Biosafety Advisory Council

07-03-2008

Secretariat

O./ref.: WIV-ISP/BAC/2008_SC_673
Email: bac@sbb.ihe.be

Title: Advice of the Belgian Biosafety Advisory Council on the notification **B/BE/07/BVW3** of the company GENimmune for deliberate release in the environment of genetically modified organisms other than higher plants for research and development

Context

The notification B/BE/07/BVW3 has been submitted by GENimmune to the Belgian Competent Authority in December 2007 for a request of deliberate release in the environment of genetically modified organisms other than higher plants for research and development according to Chapter II of the Royal Decree of 21 February 2005.

The title of the notification is: **"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".** The planned activity concerns a clinical trial where alternatively the healthy volunteers or the patients will receive intramuscular injections with a plasmid presenting the hepatitis B virus (HBV) polyepitope gene or subcutaneous injections with a highly attenuated strain of the *Vaccinia* virus genetically modified to express the polyepitope gene of the HBV. This treatment is developed as a therapeutic vaccination for patients with chronic hepatitis B.

Both volunteers and patients will be injected by trained personnel in several hospitals. The GM virus is deemed unable to replicate in human cells but virus can sometimes be found on the wound dressing covering the injection site. As the trial centres are located in Brussels and in Flanders and possibly in Wallonia, the national territory is considered as the wider potential release area of the GM *Vaccinia* virus.

The dossier has been officially acknowledged by the Competent Authority on 17 December 2007 and forwarded to the Biosafety Advisory Council for advice.
Within the framework of the evaluation procedure, the Biosafety Advisory Council, under the supervision of a coordinator and with the assistance of its Secretariat, contacted experts to evaluate the dossier. Three experts from the common list of experts drawn up by the

Biosafety Advisory Council and the Division of Biosafety and Biotechnology (SBB) answered positively to this request. The SBB also took part in the evaluation of the dossier.
The experts and the SBB assessed whether the information provided in the notification was sufficient and accurate in order to state that the deliberate release of the genetically modified organism for its intended use, would not raise any problems for the environment, animal health or human health (people coming in contact with the treated patient and/or with the GMO).

On 25 January 2008, based on a list of questions prepared by the Biosafety Advisory Council, the Competent Authority requested the notifier to provide additional information about the notification. The answers to these questions were received from the notifier on 8 February 2008 and reviewed by the coordinator and the experts. The scientists in charge of evaluating the dossier considered this additional information satisfactory.

For the purpose of this evaluation, the following legal basis has been considered:
- Annex II (principles for the risk assessment) and annex III (information required in notifications) of the Royal Decree of 21 February 2005
- Commission Decision 2002/623/EC of 24 July 2002 establishing guidance notes supplementing Annex II to Directive 2001/18/EC.

As a plasmid does not fall under the definition of a GMO, the risk assessment mainly focused on the risks related to the use of the genetically modified *Vaccinia* virus. The safety of the plasmid was however also considered in the environmental risk assessment.
The pure medical aspects concerning the efficacy of the medicinal product and its safety for the treated patient, as well as aspects related to social, economical or ethical considerations, are outside the scope of this evaluation.

In parallel to the scientific evaluation of the notification, the Competent Authority also made the dossier available on its website for the one-month public consultation foreseen in the abovementioned Royal Decree. As a result of this consultation, the Competent Authority forwarded to the Biosafety Advisory Council 1 reaction of the public relevant for the environmental and/or public health safety of the GMO.
This reaction was taken into account in the elaboration of the advice of the Biosafety Advisory Council given below. Answers are sent separately to the Competent Authority.

Summary of the Scientific evaluation

1. The characteristics of the donor, the recipient or parental organism

No major risks were identified.

The question of the Biosafety Advisory Council about the claim that the attenuated *Vaccinia* virus is not able to reproduce in mammalian cells other that BHK-21 cells was adequately answered by the notifier.

2. Information related to the vector

No major risks were identified.

3. Information related to the characteristics of the GMO

No major risks were identified.

The question of the Biosafety Advisory Council about potential toxic immune responses against the modified *Vaccinia* virus was adequately answered by the notifier.

4. The condition of release

No major risks were identified.

The Biosafety Advisory Council requested the notifier to give more information about the procedures for the removal and destruction of biohazard material in the trial centres. The notifier gave satisfactory complementary information.

5. The risks for the environment and human health

No major risks related to the modified *Vaccinia* virus were identified.

6. The monitoring, control, waste treatment and emergency plans proposed by the applicant

No major risks related to the modified *Vaccinia* virus were identified.

The question about arrangements taken to prevent organ or blood donation by the subjects involved in the clinical trial was adequately answered by the notifier:
- for both healthy volunteers and patients blood donation is excluded during the study period which encompasses a 6 months follow-up period after the last study drug administration.
- both healthy volunteers and patients will receive a pocket-size card containing details on their participation in a study with a GMO stating that this may render their organs/tissues unsuitable for transplantation until 6 months after the last study drug administration.

The question about the transfer of waste from the hospital to the company and the question about the waste treatment was adequately answered by the notifier.

7. Additional points considered by the experts of the Belgian Biosafety Advisory Council

Although out of the scope of the Directive 2001/18, the Biosafety Advisory Council drew the attention of the notifier on the following points:

- possible integration of the plasmid DNA into the genome of the cells of the treated volunteers/patients;
- presence of a kanamycin resistance gene nptII in the plasmid and putative transfer of the resistance gene from DNA vaccinated subjects to their bacterial floras;

The Belgian Biosafety Advisory Council agrees with the company that the level of residual plasmid DNA will be low and decreasing over time and that no germline transmission is expected to occur.

The Belgian Biosafety Advisory Council accepts the arguments of the company when it says that kanamycin is currently considered to be of minor clinical importance, that resistance to aminoglycoside antibiotics is widespread in the nature and that transfer of the plasmid to the patient bacterial flora is considered unlikely.

Conclusion

Based on the scientific assessment of the notification made by the Belgian experts, the Biosafety Advisory Council concludes that it is unlikely that the genetically modified *Vaccinia* virus (INX102-0557 HBV MVA) engineered to express the polyepitope gene of the hepatitis B virus and developed as a therapeutic vaccination for patients with chronic hepatitis B, will have any adverse effects on human health or on the environment in the context of the intended clinical trial.

Therefore, the Biosafety Advisory Council issues a **positive advice with the following conditions**:

- The Biosafety Advisory Council should receive a copy of the clinical trial protocol as soon as it has been finalised (including information for the patient).
- The notifier and the investigators must strictly apply the protocol, the biosafety monitoring and, if necessary, the emergency measures as described in the dossier.
- Any protocol amendment, which could have biosafety implications, has to be previously approved by the Competent Authority.
- The notifier is responsible to verify that each investigator has the required authorisations to perform the clinical trial activities inside the hospital (laboratory, pharmacy, hospital room, consultation room...) according to the Regional Decrees transposing Directive 90/219/EEC on Contained use of genetically modified organisms.
- The Biosafety Advisory Council should be informed within 2 weeks when the first healthy volunteer or patient starts the treatment and the last subject receives the last treatment.

- At the latest six months after the last visit of the last patient included in each trial, the notifier must send to the Competent Authority the final study report including a report with details concerning the biosafety aspects of the project. This report will at least contain:
    - the number of patients included in the trial
    - the list of all adverse events
    - a report on the accidental releases, if any, of the recombinant ~~*L. lactis*~~ Vaccinia virus

correction secrétariat du Conseil 27/03/2008

Dr. D. BREYER

p.o. Prof. D. Reheul
President of the Biosafety Advisory Council.

Annex 1: Compilation of comments of experts in charge of assessing the dossier B/BE/07/BVW3 (ref: BAC_2008_GT_652)

Bioveiligheidsraad
Conseil de Biosécurité

January 2008

Secretariaat
Secrétariat

O./ref.: WIV-ISP/BAC_2008_GT_652
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Compilation of comments of experts in charge of assessing the dossier B/BE/07/BVW3

Mandate for the Group of Experts: mandate of the Biosafety Advisory Council (BAC) of 23 November 2007
Coordinator: Dr. A. Fauconnier
Experts: Hubertine Heremans (KUL), SBB (WIV/ISP), Alain Vanderplasschen (ULg), Karen Willard-Gallo (ULB)
Domains of expertise of experts involved: Human medicine, gene therapy, infectious diseases, molecular genetics, design of vectors, virology, poxvirus, vaccination, veterinary medicine, wildlife disease, zoonoses, biosafety
Secretariat (SBB): Didier Breyer, Martine Goossens, Philippe Herman

INTRODUCTION

Dossier **B/BE/07/BVW3** concerns a notification of the company **GENimmune** for deliberate release in the environment of genetically modified organisms other than higher plants according to Chapter II of the Royal Decree of 21 February 2005.
The notification has been officially acknowledged on 17 December 2007 and concerns clinical trials with the modified Vaccinia virus Ankara (strain MVATGN33) which has been genetically modified to express a polyepitope of the Hepatitis B virus (HBV). This GM-medication is developed as therapeutic vaccine against HBV.

Depending on their expertise, the experts were invited to evaluate the genetically modified organism considered in the notification as regards its molecular characteristics and its potential impact on human health and the environment. The pure medical aspects concerning the efficacy of the medicinal product and its safety for the treated patient are outside the scope of this evaluation.
The comments of the experts are roughly structured as in
- Annex II (principles for the risk assessment) of the Royal Decree of 21 February 2005
- Annex III (information required in notifications) of the Royal Decree of 21 February 2005
- Commission Decision 2002/623/EC of 24 July 2002 establishing guidance notes supplementing Annex II to Directive 2001/18/EC.

List of comments received from the experts

1. INFORMATION RELATED TO THE CHARACTERISTICS OF THE DONOR, THE RECIPIENT OR PARENTAL ORGANISM

(e.g. possibility of natural transfer of genetic material to other organisms, pathological, ecological and physiological characteristics, indigenous vectors ...)

Comment 1

Has evaluated this item and has no questions/comments.

Comment 2

Has evaluated this item and has no questions/comments.

Comment 3

Pox-viruses engineered to express foreign genes are established tools for vaccine development in biomedical research. Large packaging capacity for recombinant DNA, precise virus-specific control of target gene expression, lack of persistence in the host, high immunogenicity as a vaccine, and ease of vector and vaccine production are important features of pox-viruses. Concerns about the safety of pox-viruses, including vaccinia virus as the former smallpox vaccine, have been addressed by the use of viruses that are replication defective in human cells. Among these, the European vaccinia virus strain MVA can be considered as the strain of choice for the design of novel and safe pox-virus vectors. The features that make MVA vectors suitable for clinical use and that should greatly reduce the potential hazard to working individuals and prevent transmission to non-target environment include the severely restricted host range, the avirulence in animals, and the extensive safety testing in humans. This phenotype has defined recombinant MVA as efficient and exceptionally safe viral vector.

Injection of plasmid DNA in vivo is currently controversial concerning its long term safety, mainly because either clinical studies have been performed on terminal patients or because for those using healthy individuals there has not been sufficient time to determine any long term side effects due to DNA persistence. The efficiency of transfection of host cells after injection of naked or plasmid DNA is low, but still sufficient to induce immunological responses, with the plasmid retained for the life of the cell. While the majority of transfected cells are eliminated, residual expression has been detected for longer periods with a potential risk of plasmid integration into the host genome, the acquisition of the antibiotic resistance gene in host cells or bacteria present in the gastrointestinal flora, the induction of anti-DNA antibodies and autoimmunity, and/or the induction of tolerance.

Comment 4

MVA is a highly attenuated strain of Vaccinia virus that has been attenuated by extensive passages on chicken embryo fibroblasts. This process led to the production of an attenuated strain that lost about 15% of its genes including virulence factors and genes required for efficient replication of Vaccinia in mammalian cells. Consequently, infection of human cells by MVA leads to an abortive infection and this vector should not be considered as a replicating vector.

The safety record of MVA has been demonstrated by an impressive amount of studies performed in several animal species, both in immunocompetent and immunocompromised subjects. All studies were supportive of an extremely safe profile. In human, MVA was used extensively as a vaccine against smallpox and was proved to be the safest strain of Vaccinia.

Several MVA recombinants expressing various types of antigens have already been tested. Here again all studies demonstrated an extremely safe profile, even in the human species.

I would suggest to the "Conseil de Biosécurité" to draw a list of vector for which there are important amount of data supporting their safety. MVA should definitely belong to this list. For the vectors belonging to this list, it should be relevant to establish a simplified procedure focusing the attention of the "Conseil de Biosécurité" on the intrinsic features of the GMO: site of insertion, transgene expressed.

2. INFORMATION RELATED TO THE VECTOR

(e.g. description, sequence, mobilisation ...)

Comment 1

Modified VacciniaVirus Ankara (MVA) is a highly attenuated, non replicating poxvirus, which has lost about 15% of its parental genome. The notifier refers to different publications in the technical dossier to support the claim that MVA are not able to reproduce in mammalian cells other that BHK-21 cells and that MVA are not able to produce infectious mature forms in mammalian cells, supporting the safety of this vector.
However, relevant information is reported by a recent publication, which is not referred in the technical dossier. Even though these observations only came from *in vitro* experiments, it is worth to note that Okeke *et al*. (2006) have demonstrated that another mammalian cell line, rat intestinal epithelial IEC-6 cells, supported efficient MVA multiplication. According to this publication, the fact that mammalian cell lines are non-permissive to MVA is based on the limited number of mammalian cells studied so far.
They also have shown that a limited number of mature virions were actually produced in mammalian cell lines that were so far considered to be semi- and non-permissive. This is relevant for the safety of MVA since these mature viruses could be a reservoir of infectious virions and could therefore be a potential source of infection.

Comment 2

The HBV sequences inserted were limited to a sequence designed to produce 30 CTL and HTL epitopes with the appropriate linkers, spacers and control sequences. This production of this insert was carefully controlled at each stage through to the final recombinant MVATG16997 to insure that no mutations, deletions or insertions occurred in the original sequence. They were also careful to avoid creating new novel junctional epitopes and that the protein produced is rapidly processed into the individual epitopes, which are innocuous.

The INX102-3697 HBV pDNA contains the E. coli origin of replication and a kanamycin resistance gene both of which, although limited, could present long term problems through persistence in the host.

Comment 3

Has evaluated this item and has no questions/comments.

3. INFORMATION RELATED TO THE CHARACTERISTICS OF THE GMO

3.1. Information related to the genetic modification

(e.g. methods used for the modification, description of the insert/vector construction ...)

Comment 1

Has evaluated this item and has no questions/comments.

Comment 2

Solid and standard molecular biology techniques were used to design and insert specific sequences in the vectors.

Comment 3

Has evaluated this item and has no questions/comments.

3.2. Information on the molecular characteristics of the final GMO

(e.g. number of copies of the transgenes, phenotypic and genetic stability of the transgenes, expression of the new genetic material, re-arrangements in the genome, inclusion or suppression of genetic material ...)

Comment 1

Has evaluated this item and has no questions/comments.

Comment 2

The MVATG16997 is fully dependent upon the vaccine transcriptional machinery for expression and therefore the HBV polyepitope sequence and MVA proteins should only be expressed for a short period after administration to the patient (infected cells die due to the infection within 24-48 hours). New virus mature virus particles with the potential to infect other cells or be transmitted to another host are not produced during MVA infection in humans.
While the INX102-3697 HBV pDNA is likely to persist in the host for an extended period including the life of transfected cells (GENimmune's studies show that INX102-3697 persists for at least 85

days – their test endpoint - in rabbits) the transgene itself is unlikely to cause problems because once expressed it is quickly processed into individual immunopeptides.

Comment 3

The dossier is rather detailed about these issues. The stability of the GMO has been tested under drastic condition (B/BE/07/BVW3, Part 1 A Technical Dossier) and proved to be perfectly stable. The transgene encodes an artificial polypeptide that is most probably very unstable due to inappropriate folding. Consequently, it should disappear very quickly from the expressing cells and no toxicity is foreseen from the transduced polypeptide. However, the applicant hope that the peptides derived from the transgene will be processed and expressed in MHC-I molecules to induce a cytotoxic immune response against HBV infected cells.

3.3. Considerations for human, animal or plant health

(e.g. invasiveness and virulence, toxic or allergenic effects, possibility of survival outside of receiving host, other product hazards ...)

Comment 1

Has evaluated this item and has no questions/comments.

Comment 2

Has evaluated this item and has no questions/comments.

Comment 3

MVA infection is self-limiting and therefore not a human health problem when used as a vaccine. Transmission of MVATG16997 to plants or animals from the vaccinated patients is unlikely since MVA infection does not produce infectious particles.

The INX1-2-3696 HBV pDNA poses no obvious problems for its use in the development of the MVATG16997 strain or for the polyepitope expression in the injected host. However, although the efficacy of DNA vaccination is very low, safety concerns including removal of antibiotic resistance genes from the plasmid DNA have not been adequately addressed by the DNA vaccination field in general. The long term effects of stably integrated plasmid DNA in vivo are unknown. These concerns should have been given more consideration as a potential problem in this dossier.

Comment 4

To address this point in detail, one should consider four different questions: 1/ the safety of the vector; 2/ putative modification of the vector safety due to the insertion of the transgene, 3/ putative hazardous effect of the transgene expression product and 4/ putative hazardous effect of the immune response induced by the GMO.

I have certainly no worries with questions 1 to 3. Concerning question 4, I propose to address some questions to the notifier.

Did they investigate the possibility that the GMO could induce an immune response raised against a self antigen by some mechanism of cross-reaction?

Of course, it is attractive to conclude that one should no expect any problem as the plasmid vector encoding the very same transgene was proved to be safe. I would certainly not agree with such short reasoning. Indeed, it is important to note that the plasmid vector did not induce a detectable immune response raised against the transgene. Consequently, this plasmid could be safe simply because it is unable to induce an immune response against the transgene. MVA being more immunogenic that the plasmid vector could induce an immune response against the transgene (this is the basic of the application) and this immune response could be deleterious to the vaccinated subject by inducing somr kind of autoimmune disease. Even if this possibility is very unlikely, it needs to be addressed by the applicant.

4. INFORMATION RELATING TO THE CONDITION OF RELEASE

(e.g. description of the activity, quantities of GMO to be released, workers protection measures, elimination of any contaminating material in the preparation of the GMO stock, elimination of the GMO at the end of the experiment ...)

Comment 1

Has evaluated this item and has no questions/comments.

Comment 2

Has evaluated this item and has no questions/comments.

Comment 3

It is not clear to me why they want to have the trial centers keep the used pDNA and MVA containing solutions (there is always a surplus for accurate measurement and they have 1.25 ml per vial for a 1 ml injection) in the original vials for ultimate return to GENimmune. Even in a Ziploc bag, these vials could be broken and cut through the plastic bag releasing the biological material (non-attenuated MVA or pDNA) into the immediate vicinity or into an unsuspecting individual cut by the broken vial. All of these hospitals should all have adequate procedures for the removal and destruction of biologically hazardous materials. Immediate on site disposal after the patient is injected seems to be a more prudent approach than storage and transport back to the company for destruction.

Comment 4

Has evaluated this item and has no questions/comments.

5. INFORMATION RELATED TO THE RISKS FOR THE ENVIRONMENT AND HUMAN HEALTH

5.1. Information on spread ("shedding") of the GMO from the treated patient/animal to other persons/animals or to the environment (including indirect/delayed effects due to vertical transmission to offspring).

(e.g. genetic transfer capability, routes of biological dispersal, target organisms ...)

Comment 1

Has evaluated this item and has no questions/comments.

Comment 2

Has evaluated this item and has no questions/comments.

Comment 3

While I agree that the plasmid DNA will essentially be limited to the injection site, there is not sufficient evidence to state that after injection "the vector systems are eliminated and the target environment returns to its initial state". Their own studies (pg 35 Part 1A Technical Dossier) show that the pDNA persists for at least 85 days in rabbits. While the majority of the injected plasmid DNA will never enter the cells nor be expressed, it is not known how much of the pDNA that does enter the nucleus and is expressed is also integrated into the host DNA. Their own studies suggest possible integration of the INX102-3697 HBV pDNA in 1 out of 7 samples tested from inoculated rabbits. They concluded that there was no integration because they found <10 copies in one sample and 17 copies in another, and nothing (detection limit of 10 copies) in the remaining 6 samples. Undetectable DNA or RNA does not mean that none is present, for example, HIV-1 RNA (viral genome) copy number is considered to be undetectable at less than 50 copies/ml of serum, but this is still sufficient virus to cause immune perturbation and disease.

Therefore, their repeated statement in the dossier that the presence of the pDNA is transient and therefore not a problem is UNTRUE and irresponsible. They should not simply ignore the fact that in reality any long term effects of injecting plasmids in humans are currently unknown. Studies have shown that after intramuscular injection in swine plasmid DNA was still detectable after 4 weeks (Gravier et al. 2007), in salmon both DNA and luciferase activity were detected 535 days after plasmid injection (Tonheim t al.,2007), and that both high and low dose inoculations of pDNA in mice and rabbits show persistence after 2 months (Leamy et al.,2006). Furthermore, it is well known that bacterial plasmid DNA can be stably transfected and expressed in mammalian cells, which provides the basis for DNA vaccination. While the efficiency of transfection of host cells after injection of naked or plasmid DNA is low, there are still sufficient levels to induce immunological responses, with stably integrated plasmids retained for the life of the cell. Therefore, it is untrue to say that the INX102-3697 vector will not persist in the host and can only replicate in a bacterial environment.

Our knowledge concerning long term vaccine safety is much better for MVA than plasmid DNA since a large number of Germans were vaccinated in the 1950's against smallpox using MVA.

Comment 4

Has evaluated this item and has no questions/comments.

5.2. Information on possible effects on human health resulting from interactions of the GMO and persons working with, coming into contact with or in the vicinity of the GMO release (carekeepers, patient relatives, immunocompromised people ...).

Comment 1

Has evaluated this item and has no questions/comments.

Comment 2

Has evaluated this item and has no questions/comments.

Comment 3

Again, GENimmune seems to have taken a rather cavalier attitude to the persistence of the plasmid DNA and any potential long term effects, including the antibiotic resistance gene that has not been removed from this plasmid. There is sufficient data to support the idea that the plasmid persists in the injected host and that low level of integration takes place. Studies have shown that the injection of DNA leads to a local inflammatory reaction with the recruitment of T cells to the site (He and Falo, 2006). The proliferating immune cells could be more susceptible to uptake and integration of the plasmid DNA at the injection site. Immune cells, particularly memory T cells, are long lived and continuously re-circulated throughout the body, which would take the INX102-3697 HBV pDNA well beyond the injection site.

Comment 4

Has evaluated this item and has no questions/comments.

5.3. Information on possible effects on animal health or on the environment.

Comment 1

Has evaluated this item and has no questions/comments.

Comment 2

Has evaluated this item and has no questions/comments.

Comment 3

It is unlikely that either the MVA or pDNA will be released into the environment or exposed to animals from the clinical trial setting. However, the stock vials containing the solution for injection should be disposed of according to standard biohazard procedures at the cllinical trial site and not transported back to GENimmune.

Comment 4

Has evaluated this item and has no questions/comments.

5.4. Information on selective advantages or disadvantages conferred to the GMO compared to the parental organism.

Comment 1

Has evaluated this item and has no questions/comments.

Comment 2

Has evaluated this item and has no questions/comments.

Comment 3

The introduction of the HBV polyepitope sequence into MVA does not appear to have conferred any apparent selective advantages or disadvantages to the MVATG16997 vector. The kanamycin selective marker has not been removed from INX102-3677 HBV pDNA and therefore it cannot be said that there is no risk due to the retention of this sequence in the plasmid DNA.

Comment 4

As mentioned above, transgene product is an artificial polypeptide that will be degraded very quickly in the expressing cells. It will consequently not be able to confer a selective advantage to the GMO.

5.5. Information on the possibility of the GMO to reconvert to his wild type form and possible consequences for human health or the environment.

Comment 1

Has evaluated this item and has no questions/comments.

Comment 2

Has evaluated this item and has no questions/comments.

Comment 3

MVA has been passaged more than 500 times to achieve this attenuated virus and lacks genes critical in the pathogenicity of the wild-type vaccinia virus. For MVA there is sufficient distance from the first human injections to determine that the virus cannot revert to wild-type in the human host. The jury is still out on the plasmid DNA.

Comment 4

The GMO was tested for its stability. If the GMO lose the inserted sequence, the derived strain will be safe as MVA. The possibility that MVA revert to a virulent strain is simply impossible due to the important deletion responsible for the attenuation.

5.6. Information on the possibility of the GMO to exchange genetic material with other micro-organisms and possible consequences for human health or the environment.

Comment 1

Has evaluated this item and has no questions/comments.

Comment 2

Has evaluated this item and has no questions/comments.

Comment 3

Low frequency stable transfection or maintenance of the plasmid DNA in circular form in host cells, particularly proliferating T cells recruited to the injection site, could circulate back to various locations including the gut, where a flora of bacteria, including substantial numbers of E. coli are present. Transfer of the foreign genetic material back to bacteria is therefore not impossible.

Comment 4

This is very unlikely knowing that Vaccinia has no natural reservoir. Natural poxvirus infections occurring in humans are caused by viruses that are phylogenetically distant from Vaccinia. Consequently, recombinations are not expected to occur.

5.7. Information on the possibility of gene transfer to other organisms and about the selective advantages or disadvantages conferred to those resulting organisms (possible consequences for human health or the environment).

Comment 1

Has evaluated this item and has no questions/comments.

Comment 2

Has evaluated this item and has no questions/comments.

Comment 3

Antibiotic resistance has become a major human health problem that seems destined to worsen in coming years. Resistance is likely not entirely due to the administration of antibiotics to patients but also to the inadvertent release of bacteria containing plasmids with antibiotic resistance genes from laboratories and/or patients vaccinated with plasmid DNA. We have not been injecting humans with plasmid DNA for a sufficient number of years to establish clearly whether or not there are long term effects, hence the continued controversy on this subject. GEN immune's comments that the *nptII* gene (kanamycin resistance) is only one mechanism by which bacteria could acquire resistance to this aminoglycoside may be true but that does not mean that it should be ignored or considered as unimportant. While each individual event of injection and dissemination of antibiotic resistance genes may not constitute a significant threat to humans, animals or the environment, the sum total of all these events could have a greater impact and must be considered.

Comment 4

Has evaluated this item and has no questions/comments.

6. INFORMATION RELATED TO THE MONITORING, CONTROL, WASTE TREATMENT AND EMERGENCY PLANS PROPOSED BY THE APPLICANT

6.1. Monitoring plan proposed by the notifier and possibility to identify the occurrence of non-anticipated adverse effects.

(adequation between the monitoring plan and risks identified during the risk assessment, when appropriate measures to minimize the potential risks to offspring ...)

Comment 1

Has evaluated this item and has no questions/comments.

Comment 2

Has evaluated this item and has no questions/comments.

Comment 3

Has evaluated this item and has no questions/comments.

Comment 4

Has evaluated this item and has no questions/comments.

6.2. Surveillance and control of the release

(adequation between the procedures to avoid and/or minimise the spread of the GMO and risks identified during the risk assessment...)

Comment 1

Has evaluated this item and has no questions/comments.

Comment 2

Has evaluated this item and has no questions/comments.

Comment 3

Other than leakage at the injection site and the materials associated with administration of the vaccine (vials, syringes, needles, swabs, bandages, etc) with potential exposure for the health care works involved in the trial, exposure to the MVA and pDNA vectors should remain limited to the injected individual. However, should these individuals be eliminated as potential organ or blood donors, and if so over what period of time? Although HBV patients will are not viable donors what about the healthy controls involved in testing the safety of these vectors?

Correct biohazard disposal of ALL the materials used should be done immediately at the trial site.

Comment 4

Has evaluated this item and has no questions/comments.

6.3. Information on the waste generated by the activity and its treatment.

(e.g. type of waste, amount ...)

Comment 1

Has evaluated this item and has no questions/comments.

Comment 2

Material decontamination and waste treatment are not described in the technical dossier. Local procedures at the hospital and at the company itself (since empty vials used for the treatment are collected for return to GENimmune) should be described.
The applicants should also describe the transfer of empty vials from the different hospitals enrolled in this trial back to GENimmune (storage before transfer, frequency of transfer,…).

Comment 3

See above (6.2)

Comment 4

Has evaluated this item and has no questions/comments.

6.4. If applicable, information on the emergency plan(s) proposed by the notifier.

Comment 1

Has evaluated this item and has no questions/comments.

Comment 2

Has evaluated this item and has no questions/comments.

6.5 Information related to the identification of the GMO and the detection techniques

(e.g. identification methods and detection techniques, sensitivity, reliability and specificity of the proposed tests ..)

Comment 1

Has evaluated this item and has no questions/comments.

Comment 2

Has evaluated this item and has no questions/comments.

Comment 3

While PCR and other immunological and molecular biological techniques are highly sensitive, the level of detection is not an absolute and for humans usually the tissue available for testing the presence of residual DNA is limited to blood, serum, urine, etc. Concentrations in local and sequestered tissue microenvironments may be much higher than anticipated and need to be considered.

Comment 4

Has evaluated this item and has no questions/comments.

7. OTHER INFORMATION

7.1 Do you have any other questions/comments concerning this notification that are not covered under the previous items?

Comment 1

On p. 10 of the technical dossier (Part 1 A), under the subheading 4 "Phenotypic and genetic markers' the notifier states that the MVA virus has a genome shortened by about 9 per cent. However, at the same page but under subheading 8 'Description of the geographic distribution, etc', the notifier states that MVA lost approximately 15% of its parental genome. In order to be consistent, the notifier should correct this statement according to the more recent data.

Comment 2

A/ As mentioned above, I would like to know if the notifier has any evidence that the immune response expected against the transgene will not be deleterious for the vaccinated subject. Even if the present analysis of the dossier does not attempt to address the safety of the GMO for vaccinated subjects, it is important to note that accidental inoculation of a worker with the GMO could cause the putative negative effect observed in vaccinated subject.

B/ If the immune response induced against the transgene by the vector is not deleterious in healthy volunteers, it could be deleterious in HBV carrier. Indeed, if the vector succeeds to induce a strong CTL immune response against HVB infected cells and if this immune response develops quickly after vaccination, HVB infected cells could be destroyed massively leading to some immune mediated pathology.
One suggestion could be to allow the trial on healthy volunteers and then based on the result of this first trial and on the immune response induced against HVB to reanalyse the safety of the second phase planed on HVB carrier.

References

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