GENimmune N.V.

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GMO Deliberate Release Notification

INFORMATION FOR THE PUBLIC[1]

A MULTI-CENTRE PHASE I STUDY TO EVALUATE THE SAFETY AND TOLERABILITY OF A HETEROLOGOUS PRIME-BOOST VACCINATION WITH INX102-3697 HBV PDNA/INX102-0557 HBV MVA IN HEALTHY VOLUNTEERS AND HBeAg+ CHRONIC HEPATITIS PATIENTS

European notification number
B/BE/07/BVW3

[1] *This document is in line with the "Guidelines To Compile The Public Dossier - Deliberate releases of genetically modified micro-organisms for experimental purposes (part B)" of the Biosafety Advisory Council (version of 26 February 2003). Mandatory text is presented in italics.*

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1. REGULATORY FRAMEWORK AND AUTHORIZATION PROCEDURE

The release of genetically modified organisms (GMOs) in the environment is strictly regulated at European level by directive 2001/18/EC of 12 March 2001 repealing directive 90/220/EEC and at Belgian level by the Royal Decree of 21 February 2005 regulating the deliberate release and/or marketing of GMOs or products that contain GMOs into the environment repealing the Royal Decree of 18 December 1998.

To ensure the safe use of GMOs, the provisions of the Royal Decree above stipulate that the release of GMOs for experimental aims is prohibited without prior consent from the competent Minister. The decision is based on a thorough evaluation of the biosafety of the planned release, which is conducted by the Biosafety Advisory Council, composed of different Scientific Committees grouping independent experts from Belgian universities and governmental institutes.

To acquire the necessary authorization from the competent Minister, the company GENimmune N.V. submitted an application dossier to the competent authority. On the basis of the advice of the Biosafety Council, the competent minister could grant permission to the company GENimmune N.V. to conduct experiments with transgenic modified vaccinia virus Ankara (MVA) as stipulated in the application ***B/BE/07/BVW3****.*

The release will take place at locations in Flanders / Wallonia / Brussels as a consequence of clinical trials conducted at Centre Hospitalier Universitaire Brugmann, Brussels; Cliniques Universitaires Saint-Luc, Brussels; Drug Reseach Unit Ghent (D.R.U.G.) Gent; SGS Life Science Services - Research Unit Stuivenberg, Antwerpen; Universitair Ziekenhuis Antwerpen, Antwerpen; Universitair Ziekenhuis Brussel, Brussels; Universitair Ziekenhuis Gasthuisberg, Leuven, and Universitair Ziekenhuis Gent, Gent.
It is expected to start on March 1, 2008 and to be completed by March 1, 2010.

2. DESCRIPTION OF THE GENETICALLY MODIFIED MICRO-ORGANISM (GMM)

The GMM is a therapeutic polyepitope vaccine developed to treat patients who are chronically infected with hepatitis B virus (HBV).

The gene of interest is an artificially assembled gene containing several copies of short DNA stretches, separated by linkers, encoding peptides for immune response induction (epitopes) towards HBV. The HBV polyepitope gene is presented either in a plasmid (INX102-3697 HBV pDNA) or in a modified vaccinia virus Ankara (MVA) (INX102-0557 HBV MVA).

Vaccinia virus is well-known for its role as a vaccine that eradicated the smallpox disease.
The MVA is a highly attenuated strain of vaccinia derived from a chorioallantois vaccinia virus that was first isolated in Ankara, hence its name. During the process of attenuation, after numerous passages in chicken embryo fibroblasts, MVA lost approximately 15% of its genomic genetic information, including specific functions that regulate viral host range and that are responsible for evasion of the host immune response. As a result, MVA multiplication is extremely limited in mammalian cells. One of the major safety advantages of MVA is that it does not complete its life cycle in non-permissive (human) cells resulting in the accumulation of immature viruses. The injection of MVA in human subjects is therefore not expected to result in the production of mature and infectious particles.

After injection, the genetically modified MVA strain will express the HBV polyepitope only transiently. The gene product is designed to elicit immune responses in a broad population, but has no enzymatic activity by itself. The newly synthesized protein is rapidly processed and degraded and therefore does not accumulate.

The recombinant plasmid also contains a gene for resistance to certain aminoglycoside antibiotics (e.g. kanamycin). This gene is expressed in *Escherichia coli* during plasmid production and is necessary for selection.

3. TYPE AND PURPOSE OF THE ENVISAGED TRIAL

The deliberate release is a an open-label, non-randomized, uncontrolled, multi-centre, phase I study to evaluate the safety, tolerability, and immunogenicity of a heterologous prime-boost vaccination of alternating intramuscular (IM) injections of INX102-3697 HBV pDNA and subcutaneous (SC) injections of INX102-0557 HBV MVA.

In most cases, the immune response induced by a single dose of a vaccine is not sufficiently strong or sustained to be effective. Repeated administration can enhance the immune response to a vaccine antigen, a phenomenon known as "boosting." The "prime-boost" approach is the administration of the same antigen in two different vectors given successively. Exposure to the antigen in the first vector "primes" the immune response; re-exposure to the same antigen in the second vector "boosts" the response. This approach has also been termed "heterologous boosting," to distinguish it from the traditional method (homologous boosting) in which two or more doses of the same vaccine are given successively. In this study the administration of the HBV polyepitope via the plasmid (INX102-3697 HBV pDNA) will be alternated by the administration via the MVA (INX102-0557 HBV MVA).

The trial is aimed at assessing safety, tolerability and immunogenicity of the vaccine.

The study will consist of a first part with vaccination of 15 healthy volunteers. If the vaccine is confirmed to be safe and tolerable, a second part with vaccination of 15 patients with chronic hepatitis B will be initiated.

Subjects will be injected 5 times with a solution containing either the plasmid or the recombinant MVA strain. Treatments will be separated by a period of 3 weeks. For each formulation one dose is foreseen. The persons participating in the study are aware of the treatment they receive (open-label trial).

The patients are selected according to very stringent criteria. In order to be able to recruit sufficient eligible patients a multi-centre approach is required.

A previous single-centre Phase I study with the INX102-3697 HBV pDNA plasmid has shown that this product is safe and is well tolerated. INX102-0557 HBV MVA has not been tested in a clinical trial setting before. However, several trials have been conducted in the EU and in the rest of the world with recombinant MVA vectors for a similar purpose. All results confirm that MVA is a promising safe vector for medical applications.

4. RESEARCH AND DEVELOPMENT FRAMEWORK

Hepatitis B is an inflammation of the liver and is caused by the hepatitis B virus. Globally more than 350 million people are infected resulting in about 1 million deaths annually. HBV infection is a complex disease entity that may either resolve spontaneously or manifest itself in a variety of ways. Chronic carriers of HBV are at increased risk of developing long-term complications, i.e. cirrhosis, liver failure and liver cancer. Fifteen percent to 40% of people chronically infected with HBV will have to cope with serious pathological consequences at some stage. Therefore, effective antiviral treatment is an important aspect in the management of chronic hepatitis B and its complications.

While the available drugs for treatment of chronic hepatitis B are rarely able to clear the infection, they can stop the virus from replicating. However, some individuals are much more likely to respond than others and development of viral resistance does occur.

Therapeutic vaccines to treat established chronic (viral) infections, such as hepatitis B, aim to eradicate or at least suppress the infecting agent long term. They require the induction of cytotoxic T lymphocyte (CTL) responses. CTLs are a type of white blood cells that are capable of killing virus-infected cells. To activate the CTLs an antigen comprising specific epitopes, such as the HBV polyepitope in this study, is required.

Based on an expanding amount of evidence, both from published literature and from GENimmune's own research programs, it is expected that heterologous prime-boost vaccination regimens may offer a more promising strategy to induce vigorous cellular responses in humans.

A first clinical trial (single centre Phase I study) with INX102-3697 HBV pDNA plasmid expressing the polyepitope was conducted in the USA. The trial evaluated the safety and tolerability of four monthly intramuscular injections at two dose levels compared to a placebo. There were no clinically significant differences that could be attributed to the treatment. Study medication was regarded as safe and was well tolerated at both dose levels.

The proposed Phase I clinical trial is the first evaluation of the alternating application of the polyepitope vaccine presented via a plasmid and via an MVA vector. It is entirely based on transient expression and transformation of human cells is excluded.

5. POTENTIAL BENEFITS OF THE PLANNED RELEASE

The planned release is a further step in the development of a new strategy to treat chronic hepatitis B.

Currently, treatment in general reduces viral load by several orders of magnitude. Patients still at risk of developing complications due to the persisting infection. Also, potential risks such as side effects or development of viral resistance and a person's likelihood of adhering and responding to treatment play a role.

The ultimate goal in treatment is to eradicate the virus before irreversible liver damage occurs. The proposed heterologous prime-boost vaccination approach in this study is a promising approach to that end.

6. ASSESSMENT FOR POTENTIAL RISKS FOR THE HUMAN HEALTH AND ENVIRONMENT

As MVA is a highly attenuated, host-restricted, non-replicating poxvirus strain, it is widely considered as the vaccinia virus strain of choice for clinical investigation and development for use as a potent vector system in recombinant vaccine development. Recombinant MVA vaccines have been safely administered to humans in clinical trials for melanoma, HIV, and orthopox infections. In the EU, several clinical trials with genetically modified MVA have been conducted. All cases confirmed that the MVA vectors can be safely used and do not lead to uncontrolled dispersal. This experience is highly relevant for the proposed release.

The MVA strain has several advantages in terms of safety. It is not able to multiply in most mammalian cells (MVA only multiplies efficiently in primary chicken embryo fibroblasts and baby hamster kidney cells), which reduces the risk of dissemination. It is not pathogenic for humans because it has lost several sections of genomic DNA. It cannot interact with the host genome as it remains in the cytoplasm, outside the nucleus, limiting the possibility of integration. It stays there until eliminated by normal cell processes. The expression of the transgene in the subject is therefore limited in time. Due to its attenuation, MVA is generally classified as an organism belonging to biological risk level 1. The genetic modification does not change that.

In the clinical trials, the INX102-0557 HBV MVA investigational product is provided as a solution in a sealed ampoule. The INX102-3697 HBV pDNA drug product is provided as a solution in a single-use vial for direct extraction using a hypodermic syringe.

The solution is administered by trained personnel in a trial centre room that corresponds with hospital room biosafety level 1 containment conditions. The injection site is first disinfected. After the injection, the injection site is covered with a large transparent waterproof bandage to prevent exposure to a potential ‘leak’ from the infection site. The subject remains in the hospital setting for approximately 4 hours for standard medical follow-up. The bandage is replaced before the subjects leave the site. Further release

is not expected. subjects are allowed to continue their normal activities between treatments,. No special instructions inspired by a biosafety measure will be provided.

Apart from the subject, potential exposure is limited to the medical staff involved: contact with the solution from an ampoule, syringe or bandage, or accidentally via an injury due to a broken vial, opened ampoule or needle. The chance for such an exposure is extremely limited as medical staff will wear protective clothing such as disposable gloves and a laboratory coat. All waste material will be destroyed as hazardous medical waste according to local procedures. In the event of a spill standard disinfection regimens and common sterilization procedures are effective. The medical staff will receive instructions to that end. Even when exposure occurs, it is not expected to trigger a significant reaction. While the effect of the polyepitope needs to be confirmed, there are no negative indications so far for exposure. Exposure to the MVA background could lead to an immune reaction, which in itself is not undesirable. The dose and frequency will most likely be too low to be of consequence. Furthermore, extensive experience is available on possible side effects.

Exposure to other humans, even family members, is highly unlikely.

Vaccinia virus is traditionally regarded as a laboratory virus with no natural reservoir. As MVA is unable to replicate effectively in human cells and most mammalian host cells, the risk of dissemination and transmission is reduced. A biodistribution study in mice using INX102-0557 HBV MVA confirms that MVA does not persist.

Plasmid DNA is subject to enzymatic degradation in the bloodstream and is therefore rapidly metabolized and cleared from the circulation. The fate of INX102-3697 HBV pDNA was studied in rabbits. The plasmid was not found to be integrated into genomic DNA.

There is no active dispersal mechanism and dissemination would mainly be limited to the hospital room. Gene transfer to other organisms in the environment is not expected. In MVA the HBV polyepitope gene was integrated via homologous recombination. This transfer mechanism is unlikely to happen in nature as no relevant vaccinia strains are present.

Plasmids as such are not capable of transferring genetic material since they need to be integrated in bacterial transfer mechanisms before they can be exchanged and transmitted. Under laboratory conditions much effort is generally required to transform bacteria with non-conjugative and/or non-mobilizable plasmids. An occasional uptake of plasmid by bacteria inside a human subject or in the environment is therefore very unlikely. Moreover, measures are taken to limit the exposure of plasmid DNA to bacteria at the injection site or in the environment: the injection site is disinfected before injection and any waste product is recovered and inactivated. In the unlikely event of uptake, the bacteria could temporarily gain tolerance to kanamycin as an additional characteristic. This would not add to the already widespread presence of this antibiotic resistance gene in bacterial sources in nature. The HBV polyepitope gene is not a selectable trait under natural conditions.

The HBV polyepitope is a unique, synthetic gene that can be detected using polymerase chain reaction (PCR) technology. However, given the highly unlikely event that shedding occurs except to a very limited extent at the injection site, no routine tracing and monitoring of the GMO is foreseen.

This notification concerns a deliberate release of GMM for experimental purposes. Therefore, as a general rule, the use of this material for any other purpose is prohibited.

7. RESPONSIBILITIES OF THE NOTIFIER

The consent that could be given to the notifier by the competent Minister stipulates that the notifier takes complete civilian liability regarding the damage that could be caused by the deliberate release to the health of humans, animals, or environment.

8. INSPECTION BY THE PUBLIC AUTHORITIES

Inspectors are in charge of inspecting the trials for compliance with the conditions specified in the consent and to investigate potential breaches of the consent. In case where mismanagement or fraud is identified specific sanctions will be imposed.

9. ACTIVITY REPORT

At the end of the trial an activity report prepared by the notifier needs to be delivered to the competent authority. This activity report includes at least the following data:

- *the site and period of release,*
- *the precise nature of the actually released GMMs,*
- *the aim(s) of the trial,*
- *the measures that were taken to prevent unwanted release of transgenic material,*
- *if applicable, the measures that were taken to protect the subject (patient/animal) during administration of the GMM-containing study drug,*
- *if applicable, the measures that were taken to protect the relatives of the treated patients,*
- *the measures that were taken to protect the workers who had to manipulate the GMM-containing material,*
- *the method used for the destruction of the unused or contaminated material,*
- *the results obtained during the trial,*
- *an overview of the monitoring of patient/animal for GMM shedding,*
- *an overview of the monitoring of GMM or recombinant DNA in the environment.*

10. CONTACT

If you have any comment on the public dossier or our activities or wish to obtain additional information on the deliberate release, please contact us at the following address.

Notifier:

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You can also have access to a summary of the notification (SNIF) on the web site of the Joint Research Centre of the European Commission (http://gmoinfo.jrc.it/). Comments can be addressed to the Commission via this web site.