Bioveiligheidsraad
Conseil de Biosécurité

11-09-2012

Secretariaat
Secrétariat

O./ref.: WIV-ISP/41/BAC/2012_0807

Title: Advice of the Belgian Biosafety Advisory Council on the notification B/BE/12/BVW1 of the company Theravectys for deliberate release in the environment of genetically modified organisms other than higher plants for research and development

Context

The notification B/BE/12/BVW1 has been submitted by Theravectys to the Belgian Competent Authority in May 2012 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 multi-center, randomized, double blind, placebo-controlled Phase I/II trial to compare the safety, tolerability and immunogenicity of the therapeutic THV01 vaccination at 5.10^6 TU, 5.10^7 TU or 5.10^8 TU doses to placebo in HIV-1 clade B infected patients under highly active antiretroviral therapy"**. The purpose of the study is to determine whether the therapeutic vaccine THV01-1/2, enables HIV-1 infected patients under highly active antiretroviral therapy to stop taking this therapy for a sustainable period of time and improves their quality of life. THV01-1 and THV01-2 are two life recombinant lentiviruses derived from NL4-3 strain of the HIV-1. They are non-replicative and non-pathogenic as all accessory genes from the parental HIV virus have been removed. Their sequences are limited to the sequences required to trigger a cellular immune reaction. The two GMOs, THV01-1 and THV01-2, are genetically identical but differ by their pseudotyping proteins and are thus phenotypically different.

The product THV01-1/2 or matched placebo is administered intramuscularly and each subject in the study will receive a first injection of THV01-1 followed by an injection of THV01-2 8 weeks later. Following injection, THV01-1/2 will transduce all cells and their RNA reversed into DNA will integrate into the host genome. The non replication competent lentivirus cannot be transmitted by the treated patient through sexual contact or direct blood contact. However as the treated patients are HIV infected, the probability of recombination with wild-type virus leading to formation of replication competent lentivirus was assessed.
As the trial centres are located in Brussels and in Wallonia the national territory is considered as the wider potential release area of the GM lentivirus.

The dossier has been officially acknowledged by the Competent Authority on 04 May 2012 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 (BAC) and the Biosafety and Biotechnology Unit (SBB) answered positively to this request. The SBB also took part in the evaluation of the dossier while the

Platform for Molecular Biology and Biotechnology of the Scientific Institute of Public Health evaluated the analytical procedure for the detection of THV01-1/2 submitted by the notifier.
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 7 June 2012, based on a list of questions prepared by the BAC, 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 16 July 2012 and transmitted to the secretariat of the BAC on the same day. This complementary information was reviewed by the coordinator and the experts who had also the opportunity to discuss with the notifier during a meeting which took place at the Scientific Institute of Public Health on 19 July 2012. On 20 June 2012, a list of further remarks and requirements of the BAC was send to the notifier, mainly in order to guide the notifier in further completing and improving the personnel instructions and patient information form. On 2 August 2012, the Competent Authority received amended versions of the personnel instructions (including a synopsis as requested by the BAC), of the patient's information and consent form and of the protocol for GMO detection. This information was transmitted on the same day to the secretariat of the BAC and forwarded to the experts. The amended personnel instructions and patient information still needed some improvement and an additional request of the BAC was sent to the notifier on 21 August 2012. The final versions of the documents were received on 6 September 2012.

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

The donor, recipient and parental organisms have been correctly described in the dossier.
No major risks were identified.

2. Information related to the vector

The GM lentivirus has been produced by transient transfection of HEK-293T cells by 3 different plasmids expressing the encapsidation proteins, the envelope glycoprotein and the proviral DNA encompassing the antigenic sequence. The notifier was asked to further assess the risk of mobilization, which cannot be ruled out even with a deletion of the U3 region. This has been adequately described in the answers of the notifier.

3. Information related to the characteristics of the GMO

The notifier was asked to precise i) which sequences of Gag, Pol and Nef were cloned into the transgene carried by the vector , ii) which sequences were used to optimise expression and iii) to better document the promoter sequence in terms of its potential homology with wild type HIV sequences, its potential to enhance recombination occurrence or its potential to activate upstream or downstream sequences. The notifier adequately answered these questions and the potential (non)-functionality of the fusion protein encoded by the transgene was further discussed during the expert meeting. In particular, the targeted population of HIV-1 clade B infected patients and the fact that these patients are under highly active antiretroviral therapy was taken into consideration during the assessment.

From the application it was not clear which cells were transduced to verify the identity and integrity of the GMO. This has adequately been addressed by the notifier in his answer
The BAC estimates that, although identity and integrity tests were performed correctly, the generation of new recombinant viruses in the targeted population of HIV-1 clade B infected patients can not be ruled out.

The rate and level of protein expression from the GMO had not been verified in cell culture. It has been asked to the applicant to perform this verification and to clarify the effect anticipated for the patient. In its answer, the notifier clarified that the poly-protein encodes the entire protein sequence of p24 protein which can be rendred functional upon presence of wild type HIV protease. However, taking into account that the patients are taking protease inhibitors during the vaccination period, it can be considered that the formation of functional p24 will be unlikely.

While a number of modifications have been made to the vector backbone to reduce the risk of homologous recombination with wild-type HIV, the transgene encompasses sequences with homology with wild type HIV sequences, thereby increasing again,the overall homology of the GMO with wild-type HIV and increasing the theoritical probability of recombination and /or mobilisation events. Therefore the notifier was asked to comment on the probability of recombination events in HIV positive patients.
The notifier agreed that homologous recombination and/or mobilization events can not be ruled out, especially taking into account that the target population is HIV positive. The notifier commented that the risk of spread of recombinant particles in case of recombination *in vivo* between the THV01-1 or THV01-2 vaccines and HIV is mitigated by the construct of the vectors (see above) and because the patients enrolled in the trial will maintain their anti-protease inhibitors therapy during the vaccination period. BAC could agree with the risk assessment related to the generation of recombination events and could agree with the modifications brought accordingly to the environmental risk assessment, the information for the patient and the personnel instructions so as to underline this possible risk.

The BAC agrees that the modified lentiviral vector is not pathogenic. However some insertional mutagenesis harm to the patient or personnel accidentally infected cannot be excluded.
The notifier agreed with this remark and took it into account in its revised version of the instruction for the personnel (see below).

4. The condition of release

The BAC considers that the notifier's initial statement that "there is no possibility of host to host transmission" is incorrect.
Even in patients with a very low amount of HIV circulating viruses a substantial amount of virus could still be present in the various lymphoid tissues. Lymphnodes are an important reservoir of HIV. Therefore, knowing that after injection in rats the GMO is detectable both at

the injection site and in draining lymph nodes and anticipating that some of the GMO will be trapped on the surface of immature dendritic cells and subsequently transported to the lymphnodes, it is probable that the GM vector will encounter wild-type HIV and that its entry into a latently infected cell will reactivate (or activate) wild-type virus expression in parallel with expression of the vector. Given the probability GM vector will encounter wild-type HIV, recombination events can not be ruled out, neither the formation of replication competent viruses that have the potential for human-to-human transmission through the same routes as wild-type HIV.

In its answer the notifier agreed that as THV01-1 and THV01-2 lentivectors have never been injected in human and that homologous recombination and/or mobilization events cannot be ruled out, especially taking into account that the target population is HIV positive. The BAC could agree with the theoretical estimation of probability of replication competent lentivirus formation which was adequately addressed in the answers of the notifier. On request of the BAC this potential risk has been underlined in the patient's informed consent and instructions for personnel and detailed in the environmental risk assessment.

The BAC asked also to the notifier to specify the injection site of the first and second vaccination. In its answer the notifier argued that preclinical studies in rats indicated that the site of successive injections did not have an influence on the immune response, therefore it was proposed by the notifier not to precise the location of the intramuscular injections in the patient and to leave the choice of the injection site of the first and the second vaccination up to the investigator. However the BAC considered that it could be reasonably assumed that having the first and second vaccination at opposite locations (i.e. first right upper arm, then left thigh) would be beneficial to reduce the probability that injected GMO would encounter a wild-type HIV. The notifier was requested to take into account these considerations and to modify the 'personnel instructions' accordingly. The notifier provided amended 'personnel instructions' which satisfactorily details the injection site of the first and second vaccination.

The BAC had several critics relating to the instructions prepared for the personnel. It did not contain any detailed description of the procedure how to treat contaminated syringes, needles, bandages and cottons. No further information was provided as regards the precautionary measures taken during administration and the importance of avoiding needlestick injury. Instructions regarding waste and biohazard signs were not enough detailed which was also the case for the instructions in case of accidental spill. In addition it was asked to add a caution about vortexing of the vials and to qualify the risk for the personnel more adequately.
The latest version of the document submitted on 6 September, including the synopsis, takes all these comments into account and, as requested by the BAC, it strengthen the instructions so as to underline the 'mandatory' character of measures and to improve the consistency throughout the document as regards the instructions for avoiding sharp injuries and the use of personal protective equipment.

The possibility that recombination could occur (see above) means that safe sex practices by the vaccinated patient need to be detailed and explained. The BAC asked to the notifier to amend the patient informed consent form.
The latest version of the document submitted on 6 September takes all these comments into account and, as requested by the BAC, uses a stronger language regarding safe sex practices.

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

Because the generation of recombinant viruses cannot be ruled out (see above points 3 and 4), the BAC considers that a monitoring of the patients is necessary. It is particularly important to determine whether, in HIV-infected humans, the lentiviral sequences persist longer or disseminate further from the injection site than foreseen, to use sensitive approaches to search for recombinant viruses (GMO and HIV) and to determine whether there are significant changes in the draining lymph nodes that reflect altered and unanticipated activities of the immune response or HIV.
In answer to this request the notifier refers to the study protocol: it plans to perform blood samples of injected patients at several time points during the study and to perform specific detection of the lentiviral particles allowing discrimination from the HIV. In case of vectors' sequence are still present after week 24, Theravectys could decide to add further blood sampling dedicated to study clearance of the particles within the blood circulation. In case of any positive result, full sequencing will be performed to discriminate between 'native' lentiviral vector's sequence and recombinant originating from *in vivo* recombination with HIV. The BAC judged the tests described by the notifier adequate.
To avoid as much as possible unnecessary risks, the BAC also asked the notifier to review the procedures regarding the handling of partially used, expired or empty vials. In its amended documents (e.g. 'personnel instruction'), the notifier satisfactorily adapted its procedures.

Regarding the analytical procedure proposed by the notifier to accompany the control samples that will be send to the Scientific Institute of Public Health after the start of the clinical trial, the BAC asked a detailed protocol on the conservation and analysis methods of the control sample, describing all steps needed to be able to perform a PCR starting from the transfection of permissive cells, cell harvesting, cell lysate, extraction of genomic DNA and performing a PCR using this genomic DNA. Also a protocol describing the conversion of RNA-material of lentiviral particles into cDNA has been requested.
The notifier submitted detailed analytical procedures. The Platform for Molecular Biology and Biotechnology of the Scientific Institute of Public Health requested some more precisions which were adequately provided by the notifier.

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 therapeutic vaccine THV01-1/2, made of two life recombinant lentiviruses derived from NL4-3 strain of the HIV-1 and developed for the therapeutic vaccination of HIV-1 infected patients under highly active antiretroviral therapy, will have any adverse effects on human health or on the environment in the context of the intended clinical trial and provided that all the foreseen safety measures are followed.

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

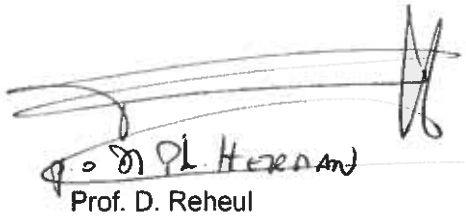
- The notifier and the investigators must strictly apply the clinical trial protocol, and all the safety instructions as described in the dossier.

- Any protocol amendment has to be previously approved by the Competent Authority.

- The notifier is responsible to verify that each study centre has qualified personnel experienced in handling infectious material and that the 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 2009/41/EC on Contained use of genetically modified micro-organisms.

- The Biosafety Advisory Council should be informed within 2 weeks when the first patient starts the treatment and the last patient 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 competent authority at the attention of the Biosafety Council a report with details concerning the biosafety aspects of the project. This report will at least contain:
  - the total number of patients included in the trial and the number of patients included in Belgium;
  - a summary 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 lentiviruses .



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/12/BVW1 (ref: BAC_2012_0574)

Bioveiligheidsraad
Conseil de Biosécurité

07-06-2012

Secretariaat
Secrétariat

O./ref.: WIV-ISP/41/BAC_2012_0574
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Compilation of Comments of Experts in charge of assessing the dossier B/BE/12/BVW1

Mandate for the Group of Experts: mandate of the Biosafety Advisory Council (BAC) of 23 April 2012
Coordinator: Manu Séverin
Experts: Katia Pauwels (WIV-ISP), Anton Roebroek (KUL), Jean-Claude Twizere (ULg), Karen Willard-Gallo (ULB)
Domains of expertise of experts involved: Human medicine, virology, lentivirus, design of vectors, therapeutic vaccination, molecular genetics, biosafety, contained use, workers protection
Secretariat (SBB): Didier Breyer, Martine Goossens, Philippe Herman, Katia Pauwels

INTRODUCTION

Dossier **B/BE/12/BVW1** concerns a notification of the company TheraVectys 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 2012 and concerns a clinical trial with THV01-1 and THV01-2, two live recombinant viral vectored vaccines derived from the NL4-3 strain of the HIV-1. The antigen encoded by these vaccines is derived from the HIV-1, clade B. It is composed of clustered epitopes of the Gag, Pol and Nef proteins under the regulation of a THERAVECTYS patented human promoter. These GM-vaccines are developed for use as therapeutic vaccination of patients infected with HIV.

♦ 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

Remark: The comments below have served as basis for a list of questions that the Competent authority forwarded on 07-06-2012 to the notifier with a request to provide additional information. The comments or remarks highlighted in grey correspond to the questions addressed to the notifier.

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

The presence of indigenous vectors has not been adequately addressed in the dossier (see comments under section 5.2). The notifier should comment on:

- the possibility of mobilization of potential integrated HIV-provirus (HIV-1 clade B infected patients)
- the presence of human endogenous retroviruses (HERVs) that may be a source of functional lentiviral sequences capable of recombining with HIV-derived LV.

Comment 3

I would advice Theravectys to do a better job concerning the description of the donor, recipient and parental organism. For instance, pathological characteristics of HIV-1 (donor, parental organism) should be documented in more details. The sentence on paragraph 1, page 12-annex III is not enough to describe the infectivity, virulence and host range of HIV-1. I would suggest some recent review papers on HIV-1 and AIDS: Montagnier, 2010; Fox and Fidler, 2010; Maggiolo, 2009; Williams and Burdo (2009); Gupta and Towers (2009).

Additional comment from the SBB
This remark is pertinent but the information given in the dossier is adequate to perform the environmental risk assessment.

Comment 4

The notifier states that “the probability for the GMOs to encounter wild - type HIV particles *in vivo*, leading to formation of replication competent viruses, is low because the intended targeted population is patients that have an undetectable level of viraemia (<50copies mL^{-1} using standard commercial assays) and thus very low amount of circulating viruses”. However, undetectable viremia in the blood is only the tip of the HIV iceberg in infected individuals, with substantial amounts of virus (principally in latently infected but also detected in actively infected CD4 T cells) still present in the various lymphoid tissues of patients on HAART (highly active anti-retroviral therapy). In addition, the notifier’s own tests found that after injection in rats the GMO is detectable both at the injection site and in draining lymph

nodes (LN). Together, this suggests that recombination to produce a replication competent virus(es) is actually quite probable.

The notifier has chosen a lentiviral vector for their GMOs to target non-dividing cells such as dendritic cells (DC); however, as designed it will not discriminate between cellular subpopulations and thus will also infect other cell types, including both productively and latently HIV-infected CD4 T cells.

Studies have shown that dendritic cells can trap HIV on their surface through DC-SIGN in addition to being infected themselves (da Silva et al., 2011;Venkatachari et al., 2009). The notifier detected vector sequences in the draining LN of injected rats and concluded that they arrived there through migrating DC, although evidence was not provided to show whether these DC (or other migratory leukocytes) are transporting and/or are transduced with the GMO. Based on current knowledge, it is reasonable to anticipate that some of the GMO will be trapped on the surface of immature DC at the injection site and subsequently transported to the LN. Lymph nodes are an important reservoir for HIV, containing productively infected CD4 T cells along with what is thought to be a, if not the, principal reservoir of latently infected CD4 T cells and to a lesser extent infected DC (von Bubnoff, 2012; Watanabe et al., 2011 ; Mens et al., 2010 ; North et al., 2010 ; Mannioui et al., 2009 ; Chun et al., 2008). Therefore, it is not only possible but also probable that LN resident CD4 T cells (uninfected or HIV-infected) will be actively infected with the GMO. This potential is further enhanced by the patients discontinuation of HAART over the 8 week vaccination period (to facilitate transduction and integration of the GMOs), which will create an even more favorable environment for cycles of wild-type HIV reactivation, replication and *de novo* infection of uninfected CD4 T cells in patients.

Thus, based on their own experiments showing that the vector is found in draining lymph nodes the statement above ("the probability for the GMOs to encounter wild-type HIV particles *in vivo*…) is incorrect. There is every reason to believe that the vector will encounter wild-type HIV and that its entry into a latently infected cell will reactivate (or activate) wild-type virus expression in parallel with expression of the vector. This will create an environment with real potential for the production of recombinant viruses (note: the notifier's efforts at codon-optimization and shuffling of the proviral sequences reduces but does not eliminate the risk), with the probability that some could be replication competent. Thus, any new recombinant viruses produced could have the potential for human-to-human transmission through the same routes as wild-type HIV, making the notifier's statement that "there is no possibility of host to host transmission" incorrect.

This problem is not addressed in the dossier nor is the clinical study designed to assess these parameters. Because there is a possibility (irrespective of the probability) that recombinant viruses will form [potentially including recombinant viruses that increase the replication competence of the GMO through recombination with the wild-type HIV promoter and/or regulatory virus sequences and/or insertion of the vector promoter sequences into wild-type HIV] when the GMOs are administered to HIV-infected individuals whose draining lymph nodes contain significant numbers of HIV-infected CD4 T cells, at the very least further monitoring of these patients is necessary. It is particularly important to determine whether, in HIV-infected humans, the lentiviral sequences persist longer or disseminate further from the injection site than foreseen, to use sensitive approaches to search for recombinant viruses (GMO and HIV) and to the extent possible determine whether there are significant changes in the draining lymph nodes that reflect altered and unanticipated activities of the immune response or HIV.

Finally, a plan for limiting dissemination of any potential recombinant viruses through safe sex practices by the patient should be detailed. There is an obligation for the notifier to acknowledge the probability that recombinant viruses could be produced in the context of this trial, to investigate this further, and find a responsible solution for insuring that any recombinants are limited to the vaccinated trial recipient.

2. INFORMATION RELATED TO THE VECTOR

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

Comment 1

Has evaluated this item and has no questions/comments

Comment 2

Has evaluated this item and has no questions/comments

Comment 3

#Vector1: HEK293 : the reference for this cell line is : Graham et al, 1977.
Page 14, annex III. Item#4: stating that “only the cytoplasmic transcription machinery is solicited to produce the final GMO” is wrong to my opinion. To produce the GMO, viral elements need to be integrated into the genome of a packaging cell line (HEK293 in this case). Integration occurs in the nucleus. For detailed information on lentiviral production, please see Mendenhall et al (2012).

#vector2: pFLAP-H01-W
-A detailed construction map should be provided in order to better understand all steps from the NL43 donor to the final vector presented on figure 3.

Note from the SBB
We do not think that this information is needed to perform the risk assessment.

- Minor comment: figure 3 legend: proviral **DNA** and not **RNA**
-coding sequences: what sequences of Gag, Pol and Nef were cloned into the vector? For instance, cytotoxic T lymphocytes epitope sequences that have been removed should be indicated. Codon optimisation should also be described and shown on a figure. What sequences were added to optimise protein expression?
-The characteristics of the promoter should be described. Is it a strong or weak promoter as compared to the 5’LTR of HIV-1? What inducible sequences are present in that promoter? Is the promoter specific to a given cell type? (dendritic, macrophages, T cell,…)
-Page 16 annex III #4: the first sentence should be corrected as follows: The pFLAP - H01 - W plasmid will be principally **transcribed** into one **viral** RNA molecule….

Comment 4

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

Both lentiviral vectors encode the same transgene derived from HIV‑1, composed of clustered peptides and epitopes of the HIV‑1 clade B, Gag, Pol and Nef proteins under the regulation of a THERAVECTYS patented human promoter. According to the notifier the promoter is devoid of any enhancer sequence and is inducible at a basal level in all cells.
The notifier is asked to better document the characteristics of the promoter sequence in terms of its potential homology with wild type HIV sequences, its potential to enhance recombination occurrence or its potential to activate sequences which are upstream or downstream the LTR sequences (insertional activation). Although deletion of the U3 region elements can reduce the risk associated with enhanced expression of genes surrounding the integrated vector genome, the internal enhancer and promoter required for transgene expression continue to pose a relevant risk of transactivation and therefore need to be carefully chosen. Cellular (internal) promoters are weaker insertional mutagens compared to retroviral promoters and using lineage or tissue specific promoters can potentially increase biosafety (Zychlinski et al., 2008).

Another comment refers to the deletion of the U3 region: the risk of mobilization is reduced with SIN vectors, however on should keep in mind that studies have shown that mobilization is not totally eliminated (Grunwald et al., 2004; Logan et al., 2004; Hanawa H. et al., 2005).

As a conclusion, the above mentioned reflections should be part of the risk assessment of the use of the GMO. However, it could be considered that it is very unlikely that these considerations will alter the conclusions of the environmental risk assessment : even in the worst-case scenario that RCL particles will be generated, the risk for the human population is not higher compared to the risks that HIV-patients may present for the human population.

Comment 3

-Page 19, #1, sentence 3: please replace “is identical” by “corresponds”: the plasmid sequence is a DNA, the genetic material inside a lentivirus is a RNA sequence.

-A figure describing the cloning strategy should be provided along with figure 3.
Note from the SBB
We do not think that this information is needed to perform the risk assessment.

-Please specify what cells were transduced to verify the GMO and the relevance of that cell line (dendritic?, macrophage?)

Comment 4

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

Has evaluated this item and has no questions/comments

Comment 3

Page 22 d. The rate and level of protein expression from the GMO should be verified at least in cell culture by infecting human cell lines (macrophages, dendritic cells or T lymphocytes). This experiment will also allow verification of the activity of the expressed polyprotein.
Question: What the notifier means by “non-functional” polyprotein?

Comment 4

A number of modifications have been made to the HIV backbone used as the lentiviral vector for these GMOs to reduce the risk of homologous recombination with wild-type HIV. While they decrease the probability of recombination with wild-type HIV they do not remove the possibility that this can occur. Therefore, this alone is not sufficient to insure containment of the GMOs to the trial recipient.

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

The notifier states on p12 of Annex IIIA that the recipient organisms (the modified lentiviral vector in this case) are not considered pathogenic or harmful. While it could be agreed that the GMO is not pathogenic, it may present some insertional mutagenesis harm to the patient. However, I agree that the risk for the human population, animal health or plant health is negligible as the GMO is replication deficient, no GMO shedding was observed in body fluids (cfr. biodistribution studies using

intramuscular injection in rats) and in the worst case scenario that generation of replication competent lentiviruses would occur, the risk would not be comparable to the risk that HIV-patients present for the human population.

Comment 3

Has evaluated this item and has no questions/comments

Comment 4

See point 1.

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

Unlike in the Summary (2011-006260-52_SNIF_BE_V1.0, page 33) and the Technical Dossier_AnnexeIII (2011-006260-52_ANNEXIII_BE_V2.0, page 41), the Personnel_Instruction (2011-006260-52_Personnel instruction_ENG_BE_V1.0) does not contain any detailed description of the procedure how to treat contaminated syringes, needles, bandages and cottons. This omission should be corrected, especially since also the handling of the left-over product and dilution vials is described in detail in order to enable proper returning to the manufacturer for future destruction.

Comment 2

Has evaluated this item and has no questions/comments

Comment 3

Has evaluated this item and has no questions/comments

Comment 4

It seems to be an unnecessary risk to have the trial centers store partially used, expired or empty vials for their ultimate return to the notifier. These vials could be inadvertently broken, releasing the GMOs into the immediate vicinity or inoculating an unsuspecting individual cut by the broken vial. These centers treat HIV-infected patients and therefore they have adequate procedures for the removal and destruction of biologically hazardous materials. Immediate on site disposal after the patient is injected is the most prudent approach for dealing with residual material. Unopened, unused vials that are still usable could ultimately be returned to the notifier for their future use.

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

-Question 1:

Did the notifier check for potential hypersensitivity responses against VSV-G envelope as it has been shown in other studies? Maloy et al (2000).

-Question 2:

The GMO will express part of NEF to induce immune response. I do not know, from the documents examined, whether or not Nef biological effects are observed from the expressed polyprotein. For instance, did the notifier verify, at least in cell culture, the following NEF effects: CD4, CD8, and MHC class I down-regulation, T cell killing, altered T-cell signaling and activation, and enhanced viral infectivity? See these reference for review on NEF: Zou et al. (2012), Hanna et al. (2006); Stove and Verhasselt (2006).

Comment 4

If no recombination occurs between the GMO and HIV, then spread is unlikely. However, the possibility that recombination could occur (see point 1) means that safe sex practices by the vaccinated patient need to be detailed and explained. The studies they performed in rats investigated the GMOs activities in a non-HIV environment. The fact that they found the vector travels to the draining lymph node in uninfected rats is of considerable concern since this creates a real potential for the vector to travel to draining LN in HIV-infected patients where it will encounter infected CD4 T cells, ultimately creating a potential for wider dispersion of the vector itself and/or production of recombinant lentiviruses derived from HIV and the GMO.

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

The probability of generation of recombinant competent lentiviral particles has been minimized due to the use of the accessory genes of the vector, the splitting of viral sequences on separate plasmids and the deletion of promoter and enhancer elements in the transfer vector itself. The notifier concludes that the probability for the GMOs to encounter wild - type HIV particles *in vivo*, leading to formation of replication competent viruses, is extremely low because the intended targeted population are patients that have an undetectable level of viraemia (<50copies mL^{-1} using standard commercially assays) and thus have a very low amount of circulating viruses. However, the following considerations related to the post release transfer of genetic material from indigenous organisms to the GMOs have not been addressed by the notifier :

- Although viraemia level is undetectable in the patients enrolled, it does not preclude the possibility of mobilization of integrated HIV-provirus, thereby leading to an increased probability of interaction with circulating wild-type viruses and potential of complementation or recombination. Findings of the first clinical trial using LV have actually revealed mobilization of lentiviral vector sequences in patients with chronic HIV infection (Modlich et al., 2009).

- Second, some human endogenous retroviruses (HERVs) have sequences that are recognized by HIV-1Rev after HIV-1 infection in a permissive cell, thereby promoting nuclear export of the HERV transcripts in these cells [Yang et al., 1999]. Moreover, RNA derived from HERVs is detectable in the plasma of HIV-1-infected individuals [Contreras-Galindo R et al., 2006a; Contreras-Galindo R et al., 2006b; Garrison et al. 2007] supporting the view that significant protein coding capacity and activity potential still exist for these endogenous retroviruses. Hence, the possibility that HERV are a source of functional lentiviral sequences capable of recombining with HIV-derived LV should be addressed.

- Third, the transgenes delivered by the LV particles warrants particular consideration. Despite the fact that the transgene is composed of a cluster of short peptides (epitopes) of the HIV-1 Gag, Pol and Nef proteins devoid of any functionality, the presence of additional wild-type sequences should be assessed in terms of increased amount of homologous sequences with HIV-1 and hence increased probability of recombination. The notifier is requested to comment on this.

However, notwithstanding that these reflections should be part of the risk assessment of the use of the GMO, it is very unlikely that these considerations will alter the conclusions of the **environmental** risk assessment: even in the worst-case scenario that RCL particles will be generated, the risk for the human population is not higher compared to the risks that HIV-patients may present for the human population.

Comment 3

Has evaluated this item and has no questions/comments

Comment 4

See point 1 concerning the practice of safe sex.

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

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

Has evaluated this item and has no questions/comments

Comment 4

Has evaluated this item and has no questions/comments

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

The presence of indigenous vectors has not been adequately addressed in the dossier (see comments under section 5.2). The notifier should comment on:

- the possibility of mobilization of potential integrated HIV-provirus (HIV-1 clade B infected patients)
- the presence of human endogenous retroviruses (HERVs) that may be a source of functional lentiviral sequences capable of recombining with HIV-derived LV.

Comment 3

Has evaluated this item and has no questions/comments

Comment 4

See point 1.

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

The potential to recombine with wild-type HIV is discussed in point 1.

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

Transmission of any recombinant HIV viruses would be via the known mechanisms for this virus and need to be addressed.

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

See point 1.

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

See point 1.

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 the personnel instruction, no further information is provided as regards the precautionary measures taken during administration (use of a safety needle, use of inactivating solution to disinfect surfaces immediately after administration (e.g. specific measures adopted to inactivate the presence of LV particles not injected but remaining on the skin, the inoculation site should be thoroughly cleaned), prophylaxis for the personnel, …).

In the SSI the notifier recommends to use ‘ a hood (such as a safety cabinet) when handling the THV01-1 and THV01-2 vaccines. A hood is not the right containment to prevent unintentional release of the GMO in the environment. The word “hood” that may confuse the staff should be deleted and it should be stressed that, if available, a biological safety cabinet (BSC) should be used,

While emergency procedures described encompasses a procedure after accidental needle stick injury, it is also important to emphasize on the importance of avoiding needle stick injury. How exactly needles are disposed? Are safer engineered needles or needless systems employed? How personnel is informed that recapping should be avoided? The personnel instruction should detail these issues and provide more clear instructions.

Personnel instructions regarding waste and biohazard signs should also be more detailed.
This is also the case for the instructions in case of accidental spill (annex III, p 42)/
For skin and eye contamination with/without injury it is prescribed to wash immediately and abundantly with tap water. Although there are waste and decontamination measures taken in general, there is no specific procedure in case of incident. For example the first step in case of skin contamination has to be soaking up all liquid with absorbent paper or other material (with appropriate inactivation afterwards), then decontaminate the skin (which product, contact time?) and then rinse with water. This to minimize the release of GMO into the environment. Contaminated clothes and used paper to absorb the most concentrated IMP must be disposed or inactivated as infectious material.
In case of eye contamination the washing product has to be collected and inactivated before disposing.

In the instructions in case of accidental spill (annex III, p 42), different concentrations of bleach are recommended. As bleach may vary in the percentage of sodium hypochlorite, which is the active component in bleach, the notifier is requested to further specify the recommended concentration in terms of percentage sodium hypochlorite.

See also comment in section 6.4.

Taking into account the above remarks, the notifier is asked to submit an amended personnel instruction document and is asked to further complete the description of the methods and procedures for controlling GMOs in case of unexpected spread.

Comment 3

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

In the instructions in case of accidental spill (annex III, p 42), different concentrations of bleach are recommended. As bleach may vary in the percentage of sodium hypochlorite, which is the active component in bleach, the notifier is requested to further specify the recommended concentration in terms of percentage sodium hypochlorite.

Comment 3

The instructions for personnel (preparation and injection of the GMO) do not include any information on follow up procedures for accidental exposure by needle stick, broken vial, contamination of the eyes, etc.). Follow up medical assessments (including precautions, potential interactions, effect on pregnancy, etc.) and their duration for an injured healthy care worker should be detailed in this instruction file.

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

Tests to determine whether any recombination between the GMO and wild-type HIV from the patient are required.
They need (as a minimum) to do something like deep sequencing or ultra sensitive qRT-PCR or RNAseq on blood leukocytes (I think that getting a biopsy of the draining lymph node would be medically difficult even if it is ideal – but you never know, it might be possible in the context of a safety trial?) to look for changes in vector and HIV sequences. While not ideal, the circulating leukocytes (CD4 and DC) do mirror at a lower frequency what is happening in the LN. And before the patients go back on HAART, the frequency of both productive and non-productively infected cells in circulation is likely to rise, increasing the chances of seeing potential recombinant viruses.

Another possible approach is to use a non-invasive approach like PET or MRI scanning (at baseline compared to 1 week after the second injection before restarting HAART) to look at the draining LN near the injection site to see if it has become either very enlarged or involuted, which could reflect abnormal activities. The problem with this is that an enlarged LN could just signal an effective response to the vaccine. An involuted LN could indicate mass destruction by HIV. That said, the greater the level of activation, cell division and/or virus production the higher the risk of recombination so detection of macro changes to the LN could justify the need to take a LN biopsy to look for recombinants.

It is not clear whether the first and second injections will be in the same location or in different locations. In the protocol, they only state that the GMO will be administered in 1-3 i.m. injections depending upon the volume and patient – it is not specified (but should be) whether they are all in the same area and if the second GMO should be administered at the same site (i.e. right upper arm, left thigh, etc) – the LN would be more reactive and a PET scan of one location would be sufficient if this was done. In any event, if possible the injection location should be specified in the protocol and if not it should be noted by the investigator.

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

The notifier needs to give more thought to the possible and probable interactions between the GMOs and HIV in the trial patients. Their determination of vector dissemination in an uninfected rat model

provides limited useful information because in humans, and in particular HIV-infected humans, the reality could actually be very different. While one can argue that the HIV-infected individual already has a multitude of quasispecies present, the possibility of recombinant viruses containing GMO and HIV sequences forming must be addressed with specific analyses proposed to assess these events.

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