

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 | Spain |
| (b) | Notification number | B/ES/17/20 |
| (c) | Date of acknowledgement of notification | 24/11/2017 |
| (a) | Title of the project | <i>Clinical study</i>
<i>64041757LUC2002: An Open-label Randomized Phase 1b/2 Study of the Efficacy and Safety of JNJ-64041757, a Live Attenuated Listeria monocytogenes Immunotherapy, in Combination with Nivolumab Versus Nivolumab Monotherapy in Subjects With Advanced Adenocarcinoma of the Lung.</i> |
| (b) | Proposed period of release | From 15/02/2018 until 15/02/2021 |

2. Notifier

Name of institution or company: sponsor name Janssen-Cilag International NV,
Turnhoutseweg 30
Beerse
B-2340
Belgium

3. GMO characterisation

(a) Indicate whether the GMO is a:

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

specify phylum, class ...

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

*JNJ-64041757 is a genetically modified version of *Listeria monocytogenes* (Lm) which is intended for use as a human cancer immunotherapeutic in the treatment of advanced adenocarcinoma of the lung. The genetic modifications result in the following changes:*

- 1. Attenuation of virulence by the deletion of the coding sequences of two wild-type virulence determinants (ActA and Internalin B)*
- 2. Heterologous gene expression by stable integration of epidermal growth factor variant III (EGFRvIII) human mesothelin (hMeso) expression cassette into the *tRNA^{Arg}* locus of the *Lm ΔactA/ΔinlB* chromosome.*

JNJ-64041757 is derived from the Live Attenuated Double-Deleted (LADD) Lm platform strain ANZ-100. In the absence of comprehensive data for JNJ-64041757, data derived from the platform strain (ANZ-100) and derived strains (CRS-207 and JNJ-64041809) are referenced in support of this notification.

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

The gene sequence encoding EGFRvIII mesothelin is stably integrated on the bacterial chromosome. The genetic stability of JNJ-64041757 has been demonstrated in up to >270 generations. For additional details regarding the genetic stability of JNJ-64041757 see Section E.2.

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

Yes (X) No (.)

If yes, insert the country code(s) *BE*
I.

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 B/././...

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 (X) No (.)

If yes:

- Member State of notification United States & Canada
- Notification number 1609-1541 (United States); NSN-18526

(Canada)

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

No environmental impact attributable to the inadvertent GMO release is expected to occur for the following reasons:

- *The GMO is a live, attenuated double-deleted (LADD) strain of Listeria monocytogenes (Lm) that does not occur naturally and has been modified in the laboratory to be 1,000 times less virulent than wild-type Lm in mice, while maintaining immunogenicity. Because the bacteria are attenuated in normal cells, no adverse effects would be expected for individuals who inadvertently come into contact with them, or for the environment. If JNJ-64041757 were to be released into the environment it would have no selective advantage over naturally occurring Lm and would remain attenuated for virulence by means of the $\Delta actA$ and $\Delta inlB$ genetic deletions and would therefore not be a risk to humans or animals exposed to it (Brockstedt 2004). As a precaution, surveillance cultures will be obtained during the treatment and follow-up phases, to confirm the clearance of JNJ-64041757 after repeated dosing, and up to 1 year after prophylactic antibiotics.*
- *JNJ-64041757 will be administered intravenously to human subjects as part of the controlled clinical study, Clinical Protocol 64041757LUC2002 in a hospital setting. Shedding via urine or faeces of subjects into the environment is not anticipated, therefore no plant or animal species are likely to be exposed (Le 2012, Brockstedt 2004).*
- *Although fecal shedding was observed in a single monkey administered at the highest dose (1×10^9 CFU) of JNJ-64041757 and urine shedding was observed in male and female monkeys at the highest dose (3×10^{10} CFU) of CRS-207, per protocol assessments of saliva, stool and urine of subjects, in clinical studies with any LADD strain (JNJ-64041757, JNJ-64041809, ANZ-100 and CRS-207) failed to document any evidence of shedding. In addition, monitoring of blood cultures suggest quick clearance of JNJ-757 from the bloodstream, with the majority of subjects having negative blood cultures 2 hours after infusion.*
- *No cases of listeriosis, Listeria (LADD) bacteremia, or Listeria (wild-type) bacteremia have been reported in subjects completing therapy with either JNJ-64041757 or JNJ-64041809. Three cases ($< 0.1\%$) of persistence of CRS-207 beyond the 7-day prophylactic oral antibiotic course have been reported. To reduce the risk of persistence, blood cultures are monitored both during and after treatment, prophylactic antibiotics administered and immunosuppressive therapies are prohibited. Any finding of positive Listeria during the study is treated as an adverse event of special interest and reported in an expedited manner to Janssen.*
- *In summary, the risk associated with administering the GMO to patients is low, and the risk to other humans and the environment is negligible.*

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

1. Recipient or parental organism characterisation:

Information related to parental organism characterization is presented.

(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 (.)
(specify phylum, class) ...

other, specify ...

2. Name
- | | | |
|-------|---------------------------------------------|-------------------------------------------|
| (i) | order and/or higher taxon (for animals) | Bacillales |
| (ii) | genus | <i>Listeria</i> |
| (iii) | species | <i>monocytogenes</i> |
| (iv) | subspecies | ... |
| (v) | strain | <i>Spontaneous streptomycin resistant</i> |
| | <i>mutant of a wild-type strain (10403)</i> | |
| (vi) | pathovar (biotype, ecotype, race, etc.) | ... |
| (vii) | common name | <i>Listeria monocytogenes</i> |

3. Geographical distribution of the organism

Wild-type Lm is ubiquitous in the environment and can be found in moist environments, soil, and decaying vegetation. In contrast, the parental strain for the GMO (CERS 382.20) is streptomycin resistant (resistance was achieved by laboratory selection and is not the consequence of genetic manipulation). The concerned GMO (JNJ-64041757) is not naturally occurring but an IMP for clinical use and will be administered to subjects with advanced adenocarcinoma of the lung under controlled conditions at clinical sites in the Member State in accordance with Clinical Protocol 64041757LUC2002. No deliberate release to the environment is expected.

(a) Indigenous to, or otherwise established in, the country where the notification is made:
Yes (X) No (.) Not known (.)

(b) Indigenous to, or otherwise established in, other EC countries:
Yes (X) No (.)

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

Atlantic	X
Mediterranean	X

Boreal	X
Alpine	X
Continental	X
Macaronesian	X

(ii) No (.)
 (iii) Not known (.)

(c) Is it frequently used in the country where the notification is made?
 Yes (.) No (X)

(d) Is it frequently kept in the country where the notification is made?
 Yes (X) No (.)
As a microbiology lab reference.

4. Natural habitat of the organism

(a) If the organism is a microorganism
 water (X)
 soil, free-living (X)
 soil in association with plant-root systems (.)
 in association with plant leaf/stem systems (.)
 other, specify *animals, foodborne pathogen; uncooked meats, uncooked vegetables, pasteurized or unpasteurized milk, foods made from milk, decaying vegetation and processed food*

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

5. (a) Detection techniques

Listeria can be detected by plating on BHI Agar plates. Multilocus sequence typing (MLST) is a reference method for global epidemiology and population biology of bacteria, including L. monocytogenes.

(b) Identification techniques

L. monocytogenes is genetically heterogeneous. To help epidemiological investigation and to define clones (groups of genetically similar isolates descending from a common ancestor), a variety of typing methods have been used, including pulsed-field gel electrophoresis, single nucleotide polymorphism typing, and multiple house keeping and virulence gene sequencing.

The streptomycin-resistant phenotype of CERS 382.20 was demonstrated by growth on selective media. The streptomycin resistant phenotype is used to facilitate identification of the clinical strain and distinguishes it from other Listeria and non Listeria species.

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: *Listeria monocytogenes (Lm)* is categorized into risk group 2

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

8. Information concerning reproduction

- (a) Generation time in natural ecosystems:

Wild-type Lm is a facultative intracellular pathogen that induces its own uptake into non-phagocytic cells and spreads from cell to cell using an actin-based motility process. Intracellular Lm grow with initial doubling times of ~40 min, which approximates the growth rate in rich media. In most cells, intracellular growth continues without causing damage to host cells and mutants that prematurely kill or damage host cells are avirulent; however, whereas cells infected with wild-type bacteria eventually die sometime after 8 h post-infection, bacteria rapidly spread to neighbouring cells and thus propagate the infection.

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

In the setting of the conduct of the proposed clinical trial, wild-type Lm would be expected to behave as described above with a shortest doubling time of ~40 minutes. However, the live, attenuated, double-deleted (LADD) Lm based immunotherapy platform has deletions of 2 genes, actA and inlB. These genes encode the virulence-determinant proteins ActA and Internalin B which facilitate cell-to-cell spread and invasion of nonphagocytic cells, in particular hepatocytes. The deletions limit virulence in the liver, a principal target organ of infection by the wild-type organism.

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

- (d) Factors affecting reproduction:

Wild-type Lm is an intracellular pathogen and virulence is associated with the ability of bacteria to move into host cells by polymerization of host cell actin at one end of the bacterium, which helps them propel through cytoplasm. The lifecycle involves early escape from the phagocytic vacuole, rapid intracytoplasmic multiplication, bacterially induced actin-based motility and direct spread to neighbouring cells, where the cycle is started again. Alteration of any of these processes reduces the ability of the bacteria to reproduce and establish infection. This will include the availability of a permissive host cell, temperature and appropriate physiological milieu.

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 | <i>Gram positive facultative anaerobic, non-spore forming bacterium</i> |

(b) relevant factors affecting survivability:

Wild-type Lm is a Gram positive facultative anaerobic, non-spore forming bacterium that is widely distributed in nature, resistant to adverse environmental conditions and is capable of invading, surviving and growing within host cells in the gastrointestinal tract or within macrophages. Lm grows at temperatures ranging from 0 to 45°C and pH 4.1–9.6 and can survive in or on foods for very long periods of time. This pathogen finds favorable growth conditions on floors, drains and equipment within food industry premises, notably in the cold and wet atmosphere of refrigerated rooms where only non-psychrotrophic bacteria can survive.

10. (a) Ways of dissemination

Vertical transmission from mother to foetus, ingestion of infected foodstuffs. Human travel, animal or food trade, wild animal migration or wind and dust may also contribute.

(b) Factors affecting dissemination

Changes to travel, trade, animal migration and climatic conditions. Different global approaches to food hygiene and medical treatment for bacterial infection and next generation drug therapies.

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)

None

C. **Information relating to the genetic modification –**

1. Type of the genetic modification

- | | | |
|-------|-------------------------------|-----|
| (i) | insertion of genetic material | (X) |
| (ii) | deletion of genetic material | (X) |
| (iii) | base substitution | (.) |
| (iv) | cell fusion | (.) |
| (v) | others, specify | ... |

2. Intended outcome of the genetic modification

1. *Attenuation of virulence by the deletion of the coding sequences of two wild-type virulence determinants (ActA and Internalin B) to render the GMO safe for human clinical use.*

2. *Insertion of an expression cassette to produce a human cancer antigen by stable integration of human mesothelin (hMeso) expression cassette into the tRNA^{Arg} chromosomal position. This enables the intracellular secretion of mesothelin and the subsequent processing for antigen presentation to the patient's immune system.*

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, partially) No (.)

Both modifications were achieved by the use of homologous recombination plasmid vectors. No element of the vector used to attenuate wild-type Lm by deleting the virulence determinants remains in the genome of the GMO. The coding sequence of the gene for human mesothelin is stably established in an expression cassette in the genome of the GMO; no other element of the transfer vector remains in the GMO.

If no, go straight to question 5.

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

- (a) Type of vector

plasmid	(X)
bacteriophage	(.)
virus	(.)
cosmid	(.)
transposable element	(.)
other, specify	...

- (b) Identity of the vector

Two plasmids, pKSV-dlactA and pKSVdlinlB, were used sequentially to create the actA and inlB gene deletions in Lm. The plasmids were constructed similarly, and contained the chloramphenicol gene for antibiotic selection, a temperature sensitive origin of replication and approximately 1 kilobase of Lm sequence from both sides of the gene to be deleted but without the wild type gene (actA or inlB). Each deletion was created by an initial homologous recombination into the host strain genome, followed by a second recombination event during non-selective passage of the cells, which resulted in complete deletion of the vector sequences and the wild type virulence gene. Clones were screened and the final clone was sequenced to confirm deletion of the actA and inlB genes.

The expression cassette was constructed on an integration plasmid, pPL1252, with an origin of replication, a genes for antibiotic selection and loxP sites to facilitate removal of unnecessary plasmid sequences. Using the same methodology for deletion

of act A and inlB, plasmid pPL1252 was inserted at the tRNA^{Arg} locus on the chromosome. After integration the integration plasmid sequences were removed, including antibiotic resistance genes, by transfer of a temperature-sensitive plasmid encoding the Cre recombinase into the strain. Clones were screened and the final clone was sequenced to confirm insertion of the expression cassette at the tRNA^{Arg} locus and deletion of unnecessary plasmid sequences.

- (c) Host range of the vector

Upon integration of plasmid into the genome the plasmid is no longer present (deletion of plasmid sequences), only the expression cassette remains. Therefore no replication of the vector can occur.

- (d) Presence in the vector of sequences giving a selectable or identifiable phenotype
 Yes (.) No (X)

antibiotic resistance (.)
 other, specify ...

Indication of which antibiotic resistance gene is inserted

No modification to the strain was genetically engineered to make the strain strep resistant. However, the strain was selected for strep resistance by growth on selective media.

- (e) Constituent fragments of the vector

Explained in 1b above.

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

- (i) transformation (.)
 (ii) electroporation (X)
 (iii) macroinjection (.)
 (iv) microinjection (.)
 (v) infection (.)
 (vi) other, specify ... *Conjugation for transfer of the expression cassette in pPL1252 from E.coli strain SM10 to the Listeria strain*

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

JNJ-64041757 was engineered by inserting an EGFRvIII human mesothelin expression cassette at the tRNA^{Arg} locus of the final Lm Δ actA/ Δ inlB strain CERS382.20. Although plasmid vectors were used in the construction of the JNJ-

64041757 investigational agent, no residual bacterial plasmid vector sequences (including those for antibiotic resistance) remain in the JNJ-64041757 strain as determined by PCR.

- (b) Source of each constituent part of the insert
Lm actA promoter (a modified amino (N)-terminal domain of the ActA protein that includes the signal sequence (ActAN100)), human EGFR (EGFRvIII), human mesothelin protein (hMeso)*
- (c) Intended function of each constituent part of the insert in the GMO
EGFRvIII was added to the human mesothelin expression cassette to enhance expression (ie, is used as a promotor). Human mesothelin was inserted as a tumor antigen to target immune response against tumors expressing mesothelin.
- (e) Location of the insert in the host organism
- on a free plasmid (.)
 - integrated in the chromosome (X)
 - other, specify ...
- (f) 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 (X)
- insect (.)
- fish (.)
- other animal (.)

(specify phylum, class) ...

other, specify: *human mesothelin protein sequence expressed from a synthetic DNA sequence that was codon-optimized for expression in Lm.*

2. Complete name

- (i) order and/or higher taxon (for animals) Primates
- (ii) family name for plants ...
- (iii) genus *Homo*
- (iv) species *sapiens*
- (v) subspecies ...
- (vi) strain ...

- | | | |
|--------|------------------------|-----|
| (vii) | cultivar/breeding line | ... |
| (viii) | pathovar | ... |
| (ix) | common name | Man |

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

Yes ☐ No ☒ Not known ☐

If yes, specify the following:

(a) to which of the following organisms:

humans	<input type="radio"/>
animals	<input type="radio"/>
plants	<input type="radio"/>
other	<input type="radio"/>

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

Yes ☐ No ☒ 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 ☐ No ☒

If yes, specify

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

Yes ☐ No ☒ Not known ☐

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 ☒ No ☐ Not known ☐

Specify: The deletion of both Lm actA and Internalin B (inlB) genes results in an attenuated strain that is unable to spread effectively from cell to cell. Alteration of normal motility processes reduces the ability of the bacteria to establish infection and/or survive by 1,000- fold in mice.

If JNJ-64041757 were to be accidentally released into the environment, it may persist in the natural habitat of Lm; however, it would remain attenuated and not be a risk to humans or animals exposed to it.

Of note, in the clinic, subjects are safely intravenously infused with 1×10^9 bacteria over one hour, a dose unlikely to be encountered through any environmental exposure.

- (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: *See point I(a), above*

- (c) is the GMO in any way different from the recipient as far as dissemination is concerned?
 Yes (X) No (.) Not known (.)

Specify: *See point I(a), above*

- (d) is the GMO in any way different from the recipient as far as pathogenicity is concerned?
 Yes (X) No (.) Not known (.)

Specify: *The deletion of both *Lm actA* and *inlB* virulence determinants results in a 1000-fold reduction in pathogenicity in mice when compared to wild-type *Lm*. The complete deletion of the coding sequences of these genes prevents reversion to a virulent phenotype and the *tRNA^{Arg}* locus of the *Lm ΔactA/ΔinlB* chromosome preserves the phenotype of the host strain.*

2. Genetic stability of the genetically modified organism

The gene sequence encoding EGFRvIII mesothelin is stably integrated on the bacterial chromosome. Stability testing of the gene deletions and nucleotide changes in the mesothelin expression cassette indicate that both are stable for >270 generations (or doublings) in culture; this is significantly larger than the 17-18 generations (or doublings) required for the manufacture of the GMO for clinical use.

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 (.)
 plants (.)
 other ...

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

*Wild-type *Lm* is a bacterial pathogen. It is a Gram-positive, facultatively anaerobic, non-spore forming rod that is commonly found in moist environments, such as soil,*

decaying vegetation, sewage, human or animal faeces and water. Pathogenic Lm causes listeriosis that affects humans and animals, usually through food-borne transmission. Direct transmission is possible but rare. Listeriosis disproportionately affects pregnant women, new-borns, those with weakened immune systems and older adults, whereas clinically apparent human infection is not commonly reported in immunocompetent, normal individuals, despite its widespread presence in the environment.

The GMO is a LADD Lm-based immunotherapy platform that has deletions of 2 genes, actA and inlB. These genes encode the virulence-determinant proteins ActA and Internalin B, two proteins that facilitate cell-to-cell spread and invasion of non-phagocytic cells, in particular hepatocytes. The deletions limit growth in the liver, a principal target organ of infection by the wild-type organism, by blocking direct hepatocyte infection via the InlB-hepatocyte growth factor receptor interaction and ActA-mediated cell-to-cell spread into hepatocytes from infected liver-resident Kupffer cells. Deletion of actA and inlB in the attenuated Lm retains the immune-stimulatory potency of the wild-type pathogen but with 1,000-fold attenuation of virulence in mice when compared with wild-type Lm. In preclinical toxicology studies in cynomolgus monkeys, liver toxicity was minimal and not dose limiting. No adverse effects are expected for individuals who inadvertently come into contact with it.

4. Description of identification and detection methods

- (a) Techniques used to detect the GMO in the environment
Quantitative culture methods (colony forming units) confirmed by PCR analysis are used to detect JNJ-64041757 in urine, saliva and faecal samples.
- (b) Techniques used to identify the GMO
Testing for streptomycin resistance is used as an identity test to confirm the background strain of the GMO. PCR analysis using primers specific for identification of actA and inlB gene deletions and the presence of the mesothelin expression cassette at the tRNA^{Arg} locus are used to confirm the identity of the GMO.

F. Information relating to the release

1. Purpose of the release (including any significant potential environmental benefits that may be expected)

JNJ-64041757 is an IMP administered to patients with advanced adenocarcinoma of the lung as part of the controlled clinical study (64041757LUC2002). In this study one dose of JNJ-64041757 contains 1×10^9 colony forming units (CFU) diluted in saline solution, to be administered intravenously (IV). The administration will take place in a hospital setting by direct administration to the patient by a Health Care Professional. There will be controlled destruction of any remaining IMP. Given the contained manner in which this IMP will be administered we anticipate significant potential environmental benefit compared to the deliberate release of an IMP

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 sites of potential release in Spain include the clinical trial sites where up to 10 patients are anticipated to participate in the Ph1b part of clinical study 64041757LUC2002. As an added precaution, a 7-day course of antibiotics is administered after the last GMO infusion. Per protocol assessments of saliva, stool, and urine of subjects, in clinical studies with any LADD strain (JNJ-64041757, JNJ-64041809 and CRS-207) failed to document any evidence of shedding. In addition, monitoring of blood cultures suggest quick clearance of JNJ-757 from the bloodstream, with the majority of subjects having negative blood cultures 2 hours after infusion. As summarized in Section J 4, there is a potential risk of persistence of the bacteria. To reduce this risk blood cultures are monitored both during and after treatment, prophylactic antibiotics are administered and immunosuppressive therapies are prohibited.

3. Information concerning the release and the surrounding area

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

The clinical trial will be conducted at the following sites:

- Hospital Universitari Quirón Dexeus de Barcelona*
- Hospital Son Llatzer de Palma de Mallorca*
- Complejo Hospitalario Regional de Málaga*
- Complejo Hospitalario de Jaén*
- Hospital General Universitario de Elche*

The GMO will be administered under controlled conditions at the clinical sites; however, as this is an outpatient study, recipients will return to their homes.

(b) Size of the site (m²): *Not applicable. The drug product is given to a patient via intravenous infusion in a hospital clinical environment. It is not anticipated that the GMO will be released into the environment.*

- (i) actual release site (m²): ... m²
- (ii) wider release site (m²): ... m²

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

No environmental sites outside the hospital room are anticipated to be affected. Containment measures during the administration of JNJ-64041757 to patients will exclude the release of the GMO into the environment. Personal protective equipment will be used to avoid exposure to the GMO to the medical personnel involved in the administration of the product.

The proximity of significant biotopes, protected areas or drinking water supplies cannot be excluded as possible potential sites of release, however, the most likely theoretical route for exposure would be via the disposal of patient urine or faeces.

As summarized in Section F2, a 7-day course of prophylactic antibiotics is administered after the last GMO infusion to eliminate residual JNJ-64041757 in

patients. No bacterial shedding has been observed in clinical studies with any LADD strain including JNJ-64041757. As summarized in Section J 4, there is a potential risk of persistence of the bacteria for which measures have been implemented to mitigate this risk.

- (d) Flora and fauna including crops, livestock and migratory species which may potentially interact with the GMO

There is a negligible likelihood of such exposure. It is not expected that subjects who receive the GMO will spread infection to others; however, subjects receiving treatment will be counselled to avoid the potential risk by practicing good health hygiene (e.g. hand-washing) as with any potential infectious agent and adhering to protocol requirements for barrier method of contraception during sexual intercourse and using caution around immunocompromised individuals or neonates to minimise the potential for direct fluid exchange in the 7-day period after administration.

4. Method and amount of release

- (a) Quantities of GMOs to be released:

Each vial contains 1×10^9 CFU/mL. For the Phase 1B at least 6 subjects will be administered JNJ-64041757 at a dose of 1×10^9 CFU IV. For Phase 2 a maximum of 140 subjects will be stratified in subgroups according to PD-L1 level and then be assigned randomly (1:1) to receive JNJ-64041757 plus nivolumab (Group A) or nivolumab monotherapy (Group B). will be administered JNJ-64041757 at a dose of 1×10^9 CFU IV. In Spain we anticipate upto 10 subjects to be treated with JNJ-64041757 during Ph1b of this study.

- (b) Duration of the operation:

Each administration of JNJ-64041757 is anticipated to take up to 60 minutes. We anticipate subjects to be treated with JNJ-64041757 for a maximum of 2 years.

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

Janssen is providing a Safety Data Sheet (SDS) on the safe handling directions for JNJ-64041757, measures in case of accidental spills, personal protective equipment, first aid, decontamination and disposal. These measures are in place in order to avoid any release of JNJ-64041757 into the environment.

JNJ-64041757 is transported to the receiving site in sealed cryogenic vials. Each vial is individually labelled identifying the product information (contents and quantity of material) storage conditions and manufacturers information. In addition, the label includes the following text: 'This product contains genetically modified organisms' for sites outside of the US. The vials stored on dry ice or equivalent shipping conditions inside an insulated shipping container. The shipping containers are insulated to prevent vial breakage and vial thaw.

The main hazard for the shipment of these vials relates to vial breakage. If a vial were to be received cracked it is possible for the product to leak out from the vial and contaminate the shipping components. JNJ-64041757 is an attenuated L.

monocytogenes commonly found as a soil bacterium. The product is easily removed from surfaces using standard antimicrobial cleaning agents. The detectability of the vial breakage is high, because vial cracks would be visible. The environmental risk caused by vial breakage is low.

Summary of JNJ-64041757 handling procedures are:

- No primary barriers required
- Standard Precautions are to be observed (e.g., laboratory coats and gloves; eye, face protection, as needed)
- Laboratory bench and sink required

The GMO is administered through an IV infusion. Investigational sites will disinfect equipment and surfaces in accordance with standard medical practices. The single-use vial and syringe used to draw up and add the GMO to the saline bag will be disposed of in a Sharps container. The saline bag is disposed of in accordance with the institutions' biosafety policies.

5. Short description of average environmental conditions (weather, temperature, etc.)
Given that the GMO is prepared for administration and given to patients in hospital clinical environment and that hospital rooms have to fulfill hygiene conditions required for the treatment of patients, it is not anticipated that the GMO will be released into the environment, however, should release occur the environmental conditions will be those of the sewage system.
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.
As described in Section A 3(b), JNJ -64041757 is derived from the platform LADD Lm strain ANZ-100. These LADD Lm strains have been administered to more than 485 human subjects with advanced cancers (including 9 subjects treated with JNJ-64041757). As summarized in A7, to date, no shedding has been observed in clinical studies for all the LADD Lm strains tested including JNJ-64041757, JNJ-64041809 and CRS-207. As summarized in Section J 4, there is a potential risk of persistence of the bacteria for which measures have been implemented to mitigate this risk. There have been no environmental or human health impacts from the release of LADD-based immunotherapies.

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	...
(iii) genus	<i>Homo</i>
(iv) species	<i>Homo sapiens</i>
(v) subspecies	...
(vi) strain	...
(vii) cultivar/breeding line	...
(viii) pathovar	...
(ix) common name	man

2. Anticipated mechanism and result of interaction between the released GMOs and the target organism (if applicable)
Human patients will be administered an IV infusion of the GMO. Once in the blood stream, Lm induces a cellular immune response comprised mainly of cytotoxic T lymphocytes (CTL). The GMO is engineered to express mesothelin antigens that are present in high numbers on the surface of tumour cells, so the CTLs induced by administering the GMO are specifically designed to recognise and selectively destroy the tumour cells that over-express the mesothelin antigen and, as such, alleviate the disease.

3. Any other potentially significant interactions with other organisms in the environment
The GMO-induced CTLs are stimulated by Lm to recognise Lm-infected cells in the infected host as foreign and destroy them, thus clearing the bacteria from the body.

4. Is post-release selection such as increased competitiveness, increased invasiveness for the GMO likely to occur?
 Yes (.) No (X) Not known (.)

Give details:

Compared with the wild-type Lm, the GMO is reduced in its pathogenic capacities. The deletion of the actA and inlB genes that facilitate cell-to-cell spread attenuate the pathogenicity of LADD by 1000-fold in mice, without diminishing immunogenicity. As the GMO is less virulent than wild type Lm it is expected that the LADD strain would be out-competed by wild type Lm in its natural milieu.

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

Though the GMO is administered intravenously via a controlled clinical trial study, there is a theoretical possibility that the GMO could be shed from patients in body fluids and excreta into the surface water ecosystem. However, following IV administration, the GMO is rapidly cleared from the blood. A 7-day course of antibiotics is administered after the last GMO infusion to ensure total clearance. As summarised in Section J 4, there is a potential risk of persistence of the bacteria. To reduce this risk blood cultures are monitored both during and after treatment, prophylactic antibiotics are administered and immunosuppressive therapies are prohibited.

In addition, as summarized in Section H 6 surveillance blood cultures will be collected during the Treatment Phase, at the End of Treatment visit and for up to 12 months after the last dose of JNJ-64041757. As summarized in Section A7, there have been no reports of shedding of the bacteria to the environment from LADD-based therapy in clinical studies. As per Section E1(a), if JNJ-757 were to be accidentally released into the environment, it may persist in the natural habitat of Lm. however, it would remain attenuated and not be a risk to humans or animals exposed to it. Finally, as per Section G 4, the GMO is less “fit” than the wild-type host.

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
Not relevant

- | | | |
|--------|-----------------------------------------|-----|
| (i) | order and/or higher taxon (for animals) | ... |
| (ii) | family name for plants | ... |
| (iii) | genus | ... |
| (iv) | species | ... |
| (v) | subspecies | ... |
| (vi) | strain | ... |
| (vii) | cultivar/breeding line | ... |
| (viii) | pathovar | ... |
| (ix) | common name | ... |

7. Likelihood of genetic exchange in vivo

- (a) from the GMO to other organisms in the release ecosystem:
The GMO will be administered to subjects in a clinical environment. The GMO does not contain plasmids or transposons which could enable transfer of genetic material to other listeria spp. or other permissive bacterial spp. harboured by clinical trial subjects. Spontaneous transfer to or recombination with the genomes of other organisms is extremely unlikely in the setting of the proposed release.
- (b) from other organisms to the GMO:
JNJ-64041757 is administered intravenously to patients in a clinic environment. Spontaneous transfer from or recombination with the genomes of other organisms is extremely unlikely in the setting of the proposed release.
- (c) likely consequences of gene transfer:
The deletion of both the actA and inlB genes results in an attenuated strain that is unable to spread from cell to cell and has a reduced capacity to infect hepatocytes and other non-phagocytic somatic cells directly or indirectly. The expression cassette utilises prokaryotic transcriptional and translational elements for expression of mesothelin and, therefore, no recombinant DNA that is functional in a eukaryotic host cell will be directly transferred in vivo.

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

Not applicable

9. Possible environmentally significant interactions with biogeochemical processes (if different from the recipient or parental organism)
Lm is not known to contribute to any major biochemical cycle (carbon, sulphur, nitrogen). The traits introduced into the organism are not expected to affect this aspect. No interaction with biogeochemical processes is expected.

H. Information relating to monitoring

1. Methods for monitoring the GMOs
EGFRvIII human mesothelin expression cassette (hMeso38) can be identified in the GMO using PCR.

2. Methods for monitoring ecosystem effects
No ecosystem effects are expected.
3. Methods for detecting transfer of the donated genetic material from the GMO to other organisms
Transfer of donated EGFRvIII human mesothelin expression cassette (hMeso38) can be identified using PCR
4. Size of the monitoring area (m²)
N/A
5. Duration of the monitoring
3 years
6. Frequency of the monitoring
Monitoring is planned at baseline , 18-24 hours (Day 1), Day 4, Day 7, and 28 days after last GMO infusion. Among subjects who receive JNJ-64041757, surveillance blood cultures will be collected as follows: at the first 4 visits of the Treatment Phase; on Day 1 of any subsequent cycle with a disease assessment, or as clinically indicated; at the End of Treatment visit (before prophylactic antibiotic therapy); and 3, 6, 9, and 12 months after the last dose of JNJ-64041757.

I. Information on post-release and waste treatment

1. Post-release treatment of the site
The GMO is administered through an IV infusion. Investigational sites will disinfect equipment and surfaces in accordance with standard medical practices, thoroughly with ethanol or water with detergent. The single-use vial and syringe used to draw up and add the GMO to the saline bag will be disposed of in a Sharps container. The saline bag is disposed of in a receptacle for biohazardous waste. Additional instructions relating to the handling of the IMP including the decontamination of surfaces is described in the SDS. The SDS is provided to each clinical site and provides information on how to handle spills or accidental exposure to JNJ-64041757.
2. Post-release treatment of the GMOs
After administration, the GMO is rapidly cleared from the blood and there has been no evidence of shedding into the environment following LADD administration to >400 patients.
3. (a) Type and amount of waste generated
Biohazard, one product vial/ IV infusion set per patient per dose. Disposable materials that have been exposed to bacterial material (e.g. empty containers, disposable gloves, wipes, etc.)

 (b) Treatment of waste
Waste should be disposed in a receptacle for biohazardous waste. Waste will be decontaminated before disposal (steam sterilization, chemical disinfection, and/or incineration). Urine, faeces and faecal traces, hygienic wipes, disposed of in the sewage system. The biological containment does not require additional treatment. Furthermore, the sewage treatment system is designed to eliminate bacteria.

J. Information on emergency response plans

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

The GMO is an IMP intended for use in a controlled clinical trial to be conducted at qualified medical facilities and under controlled conditions and handling procedures. JNJ-64041757 is transported to the receiving site in sealed cryogenic vials. Each vial is individually labelled identifying the contents and quantity of material. The vials stored on dry ice or equivalent shipping conditions inside an insulated shipping container. The shipping containers are insulated to prevent vial breakage and vial thaw. The main hazard for the shipment of these vials relates to vial breakage. If a vial were to be received cracked it is possible for the product to leak out from the vial and contaminate the shipping components. The product is easily removed from surfaces using standard antimicrobial cleaning agents.

The probability for vial breakage is low for several reasons. The product is shipped within insulated shipping containers, designed to prevent vial breakage. The detectability of the vial breakage is high, because vial cracks would be visible. The environmental risk caused by vial breakage is low. Handling of such incidents is covered by the SDS which will be provided with the IMP.

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

For crushed or broken vials, site personnel are instructed not to raise dust, mist or aerosols and to surround the spill with absorbents to minimize entry into the air, capturing all remaining liquid into spill absorbent materials and place them into a leak-proof container suitable for disposal in accordance with applicable waste disposal regulations. Decontamination procedures are provided in the SDS

If inhaled:

If breathed in, move person into fresh air. Consult a physician.

In case of skin contact:

Take off contaminated clothing and shoes immediately. Wash off immediately with plenty of water. Consult a physician. Wash contaminated clothing before re-use.

In case of eye contact:

Rinse immediately with plenty of water, also under the eyelids, for at least 15 minutes.

Remove contact lenses.

Consult a physician.

If swallowed:

If swallowed, rinse mouth with water (only if the person is conscious).

Call a physician immediately.

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

Not applicable

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

Direct human to human spread of wild-type Lm is limited; it almost exclusively transmitted by ingestion of contaminated foodstuffs. The GMO (JNJ-64041757) has been modified in the laboratory to be less virulent than wild-type Lm so no adverse effects would be expected for individuals who inadvertently come into contact with it.

Procedures for handling JNJ-64041757, include thorough hand washing after handling and the use of protective gloves, eye protection, and face protection. Individuals who prepare JNJ-64041757 for injection must take appropriate precautions (e.g., gloves, laboratory coat, face protection, needle stick or sharps precautions) to avoid contamination or direct contact with the agent. After it is prepared for injection, the chance for direct exposure to JNJ-64041757 by study personnel is greatly diminished. After each infusion is completed, injection site must be handled according to applicable institutional disinfection method such as wiping with rubbing alcohol and closed dressing materials on injection site.

To date, these LADD Lm strains have been administered to more than 485 human subjects with advanced cancers with no signs of chronic infection reported. In the event of an undesirable effect, standard isolation precautions are recommended and JNJ-64041757 is susceptible to most antibiotics commonly used to treat listeria infections. In addition, avoiding direct contact with individuals who are risk of listeriosis is recommended to subjects participating in the study and receiving JNJ-64041757.

Patients who receive the treatment with the product will be monitored for the occurrence of adverse events and serious adverse events (SAE) according to the clinical protocol. Each SAE will be recorded and assessed by the hospital staff and the study sponsor, and Health Authorities will be notified when applicable. After the last JNJ-64041757 infusion, mandatory prophylactic antibiotic therapy must be administered for 7 days with amoxicillin 500 mg orally 3 times per day (or trimethoprim/sulfamethoxazole 160 mg/800 mg orally 2 times per day for subjects with penicillin allergy) as a precaution. The antibiotics should be initiated on the day of the End-of-Treatment visit, after collection of End-of-Treatment blood cultures (through peripheral vein and indwelling venous access device, if applicable) have been collected. Subjects who have a venous access device should have their first dose of antibiotic administered as an intravenous dose through the venous access device (2g ampicillin or 5 to 10mg/kg trimethoprim/sulfamethoxazole [based on trimethoprim component] for subjects with penicillin allergy), followed by 6 days of oral antibiotic prophylaxis.

To date, there have been no cases of listeriosis, Listeria (LADD) bacteremia, or Listeria (wild-type) bacteremia reported in subjects completing therapy with either JNJ-64041757 or JNJ-64041809. Of more than 485 subjects treated within the broader category of LADD Lm-based therapies (including 9 subjects treated with JNJ-757), three cases (< 0.1%) of persistence of CRS-207 beyond the 7-day prophylactic oral antibiotic course have been reported. An indwelling port is suspected to be the source of persistence that led to these positive cultures. In all instances, subjects recovered after being successfully treated with antibiotics and follow-up cultures were all negative for Lm. However, as a result of these cases, to reduce the risk of persistence, Janssen has instituted changes to the conduct of studies investigating JNJ-64041757 relating to monitoring of blood cultures both during and after treatment, administration of prophylactic antibiotics, and prohibition of immunosuppressive therapies. Any finding of positive Listeria during the study is treated as an adverse event of special interest and reported in an expedited manner to Janssen.

References

1. Brockstedt, D.G., et al., *Listeria-based cancer vaccines that segregate immunogenicity from toxicity*. Proc Natl Acad Sci U S A, 2004. **101**(38):13832-13837.
2. Le, D.T., et al., *A Live-attenuated Listeria Vaccine (ANZ-100) and a Live-attenuated Listeria Vaccine Expressing Mesothelin (CRS-207) for Advanced Cancers: Phase I Studies of Safety and Immune Induction*. Clinical cancer research, 2012. **18**(3):858-868.