This document contains 2 advices:

- the original advice dated of 16 March 2004,
ref: BAC_2004_SC_112

- a second advice dated of 5 July 2004 after comments were received from the notifier,
ref: BAC_2004_SC_147

Biosafety Advisory Council

26 March 2004

Secretariat

O./ref.: WIV-ISP/BAC/2004_SC_112[1]
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Title: Advice of the Belgian Biosafety Council on the notification B/BE/03/B3 of the company Transgène for deliberate release in the environment of genetically modified organisms other than higher plants for research and development

Context

The notification B/BE/03/B3 was submitted by Transgène to the Belgian Competent Authorities in January 2004 for a request of deliberate release in the environment of genetically modified organisms other than higher plants for research and development according to Part B of Directive 2001/18/EC and the Royal Decision of 18 December 1998.

The dossier has been officially acknowledged on February 2, 2004.

The planned activity is a clinical trial on cancer patients with a genetically modified adenovirus designed to treat cancer patients. The title of the study is: **"Phase I/II multicentre study of TG1024 (Adenovirus Interleukin 2) in patients with metastatic melanoma or other advanced solid tumor cancers."**. The Belgian patients will be treated at the University Hospital Erasme in Brussels, where Prof. Thierry Velu, Department of Medical Oncology, will be the principal investigator. The study is already running in Switzerland. No other European country in involved in this trial.

Scientific evaluation

The dossier has been evaluated by a group of experts of the Biosafety Council. They answered a list of questions which were mainly based on Annex 2 D1 of the European Directive 2001/18/EC and its guidance notes (2002/623/EC) relative to the risk assessment of genetically modified organisms. The evaluation of the experts is summarised below.

[1] revised version of document BAC_2004_SC_109 as approved on 25 March 2004

1. Human adenovirus type 5 is a virus of the low biorisk class 2. The transmission of human adenovirus infection and disease varies from sporadic to epidemic. Direct or indirect transmission occurs from throat, faeces, eye or urine, depending on the virus serotype. Wild type adenoviruses cause harmless respiratory infections in humans and animals but adenoviruses have been isolated from immunocompromised patients and have contributed to their morbidity and mortality.

2. The vector is made replication-defective by deletions in E1 and E3 regions of the wild type adenoviral genome: all the necessary genes for correct replication and propagation have been deleted. E3-deleted vectors should be recognized and eliminated more readily by the immune system, hereby reducing vector persistence.
In the deleted E1 region, a fragment of DNA coding for Interleukin 2 (IL2) has been inserted. The sequence was obtained from a DNA fragment extracted from human peripheral blood lymphocytes. Interleukine 2 should simulate the immune system of the patient to eliminate cancer cells.

3. Due to the production method used the risk of generating replication-competent adenoviruses (RCA) is unlikely. Besides, all batches are rigorously tested for the presence of RCA by Transgène.

4. At the present time, 25 patients have been treated with TG1024 in Switzerland. Analysis has shown the good safety of the product administered every three weeks.
Up to the present, the principal side effects associated with the intra-tumour administration of TG1024 reported by patients are: fatigue, erythema and pain at the injection site, fever, chills, nausea and vomiting, loss of appetite, headaches and dizziness. The majority of the transduced adenoviral vector genomes essentially remain episomal, a state that minimizes the risk of insertional mutagenesis. In addition, since adenoviral vectors do not integrate, dividing cells will gradually loose the adenoviral vector.
For the patient, the risk due to the toxicity of IL-2 administrated locally in the tumour should be considered acceptable within the goals of this trial, as long as the IL-2 remains concentrated in the tumour.

5. If present, the low number of virus particles that become excreted may infect a few cells of the persons that are in contact, but this will cause no problem. No new virus will be produced and the low doses of IL2 that could be produced will cause no harm.
However, it cannot be ruled out that the recombinant adenovirus can exchange its genetic material during co-infection of the same human cell by a wild-type adenovirus and thus reacquires a replication capacity generating RCA. The probability of occurrence of this event is extremely low and would involve only a limited number of viral particles which would be rapidly eliminated by the immune system, and consequently would have no effects on health of the persons in contact with the treated patient after a putative horizontal transmission. In addition, the respect of confinement, carrying out of protection, control and monitoring measures could reduce significantly the likelihood of post-release dissemination of the vector to other persons. Since the presence of recombinant adenovirus has been demonstrated in

body fluids, mainly within the 24 hours following administration, the treated patient should be hospitalised for 24 hours and visits should be restricted to health care workers who should avoid any direct contact with body fluids and secretions.

6. TG1024 cannot be found in the natural environment due to its non replicative character (E1 deletion) and its incapacity to propagate in the natural environment. In addition, the human adenoviruses, namely HAdV-5 from which Ad-IL2 vectors are derived, is not pathogenic to animals and does not form tumours even in permissive animal models.

7. In the current study, intra-tumoral administration confines the Ad-IL2, at least the large amount of the latter, to a limited area near the injection site, limiting the risk of horizontal transmission i.e. transmission to other humans.
If inadvertent horizontal transmission occurs with replication-deficient adenoviral vectors or even with RCA, the risks would be minimal.

8. Based on preclinical studies, the risk of inadvertent germline gene transfer in patients enrolled in the further clinical trials with TG1024 is expected to be very low if any.

9. The possibility of the GMM to reconvert to his wild type form is extremely remote and unlikely to occur.

10. Gene transfer to other micro-organisms cannot be completely excluded but the risk is very low. Consequently the risk for human health and the environment is very low.

11. The magnitude of the as above identified potential risks is very low.

12. The monitoring, waste and emergency plans proposed by the applicant addresses the risks concerning potential adverse effects.

13. It would be advisable to reduce the person contacts of the patient to a minimum (no visit, especially with naive children) during a period of two weeks after the first injection, in order to exclude the possibility of the spread of a recombinant virus, that theoretically may be formed, to another person. During this period, we expect an appropriate immunological response shortly after the first injection that will eliminate eventually formed recombinant virus.
For broken ampoules, contacts with the skin and wounds, it would be the best to propose disinfectants which have been proven to be effective against adenoviruses.

Conclusion

Based on the scientific assessment, the Biosafety Advisory Council concludes that the risk of using this GMM in this clinical trial is, for human health and the environment, very low. It could still be reduced if some extra precautions are taken. Therefore, the dossier receives a positive advice under the following conditions:

1. The notifier and its investigators applies the protocol, the biosafety, monitoring and, if necessary, emergency measures as described in the dossier.

2. After each injection, the patients stays at least 24 hours in an individual room at the hospital until the level of viral vector in his body fluids has begun to decrease.

3. When back home, the patient is instructed to apply strict hygiene measures (hand washing, no exchange of dishes,..) during all the time of his participation in the study and, at least after the first injection, to reduce contacts with other persons to a minimum (no extra visits during the first 2 weeks, especially with naive children).

4. The waste placed in closed containers treated according to regular hospital procedure for infectious material is not placed in the same environment that the ones from virology department in order to avoid any contact with other viruses. It will be decontaminated separately but according to the same hospital procedure for infectious waste.

5. A complete and accurate treatment plan in the unexpected event of exacerbated immune or inflammatory reactions as in the Gellsinger's case is provided by the sponsor to the investigators.

6. The list of disinfectants proven to be effective against adenoviruses is provided by the sponsor to the investigator. A disinfectant from this list is made available in the rooms where the study medication will be handled.

Prof. Dr. ir. Dirk REHEUL

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

Annex : Expertise report. (ref: BAC_2004_GT_111)

Biosafety Advisory Council

Secretariat

5 July 2004

O./ref.: WIV-ISP/BAC/2004_SC_147[1]
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Title: Advice of the Belgian Biosafety Council in response to the letter of 4 June 2004 of the notifier following the authorisation delivered for notification B/BE/03/B3 of the company Transgene for deliberate release in the environment of genetically modified organisms other than higher plants for research and development

Context

The notification B/BE/03/B3 was submitted by Transgene to the Belgian Competent Authority in January 2004 for a request of deliberate release in the environment of genetically modified organisms other than higher plants for research and development according to Part B of Directive 2001/18/EC and the Royal Decision of 18 December 1998.

The dossier has been officially acknowledged on 2 February, 2004.

The planned activity is a clinical trial on cancer patients with a genetically modified adenovirus designed to treat cancer patients. The title of the study is: **"Phase I/II multicentre study of TG1024 (Adenovirus Interleukin 2) in patients with metastatic melanoma or other advanced solid tumor cancers."**. The Belgian patients will be treated at the 'CUB Hôpital Erasme' in Brussels, where Prof. Thierry Velu, Department of Medical Oncology, will be the principal investigator. The study is already running in Switzerland. No other European country is involved in this trial.

The Belgian Biosafety Council has sent its advice (advice of 26 March 2004 with reference BAC_2004_SC_112) on 2 April 2004 to the Competent authority and on 29 April 2004 the authorisation was delivered to the notifier on the conditions proposed by the Biosafety Council in its advice.

On June 4th 2004 the notifier has sent comments to the Competent authority about the conditions of the authorisation and asks to the Council and its experts to alleviate condition 2

[1] revised version of document BAC_2004_SC_145 as approved on 5 July 2004

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where it is required that *'after each injection, the patients stays at least 24 hours in an individual room at the hospital until the level of viral vector in his body fluids has begun to decrease'*. The notifier accompanies his request with data about 'Viral Dissemination in Blood in Patients receiving Repeated Injections of Ad-IL2 (Clinical Study Referred TG1042.01)' and 'Adenoviral Detection in Biological Fluids of Patients Treated with Ad-IFNγ in the Phase I Clinical Study Referred TG1042.01'. These data were already present in the original dossier acknowledged on 2 February 2004.

In a letter dated of 10 June 2004 the competent authority asks the advice of the Council on the above question of the notifier and on the biosafety implications of the new protocol amendment (amendment nr. 5) that was also notified on June 4th, 2004.

Scientific evaluation

The previous scientific evaluation (see expertise report accompanying the advice of 26 March 2004 - ref: BAC_2004_GT_111) concluded that:
- If present, the low number of virus particles that become excreted may infect a few cells of the persons that are in contact, and that this will cause no problem. No new virus will be produced and the low doses of IL2 that could be produced will cause no harm.
- However, it cannot be ruled out that the recombinant adenovirus can exchange its genetic material during co-infection of the same human cell by a wild-type adenovirus and thus reacquires a replication capacity generating RCA. The probability of occurrence of this event is extremely low and would involve only a limited number of viral particles which would be rapidly eliminated by the immune system, and consequently would have no effects on health of the persons in contact with the treated patient after a putative horizontal transmission.
- In addition, the respect of confinement, carrying out of protection, control and monitoring measures could reduce significantly the likelihood of post-release dissemination of the vector to other persons and since the presence of recombinant adenovirus has been demonstrated in body fluids, mainly within the 24 hours following administration, the treated patient should be hospitalised for 24 hours and visits should be restricted to health care workers who should avoid any direct contact with body fluids and secretions.

The experts estimate that with successive injections the risk of the patient to excrete virus particles will decrease. Patients who were already immunised against adenovirus will have had their immune system reactivated by the first injection. Patients who were not yet immunised against adenovirus will have their immunisation induced by the first injection and they will become each time more reactive against the viruses disseminated in their blood. Therefore taking into account the precaution principle, a 24 hours hospitalisation should be advised after the first injection of Ad-IL2. For the following injections, 6 to 8 hours hospitalisation should be enough to minimise the risk of post-release dissemination of the vector to other persons.

Conclusion

Based on the scientific assessment and knowing that, as stated in its advice of 26 March 2004, the risk of using this GMM in this clinical trial is, for human health and the environment, very low, the Biosafety Advisory Council considers that, without increasing the risk for human health and the environment, the condition 2 of its advice of 26 March 2004 can be restricted for each patient to the first injection of Ad-IL2. Therefore this condition can be interpreted in a less restrictive way and it becomes:

- After the first injection, the patients stays at least 24 hours in an individual room at the hospital until the level of viral vector in his body fluids has begun to decrease. For the following injections the length of the hospitalisation should be at least 6 hours in absence of any signs or symptoms of active concomitant respiratory tract infections.

The Biosafety Advisory Council has no comments about the new protocol amendment which has not any impact on the biosafety of the project.

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