (.)

If yes, specify

*L. reuteri* is classified as a non-pathogenic, biosafety level-class I organism. Meaning that they are not known to cause disease or infection in healthy adults and additionally pose no threat to the environment. No special care or consideration is deemed necessary outside of normal good-hygiene practices (i.e. proper hand-washing). It has been successfully marketed as a probiotic for decades. Since 2008, *Lactobacillus reuteri* have been listed on the FDA’s inventory of GRAS organisms (Generally Regarded As Safe) notices under 62 FR 18938; April 17, 1997 proposed rule and the 81 FR 54960; August 17, 2016 final rule. It has also cleared the EFSA updated list of QPS (qualified presumption of safety) organisms (Biohaz et al., 2017). Generally, this concept is used in relation to food and feed safety assessments. However, the common characteristic among the organisms on this list is one of non-pathogenic behavior, meaning that no disease or adverse reactions are commonly associated with these GRAS designated organisms.

Additionally, R2LC specifically is a wild type strain (a native isolate from the gut of rats) and it is reasonable to assume that it already has a natural and native presence in the environment. Shedding of *L. reuteri* R2LC, would therefore happen naturally in most types of environmental habitats since rats are free-living and there is no control over where they choose to roam. Historically and currently, there is no adverse risk associated with the natural shedding of this microorganism in its wild type form.

7. Is the recipient organism significantly pathogenic or harmful in any other way (including its extracellular products), either living or dead?

Yes (.) No (X) Not known (.)

If yes:
(a) to which of the following organisms:

humans (.)
animals (.)

plants (.)

other (.)

(b) give the relevant information specified under Annex III A, point II. (A)(11)(d) of Directive 2001/18/EC

*L. reuteri* has been commercially available in the form of probiotic supplements for several decades. It is a natural isolate of the mammalian gastrointestinal tract, but has also been isolated from different areas of the body, such as breast milk, vagina and skin. It has been isolated from a number of different mammals and birds, including mouse, pig, chicken, rat and human. *L. reuteri* has been involved in numerous studies designed to reduce colic symptoms in infants and no adverse effects associated with *L. reuteri* have ever, to the Notifier’s knowledge, been reported. The specific *L. reuteri* strain (*L. reuteri* R2LC) in focus in this document was first isolated and characterized from rats by Molin *et al* (1992) (*Molin* *et al*., 1992). Since then, *L. reuteri* R2LC has been used in a number of colitis mouse/rat studies with promising results. Positive health benefits regarding colitis models are based on the evidence that *L. reuteri* R2LC reduces pro-inflammatory cytokines while reducing the permeability of the intestinal barrier to translocation of pathogenic microbes. Since *L. reuteri* has a long history of beneficial, symbiont contact with warm-blooded animals and humans, adverse effects associated with the wild type organism are purely hypothetical.

8. Information concerning reproduction

(a) Generation time in natural ecosystems:

Unknown, but *L. reuteri* R2LC is a native isolate of rats and it is reasonable to assume that it has a natural presence in the environment, however; specific information on generation time would require a massive environmental/ecological study and, in fact, is unknown for most bacterial species (*Gibson et al*., 2018). Under optimal growth conditions (laboratory studies), a generation time between 1.5 and 2 hours has been measured for *L. reuteri* R2LC.

(b) Generation time in the ecosystem where the release will take place:

Unknown, but the experimental data indicate that there is no growth of *L. reuteri* R2LC in the purposed ecosystem (i.e. volunteer subjects).

(c) Way of reproduction: Sexual (. ) Asexual (X)

(d) Factors affecting reproduction:
For substantial growth and reproduction, the minimally required nutrients have been ascertained. The growth requirements are highly fastidious and include a combination of certain amino acids, vitamins and trace minerals. *L. reuteri* R2LC is considered anaerobic, but has some oxygen tolerance. They are extremely sensitive to dry conditions and die rapidly without moisture. The strong surface adhesion ability of *L. reuteri* R2LC is believed to be tightly connected with their ability to adhere to the unique forestomach of rats and rodents underlying host-specific colonization (Duar *et al.*, 2017).

9. **Survivability**

(a) **Ability to form structures enhancing survival or dormancy:**

(i) endospores (.)

(ii) cysts (.)

(iii) sclerotia (.)

(iv) asexual spores (fungi) (.)

(v) sexual spores (funghi) (.)

(vi) eggs (.)

(vii) pupae (.)

(viii) larvae (.)

(ix) other, specify…

*L. reuteri* is non-spore forming and no additional structures for enhancing survival or dormancy are known. However, *L. reuteri* are normally known to form biofilms.

(b) **Relevant factors affecting survivability:**

*L. reuteri* requires a moist to wet substrate consisting of specific amino acids, vitamins and trace minerals. They can grow normally between 30-45°C, with 37-42°C being generally regarded as optimal. They grow poorly below 30°C and not at all below 25°C. They are described as obligately heterofermentative, meaning that they produce several by-products as a result of fermentation. Also it refers to their oxygen requirements, in this case, grows without oxygen (anaerobic).
Although, R2LC has some oxygen tolerance. Other characteristics allow it to be an efficient host benefit, by high tolerance to both bile and pH levels. In general, *L. reuteri* has shown strong colonizing ability due to the presence of mucus binding and cell surface proteins, although R2LC has to our knowledge, never been specifically isolated from humans even though it has been experimentally inoculated. This strong colonizing ability has been shown in rats and other rodents indicating a host-specific characteristic that is implied by several studies. *L. reuteri* is believed to have an evolutionarily reduced genome that has been host-adapted in parallel with the evolution of warm-blooded animals. It is considered dependent on its natural host for long-term survival and proliferation.

10. **Ways of dissemination**

The natural route of dissemination throughout the environment is likely through rat feces. The route of dissemination in the clinical setting is intended only through the wound dressings/bandaging waste and the potential for washing and cleaning the wound area (only if the presence of an infection is suspected for safety reasons). Experimental evidence suggests that most of the GMO bacteria will be dead in the first 1 hour after treatment when applied to wounds not covered by a dressing. In mice, no GMO bacteria have been recovered from swabs of the wound bed after 24 hours post-treatment. ILP100 can survive for 48 hours on the skin immediate to the treated wound (when a film dressing is placed on the wound keeping a moist environment) of mini-pigs, but at a drastically reduced number compared with treatment dose (0.001% survival).

(b) **Factors affecting dissemination**

Survival time on the wounds and attached to wound dressing is very short (refer to 10 (a) above). In very dry conditions, the bacteria are expected to die even faster (e.g. <24 hours). In the unlikely event that the GMO would be washed into the sink (without the use of soaps or disinfectants), it is conceivable that the bacteria would survive for some time (days) in the moist water drainage pipes. If ILP100 is shed into the drainage system, the most likely place for it to end up is in the sewage treatment plant, wherein it will not survive due to the three-step water purification strategy in Uppsala (Uppsala Vatten, Kungsängsverket).

11. **Previous genetic modifications of the recipient or parental organism already notified for release in the country where the notification is made (give notification numbers)**

..., B/.../.... Not previously notified

There are no previous notifications involving the specific strain, *L. reuteri* R2LC. However, another similar GMO, involving a *Lactococcus lactis* bacterial strain (European notification number; B/BE/18/BVW5) is currently under release evaluation and may be considered relevant.
C. **Information relating to the genetic modification**

1. **Type of the genetic modification**
   (i) insertion of genetic material  (X)
   (ii) deletion of genetic material  (.)
   (iii) base substitution            (.)
   (iv) cell fusion                  (.)
   (v) others, specify…             (.)

2. **Intended outcome of the genetic modification**

   The outcome of the genetic modification is meant to reliably express human chemokine, CXCL12-1α from *L. reuteri* R2LC. The human gene sequence for CXCL12 was codon-optimized for expression in *L. reuteri* R2LC and an additional secretion signal, native to *L. reuteri* R2LC was added in frame with the gene for CXCL12. This modification was inserted into a *Lactobacillus* expression vector, pSIP411 using standard laboratory methods (Sørvig *et al.*, 2005; Vågesjö *et al.*, 2018). The purpose of the secretion signal is meant to direct the recombinant CXCL12 to the outside of the bacterial cell, and by that reach the local environment of the wound bed, wherein it will initiate the signal for the wound healing cascade.

3. **(a) Has a vector been used in the process of modification?**
   
   Yes (X)        No (.)

   If no, go straight to question 5.

   **(b) If yes, is the vector wholly or partially present in the modified organism?**
   
   Yes (X)        No (.)

4. **If the answer to 3(b) is yes, supply the following information**

   **(a) Type of vector**
   
   plasmid  (X)
   bacteriophage  (.)
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(b) Identity of the vector ...

The plasmid, pSIP411, is an inducible expression vector from the pSIP400 series (information on which can be found in Sørvig et al., 2003 and 2005). The pSIP411 was engineered as an expression vector specifically for *Lactobacillus*. The DNA sequences for human CXCL12 have been codon-optimized for translation in *L. reuteri* R2LC and is therefore genetically not recognized by human transcription/translation machinery. The transgene, CXCL12, responsible for expression of the bacterial-derived, recombinant protein is identical in amino acid sequence to the endogenous human CXCL12. This new plasmid is called pILP100_hCXCL12opt plasmid.

(c) Host range of the vector...

The pSIP411 expression vector uses the replicon region from the pSH71, a vector isolated from *Lactococcus lactis* subsp. *lactis* 712. This vector has been shown to sustain replication, in addition to *Lactobacillus* and *Lactococcus*, in *Bacillus*, *Staphylococcus aureus*, *Streptococcus*, *Leuconostoc*, *Pediococcus* and *Propionibacterium* as well as a few *Escherichia coli* strains.

(d) Presence in the vector of sequences giving a selectable or identifiable phenotype

<table>
<thead>
<tr>
<th>Yes (X)</th>
<th>No (.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Antibiotic resistance</td>
<td>(X)</td>
</tr>
</tbody>
</table>

Other, specify…

Indication of which antibiotic resistance gene is inserted

*ermB*; erythromycin resistance and cross-resistance to clindamycin and possibly linezolid

The *ermB* gene is found naturally among isolates of host-specific *Lactobacillus*, including human isolates. The *ermB* gene is a natural isolate from *Lactobacillus reuteri* 1063. It is normally located on a plasmid called pLUL631 which is found in several isolates of *L. reuteri*. 
Constituent fragments of the vector

The main features of the pILP100_hCXCL12opt plasmid are listed in Table 1 and Figure 1 below.

**Table 1: Main Features of ILP100 GMO plasmid.**

<table>
<thead>
<tr>
<th>Feature</th>
<th>Function</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>TpepN</td>
<td>Transcriptional terminator of reporter gene (CXCL12)</td>
<td><em>Lactococcus lactis</em>&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>SH71 replicon (&lt;i&gt;copG&lt;/i&gt; transcriptional regulator, &lt;i&gt;repA&lt;/i&gt;, putative, copy-number control gene)</td>
<td>Sustains replication in a broad-host range including lactococci and lactobacilli</td>
<td><em>Lactococcus lactis</em> subsp. &lt;i&gt;lactis&lt;/i&gt; 712&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Leader peptide to &lt;i&gt;ermB&lt;/i&gt;</td>
<td>Contains transcriptional control elements, including ribosomal binding site and promoter for &lt;i&gt;ermB&lt;/i&gt; (methylation activity)</td>
<td><em>Lactobacillus reuteri</em> 1063; native plasmid pLUL631&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>&lt;i&gt;ermB&lt;/i&gt;</td>
<td>Erythromycin resistance marker gene</td>
<td><em>Lactobacillus reuteri</em> 1063; native plasmid pLUL631&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>&lt;i&gt;P&lt;/i&gt; &lt;i&gt;sppIP&lt;/i&gt;</td>
<td>Inducible promoter</td>
<td><em>Lactobacillus sakei</em>&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>&lt;i&gt;sppK&lt;/i&gt;</td>
<td>Gene coding for histidine protein kinase -activates regulator</td>
<td><em>Lactobacillus sakei</em>&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>&lt;i&gt;sppR&lt;/i&gt;</td>
<td>Gene coding for response regulator -activates transcriptional promoters*</td>
<td><em>Lactobacillus sakei</em>&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>&lt;i&gt;P&lt;/i&gt; &lt;i&gt;orpX&lt;/i&gt;</td>
<td>Inducible promoter</td>
<td><em>Lactobacillus sakei</em>&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>&lt;i&gt;hCXCL12&lt;/i&gt;</td>
<td>Gene coding for human chemokine</td>
<td></td>
</tr>
</tbody>
</table>

<sup>a</sup>Reference (Tan et al., 1992)
<sup>b</sup>Reference (Hayes et al., 1990)
<sup>c</sup>Reference (Axelsson et al., 2003; Sørvig et al., 2003)
<sup>d</sup>Reference (Sørvig et al., 2003; Sørvig et al., 2005)

*transcriptional terminator created as a fusion between <i>sapAsaiA</i> (sakacin A gen cluster) and the cat194 terminator [Cm (chloramphenicol) resistance gene present in many plasmids of Gram positive bacteria]. Located between <i>sppR</i> and <i>PorfX</i>. 
Figure 1: Plasmid Map of pILP100_hCXCL12opt Plasmid ILP100.

(f) Method for introducing the vector into the recipient organism

(i) transformation (.)
(ii) electroporation (X)
(iii) microinjection (.)
(iv) microinjection (.)
(v) infection (.)
(vi) other, specify ...
5. If the answer to question B.3(a) and (b) is no, what was the method used in the process of modification?

(i) transformation
(ii) microinjection
(iii) microencapsulation
(iv) macroinjection
(v) other, specify …

6. Composition of the insert

(a) Composition of the insert

i) SH71 replicon

ii) ermB (including leader peptide and methylase gene)

iii) P\textsubscript{sppIP} inducible promoter

iv) sppK/ sppR two-component signal response regulator element

v) P\textsubscript{orfX} inducible promoter

vi) hCXCL12 1 alpha codon-optimized human chemokine

vii) TpepN transcription terminator for reporter gene (hCXCL12)

(b) Source of each constituent part of the insert

i) SH71 replicon; Lactococcus lactis subp. lactis 712

ii) ermB Lactobacillus reuteri 1063; native plasmid pLUL631

iii) P\textsubscript{sppIP} promoter; Lactobacillus sakei

iv) sppK/ sppR two-component response regulator; Lactobacillus sakei

v) P\textsubscript{orfX} promoter; Lactobacillus sakei

vi) hCXCL12; human chemokine
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vi) *TpepN* transcription terminator; *Lactococcus lactis*

(c) Intended function of each constituent part of the insert in the GMO

i) SH71 replicon; allows replication of plasmid

ii) *ermB*; selection marker for GMO, erythromycin resistance

iii) *P*<sub>spp</sub><sup>IP</sup> inducible promoter; promotes expression of hCXCL12 through activation of the *sppK/sppR* two-component system

iv) *sppK/sppR* two-component signal response regulator element; senses and activates transcriptional promotor of hCXCL12

v) *P*<sub>orf</sub><sup>X</sup> inducible promoter; further promotes expression of hCXCL12

vi) hCXCL12 codon-optimized human chemokine

vii) *TpepN* transcription terminator for reporter gene (hCXCL12)

(d) Location of the insert in the host organism

- on a free plasmid (X)
- integrated in the chromosome (.)
- other, specify...

(e) Does the insert contain parts whose product or function are not known?

Yes (.), No (X)

If yes, specify ...

D. Information on the organism(s) from which the insert is derived

1. Indicate whether it is a:

   viroid (.)

   RNA virus (.)

DNA virus
(.)
bacterium
(.)
fungus
(.)
animal
(.)
  - mammals
  (.)
  - insect
  (.)
  - fish
  (.)
  - other animal
  (specify phylum, class) ...

- other, specify

The free plasmid is a group of interest (GOI) element and is described according to all of the expressed proteins carried on the plasmid. All expressed proteins part of the plasmid are from *Lactococcus lactis*\(^{(a/b)}\), *Lactobacillus reuteri* 1063\(^2a\), *Lactobacillus sakei* Lb790\(^2b\) or Human (chemokine, CXCL12-1a)\(^3\).

2. 1) Complete name (*Lactococcus lactis*; 2 different strains represented)

| (i) order and/or higher taxon (for animals) | N/A |
| (ii) family name for plants                 | N/A |
| (iii) genus                                 | *Lactococcus* |
| (iv) species                                | *lactis* |
| (v) subspecies                              | \(^a^{cremoris}/^{b}lactis\) |
| (vi) strain                                 | \(^aL. lactis\) \(^bL. lactis\) subsp *cremoris* MG1363 \(^bL. lactis\) subsp *lactis* 712 |
| (vii) cultivar/breeding line                | N/A |
| (viii) pathovar                             | N/A |
(ix) common name

2) Complete name (\textit{Lactobacillus reuteri}; \textit{Lactobacillus sakei})

(i) order and/or higher taxon (for animals) \textit{Lactobacillales}

(ii) family name for plants \textit{Lactobacillus}

(iii) genus \textit{Lactobacillus reuteri; Lactobacillus sakei}

(iv) species \textit{Lactobacillus reuteri; Lactobacillus sakei}

(v) subspecies \textit{Lactobacillus reuteri; Lactobacillus sakei}

(vi) strain \textit{Lactobacillus reuteri 1063; Lactobacillus sakei Lb790}

(vii) cultivar/breeding line \textit{L. reuteri 1063; L. sakei Lb790}

(viii) pathovar \textit{N/A}

(ix) common name \textit{L. reuteri 1063; L. sakei Lb790}

3) Complete name

(i) order and/or higher taxon (for animals) \textit{Primates}

(ii) family name for plants \textit{N/A}

(iii) genus \textit{N/A}

(iv) species \textit{Homo}

(v) subspecies \textit{Sapiens}
3. Is the organism significantly pathogenic or harmful in any other way (including its extracellular products), either living or dead?

Yes (.)  No (X)  Not known (.)

If yes, specify the following:

(a) to which of the following organisms:

humans  (.)
animals  (.)
plants  (.)
other ..

(b) are the donated sequences involved in any way to the pathogenic or harmful properties of the organism

Yes (.)  No (X)  Not known (.)

If yes, give the relevant information under Annex III A, point II(A)(11)(d):

4. Is the donor organism classified under existing Community rules relating to the protection of human health and the environment, such as Directive 90/679/EEC on the protection of workers from risks to exposure to biological agents at work?

Yes (X)  No (.)  Not known (.)

*L. reuteri* and *L. lactis* are both classified as a non-pathogenic, biosafety level-class I organism. Meaning that they are not known to cause disease or infection in healthy adults and additionally pose no threat to the environment. No special care or consideration is deemed necessary outside of normal good-hygiene practices (i.e. proper hand-washing). It has been successfully marketed as a probiotic for decades. Since 2008, *Lactobacillus*
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*reuteri* have been listed on the FDA’s inventory of GRAS organisms (Generally Regarded As Safe) notices under 62 FR 18938; April 17, 1997 proposed rule and the 81 FR 54960; August 17, 2016 final rule. *L. reuteri* and *L. lactis* have also cleared the EFSA updated list of QPS (qualified presumption of safety) organisms (Biohaz et al., 2017). Generally, this concept, is used in relation to food and feed safety assessments. However, the common characteristic among the organisms on this list is one of non-pathogenic behavior, meaning that no disease or adverse reactions are commonly associated with these GRAS/QPS designated organisms.

No such Community rules relating to the protection of human health and the environment, and/or on the protection of workers from risks to exposure to biological agents at work are relevant for the human component.

5. **Do the donor and recipient organism exchange genetic material naturally?**

   Yes (.)
   No (X)

   If yes, specify ...

   There are no known occurrences of genetic exchange between human and *Lactobacillus* or *Lactococcus*. Between *Lactobacillus* and *Lactococcus* species there is the possibility for genetic exchange. However, in attempts to test the GMO capacity to transfer genetic material, by the generally recognized method of choice for bacteria in nature, conjugation, this appears extremely unlikely. Several attempts to conjugate *L. reuteri* R2LC with a well-known, highly conjugatable plasmid (pAMβ1) from a *Enterococcus faecalis* strain recognized for its capacity to be an efficient donor or recipient of mobilized genetic material failed to generate positive transconjugants (*L. reuteri* R2LC positive for pAMβ1). The same conjugation experiments were performed between ILP100 and several wound isolated strain of *Streptococcus* strains with no detected transconjugants.

   Regarding the transgene, hCXCL12, the DNA codon-optimized sequence does not exist in humans and human protein expression systems would not recognize it.

E. **Information relating to the genetically modified organism**

1. **Genetic traits and phenotypic characteristics of the recipient or parental organism which have been changed as a result of the genetic modification**

   (a) **is the GMO different from the recipient as far as survivability is concerned?**

      Yes (X)
      No (.)
      Not known ()
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Specify:

It is highly likely that the fitness burden that comes with the GMO plasmid gives ILP100 a growth burden and therefore the GMO cells (that keep the plasmid) will have a growth disadvantage. Measurements of the generation time during fermentation events, estimate a reduction of up to 25% for the GMO strain compared to the wildtype. This is corroborated with internal plasmid stability data, which estimates plasmid loss in a range of 25-50% within the first 10 generations (doubling-times) in an optimal growth environment in the absence of the antibiotic selection pressure (erythromycin). Thus, the wildtype (cell that lose the GMO plasmid) counterpart will out-compete the population and the GMO population will eventually be eliminated. The only scenario where this would not happen is if the GMO were under the immediate selection pressure of antibiotic treatment with erythromycin (and possibly clindamycin/linezolid). The erythromycin would kill the wildtype and allow for the GMO to take over the population instead. However, this is a hypothetical scenario as the severe fastidiousness of *L. reuteri* would prevent it from surviving for long in most non-native environments.

(b) is the GMO in any way different from the recipient as far as mode and/or rate of reproduction is concerned?

Yes (X)  No (.)  Unknown (.)

Specify:

There is likely a fitness burden to carrying the GMO plasmid and this would have an effect leading to a reduced rate of the bacterial replication/division, i.e. mode of asexual reproduction. This would mean that in a natural setting, at least wherein erythromycin/clindamycin/linezolid in not present, the ILP100 would be at a survival disadvantage compared to its wild type parent. See also section above; E.1.(a).

In the hypothetical event of unexpected colonization, direct exposure to erythromycin or clindamycin would give ILP100 a survival advantage that is lacking in the wildtype *L. reuteri* R2LC.

(c) is the GMO in any way different from the recipient as far as dissemination is concerned?

Yes (.)  No (.)  Not known (X)

Specify:
There is no indication that *L. reuteri* R2LC would be capable of survival, or dissemination in anything other than a rat or perhaps other rodents (Oh *et al.*, 2009; Duar *et al.*, 2017; Duar *et al.*, 2017). In the hypothetical event of unexpected colonization, direct exposure to erythromycin or clindamycin/linezolid would give ILP100 a survival advantage that is lacking in the wildtype *L. reuteri* R2LC.

(d) is the GMO in any way different from the recipient as far as pathogenicity is concerned?

Yes (.) No (X) Not known (.)

Specify:

Neither wildtype *L. reuteri* R2LC nor ILP100 are pathogenic to humans or mammals. It is also not recognized as being harmful to the environment.

2. Genetic stability of the genetically modified organism

The evolutionary genomic history indicates a long, host-specific adaptation to most *L. reuteri* strains, including strains isolated from rodents. This long-term evolution has led to that different strains are highly adapted and not likely will undergo sudden genetic changes (Duar *et al.*, 2017).

Therefore, chromosomal stability and native genetic elements are expected to be equivalent to the wildtype *L. reuteri* R2LC, however; due to the energy burden of foreign plasmid maintenance, the GMO inserted plasmid is unstable and is steadily lost during growth and replication without the specific selection marker. Internal plasmid retention studies indicate GMO plasmid loss rate estimated between 25%-50% in ≤10 generations (internal data report no. 20078 [cell banks], Karimi *et al.*, 2016). This ultimately results in the GMO returning to its wildtype state. During production, wherein the selection pressure is provided during the entire process, plasmid retention is quite stable (>90%, internal data Doc.no.20213).

3. Is the GMO significantly pathogenic or harmful in any way (including its extracellular products), either living or dead?

Yes (.) No (X) Unknown (.)

(a) to which of the following organisms?

humans (.)

animals (.)
(b) give the relevant information specified under Annex III A, point II(A)(11)(d) and II(C)(2)(i)

The pathogenic capacity of ILP100 is as low as the non-pathogenic wildtype. Additionally, ILP100 has successfully completed a repeat-dose toxicity study in mini-pigs with no adverse events reported. The development of anti-drug antibodies (ADAs) to the bacterial-derived CXCL12 recombinant protein is a potential hazard associated with all biologic medical products, but there is no evidence in pre-clinical or toxicity studies that indicates a risk to acceptable safety or tolerance in association with ILP100. Nevertheless, this will be monitored in all subjects admitted to the FIH trial.

4. Description of identification and detection methods

(a) Techniques used to detect the GMO in the environment

- \textit{L. reuteri} R2LC normally expresses an orange pigment (colony growth on specific agar medium)

- Most lactobacilli are generally and inherently resistant to vancomycin (colony growth on specific agar medium containing vancomycin)

- CXCL12 chemokine expression: ELISA

- PCR with strain and GMO specific primers

- 16S RNA sequencing

(b) Techniques used to identify the GMO

- CXCL12 chemokine expression: ELISA

- PCR with strain and GMO specific primers

- 16S RNA sequencing

F. Information relating to the release

1. Purpose of the release (including any significant potential environmental benefits that may be expected)
The general purpose of this First-in-Human (FIH) study is to evaluate safety, tolerability, exposure and preliminary efficacy of single and multiple ascending doses of *L. reuteri* expressing CXCL12 (ILP100) administered topically to experimentally induced skin wounds in healthy subjects. The study comprises a single ascending dose (SAD) part, a multiple ascending dose (MAD) part and a 5-year long-term follow-up part.

2. Is the site of the release different from the natural habitat or from the ecosystem in which the recipient or parental organism is regularly used, kept or found?

   Yes (X)  No ( )

   If yes, specify

   The parental strain is a native isolate of the rat gut. The site of the release is a controlled, clinical setting, wherein the GMO will be applied to healthy, human wound punches produced on the inner arm of volunteer subjects. The GMO is not expected to survive or thrive in this non-native environment due to strong host-specificity (to rats) and also due to the harsh micro-environment of the wound site.

   The site of release is the clinic where the clinical trial is performed. The wounds are covered during the trial and the research subjects included in the study are accordingly informed not to remove dressings in between visits, however the Notifier cannot control for the test subjects in between visits.

3. Information concerning the release and the surrounding area

   (a) Geographical location (administrative region and where appropriate grid reference):

   The GMO is not released in the environment as the wounds will be treated in a controlled area (clinical site) and then covered with secure dressings and bandages. The removal of the dressings and bandages will take place at the clinical site as outlined in the clinical protocol. The wound waste will be treated as medical waste and appropriately destroyed, or collected as research samples. The study will take place in Uppsala, Sweden.

   (b) Size of the site (m²):

   (i) actual release site (m²):

   Not applicable

   (ii) wider release site (m²):

   Not applicable
4. Method and amount of release

(a) Quantities of GMOs to be released:

Maximum release per subject is $(0.2) \times 6 \times 10^9$ units of bacteria added to 4 wounds with a maximum of 10 doses. Shedding is not expected to occur as each wound will be covered with tight dressings. The removal of the dressings and bandages will take place at the clinical site as outlined in the clinical protocol. The wound waste will be treated as medical waste and appropriately destroyed, or collected as research samples.

(b) Duration of the operation:

The complete trial procedure will last 21 days. At each dose visit the subject will remain at the clinical site for 4 hours.

(c) Methods and procedures to avoid and/or minimize the spread of the GMOs beyond the site of the release:

Product will be supplied and stored at the clinical site for the duration of the study. All involved personnel on the site will be trained in best biosafety practices to be applied during preparation in the pharmacy, transport to the administration room, precautions during administration and disposal of any biological waste. Such training involves, among others, wearing adapted protective clothing and gloves, the immediate wiping up and disinfecting of spill areas and the decontamination of waste prior to disposal.

5. Short description of average environmental conditions (weather, temperature, etc.)

Clinical treatment rooms and normal, ambient indoor conditions are expected for clinical trial staff and subjects. The investigational medicinal product will be stored at $+2-8\, ^{\circ}\mathrm{C}$, until just before rehydration with activation peptide buffer, which will be stored $+2-8\, ^{\circ}\mathrm{C}$. 

Proximity to internationally recognized biotopes or protected areas (including drinking water reservoirs), which could be affected:

Not applicable considering that shed material, if any at all, is non-infectious.

Furthermore, the GMO is considered contained (not released) as the wounds will be controlled with securely placed dressings and bandages. Additionally, internal experiments show poor survival in clean water, supporting no added danger to any local drinking water reservoirs.

Flora and fauna including crops, livestock and migratory species which may potentially interact with the GMO:

Not applicable considering neither \textit{L. reuteri} \textit{R2LC} wildtype, and consequently the GMO \textit{ILP100}, are not known to interact with any host species except rats and possibly other rodents.
The ILP100 will undergo a brief incubation period at room temperature, before application to wounds.

6. Relevant data regarding previous releases carried out with the same GMO, if any, specially related to the potential environmental and human health impacts from the release.
   None available

G. Interactions of the GMO with the environment and potential impact on the environment, if significantly different from the recipient or parent organism

1. Name of target organism (if applicable)

   (i) order and/or higher taxon (for animals)  Primates
   (ii) family name for plants  N/A
   (iii) genus  Homo
   (iv) species  Sapiens
   (v) subspecies  Sapiens
   (vi) strain  N/A
   (vii) cultivar/breeding line  N/A
   (viii) pathovar  N/A
   (ix) common name  Human

2. Anticipated mechanism and result of interaction between the released GMOs and the target organism (if applicable)

   The MOA (Mode of Action) proposed is a result of continuously produced and locally delivered CXCL12 chemokine by the L. reuteri bacteria directly to the wound bed. The delivery system of the CXCL12 was designed for continuous delivery on the notion of the short half-life and the abundance of proteases in wounds, e.g. CD26 (dipeptidyl peptidase, prolyl oligopeptidase) that inactivates CXCL12 by cleaving the NH₂-terminus (Mortier et al., 2015; Pucar et al., 2017; Saboo et al., 2016). The activity of CD26 is pH-dependent
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(Ohtsuki et al., 1998; Proost et al., 1998), and this enzyme, with the proposed system, is
inhibited by the local pH reduction by bacterial-produced lactic acid, resulting in a higher
bioavailability of the Lactobacillus-delivered CXCL12. The co-production of lactic acid
by ILP100 bacteria, lowers pH levels locally in the wound, thereby inhibiting the activity
of the enzyme CD26, which otherwise cleaves and inactivates CXCL12 in wounds. For
treated subjects, ILP100 is expected to accelerate wound healing through bacterial-
delivered CXCL12 induces proliferation and phenotypic shift of local macrophages in the
dermis immediate to the wound, whereby these macrophages start to express Transforming
Growth Factor-beta (TGF-β) faster and in higher concentrations as compared to wounds
receiving no treatment or treatment with control Lactobacillus. Safety and tolerance of
ILP100 drug product will be closely monitored and is considered a primary end point of
this study.

3. Any other potentially significant interactions with other organisms in the environment
None expected

4. Is post-release selection such as increased competitiveness, increased invasiveness for the
GMO likely to occur?

   Yes (.)  No (X)  Not known (.)

The GMO, ILP100, has been shown to have a slower generation time and plasmid
instability, strongly indicating a less fit (compared to wildtype) organism, resulting in a
survival disadvantage.

5. Types of ecosystems to which the GMO could be disseminated from the site of release
and in which it could become established

Even in the remote event that IP100 GMO manages to survive on the wound for more
than 24-48hrs, and/or survivors get rinsed into the clinical sinks, no long-term
persistence is expected. (Certainly no permanent establishment is expected.)

6. Complete name of non-target organisms which (taking into account the nature of the
receiving environment) may be unintentionally significantly harmed by the release of the
GMO

None

(i) order and/or higher taxon (for animals) ...

(ii) family name for plants ...

(iii) genus ...

(iv) species ...
7. Likelihood of genetic exchange \textit{in vivo}

(a) from the GMO to other organisms in the release ecosystem:

Highly unlikely. Since ILP100 carries the \textit{ermB} gene (erythromycin resistance) there is understandable concern that there could be transfer of the element \textit{ermB}, and therefore a contribution to the spread of antibiotic resistance. It is common that transfer of genetic material comes at a fitness cost (energy burden) for bacteria. Even a very low fitness decrease can ensure that individual bacteria, that might happen to take-up foreign DNA, would be outcompeted in a natural setting. This fitness decrease can be compensated by an immediate benefit (like acquisition of a gene that gives an appropriate level of tolerance or resistance to a condition in which they are currently battling (i.e. selection pressure) and importantly, does not render them too weak in growth and sustenance ability (i.e. fitness) to survive despite the acquisition of a beneficial trait (Baltrus 2013). This has been well-studied in the case for antibiotic resistance genes which often travel on plasmids throughout the environmental bacterial world and which, as we know now, is a great clinical challenge with ever-increasing antibiotic resistant pathogenic bacteria. However, without the immediate benefit (or selective advantage) of antibiotic resistance to erythromycin (selection pressure), it is likely that the cost of the plasmid would not be tolerated by the bacteria and be consequently lost from the population (Vogwill & MacLean 2015). This would manifest itself as plasmid instability (McLoughlin 1994). In fact, a literature search of relative measured fitness revealed that the fitness cost of carrying any given plasmid does not change that much between different host species. This means that the fitness cost causing the instability of the GMO pILP100 would be expected to be approximately the same if taken up by another host (Vogwill & MacLean 2015).

In order to specifically address horizontal gene transfer (HGT), through what is generally accepted as the principle method of transfer (namely conjugation), experiments have been performed to examine the possibilities of the \textit{ermB} resistance gene through the standard laboratory procedure of conjugation through filter-mating (Smith & Guild 1980; Tannock 1987). The results, in brief, have not yielded any evidence that the pILP100 GMO plasmid is capable of transferring the \textit{ermB} gene to another species. The findings thus far can be found in the Conjugation
Conjugation is generally regarded as a requirement for long-term foreign plasmid persistence within a given population of bacteria. Since the conjugative capacity appears to be undetectable for ILP100, the overall likelihood that the GMO plasmid will be transferred by conjugation must be considered negligible.

(b) from other organisms to the GMO:

Highly unlikely. Several attempts to conjugate *L. reuteri* R2LC with a well-known, highly conjugatable plasmid (pAMβ1) from an *Enterococcus faecalis* strain recognized for its capacity to be an efficient donor or recipient of mobilized genetic material failed to generate positive transconjugants (*L. reuteri* R2LC positive for pAMβ1). The same conjugation experiments were performed between ILP100. Additionally, several conjugation attempts were conducted with ILP100 and three wound-isolates of *Streptococcus* strains with no confirmed transconjugants.

(c) likely consequences of gene transfer:

The *ermB* gene in question comes from a natural isolate *Lactobacillus reuteri* 1063. It is normally located on a plasmid called pLUL631, which is found in several strains of *L. reuteri*. The presence of pLUL631 in more than one strain of *L. reuteri* supports the notion that the naturally occurring pLUL631 carrying the *ermB* resistance gene has been spread through gene transfer, but to the Notifier’s knowledge, genetic transfer of the *ermB* from the naturally occurring pLUL631 has not been demonstrated nor documented to be true for any other bacterial species (Ahrné et al., 1992). Furthermore, it is important to note, that several human isolates of *L. reuteri* harbor resistance genes naturally. Most of the these are carried by naturally occurring plasmids including several isolates that harbor *tetW*, a resistance gene against tetracycline and at least one human isolate from the intestine (8557:1) that naturally carries *ermB* on a naturally occurring plasmid (Egervärn et al., 2009). Therefore, the overall consequential magnitude is considered to be low.

8. Give references to relevant results (if available) from studies of the behavior and characteristics of the GMO and its ecological impact carried out in simulated natural environments (e.g. microcosms, etc.):

**Survival in water:**

The ability of ILP100 to survive if treated wounds were to be rinsed into the sink directly after treatment and therefore end up in the water drainage systems. A survival time course experiment in 1) clean water/clean pipes and 2) stagnant water in two different dirty/used pipes was performed. In clean pipes with clean tap water the survival rate of the ILP100 is <0.003% with an insignificant difference based on presence or absence of erythromycin.
In one of the dirty pipes the 10 day recovery range was 0.8-4% and in the second pipe the 10 day recovery range was 20-30% when calculated from Day 0 recovery. In the second dirty pipe, the overall numbers reduced only slightly, meaning that similar numbers of bacteria were recovered after 10 days as were inoculated at the start of the experiment. Therefore there is a small risk of ILP100 survival in the drainage pipes.

Survival on Wound Dressings
ILP100 was applied to three wound dressing conditions, 1) dry, 2) moist with saline buffer, and 3) most with nutrient source. The bacteria die quickly on dressings, even when supplemented with a nutrient source. Since almost the entire treatment (bacterial dose) dies in the first 24 hours on wound dressings, the likelihood of reaching the water drainage system is minimal.

Regarding the risk for potential spread of the *ermB* antibiotic resistant gene, the Notifier has conducted several experimental attempts at conjugation in an effort to test the GMO capacity to transfer genetic material, by the generally recognized method of choice for bacteria in nature, conjugation, and the results indicate that this appears extremely unlikely. Several attempts to conjugate *L. reuteri* R2LC with a well-known, highly conjugatatable plasmid (pAMβ1) from an *Enterococcus faecalis* strain recognized for its capacity to be an efficient donor or recipient of mobilized genetic material failed to generate positive transconjugants (*L. reuteri* R2LC positive for pAMβ1). The same conjugation experiments were performed between ILP100. Additionally several conjugation attempts were conducted with ILP100 and three wound-isolates of *Streptococcus* strains with no confirmed transconjugants.

Thus, survival in predicted areas at risk for spread of GMO is considered unlikely and even if it were to survive, the GMO are likely at a fitness disadvantage and furthermore; experimental conjugation attempts to transfer the *ermB* gene have failed. Therefore, spread of the GMO is extremely unlikely making transfer of the *ermB* gene highly improbable under the confinement strategies set by the current release specifications.

9. Possible environmentally significant interactions with biogeochemical processes (if different from the recipient or parental organism)

None known or predictable since *L. reuteri* R2LC wild type is not known to be involved in any biogeochemical process.

H. Information relating to monitoring

1. Methods for monitoring the GMOs

Regarding the monitoring of the clinical site and subjects, collection of blood, feces, skin swabs and wound biopsies will be taken according to the clinical protocol and risk management plan. These samples will be appropriately cultured or analyzed (ELISA, PCR,
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microscopy, etc.) as needed. Additionally, due to the small risk of survival in dirty water clinical sink pipes, the water in the water-lock pipes on all of the clinical sinks will also be collected and analyzed for ILP100.

2. Methods for monitoring ecosystem effects

No monitoring is considered necessary

3. Methods for detecting transfer of the donated genetic material from the GMO to other organisms

PCR identification from colonies cultured from samples

4. Size of the monitoring area (m²) ... m²

Not applicable. The subject’s blood, feces, skin and wound will be monitored after administration. In addition, the water in the water-lock pipes of all of the clinical sinks will also be collected and analyzed for ILP100.

5. Duration of the monitoring

Collection of samples from the subjects will be monitored until the end of the study. Likewise, the collection of the water drainage pipes will occur after completion of the study.

A follow-up with each subject with a physical examination is planned for six weeks (post first treatment), 6 months and 12 months plus a 5 year follow-up after cessation of the clinical study. At each follow-up, the skin area (corresponding to wound area) will be swabbed and analyzed or ILP100 presence.

6. Frequency of the monitoring

At planned intervals according to the clinical protocol 1.5, 6, 12 months and 5 years after cessation of the clinical study.

I. Information on post-release and waste treatment

1. Post-release treatment of the site

Decontamination of the IMP administration room by standard procedures will be used after administration. Any material or surface in contact with the product will be decontaminated or autoclaved. Any other disposable instruments or other materials used during the dose preparation procedure will be disposed of in a manner consistent with the standard practice of the institution for potentially biohazardous materials.
2. Post-release treatment of the GMOs

No unused product should remain at the clinical site after the end of trial period. Any open vials or unused material (that has not been returned to Ilya Pharma) will be destroyed by decontamination according to local biosafety guidelines.

3. (a) Type and amount of waste generated

Empty and/or used vials and equipment used for administration (needles, syringes, wound punch device), gauzes, wound dressings, and bandaging along with personal protective equipment (e.g. gloves etc.) and components used for collecting body fluids samples after administration.

(b) Treatment of waste

Any sharps (i.e. needles) will be disposed of in adequate sharp containers and incinerated. All waste will go into appropriate waste and sent for incineration according to local biosafety guidelines.

Likewise, all surgical materials (tools, linens, etc.) and surgical waste (gloves, gauzes, etc.) will be collected and autoclaved before washing and sterilization or incineration. All non-disposable surgical equipment will be cleaned using a chemical disinfectant and then sterilized by autoclaving according to standard practices of the institution.

All waste generated from the wounds (i.e. dressings, bandaging, etc.) will be thrown into specifically designated safety boxes that will be picked-up by an waste courier (informed about the GMO nature of the waste) and sent for immediate disposal by incineration.

J. Information on emergency response plans

1. Methods and procedures for controlling the dissemination of the GMO(s) in case of unexpected spread

The rehydrated ILP100, and further dilution steps required for dosing for application to induced wound punches will be prepared by trained clinical staff members in a contained area. In case of spillage, the affected area will be disinfected with protocol approved disinfectants and waste will be collected and incinerated according to standard protocol.

2. Methods for removal of the GMO(s) of the areas potentially affected

Should clinical staff working with the GMO come into direct contact with the GMO (through a spill or accidental needle puncture during administration, or via subject sample
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collection), no immediate and/or delayed effects different from those expected for the recipients (test subjects) are expected.

In case of accidental eye exposure of the GMO, the eye will be rinsed with eyewash for 15 minutes and the patient will then report to the hospital emergency room for evaluation. In case of accidental skin puncture of material containing the GMO, bleeding of the wound will be encouraged, the area will be washed well with soap and water and the patient will report to the hospital emergency room for evaluation.

3. Methods for disposal or sanitation of plants, animals, soils, etc. that could be exposed during or after the spread

Not applicable since exposure of plants or animals is not expected.

4. Plans for protecting human health and the environment in the event of an undesirable effect

No undesirable effects are expected. In the remote event that the GMO bacteria needs to be inactivated, there are a number of antibiotics (Penicillin, Ampicillin, Imipenem, Meropenem, and Daptomycin) that known to be effective.

REFERENCES:


BIOHAZ, E.P.O.B.H. et al., 2017. Scientific Opinion on the update of the list of QPS-recommended biological agents intentionally added to food or feed as notified to EFSA. EFSA Journal, 15(3).


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