**Application form**

**Assessment of clinical research involving gene therapeutics in the Netherlands**

**-**

***Bacteria***

**November 2018**

 **Application form**

**Assessment of clinical study involving genetically modified bacteria**

Part A: Bio-safety aspects

 A1. General information

 A2. Construction and composition

 A3. Production

 A4. Research description

 A5. Environmental risk assessment

 A6. Conclusions about possible environmental effects

Part B: Patient-related aspects (*not included in this form*)

If you have any questions, please get in touch with the Gene Therapy Office

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November 2018

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# Part A. Bio-safety aspects

This part of the application form provides the information needed for the Ministry of Infrastructure and Water Management (IenW) to grant the necessary licenses.

All information provided in this form and the accompanying documentation constitutes part of the decision to be made and for this reason is in principle open to inspection by the public; the information will also be available for such public inspection during the procedure.

The applicant may ask for parts of the information provided to be kept confidential. In that case, the applicant must give reasons why the information is of a confidential nature as well as a convincing explanation that the lifting of confidentiality will adversely affect the applicant’s competitive position. A publicly available summary of confidential information must be given, containing enough information for a clear general understanding of the application and in order to assess the risk analysis as described in the application and the decision. Confidential information must be included in a separate attachment marked ‘confidential’.

An application does not need to be limited to the specific clinical protocol that the applicant wishes to perform. If there are no consequences for the risk analysis, the application can be drawn up with a wider scope, such as for a larger number of patients.

The aim is to draw up the final decision in such a way that several clinical protocols can be performed under it, using the information described in this application. Naturally these activities must be covered by the description of the experiment and the risk analysis provided. Before submitting such a broader application, you are advised to contact the GMO office for an informal discussion of the options.

The term ‘test subjects’ as used in this form means patients or volunteers taking part in the study.

This Notification Form may contain some questions that are not relevant to your case. We would ask you NOT to answer in your notification any questions that are irrelevant to the activities for which you are applying.

Specific issues:

* Literature that is referred to has to be sent in together with the application form.
* Confidential information has to be marked as such and has to be sent in separately.
* A SNIF B (other GMO) form has to be completed and to be sent in as an electronic file.

## A1. General application details

### General information

**A1.1. Application title:**
*Please give a descriptive title that provides sufficient information on the objectives and aspects relating to the genetic modification. The title should state the type of GMO(s) and insertion(s) used and the nature of the application(s).*

**A1.2. The objective of the study for which the application is submitted:**

 *Provide a short description of the study’s objective. For example: ‘The objective of this Phase I clinical trial is to study the safety of* Clostridium tetani *for the purpose of developing a new therapy to treat skin cancer.’*

**A1.3. Describe briefly the contents of the application, the objective of the study being aimed for and the intended application of the results** *Provide a short description – of no more than half a page – of the genetically modified organisms (GMOs) applied, or of the GMOs that may thus be created, such as via recombination of genetic information between organisms or by integration of genetic material into a genome. Describe the expected action of the gene products of the transgenes and give an explanation of the biological mechanism. Also describe the scientific and public importance of the study, and state how the results of the study may be used in this context.*

*The information provided will be used as the basis for a brief description of the study in the decision.*

**A1.4. Describe briefly the intended work.***State in chronological order which types of procedures will be carried out, and for which a license is being applied for (e.g. production, transport, storage and administration of the vector, observation of patients, sampling, transport, storage and processing of samples, waste treatment). Where applicable, also indicate for which part of the study a Contained Use (IG) permit applies, and provide the number of the GMO permit concerned.*

**A1.5. Intended start and end date:**

*The decision must state a time period within which the procedures will be carried out, and so an end date must be given. The chosen end date will be included in the decision. It is possible to obtain an extension to the decision; please note, however, that any extension procedure must be completed before the decision end date has passed.*

**A.1.6. Do you want to keep other information confidential? If so, please specify in concrete terms how the release of the information would harm your competitive position.**

 *Unless marked "Confidential", all the information contained in the notification and its appendices may enter the public domain when the notification is publicly processed and the decision is published.*

 *For the sections marked "Confidential", you are requested to give a publishable summary that contains enough information to ensure a good general understanding of the notification. Furthermore, give a reason why certain information is marked "Confidential".*

### Details of applicant

*Only the legal entity that has final responsibility for the work to be carried out may act as the applicant. This means that the applicant will normally be the Board (management) of the hospital (institution) where the treatment will be given. The license holder must be able to enforce compliance with the license regulations when carrying out the work. In order to do so, it is necessary for the employees involved in the clinical procedures to come under the authority of the license holder. For this reason, employees must be directly employed by the license holder. In those cases where an employee does not come under the authority of the license holder, such as where a treating doctor is part of a partnership that is independent of the license holder, an employment contract must be arranged for carrying out work under the license, such as through a zero-hours contract with the license holder. A contract must be concluded with the party or parties carrying out this work for non-clinical procedures that are not carried out in the institution in question, in such a way that final responsibility continues to rest with the license holder.*

**A1.7. Name of legal entity:**

**A1.8. Chamber of Commerce (KvK) number:**

**A1.9. Visiting address of legal entity:**

**A1.10. Postal code and town/city of location of legal entity:**

**A1.11. At which locations will the intended work take place?**

*Since the work applied for may only be carried out under the direct control of the license holder, it is only possible to carry out work at several locations if the license holder has full control of the way in which the work being applied for is carried out at all locations, in such a way that the license conditions are complied with. In that case you must state for each location what work will be carried out at which address and in which building. To clarify: you must state for all activities at which location they will be carried out. Apart from the location for the clinical activities with the GMO, you must also state the location or locations of laboratories in which activities with the GMO are carried out under the terms of this license application, such as procedures with patient samples.
In cases where central control is not possible, such as with a multi-centre study, a separate application must be submitted for each location.*

## A2. Construction and composition of the GMO

### Bacterial strain from which the genetically modified organism was derived

**A2.1. To which bacterial species does the strain belong that was used as the original strain in the construction of the GMO?**

 *Provide, where applicable, the full scientific name, a trivial name (e.g. the commercial name), the sub-species and collection numbers.*

**A2.2. Is the original strain a GMO?**

  *If so, provide a detailed description of the genetic modification. If the strain was developed or used in the Netherlands, then provide the number or numbers of the permits under which the work was carried out.*

**A2.3. Describe the method of identification of the original strain.**

 *The properties of the original organism form the basis for the environmental risk assessment. Confirmation of the identity is therefore important. Describe the characteristics that determine the identity of the original strain. This may be based on bio-information analyses, such as sequence analysis of a characteristic part of the genome, alignments or phylogenetic analysis. Information on the origins of the original strain, such as a micro-organism originating from the American Type Culture Collection (ATCC), may also be submitted.*

**A2.4. What is the natural niche of the bacterial strain?**

  *Describe the niche in which the original strain naturally occurs. For pathogenic or commensal bacteria, indicate the hosts in which they occur, and, for pathogens, the hosts that may serve as carriers.*

**A2.5. Provide relevant data on pathogenicity and possible attenuation and biological restrictions of the original strain.**

 *If this concerns a pathogenic strain, what is its class of pathogenicity? Also describe the possible route or routes of infection.*

 *If this concerns an opportunistic pathogen, state the grounds on which the bacterium is classified as an opportunistic pathogen.*

 *If this concerns a pathogen-derived attenuated strain, describe the grounds on which the strain has been given a lower pathogenicity classification.*

 *If the organism is biologically restricted in another way, the grounds for the biological restriction must be substantiated.*

**A2.6. Provide information about reproduction and survival of the bacterial strain in natural hosts.**

*Provide information about generation time under natural circumstances in natural hosts, and about possible survival or transmission structures formed by the original strain.*

**A2.7. What are the possibilities of survival, reproduction and transmission under environmental circumstances other than in natural hosts?**

 *Provide all the observed and assumed transmission routes of the original strain, and indicate the effectiveness of the transmission and the role of the aforementioned transmission or survival structures.*

**A2.8. Is the strain able to exchange material with other organisms?**

 *Provide information about the strain’s self-transmissible elements, mobilizing plasmids, transposons or other sequences involved in the transmission of DNA. Provide information about the incompatibility class and the host range of these elements.*

### The genetically modified bacterium

**A2.9. Has a vector been used in the genetic modification?**

 *Is the vector fully or partly present in the GMO? Is the vector self-transmissible or could it become mobilized, or are these sequences involved in the transmission of DNA?*

**A2.10. Provide a description of the method of production of the genetically modified bacterium from the original strain and the original vectors.**

*Answer this question preferably using a diagram that describes the various steps of the production process.*

**A2.11. Provide a full description of the genetic material inserted into the bacterium.**

*The description should contain an elaboration of the following aspects, in particular:*

 *- regulatory sequences, such as promoter, terminator and enhancer sequences;*

 *- in case of insertion of a transgene: the function of the coded protein in the donor organism (the donor organism is the organism from which the gene was originally isolated, or in which it occurs naturally), the expected function in the genetically modified bacterium, and, if applicable, the expected function in the test subject;*

 *- in case of deletion: the function of the deleted genetic material in the original organism;*

 *- in case of point mutation(s): the effect of the point mutation or mutations on the function of the modified genetic material in the original organism;*

 *- whether the inserted DNA contains elements of which the origin or function is unknown.*

**A2.12. Provide a molecular characterization of the genetically modified bacterium.**

 *A sequence analysis must be supplied for all inserted or deleted sequences, so that the precise location of the modification in the GMO and, in case of insertion, the number of copies present and the orientation of the insertion cassette can be determined. The sequence to be submitted must contain the region of the intended modification as well as the recombination sequences used, also including the flanking sequences of the genome of the original organism. In the case of biological unrestricted GMOs, newly created fusion ORFs must be described and subjected to a bio-information analysis.*

 *The complete genome of the genetically modified bacterium must be characterized on a molecular level. This may be done according to a sequence analysis or a Southern blot analysis. Observed anomalies in the genome compared with the expected sequences, such as unexpected deletions, mutations or recombinations, must be described and interpreted. Phenotypic analysis or bio-information data may be used to support the data on the molecular characterization. If the GMO is to be based on a non-pathogenic bacterium or on a pathogenic bacterium from which the virulence factors have been removed, an elaborate analysis of the genome regarding deletions and recombinations is not necessary.*

 *The sequence of the complete genome does not need to be submitted. A bio-information analysis must be carried out for the sequences, and the annotated results must be presented. For more information, please refer to the COGEM advisory report CGM/130227-05.*

**A2.13. Summarize the data under A2.10 to A2.12 in a diagram (‘map’) of the genetically modified organism.**

 *Present clear maps of the genetically modified organism, such as plasmid maps, on which all the constituent parts of the vector are shown. In this schematic depiction, the construction of the GMO must be clearly indicated.*

**A2.14. What physiological (including pathogenic) effects may be caused by the genetically modified bacterium; and what are the available treatments?**

 *Indicate what physiological processes may occur following the application of the GMO in the host, and to which phenotypes this could lead. In addition, describe the degree to which the genetic modification will affect the pathogenic properties of the GMO. A comparison must be made between the possible pathogenic properties of the GMO and those of the original strain. In particular, the pathogenic properties that may be created specifically by the genetic modification must be considered.*

**A2.15. Indicate the degree to which the virulence of the genetically modified bacterium is or may be altered, compared with that of the original strain.**

 *When answering this question, provide an argument that elaborates on the virulence of the genetically modified bacterium, compared with that of the original strain. Also consider any modifications that were made in order to achieve the original strain.*

**A2.16. Indicate the possible transmission routes of the genetically modified bacterium.**

*Make a comparison with the original strain. Argue whether or not and how the genetic modification may affect the aforementioned aspects regarding host range and transmission routes.*

## A3. Production of the GMO

**A3.1. State under whose responsibility the production of the GMO is carried out.**

**Answer:**

O Production will be under the responsibility of the applicant and forms part of this license application.

O Production will be by and under the responsibility of the applicant but does not form part of this license application:

O A separate application for production will be submitted for contained use

O Reference is made for the production to an existing license for contained use:
(State here the number of the relevant GMO license)

O Production will be under the responsibility of third parties. If production is in the Netherlands, please state the number of the relevant GMO license. Please state if production is outside the Netherlands.

**A3.2. During which steps of the production process does quality control take place, which test methods are used and how are the tests carried out?**

*Give an overview of the production process of the GMO and describe the points in the production process at which quality control takes place. State which controls are carried out, the sensitivity of the tests and which methods are used for the controls.*

**A3.3. Which criteria are imposed on a batch of the GMO before it is released for the application in question?**

*State which criteria are used to reject a batch.*

## A4. Description of the research

### Administration

**A4.1. How many test subjects will take part in the study?**

 *Here, the maximum number of test subjects to be treated should be indicated. This number can be higher than the intended number of test subjects. Please be aware of the fact that the number you enter limits the permit. For example, if you indicate that there will be 50 test subjects, then no more than 50 can be included in the study.*

**A4.2. Describe how the batch containing the GMO will be transported and prepared for administering to the test subjects.**

 *Describe how, after delivery, the GMO preparation will be transported to the hospital pharmacy for example. Also describe the way in which the GMO preparation will be handled, as well as the conditions under which they will be handled, in order to get the preparation ready to be administered. If this handling will be carried out under an existing permit for Contained Use (IG), the number of the GMO permit concerned must be stated. In addition, describe the way in which the GMO preparation will be transported to the test subject.*

**A4.3. How will the GMO preparation be administered to the test subject?**

 *State via which route and how the GMO is administered. Also state what aspects may affect the safety of human beings and the environment.*

**A4.4. Which doses will be administered and at what time points during the study will they be administered?**

 *Indicate which doses of the GMO will be administered. Also provide an administration diagram, showing at what times they will be administered.*

**A4.5. What other medication will be administered to the test subject that may possibly affect the GMO to be administered?**

 *Any medication that would affect the GMO and possibly also the environmental risk assessment should be named here. If, for example, a vaccination study is conducted, this may also include challenge with the wild-type bacterium.*

### Sampling

**A4.6. Describe which of the samples taken from the test subject may contain GMOs.**

  *Provide an overview of the samples and indicate whether GMO material is expected to be present in them.*

**A4.7. Describe the method of sampling and how the samples will be subsequently processed.**

 *In answering this question, also indicate how transmission of the GMO during sampling and testing will be prevented. For the subsequent processing, indicate what physical restrictions apply. In case the work concerned will be carried out in the Netherlands but is not part of the current permit application, then refer to the permit for work under Contained Use and provide the number of the GMO permit this concerns.*

**A4.8. How is the GMO preparation detected after being administered?**

 *State, if applicable, when GMO components are detected during or after administration and why detection at that particular moment of the test is regarded as important. Describe the nature of the samples that are tested, the method used and the detection limit that can be achieved.*

### Waste management

**A4.9. Give an overview of the nature and quantity of the waste produced and describe how the waste will be disposed of.**

  *State which waste flows can be distinguished. State which waste flows may potentially contain the GMO and how the GMO is prevented from being released into the environment via the waste flows.*

## A5. Environmental risk analysis

### Environment-related information originating from earlier experiments

**A5.1. Describe the results originating from earlier (pre-) clinical studies with the GMO and which are important for the environmental risk assessment.**

 *In answering this question, you should elaborate on results that were achieved using an identical or similar GMO, if these are relevant for the environmental risk assessment of the present application. Important data include data on shedding, duration of latent presence of the vector/GMO, transmission of the vector/GMO, and possible interaction with other micro-organisms (including viruses). For each of these results, describe the trial set up (e.g. doses used, method of administration, detection test and detection limit) and the relevance to the work in the present permit application.*

### Risk analysis

**This is the most important aspect of the whole application!**

*Give a detailed assessment of the expected effects of the GMO on human health and the environment on the basis of the answers to the above questions and in accordance with Appendix II of EU Directive No. 2001/18/EC and the corresponding guidance notes of the European Commission (2002/623/EC). Please take into account any direct, indirect, immediate and delayed effects of the GMO on human health and the environment.*

*A risk analysis should be carried out for each GMO included in this notification, as well as for combinations of the GMOs, if any. The risk analysis must cover the effects of the GMOs that are due to interactions between the GMOs and the environment(s) where they are introduced or where they may end up under the present activities. The effects in question are those which are relevant to safety to human health and the environment. Section A6 of this form describes those aspects which must at least be taken into consideration.*

*The risk analysis should include at least the aspects mentioned in Annex 1 of this form. The risk analysis includes the following sections, which should be given in the same order as shown below (see questions A5.2 – A5.5):*

1. *List of the likely adverse effects;*
2. *Estimate of the likelihood of these effects actually taking place;*
3. *Evaluation of the risks and an estimate of the severity of the effects, based on Items 1 and 2 above. The severity can be estimated by comparing it with the severity assigned to similar risks, such as for example the effects that occur with non-GMOs in similar situations ('baseline principle');*
4. *If you have concluded in Point 3 that the risk is high, you are requested to examine what measures can be used to mitigate the risk;*
5. *Final conclusion of the risk analysis, stating the risk management measures that will be employed, and a conclusion as to the acceptability of the risks when these measures are put into operation.*

**A5.2. State which potentially harmful effects may be linked to exposure of human beings or the environment to the GMO.**

 *Describe here the effects on human beings and the environment that could occur as a consequence of the use of the characteristics of the GMO described in earlier sections of this application. The aim here is identifying the hazards; the following questions deal with the chance that these hazards will actually occur.*

**A5.3. State according to which scenario the GMO can disperse from the test subject into the environment.**

  *State in your answer the scenarios in which the GMO may disperse in the environment. Explain the level of risk that spread will actually occur. Also describe in your answer whether the number of test subjects and/or the dosage to be administered affects the risks to be identified.*

**A5.4. Give an estimate of the chance that the adverse effects described in A5.2 could actually occur.**

 *Give a reasoned estimate of the chance (likelihood) of the aspects described in A5.2 and A5.3, also taking account of the number of test subjects and the dosage.*

**A5.5. Describe the risks that could occur as a consequence of the application of the GMO, taking into account the impact of any risk management measures taken.**

 *Describe the risks in such a way that makes clear how the risks can be reduced through risk management. If risk management measures are necessary in order to limit the risks, these should be specified further in the questions below.*

### Risk management

**A5.6. Which inclusion and exclusion criteria are adopted in the selection of test subjects and what is the effect of these criteria on environmental safety?**

 *Give an overview of the inclusion and exclusion criteria that are only necessary for the protection of the environment or which criteria might possibly have an effect on safety for human beings and the environment.*

**A5.7. Describe which measures are provided for in respect of the hospitalization of the test subject.**

*When answering this question, please emphasize those aspects that are important in preventing spread in the environment of the test subject. Also indicate if, apart from medical reasons, hospitalization is prescribed as a way of protecting against possible effects for humans and the environment.*

**A5.8. If the test subject is to be hospitalized, what are the criteria for his or her release from hospital?**

  *Describe the criteria on the basis of which the test subject will be released from hospital.*

**A5.9. Describe which measures will be taken to prevent the spread of the GMO to third parties (including medical personnel).**

 *For example, give an overview of relevant (hospital hygiene) measures that will be taken. In case existing guidelines will be used, please indicate what they are (such guidelines must be attached to this application). Additional or deviating measures also must be described.*

### Procedure in case of unexpected situations and serious incidences

**A5.10. Describe the procedures to be followed if changes in the risk management are required for medical reasons.**

 *This may concern situations in which a test subject needs to be removed from isolation; for example because he or she requires intensive care, or in situations when unexpected effects are being observed, including Suspected Unexpected Serious Adverse Reactions (SUSARS) or Serious Adverse Events (SAEs). Also consider the situation of a test subject having died and an autopsy being required.*

 *All unexpected situations and serious incidences must be reported immediately. See the 'Procedure for unwanted incidences' (www.loketgentherapie.nl)*

**A5.11. Describe what aftercare will be given if a test subject ends his/her participation in the study prematurely.**

 *Also state to what extent the aftercare deviates from the aftercare for test subjects who have completed the entire study.*

### Monitoring

**A5.12.** **Describe how the monitoring will be set up to identify any spread of the GMO.**

 *In answering this question, pay attention to the method followed, but also to the period during which any positive result can be expected in connection with the applicable scenario. Also state in this context during which period monitoring will take place.*

## A6. Conclusions of the possible environmental effects

*Directive 2001/18/EC Annex II under Point D.1 gives a number of aspects that should be used whenever applicable as the basis of the conclusions about the possible environmental effects of the introduction of the GMP into the environment. All these points should be taken into account when drafting the conclusions of the risk analysis.*

1. **Likelihood of the GMO to become persistent and invasive in natural habitats under the conditions of the proposed release(s).**
2. **Any selective advantage or disadvantage conferred to the GMO and the likelihood of this becoming realized under the conditions of the proposed release(s).**
3. **Potential for gene transfer to other species under conditions of the proposed release of the GMO and any selective advantage or disadvantage conferred to those species.**
4. **Potential immediate and/or delayed environmental impact of the direct and indirect interactions between the GMO and non-target organisms (if applicable).**
5. **Possible immediate and/or delayed effects on human health resulting from potential direct and indirect interactions of the GMO and persons working with, coming into contact with or in the vicinity of the GMO release(s).**
6. **Possible immediate and/or delayed effects on animal health and consequences for the feed/food chain resulting from consumption of the GMO and any product derived from it, if it is intended to be used as animal feed.**
7. **Possible immediate and/or delayed effects on biogeochemical processes caused by possible direct or indirect interaction between the GMO and the target and non-target organisms in the vicinity of the GMO introduction(s).**
8. **Possible change in the current medical practice.**