Bioveiligheidsraad
Conseil de Biosécurité

09-09-2009

Secretariaat
Secrétariat

O./ref.: WIV-ISP/15/BAC/2009_01261

Title: Advice of the Belgian Biosafety Advisory Council on the notification B/BE/09/BVW1 of the company Roche for deliberate release in the environment of genetically modified organisms other than higher plants for research and development

Context

The notification B/BE/09/BVW1 has been submitted by Roche to the Belgian Competent Authority in May 2009 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 planned activity concerns a clinical trial and the title of the notification is: **"A randomized, double blind, placebo controlled, parallel group, multicenter study of the safety and response rate of 3 subcutaneously administered doses of 5 x 10^7 pfu (plaque forming units) of RO5217790 in patients with high grade cervical intraepithelial neoplasia grade 2 or 3 associated with High Risk HPV infection."** The purpose of the study is to evaluate the ability of the highly attenuated strain of the *Vaccinia* virus genetically modified to express the genes of the E6 and E7 proteins of the human papilloma virus (HPV) and of the human interleukin 2 (hIL2) to cause regression at 6 months of lesions on the cervix

Each subject in the study will receive three doses, administered sub-cutaneously, of either RO5217790 or matched placebo, at intervals of 1 week. 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. Given the dose schedule it is not possible for the subjects to remain within contained facilities for the duration of the study. As the trial centres are located in Brussels and in Flanders and as the patients will be treated ambulatory, 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 04 May 2009 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. Two 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 26 June 2009, 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 from the notifier to these questions were received by the Competent Authority on 24 July 2009 and transmitted to the secretariat of the Biosafety Council on 18 August 2009. This complementary information was reviewed by the coordinator and the experts.

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.

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. The Competent Authority didn't receive any reaction of the public relevant for the environmental and/or public health safety of the GMO.

Summary of the Scientific evaluation

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

No risks were identified.

2. Information related to the vector

The Biosafety Council requested more information concerning the molecular characterisation of the vector.
This information was received and judged satisfactory.

3. Information related to the characteristics of the GMO

No major risks were identified.

The Biosafety Council requested the results of the stability tests and data on the immortalization assays referred to in the dossier.
The information and needed documents were provided by the notifier and judged acceptable.

The Biosafety Council estimates that the testing performed by the notifier to rule out horizontal transmission (i.e. transmission from the treated patient to another person) of the GM virus is not enough reliable having a limit of detection judged too high.
In his answer, the notifier refers to results of PCR measurements for another MVA based product at similar dose levels and via the same route of administration. The Biosafety Council agree that these results support the notifier's assertion that the risk of horizontal transmission of the GM vaccinia virus is extremely low.

4. The condition of release

Amongst the worker protection measures, the Biosafety Council considers the use of gloves as an absolute requirement. This was agreed by the notifier who will instruct the study sites accordingly.

From the technical dossier it was not clear which site of injection was chosen to administer the study drug. This was clarified by the notifier and the choice of the site (skin of the thigh) was adequately justified.

5. The risks for the environment and human health

No major risks were identified.

Yet, it is not known precisely how long the drug product stays at the site of injection after subcutaneous injection. The Biosafety Council takes note that the sponsor will conduct a further pre-clinical study looking at vector dissemination with a more sensitive qPCR assay than previously employed and plans to evaluate the presence and persistence of the transcription and translation products of the transgenes in a cohort of a phase 1 study in healthy volunteers currently being conducted as well as in the pre-clinical study.

The Biosafety Council questioned the notifier about the possibility of the vaccine to recombine with the HPV wild-type (present in the patient) and regain the wild-type E6 and E7 proteins that have an oncogenic potential.
The arguments advanced by the notifier in his answer were judged convincing: HPV infection is confined to the cells of the cervical epithelium and after subcutaneous injection the GM vaccinia virus is not expected to be detected beyond the injection sites. Moreover, the Biosafety Council takes note that the sponsor will conduct a MVA biodistribution study in mice following subcutaneous injection.

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

The Biosafety Council takes note that the sponsor will instruct the study sites that labcoats, goggles, patient gown and bedding should be systematically decontaminated or discarded as appropriate after use.

From the technical dossier it was not clear where in the hospital the drug will be prepared and in which room the patients will be treated with this vaccine. This question was adequately answered.

In case of accidental projection of the study drug into the eye, the Biosafety Council considers that washing the eye abundantly with water of physiological liquid is a better measure than the application of an anti-viral drug (ribavirin). The sponsor agrees with this and considers the use of ribavirin ophthalmologic solution, if available, as a further conservative and reassuring measure.

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 some points concerning the efficacy and safety of the product.
The questions of the Council and the answers received from the notifier are given in annex II.

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 (RO5217790) developed as a therapeutic vaccination for female patients with high grade cervical intraepithelial neoplasia which contains modified versions of the E6 and E7 genes of HPV that no longer have immortalizing capacities and is genetically modified to express the gene of the human interleukin 2, 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 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 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 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 the trial, the notifier must send to the Biosafety Council 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 marked by the investigators as probably or definitely related to the study medication;
    - a report on the accidental releases, if any, of the recombinant Vaccinia virus.

p.o.

Prof. D. Reheul
President of the Belgian Biosafety Advisory Council

Annex 1: Compilation of comments of experts in charge of assessing the dossier B/BE/09/BVW1 (ref: BAC_2009_953)
Annex 2: Additional comments related to the safety and efficacy of the product and answers given by the applicant.

Bioveiligheidsraad
Conseil de Biosécurité

June 2009

Secretariaat
Secrétariat

O./ref.: WIV-ISP/BAC_2009_953
Email: BAC@sbb.ihe.be

Compilation of Comments of Experts in charge of assessing the dossier B/BE/09/BVW1

Mandate for the Group of Experts: mandate of the Biosafety Advisory Council (BAC) of 17 March 2009
Coordinator: Prof. P. Hermans
Experts: Annick Brandenburger (ULB), Alain Vanderplasschen (ULg), Céline Verheust (WIV/ISP - SBB)
Domains of expertise of experts involved: Virology, poxvirus, gene therapy, therapeutic vaccination, design of vectors, veterinary medicine, wildlife disease, zoonoses, biosafety, risk assessment
Secretariat (SBB): Didier Breyer, Martine Goossens, Philippe Herman

INTRODUCTION

Dossier **B/BE/09/BVW1** concerns a notification of the company **Roche** 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 04 May 2009 and concerns a clinical trial with the modified Vaccinia virus Ankara which has been genetically modified to express the human cytokine IL2 (hIL2). This GM-medication is developed as a therapeutic vaccine to treat cervical intraepithelial neoplasia grade 2 or 3 associated with high risk HPV infection.

♦ INSTRUCTIONS FOR EVALUATION

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

Has evaluated this item and has no questions/comments.

Comment 4

RO5217790 also named as TG4001, MVA-HPV-IL2, MVATG8042 is a recombinant vaccinia viral vector containing the Modified Virus of Ankara (MVA) genome (defined as the recipient) containing inserted transgenes that encode three proteins: human cytokine IL-2 (hIl2), and mutated forms of the E6 and E7 proteins from the human papilloma virus (HPV) defined as the donors. The vector is defined as the transfer plasmid pTG8042.
MVA as the recipient replicates in the cytoplasm and thus is highly unlikely to integrate with the host genome (never shown), does not persist for an extended period of time, does not replicate in primary human cells and has not been reported to have any significant toxic effects in normal and immunocompromised animals and humans. MVA is a well characterized viral vector not known to cause disease in healthy or immunocompromised adults and has minimal potential hazard to laboratory personnel and the environment.

2. INFORMATION RELATED TO THE VECTOR

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

Comment 1

Has evaluated this item and has no questions/comments.

Comment 2

The sequence of the pTG8042 has been given but with no further indication such as gene location (E6, E7 genes, BRD3 and BRG3 sequences, …). A more detailed map would have been more useful.

Comment 3

Has evaluated this item and has no questions/comments.

Comment 4

The vector in this GMO construct is defined as the pTG8042, a 9041 bp in a circular DNA form. Its sequence is completely available in the annex. It contains the three genes, two modified sequences encoding the E6 and E7 proteins from the HPV derived from the CaSki cell lines and the hIl2. The modifications of the E6 and E7 genes strongly reduce and/or abolish their ability to interfere with regulatory proteins such as p53 for E6 and pRb for E7. The deletions in both genes in this GMO showed a similar transforming activity than a MVA control assay without an expression cassette. The Il2 gene is derived from human mononuclear cells.
The mutated E6 and E7 coding sequences were fused to heterologous sequences encoding secretion signal- and membrane anchoring-domains from the measles virus and rabies virus, respectively, to improve the immunogenicity of the antigens. Why the manufacturer added two additional and different viral substrates and is not using an appropriate adjuvant which could improve immunogenicity? Being not in the "placebo" groups, these additional sequences might interfere with interpretation of clinical results. How to exclude any role for these added sequences?

The h IL2 cDNA lacks the coding sequence for the signal peptide. The sequence coding for the signal peptide is synthetically reconstituted. As mentioned by the manufacturer, the plasmid containing the reconstituted human IL2 cDNA was named pTG26 and was used in the construction of the final vector pTG8042. IL2 is a human cytokine secreted by activated T cells stimulated by antigen presenting cells, in response to antigenic stimulation and play a key role in the activation of the immune system.
Inserted in the recipient, hIl2 is detected by ELISA and is shown to be functionally active. The manufacturer write that dosages of hIl2 delivered by the vaccine is markedly reduced when compared with doses given in the treatment of kidney cancers. In table 3 of the analyses on stability, functional hIl2 is supposed to be up to 450 IU/10^4 PFUs. Can we suggest that 10^7 PFUs could produce 450000 IU of hIl2 during the first 24 hours? An experiment on rats showed that such doses of hIl2 should not be found in their plasma: what is the pharmacology of the hIl2 produced by the vaccine? (including distribution, half-life,..).Could the manufacturer provide more precision on doses (locally and systemically measured) observed in the clinical trial with the vaccine, and its relationship with the doses of vaccines ?

E6 gene is fused at the 5' terminal sequences coding for the signal peptide of the F glycoprotein of measles virus and to a sequence encoding a transmembrane domain of the glycoprotein at the 3' end. The E7 gene is fused at the 5' terminal sequences coding for the secretory signal of rabies virus glycoprotein and to a sequence encoding a transmembrane domain of a rabies virus glycoprotein at the 3' end. Once again, the manufacturer should justify the use of additional sequences derived from measle and rabies viruses in order to increase the immune response in place of using specific adjuvant.
Other gene regulatory sequences inserted in the vector (pTG8042) are presented in Table 2, page 26/83 of the annex IIIA.

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

Has evaluated this item and has no questions/comments.

Comment 3

Has evaluated this item and has no questions/comments.

Comment 4

Well described in the annex IIIA. No question except those already mentioned above.

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

The following sentence is ambiguous (annexIIIA P41):
" For all MVA-based viral vectors currently produced in the manufacturing facility differences in the restriction patterns are observed in comparison to RO5217790 using at least two restriction enzymes."
I suppose that "…differences in restriction patterns are "looked for"…" but not observed.

Comment 2

The stability tests performed have been well described but no results of these tests have been included in the section or in table 3. Only references to other study reports are indicated. Results should have been added for more clarity. Furthermore, pictures illustrating the stability tests by PCR and restriction should have been included, also for more clarity.

Comment 3

Has evaluated this item and has no questions/comments.

Comment 4

Why having chosen only 3 passages before testing expression, function and stability of the inserts? (see table 3, page 37/83 in the annex IIIA. Little information is given on the stability of the measles and rabies derived sequences.

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

Except for the significance of the ELISPOT-method to evaluate T-cell responses (annexIIIA P45).

Comment 2

Very few information has been given in the dossier about the 2 mutated HPV proteins E6 and E7 and the risk associated with their expression. No reference about the deletion of E7 protein is cited. Recent immortalization assays demonstrating the safety of the mutated E6-E7 proteins have apparently been conducted. However, no results and reference are described in the dossier. The notifier should complete this section since it is of great importance to prove the non-oncogenic potential of these two mutated proteins.

Comment 3

The MVA vector has been documented as an extremely safe vector. The safety of the backbone vector should not longer be an issue in a biosafety dossier. One should concentrate on the putative effects of transgene expression by the recombinant vector. Two main questions related to this topic should be addressed:

1/ The RO5217790 recombinant vector encodes mutated forms of HPV16 E6 and E7 proteins. It is stated in the dossier that, as a consequence of motif deletion and fusion to transmembrane domain, the recombinant proteins have reduced capability to interact with cellular pRb and p53. Consequently, RO5217790 should not exhibit immortalization capability. In this dossier, the authors stated that "Recent immortalization assays have demonstrated that the transforming activity observed following infection of cells with RO5217790 did not differ from that observed after infection of cells with a negative control virus (empty MVA vector). This is stated at least twice in the dossier (page 16 and page 43). Even if it is very likely that the mutations applied to the E6 and E7 proteins should abolish their transforming activity, the data mentioned by the applicant should be available in the dossier. They are not.

2/ IL-2 expression by the recombinant vector.
Phase I studies described briefly by the applicant revealed a surprisingly low level of responders (anti-E6, -E7 and –vaccinia antibody production). While these results could reveal HLA polymorphism affecting the efficacy of the vaccine (as suggested by the applicant) they could also be explained by some adverse effect of IL2 expression in particular conditions. Indeed, recent studies have suggested

that IL-2 expression could contribute to T reg population production (Sharfe et al., 1997; Almeida et al., 2002; Malek et al., 2002). The applicant should investigate this possibility when analysing their data. The readout described in the dossier will provide the data required to investigate this hypothesis. Even, if the hypothesis described above is unlikely, it should be addressed.

Comment 4

No questions. Most of the questions are well addressed in the annexes. However, horizontal transmission can not be ruled out since the sensibility of the testing is low (4000 PFUs/ml for blood !!). the manufacturer should provide more sensitive data in order to exclude this important issue when treating with GMOs.

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

- In page 48 of the Annex IIIA, the worker protection measures are: safety glasses or goggles and laboratory coat must be worn, gloves are recommended. Considering the potential risk of contamination by the skin, even though it is negligible, and the low inconvenience of this protection measure, gloves should be required during administration of the drug.
- The site of injection of the drug product (skin of the thigh) is only indicated in the “model informed consent form”. This should be indicated in the Annex IIIA under point III. Information relating to the conditions of release.
- There is no information indicating if the drug product is prepared in the room of the patient. If the drug is prepared outside of the room of the patient, how is the drug transferred from one local to another?

Comment 3

Has evaluated this item and has no questions/comments.

Comment 4

See above.
In addition, measures for workers are well described (gloves, safety glasses, ..) and no question arises.

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

How long does the drug product stay at the site of injection? Is contamination of other parts of the body by skin contact possible?

Comment 3

Has evaluated this item and has no questions/comments.

Comment 4

Shedding has already been discussed (see above) and should be clarified.

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

Has evaluated this item and has no questions/comments.

Comment 4

Safety procedures are well designed but should be monitored during the study period.

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

Has evaluated this item and has no questions/comments.

Comment 4

The risk is extremely low.

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

See questions under point 3.3.

Comment 4

Procedures to reduce risks are well described in the annex IIIA.

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

Conversion of the vaccine to the wild-type form of MVA is highly unlikely. However, could the vaccine recombine with the HPV wild-type (present in the patient) and regain the wild-type E6 and E7 proteins that have an oncogenic potential? The notifier should comment on this point.

Comment 3

Has evaluated this item and has no questions/comments.

Comment 4

This risk is extremely low and should not be reached in real life.

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

Has evaluated this item and has no questions/comments.

Comment 4

Since stability data are obtained after only 3 passages, it can not be ruled out, even if low. Additional data after more passages could reduce any potential risk.

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

Has evaluated this item and has no questions/comments.

Comment 4

Same comment as for point 5.6

6. INFORMATION RELATED TO THE MONITORING, SURVEILLANCE AND 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

Too light in absence of additional data. See below.

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

Has evaluated this item and has no questions/comments.

Comment 4

As a new GMO under investigation, this trial should be performed in more contained area. The pharmacist or the investigator should prepare the vaccine under specific, appropriate and well monitored conditions and patients should be hospitalised. Information for waste should be more stringently traced both in the hospital area and at home, once the patient will be back. Nothing is said about their relatives.
Among exclusion criteria, individuals in close contact with avian species should be excluded if they belong chicken, or other species (turkey,..).

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

In Table 5 of Annex IIIA, it is mentioned that labcoats, goggles, patient gown and bedding should be decontaminated if they are contaminated by the study drug. Decontamination of these materials, once used, should be systematic.

Comment 3

Has evaluated this item and has no questions/comments.

Comment 4

All devices, clothes, gloves… must followed the strict procedures of infectious agents and be destroyed accordingly. Since trace of the vaccine has been found in urine and blood, even at low level but with a non sensitive assay, all samples of urine, blood and other human secretions should be contained and destroyed separately.

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

No emergency plan has been proposed. However, decontamination processes in case of accidental release or human contamination (small volumes) are well described in the dossier.

Comment 3

1/ Comment concerning point 4 First aid measures
It is proposed in case of projection into the eye to maintain the eyelips open while moving the eyeball and to apply drop of a solution of ribavirin. What is the point of using an anti-viral molecule on a non replicative infection. Washing the eye abundantly with water of physiological liquid should be much more beneficial for the contaminated subject.

2/ MVA production may contain trace of egg antigens. As allergy against egg antigens is quite frequent in the human population, the applicant should make sure that anti-choc treatments are available when performing vaccination.

Comment 4

No emergency plan is proposed by the notifier, providing that no risk of spreading should be discussed based on a little experience performed during previous studies. No investigation will be done on the healthcare workers in contact with the vaccine nor among relatives in order to exclude any potential spread of the vaccine prep. As mentioned, it is too short.

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

Has evaluated this item and has no questions/comments.

Comment 4

The notifier provides no information on that topic assuming that there will be no release. It is clearly too short!

7. OTHER INFORMATION

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

Comment

As proposed earlier, a shorter dossier should apply to a well characterized vector such as MVA. The safety of the vector on its own does not longer need to be addressed. One should concentrate on the effect of the insertion and the expression of the trangene(s).

Comment 2

As required by several countries, this trial should be performed under more stringent rules, as defined in a containment 2 environment. The notifier should provide answers to most of the questions raised before any approval.

References

Almeida AR, Legrand N, Papiernik M, Freitas AA (2002) Homeostasis of peripheral CD4+ T cells: IL-2R alpha and IL-2 shape a population of regulatory cells that controls CD4+ T cell numbers. J Immunol 169: 4850-4860.

Malek TR, Yu A, Vincek V, Scibelli P, Kong L (2002) CD4 regulatory T cells prevent lethal autoimmunity in IL-2Rbeta-deficient mice. Implications for the nonredundant function of IL-2. Immunity 17: 167-178.

Sharfe N, Dadi HK, Shahar M, Roifman CM (1997) Human immune disorder arising from mutation of the alpha chain of the interleukin-2 receptor. Proc Natl Acad Sci U S A 94: 3168-3171.

Annex 2: Additional comments related to the safety and efficacy of the product and answers given by the applicant.

Point No. 1:
The mutated E6 and E7 coding sequences were fused to heterologous sequences encoding secretion signal- and membrane anchoring-domains from the measles virus and rabies virus, respectively, to improve the immunogenicity of the antigens. Why did the manufacturer add two additional and different viral substrates and is not using an appropriate adjuvant which could improve immunogenicity? Absent in the "placebo" groups, these additional sequences might interfere with interpretation of clinical results. How to exclude any role for these added sequences?
Response to point No. 1:
The deletion modified E6 and E7 coding sequences were fused to the heterologous sequences encoding the secretion signal- and membrane anchoring-domains to improve the immunogenicity of the antigen. Targeting of the tumor antigens to the cell membrane 1) is hypothesized to enhance the immunogenicity of the HPV targeted immunotherapy through an improved extracellular antigen presentation; 2) avoids interaction of these antigens with their cognate binding nuclear proteins, pRB and p53, thereby further minimizing any undesired (i.e. oncogenic) activity of the deletion modified antigens.
The Tm (transmembrane) domains in the transgene cassette confer better survival of TC1 tumor-bearing C57 mice as compared to the transgene cassette lacking the Tm domain in both prophylactic and therapeutic treatment regimens.
Moreover, presence of the Tm domains resulted in detection of the modified E6 and E7 by IHC on the cell membrane of BHK cells transduced with RO5217790, whereas no E6 or E7 protein was detected on the cell membrane of BHK cells transfected with MVA + deletion modified E6 + E7 +hIL2 , lacking the Tm domain. However, the precise role of this trafficking of deletion modified E6 and E7 in the immunogenicity of the antigens is not yet elucidated.
Based on these pre-clinical data we believe that these heterologous viral sequences may contribute to enhanced immunogenicity and further minimize the risk of oncogenicity of RO5217790.
The goal of this clinical trial is to assess the efficacy of the combined transgene cassette and the MVA vector as present in all patients receiving active treatment. Placebo treated patients will receive only buffer so no element of the transgene cassette or MVA will be present. The clinical utility of whole product can therefore be effectively evaluated in the proposed clinical trial.
Although we cannot dissect out the individual contributions of the heterologous viral sequences and deletion modified E6/7 and IL-2 we believe the pre-clinical data provides support for this design.

Point No. 2:
The hIL2 cDNA lacks the coding sequence for the signal peptide. The sequence coding for the signal peptide is synthetically reconstituted. As mentioned by the manufacturer, the plasmid containing the reconstituted human IL2 cDNA was named pTG26 and was used in the construction of the final vector pTG8042. IL2 is a human cytokine secreted by activated T cells stimulated by antigen presenting cells, in response to antigenic stimulation and play a key role in the activation of the immune system.
Inserted in the recipient, hIL2 is detected by ELISA and is shown to be functionally active. The manufacturer write that dosages of hIL2 delivered by the vaccine is markedly reduced when compared with doses given in the treatment of kidney cancers. In table 3 of the analyses on stability, functional hIL2 is supposed to be up to 450 IU/104 PFUs. Can we suggest that 10' PFUs could produce 450000 IU of hIL2 during the first 24 hours? An experiment on rats showed that such doses of hIL2 should not be found in their plasma: what is the pharmacology of the hIL2 produced by the vaccine? (including distribution, half-life,..). Could the manufacturer provide more precision on doses (locally and systemically measured) observed in the clinical trial with the vaccine, and its relationship with the doses of vaccines?
Response to point No. 2:

Measurements either of systemic levels or local injection site levels of IL2 have not been attempted to date in humans so we cannot relate RO5217790 dose to production of IL-2. However, in prior phase 1 clinical studies where levels of anti-IL-2 antibodies were measured in peripheral blood, no significant increases in anti-IL2 over baseline were observed.
In a repeat dose toxicology study in rats, no detectable levels of IL2 could be found in blood samples taken. Tissue from the immediate injection site was not sampled in this study.
An additional 'mechanism of action' preclinical in vivo mouse study is planned and together with new data from the planned Phase 1 and 2 clinical trials, the sponsor aims to be able to better characterize IL2 expression following treatment with RO5217790.

Point No. 3:
Phase I studies described briefly by the applicant revealed a surprisingly low level of responders (anti- E6, -E7 and –vaccinia antibody production). While these results could reveal HLA polymorphism affecting the efficacy of the vaccine (as suggested by the applicant) they could also be explained by some adverse effect of IL2 expression in particular conditions. Indeed, recent studies have suggested that IL-2 expression could contribute to T reg population production (Sharfe et al., 1997; Almeida et al., 2002; Malek et al., 2002). The applicant should investigate this possibility when analysing their data. Has the notifier addressed this question ?
Response to point No. 3:
In the Phase 1 studies previously performed, increasing antibody production to vaccinia following treatment was detectable in 5/6 CIN3 patients in one study and 2/3 patients with cervical carcinoma in a second study, although antibodies to vaccinia could be detected in all 9 patients. In the only other Phase 1 study, 5/9 patients had detectable antibody to vaccinia although this trial involved patients with advanced stage malignancy that may have affected immunocompetence (1, 2). However, in these same studies, antibodies to deletion modified E6 and E7 were detected in only 5/18 following treatment.
Several mechanisms might account for the low level of immune response to deletion modified E6 and E7 detected in previous studies including variability of host immune response due to acquired conditions or genetics. Other factors include decreased intrinsic immunogencity of expressed trangenes (may be less antigenic than MVA-derived proteins).
As the reviewer mentions, another mechanism which may modify immune responses to antigen is production of regulatory T cells, and the sponsor is aware of the potential for immune enhancing (CD8+ proliferation) and immune suppressive (T reg proliferation) effects of IL2. Study of T reg populations (in addition to CD8+ cells) are included as a subset of the cellular immunology assessments to be performed during the course of this study. These will include use of flow cytometry to measure changes in numbers of T regs following treatment and antigen stimulation assays with T reg depleted vs. non-depleted PBMC preparations to assess functionality.

1. Fiander AN, Adams M, Evans AS, Bennett AJ, Borysiewicz LK (1995) Int J Gynecol Cancer.Nov;5(6):438-442.
2. Pillai MR, Balaram P, Padmanabhan TK, Abraham T, Hareendran NK, Nair MK. (1989) Cancer. Nov 1;64(9):1853-8.

Point No. 4:
MVA production may contain trace of egg antigens. As allergy against egg antigens is quite frequent in the human population, the applicant should make sure that anti-choc treatments are available when performing vaccination.
Response to point No. 4:
Patients with a known or suspected allergy to egg antigen will be excluded from the trial.
Administration of a product produced in chick embryo fibroblasts does not always result in an allergic or anaphylactic response even in patients with known allergy to egg antigens (James et al., 1995 NEJM). Finally, clinical sites administering vaccines would typically have access to anti-histamine and epinephrine treatments used for systemic hypersensitivity and anaphylaxis.