**Application form**

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

**-**

***Standard procedure***

***naked DNA***

**November 2018**



**Application form**

**Assessment of clinical study involving naked DNA – *Standard procedure***

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

(E-mail: rik.bleijs@rivm.nl, phone: +31-30-2747569).

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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 plasmid(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 a naked DNA plasmid 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 nucleic acid preparation 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 nucleic acid preparation, you must also state the location or locations of laboratories in which activities with the nucleic acid preparation 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-center study, a separate application must be submitted for each location.*

## A2. Construction and composition of the nucleic acid preparation

### Description of the nucleic acid

**A2.1. Describe the genetically modified nucleic acid to be used.**

*In your description please give details in particular of the following aspects:*

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

*- Function of the coded proteins in the donor organism (the organism from which the gene was originally isolated or where it naturally occurs is referred to as the donor organism) and the expected function in the test subject;*

*- Whether the vector or the DNA inserted in the vector contains elements whose origin or function is unknown.*

**A2.2. Provide a description of any antibiotic-resistance genes present.**

*Provide a description of the antibiotic-resistance genes that are present, and indicate the degree to which they affect the environmental risk assessment.*

**A2.3. Provide a molecular characterization of the genetically modified nucleic acid.**

*The complete nucleotide sequence of the genetically modified nucleic acid must be outlined molecularly in a sequence analysis. Sequences must be annotated. Any deviations between the obtained nucleotide sequence and the theoretically expected nucleotide sequence must be described, and the related possible impact on the environmental risk assessment must be elaborated. Further information can be obtained from the COGEM advisory report CGM/130227-05.*

**A2.4. Provide a diagram (map) and a description of the structure of the genetically modified nucleic acid.**

*Provide plain and clear diagrams of the genetically modified nucleic acid, such as in plasmid diagrams that show all the combining plasmid components. In such a schematic depiction, the method of construction of the genetically modified nucleic acid must be clearly indicated.*

**A2.5. Provide a description of the viral sequences and transposons that may interact with the genomes of viruses or other micro-organisms.**

*Describe the sequences that may interact with genomes of viruses or other micro-organisms. Provide a deliberation on the likelihood of this interaction leading to the formation of a new GMO. If a GMO could be created, then discuss the possible routes of transmission, the likelihood of such transmission, and its consequences.*

## A3. Production of the nucleic acid preparation

**A3.1.** **State under whose responsibility the production of the nucleic acid 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 nucleic acid preparation 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 nucleic acid 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 nucleic acid 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 nucleic acid 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 nucleic acid preparation will be transported to the test subject.*

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

*State via which route and how the nucleic acid preparation 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 nucleic acid preparation 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 nucleic acid to be administered?**

*Any medication that would affect the nucleic acid preparation 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 virus.*

### 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 or nucleic acid preparations are 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 or nucleic acid preparations 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 nucleic acid preparation detected after being administered?**

*State, if applicable, when GMO components or nucleic acid preparations 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 or nucleic acid preparations and how the GMO or nucleic acid preparations are 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 nucleic acid preparation 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 nucleic acid preparation, 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 nucleic acid preparation, transmission of the nucleic acid preparation, 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 nucleic acid preparation 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 GMP on human health and the environment.*

*After administration to a human test subject, the nucleic acid preparation will remain inside the body for some time. During this time, the following interactions between the nucleic acid preparation and other organisms may occur, possibly leading to the formation of a GMO:*

*1. After the nucleic acid preparation has been administered, genetically modified human cells may form in the test subject that could be released from the body and dispersed within the environment. There is also the possibility of the nucleic acid preparation itself being released into the environment and coming into contact with other eukaryotic cells.*

*2. If the nucleic acid preparation comes into contact with germ cells, then genetically modified germ cells may be formed.*

*3. The administered nucleic acid preparation may become integrated, either fully or partly, into the genome of a virus; for example, through recombination.*

*4. During or following administration, the nucleic acid preparation may come into contact with bacteria. Within these bacteria, DNA could be preserved as plasmid or by integration into the genome. It may disperse within the environment together with the bacteria, or by horizontal gene transfer to other bacteria.*

*A risk analysis should be carried out for each GMO or nucleic acid preparation 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 contains a description of those aspects that 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 nucleic acid preparation.**

*Describe here the effects on human beings and the environment that could occur as a consequence of the use of the characteristics of the nucleic acid preparation 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 nucleic acid preparation can disperse from the test subject into the environment.**

*State in your answer the scenarios in which the nucleic acid preparation 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 nucleic acid preparation, 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 nucleic acid preparation 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 nucleic acid preparation.**

*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.**