Adviesraad voor Bioveiligheid
Conseil consultatif de Biosécurité

Advice of the Belgian Biosafety Advisory Council on the notification B/BE/18/BVW7 of the company uniQure biopharma BV for deliberate release in the environment of genetically modified organisms other than higher plants for research and development

11/12/2018
Ref. SC/1510/BAC/2018_1082

Context

The notification B/BE/18/BVW7 has been submitted by uniQure biopharma BV, LLC to the Belgian Competent Authority in October 2018 for a request of deliberate release in the environment of genetically modified organisms (GMOs) 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: *"Phase III, open-label, single-dose, multi-centre multinational trial investigating a serotype 5 adeno-associated viral vector containing the Padua variant of a codon-optimized human factor IX gene (AAV5-hFIXco-Padua, AMT-061) administered to adult subjects with severe or moderately-severe haemophilia B".*

Congenital haemophilia B is an X-linked recessive inherited bleeding disorder characterized by a partial or complete deficiency of the essential blood coagulation Factor IX (FIX). Current treatment consists of intravenous injection of recombinant or plasma-derived FIX products. The somatic gene therapy investigated with this study offers the potential for a shift of the disease severity from severe to a moderate or mild haemophilia phenotype or complete amelioration through continuous production of stable FIX levels after a single administration of the investigational medical product (IMP), AMT-061. The IMP is expected to preferentially localize to the liver.The primary objectives of this Phase III study is to demonstrate the effect of AMT-061 on endogenous blood coagulation Factor IX activity 6 months after a single AMT-061 treatment in subjects with congenital haemophilia B. Efficacy and safety aspects upon systemic administration of AMT-061 belong to the secondary objectives.

AMT-061, identified as *AAV5-hFIXco-Padua,* is a recombinant, replication-deficient adeno-associated virus-based vector (AAV5-based vector) containing the Padua variant of a codon-optimized human FIX complementary deoxyribonucleic acid (cDNA) under the control of a liver-specific promoter. The codon-optimized hFIX-Padua sequence is designed based on a naturally occurring FIX-Padua variant with enhanced protein expression. Compared to its parental counterpart, the AAV5-based vector lacks Rep and Cap viral sequences causing the loss of replication ability and the possibility to produce viral particles. If AMT-061 is not shed, the vector DNA is expected to persist in the transduced cells by the formation of episomal concatemers.

AMT-061 is administered in a solution for intravenous (IV) infusion with a single administered dose of 2 x 10^{13} gene copies/kg. After administration, subjects will be monitored at the clinical trial site for three hours for tolerance to the IMP and detection of potential immediate adverse effects. Subjects will then be followed for 5 years in the post-treatment follow-up phase with weekly visits for the first 12 weeks followed by monthly visits from month 4 to 12 and visits twice a year from month 12 to 60.

It is planned to conduct the trial in clinical sites located in Brussels and in the Flemish Region.

The dossier has been officially acknowledged by the Competent Authority on 12 October 2018 and forwarded to the Biosafety Advisory Council (BAC) for advice.
Within the framework of the evaluation procedure, the BAC, 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 BAC and the Service Biosafety and biotechnology (SBB) of Sciensano answered positively to this request. One expert from the SBB also took part in the evaluation of the dossier.
The experts 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 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) in the context of its intended use. See Annex I for an overview of all the comments from the experts.

The scientific evaluation has been performed considering following legislation:
- 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 patients, as well as aspects related to social, economic or ethical considerations, are outside the scope of this evaluation.

In parallel with 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 received from one respondant two published papers evoking possible immunogenic difference between the wild type factor IX and the Padua Factor IX. While the efficacy and patient safety assessment is out of the remit of the BAC, the BAC notes that the applicant has considered this aspect in its biosafety dossier and agrees with the applicant that immunogenic difference between the wild type factor IX and the Padua Factor IX is insignificant.

Summary of the scientific evaluation

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

The donor, recipient and parental organisms were found to be adequately described in the dossier.

2. Information related to the characteristics of the GMO and the medication

Information related to the molecular characteristics of AMT-061 including phenotypic and genetic stability of the transgenes were adequately described in the dossier.

3. The conditions of the release

The GMO will be administered intravenously to haemophilia B patients in hospital centres. Shedding of vector DNA is expected to occur in body fluids/ excreta for several days after administration.
Taken together, the information related to the conditions of the release were found to be adequately described in the dossier.

4. The risks for the environment or human health

The GMO is a recombinant, replication deficient adeno-associated virus-based vector not harbouring any antibiotic or other resistence genes. Like its parental strain it is not known to be pathogenic. The genetic modification introduced in the AAV5-based vector (AAV5-based vector) does not confer the GMO with properties that could confer risks to the human population or the environment.

Shedding of AMT-061 in body fluids/excreta is anticipated from several days to several months after administration as observed in Phase I/II studies with a vector (AMT-060) that is very similar to AMT-061. However, while spread of infectious GMO into the environment through nasal secretions, saliva, urine or faeces cannot be ruled out, a study in a nonhuman primate by Favre *et al.*[1] showed that rAAV vector genome was found in various biological fluids for up to 6 days and infectious particles exclusively in the serum during the first 48–72 hours. It can be reasonably assumed that the amount of shed infectious particles will be extremely low and only a minute fraction of the applicable dose. Taken together with the fact that material shed will be replication-deficient, the likelihood of transmission to third parties and further propagation should be considered highly unlikely. No potential adverse effects are anticipated and the environmental risk is considered negligible.

There is only a remote possibility of homologous recombination between the ITR-sequences of AMT-061 and wild-type AAV2 in case a co-infection in exposed persons occurs. Such recombination event would result in gain of functional genes of AAV2 required for replication and encapsidation but would in turn lead to the loss of the transgene. It also remarked that the genetic material from Rep and Cap genes together with the transgene would be too large in size to be packed in AAV capsid.

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

With respect to monitoring, serum and sperm of treated subjects will be monitored by qPCR until three consecutive samples are found negative for the presence of vector DNA. The BAC is of the opinion that the information on waste treatment is sufficient and does not raise safety concerns.
The BAC further remarked that the description of the procedures for the management of accidental spills still leaves room for improvement and proposes to add the following procedure:

[1] Favre *et al*. Immediate and Long-Term Safety of Recombinant Adeno-associated Virus Injection into the Nonhuman Primate Muscle. Molecular Therapy 4, 559-566, 2001

In case of accidental spills or breakage of a vial containing the GMO, the medical staff should alert people in the area of the spill, remove contaminated clothes and leave the area for 30 min. He/she should close the area and post "DO NOT ENTER". After 30 min, he/she must wear a clean lab coat and wear gloves, glasses, over-shoes and a mask. He/she must cover the spill with towels and other absorbent material starting from the edge toward the centre. He/she must carefully pour the appropriate disinfectant over the absorbent material starting from the edge to the centre. It must allow a sufficient contact time for the disinfectant to inactivate the GMO. After that, he/she must remove the paper towels and broken vials with tongs or forceps and discard in a biohazard waste bag. This procedure with absorbent materials and disinfectant should be performed twice. The Personal Protective Equipment (PPE) should be discarded in the biohazard bag. The lab coat should be decontaminated before disposal. The medical staff should report the incident to the responsible of the site.
Strict procedures should be provided for medical staff and persons in contact with the patient during the release of the viral vector. These procedures should be posted in the hospital room where the treatment should take place.
A spill kit should be available in the facility, this spill kit should contain appropriate disinfectant, PPE (i.e. gloves, safety glasses, laboratory coat, mask, over-shoes), tongs or forceps in order to take broken vials, absorbent paper towels, biohazard waste bags.

The BAC further remarks that the applicant declared to provide a control sample and the scientific documentation fifteen days, at the latest, before the start of the trial. In meeting this requirement, the applicant is invited to consider a guideline[2] describing the data to be presented by the notifier and further information on contact points relative to reference material disposition.

Conclusion

Based on the scientific assessment of the notification made by the Belgian expert, the Biosafety Advisory Council concludes that it is unlikely that AMT-061 developed to treat haemophilia B patients by means of endogenous production of FIX-Padua variant protein will have any adverse effects on human health or on the environment in the context of the intended clinical trial 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.

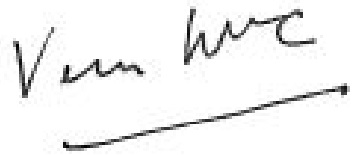
- Any protocol amendment has to be previously approved by the Competent Authority.

- 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 authorizations 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.

[2] Guideline is available at https://www.biosafety.be/content/notification-procedures-clinical-trials-gmos-human-or-veterinary-use

- The Biosafety Advisory Council should be informed within two 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 Advisory 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 AMT-061.



Dr. Corinne Vander Wauven
President of the Belgian Biosafety Advisory Council

Annex I: Compilation of comments of experts in charge of evaluating the dossier B/BE/18/BVW7 (ref. SC/1510/BAC/2018_1006)

Adviesraad voor Bioveiligheid
Conseil consultatif de Biosécurité

Compilation of comments of experts in charge of evaluating the dossier B/BE/18/BVW7

27 November 2018
Ref. SC/1510/BAC/2018_1006

Mandate for the Group of Experts: Mandate of the Biosafety Advisory Council (BAC) of 26 September 2018.
Coordinator: Jozef Anné (KUL)
Experts: Anton Roebroek (KUL), Liliane Tenenbaum (CHUV), Aline Baldo (Sciensano, SBB)
SBB: Katia Pauwels.

INTRODUCTION

Dossier **B/BE/18/BVW7** concerns a notification of the company uniQure biopharma BV 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 12 October 2018 and concerns a Phase III multinational trial investigating an adeno-associated viral vector containing human factor IX gene administered to adult subjects with severe or moderately-severe hemophilia B.

♦ 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/questions 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 not evaluated this item.

Comment 3

Has evaluated this item and has no questions/comments.

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 not evaluated this item.

Comment 3

Has evaluated this item and has no questions/comments.

3. INFORMATION RELATED TO THE CHARACTERISTICS OF THE GMO

3.1. Information related to the genetic modification

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

Comment 1

Has evaluated this item and has no questions/comments.

Comment 2

Has not evaluated this item.

Comment 3

Has evaluated this item and has no questions/comments.

3.2. Information on the molecular characteristics of the final GMO

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

Comment 1

Has evaluated this item and has no questions/comments.

Comment 2

Has not evaluated this item.

Comment 3

In the document ERA Belgium, page 4, it is discussed whether Baculovirus sequences present in the AAV batch constitute a risk for the environment. The applicants declare that indeed low levels of baculovirus DNA and of DNA from insect cells are present in AMT-061. They claim that these sequences "*are not integrated into the AMT-061 vector genome*". Does it mean that these sequences are co-encapsidated into the AAV5 capsid but are not linked to ITR sequences? The demonstration of this statement is lacking in the dossier.
At which frequency are these sequences found? Furthermore, can the applicants rule out that these sequences could code for peptides which could elicit an immune response? They state that in other clinical trials using baculoviruses to produce rAAV batches, "no harmful effects that could possibly be related to either of these DNA impurities have ever been observed" and conclude "that a risk related to residual insect cell DNA and residual baculovirus DNA can be considered negligible": Is the number of patients treated sufficient to support this conclusion? A more quantitative evaluation of this part would be appreciated.

SBB comment :

With respect to the environmental risk assessment (risk for human population at large and the environment) and the potential hazard associated to the possible presence of baculovirus DNA or DNA from insect cells in the IMP, the applicant briefly refers to the experience gained with several other AAV gene therapy vectors manufactured by means of baculovirus-based platforms (p 23 –technical dossier). Is it acceptable that the environmental risk assessment aspects associated to the presence of baculovirus sequences are not addressed more into depth by the applicant, is it proportionate to the possible risks ?
It should be noted that the assessment of immune responses of subjects receiving the IMP is out of the remit of the assessment conducted by the Biosafety Advisory Council.

Coordinator's comments :

With respect to the question whether Baculovirus sequences are co-encapsidated into the AAV5 capsid and not linked to the ITR sequences, the applicant states (p22, technical dossier) that *it is assumed that*

these sequences are co-packed with the vector genome into vector particles due to some degree of promiscuity of the packaging Rep protein. Resultantly, these DNA impurities are co-purified with the vector particles. As AMT-061 is constructed in insect cells, low levels of DNA originating from these cells are a second known DNA impurity of AMT-061. However, these sequences are short and random, and not integrated into the AMT-061 vector genome.
Baculovirus promoters are not functional in human cells. How can peptides be produced from the contaminating Baculovirus DNA, as this DNA cannot enter the nucleus?
In addition, as indicated on p28 of the technical dossier, DNA impurities are controlled during quality testing. For previous vectors produced using the baculovirus expression system **it was shown that these fragments do not harbour coding sequences**.

With regards the environmental risks associated to the presence of baculoviruses during the construction of AMT-061 in insect cells, it should be noted that although wild type baculoviruses could infect and pose a potential hazard to lepidopteran species in the environment, most baculoviral vector systems are attenuated by virtue of deletions in the *polyhedrin*, *basic* or *p10* genes. While these deletions permit baculoviruses to replicate efficiently in insect cell culture, it renders them incapable of establishing a productive infection in the host organism. Vector systems such as these are inherently very safe and will require minimal containment. In the expression system used, the polyhydron gene is deleted.

3.3. Considerations for human, animal or plant health

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

Comment 1

Has evaluated this item and has no questions/comments.

Comment 2

Has evaluated this item and has no questions/comments.

Comment 3

Has evaluated this item and has no questions/comments.

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

The applicant says in the environmental risk assessment part of the dossier that a spill kit will be available at all times during the administration procedure. Details will be provided in the IMP Handling Manual. This IMP Handling Manual is not available in the dossier.
In case of accidental spills, the medical staff should alert people in the area of the spill, remove contaminated clothes and leave the area for 30 min. He/she should close the area and post “DO NOT ENTER”. After 30 min, he/she must wear a clean lab coat and wear gloves, glasses and a mask. He/she must cover the spill with towels and other absorbent material starting from the edge toward the center. He/she must carefully pour the appropriate disinfectant over the absorbent material starting from the edge to the center. It must allow a sufficient contact time for the disinfectant to inactivate the GMO. After that, he/she must remove the paper towels and broken vials with tongs or forceps and discard in a biohazard waste bag. The PPE should be discarded in the biohazard bag. The lab coat should be decontaminated before disposal. The medical staff should report the incident to the responsible of the site.

SBB comment :

The applicant has provided an IMP handling manual (see doc 3.16 CT-AMT-061-02_IMPHM with attachments). In this document the applicant indicates that instructions for what to include in the spill kit will be sent with the IMP shipment.

Comment 3

Has evaluated this item and has no questions/comments.

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

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

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

Comment 1

Has evaluated this item and has no questions/comments.

Comment 2

Has evaluated this item and has no questions/comments.

Comment 3

Has evaluated this item and has no questions/comments.

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

Comment 1

Has evaluated this item and has no questions/comments.

Comment 2

Has evaluated this item and has no questions/comments.

Comment 3
Risks immune responses against baculovirus- or insect cells- DNA should be quantified (see above).

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 not evaluated this item.

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.

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

Comment 1

Has evaluated this item and has no questions/comments.

Comment 2

Has evaluated this item and has no questions/comments.

Comment 3

Has evaluated this item and has no questions/comments.

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.

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.

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 not evaluated this item.

Comment 3

Has evaluated this item and has no questions/comments.

6.2. Surveillance and control of the release

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

Comment 1

Has evaluated this item and has no questions/comments.

Comment 2

Has not evaluated this item.

Comment 3

Has evaluated this item and has no questions/comments.

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

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

Comment 1

Has evaluated this item and has no questions/comments.

Comment 2

The applicant does not describe waste management. Waste should be inactivated before disposal (by incineration).

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

Has not evaluated this item.

Comment 3

Has not evaluated this item.

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 not evaluated this item.

Comment 3

Did not find the description of the methods to quantify baculovirus- and insect cells DNA impurities in AMT-061 as well as their detection threshold

Coordinator's comments :
QPCR mentioned for AMT060; see detection threshold in Tech Dossier p13.
See also p27 Tech Dossier "**Residual baculovirus DNA** AMT-061 is also assessed for residual baculovirus DNA by means of a QPCR-based method. The test was qualified for use with a limit of quantitation well below the levels detected in the IMP.

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
None

Comment 2
None

Comment 3
None

References

HSE, SACGM compendium of guidance, Part 2: Risk assessment of genetically modified microorganisms (other than those associated with plants). Available at http://www.hse.gov.uk/biosafety/gmo/acgm/acgmcomp/)