Bioveiligheidsraad
Conseil de Biosécurité

23 January 2013

Secretariaat
Secrétariat

O./ref.: WIV-ISP/41/BAC/2013_0041

Title: Advice of the Belgian Biosafety Advisory Council on the notification B/BE/12/BVW2 of the company Celladon for deliberate release in the environment of genetically modified organisms other than higher plants for research and development

Context

The notification B/BE/12/BVW2 has been submitted for Celladon by Harrison Clinical Research Benelux to the Belgian Competent Authority in July 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 Phase 2b, Double-Blind, Placebo-Controlled, Multinational, Multicenter, Randomized Study Evaluating the Safety and Efficacy of Intracoronary Administration of MYDICAR® (AAV1/SERCA2a) in Subjects with Heart Failure"**. The purpose of the release is a clinical trial to investigate the safety and efficacy of AAV1/SERCA2a in human subjects with advanced heart failure.
AAV1/SERCA2a is a gene transfer medicinal product. It is a recombinant, not replication competent, adeno-associated viral (rAAV) vector expressing the human SERCA2a, an intracellular calcium pump involved in regulation of the heart contraction/relaxation cycle. The expression of SERCA2a is under control of an added ubiquitous cytomegalovirus (CMV) enhancer/promoter (CMVie). In cardiac cells of patients with moderate to advanced heart-failure SERCA2a is expected to have a therapeutic effect.
The production of AAV1/SERCA2a on cell lines is induced by adenovirus type 5 (Ad5) as helper virus. This helper virus is later eliminated during the purification process.

MYDICAR or matched placebo is administered by a single intracoronary infusion and during at least one week after the infusion there will be possible shedding of rAAV via secretions such as saliva, urine, faeces,…AAV is resistant to desiccation and able to persist in the environment. It is destroyed by bleach and other cleaning agents.

As the trial centres are located in Brussels and in Flanders the national territory is considered as the wider potential release area of the rAAV.

The dossier has been officially acknowledged by the Competent Authority on 16 October 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. Four 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 AAV1/SERCA2a 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). See Annex II for an overview of all the comments from the experts.

On 22 November 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, including an amended risk assessment and new personnel instructions, were received by the Competent Authority on 18 December 2012 and transmitted to the secretariat of the BAC on the same day. This complementary information was reviewed by the coordinator and the experts.

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

The pure medical aspects concerning the efficacy of the medicinal product and its safety for the treated patient, as well as aspects related to social, economical or ethical considerations, are outside the scope of this evaluation.

In parallel to the scientific evaluation of the notification, the Competent Authority also made the dossier available on its website for the one-month public consultation foreseen in the abovementioned Royal Decree. The Competent Authority didn't receive any reaction of the public relevant for the environmental and/or public health safety of the GMO.

Summary of the Scientific evaluation

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

The donor, recipient and parental organisms have been correctly described in the dossier.

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

The presence in the medication of adenoviral (AV) sequences, even at low frequency could, if expressed, elicit an immune response. It is therefore important to be sure that the medication is clear of AV sequences. A monitoring of the batches is foreseen at the end of the production process and is based on a qPCR test targeting the E2a sequence of AV.
In answer to a question of the experts the notifier provided extra qPCR results which convincingly demonstrated that there are residual full wild-type adenovirus particles in their rAAV stocks. However fragments of adenoviral genome could have been encapsidated together with AAV vector sequences which might if expressed elicit a immune response in adenovirus-primed individuals. The absence of inflammation markers in the pigs studies however, are convincingly suggesting that there was no immune reaction to the rAAV stocks in immunologically naïve animals. In addition there was no sign of inflammation in the first human clinical study performed by the applicant.

Regarding the CMV promoter added in the rAAV it was asked to the notifier 1) to better characterize it in relation to its potential to activate sequences of rAAV randomly integrated in the host genome and 2) to explain this choice of an ubiquitous CMV promoter instead of a heart-specific promoter, knowing that the CMV promoter expresses constitutively the gene(s) under its regulation, hence possible expression of the gene (insert) in other tissues than targeted in the patients as well in tissue infected with an accidental exposure.

The notifier explains that the CMV promoter has been chosen because the heart specific promoter did not provide as high of levels of expression of the SERCA2a protein and because pre-clinical studies have shown that after intracoronary administration in large mammals such as pigs and sheep the expression of the transgene is limited to the cardiac region. uncertainty remains regarding the ability of the CMV promoter to activate sequences of rAAV randomly integrated in the host genome and this has adequately been addressed by the notifier in its risk assessment.

3. The condition of release

The risk of unintended release via shedding of viral particles by the treated patient is low, but cannot be excluded. The notifier was asked to clarify the biosafety measures recommended to the patient.
This has been adequately described in the answers of the notifier.

The BAC had several criticisms relating to the instructions prepared for the personnel. The pharmacy manual focuses on preparation and recording and not on biosafety. In the initial dossier no clear information was provided to the health care workers involved in preparing and administering MYDICAR detailing the correct procedures to prevent accidental exposure.
The notifier has been asked to submit amended Personnel Instructions with proper workers protection measures, measures to avoid accidental exposure and accidental release of the GMO and with complete instructions regarding waste handling and the necessity to affix biohazard signs. In addition it was requested to have a synopsis of these instructions for the health care workers to have on hand.
Two new documents were submitted by the notifier on 18 december: Personnel Instructions and a shortened 1-page version of it. The new documents takes most of the above comments into account but could be further improved (see suggestion in annex 1).

4. The risks for the environment and human health

The notifier was asked to give more information regarding the AAV1/SERCA2a potential for insertional mutagenesis and or/possible recombination between the vector and the ATP2A gene.
The notifier agrees that there is a theoretical risk of recombination but it would be limited to dividing cells while cardiomyocytes, target of the treatment, that do not replicate at appreciable levels. However uncertainty remains regarding the risk of vector insertion into the genome of any cell in a patient (or personnel after accidental injection) and this has been addressed in the risk assessment.

The potential presence of adenoviral sequences (see above), even at low frequency could, if expressed, elicit an immune response but it is unlikely that hospital personnel exposed directly or after shedding will be at risk of a significant immune response in normal conditions and if wearing adequate personnel protection equipment. Risk is increased when performing procedures that can cause accidental needle sticks. This is clearly stated in the amended personnel instructions.

The notifier was also requested to evaluate more in depth the risk of re-activation of productive infection. (1) What is the risk in case of co-infection by both wild-type AAV and a helper virus? (2) What is the risk that this non-replicating rAAV starts replicating in the presence of adenoviral helper virus?
The notifier recognizes that these theoretical risks exist but can be considered to be low because it requires for multiple components being present simultaneously within the same cell inside the body. However the precise probability of such events occurring is not known. This has adequately been addressed by the notifier in its amended risk assessment.

Unintended expression of the SERCA2a protein in non-target organs such as lung or liver or in offsprings of the treated patient could have potentially serious adverse effects.
The notifier provided additional data from animal tests where the expression of SERCA2 protein was investigated from an expanded number of tissues. After intra coronary infusion of MYDICAR to minipigs an increased SERCA2 protein expression was only found near the infusion site: cardiac muscle, diaphragm, coronary artery, aorta, pulmonary artery.
The risk assessment has been amended to take into account the risk of vertical transmission to an embryo or foetus. The BAC agrees with the notifier that this risk is correctly managed by the instructions for contraception given to the patients.

Finally, in its initial assessment the notifier did not take into account potential risks for immunocompromised people who come into contact with the patients during the period where virus shedding is highest.
Based on the results of a nonclinical study where the notifier evaluated the levels and safety of AAV1/SERCA2a at approximately three times the clinical dose after intravenous administration in minipigs with and without pre-treatment with immunosuppressive therapy, the BAC agrees with the notifier that there is no particular risk for immunocompromised people who come into contact with the recombinant virus.

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

Even if the risks are low some uncertainty remains (see above). This stresses the importance of biosafety precautions to avoid unintended dissemination of the GMO that could lead to unknown adverse effects.

As requested by BAC the notifier provided precise personnel instructions that details the needed personal protective equipment, measures to avoid accidental exposure, emergency procedure in the event of an accident or a spill completed with a clear and concise plan of action to be followed in the event of a serious accidental exposure, including necessary medical follow up.
A one-page synopsis has been written in order to inform all people involved how to deal with contaminated waste, accidental spills and needle stick accidents etc.
These documents could be further improved as suggested in Annex 1.

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 AAV1/SERCA2a gene transfer medicinal product developed for the treatment of moderate to advanced heart failure, 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 also taking into account the suggestions from the Biosafety Advisory Council for improvement of the personnel instructions and its synopsis.

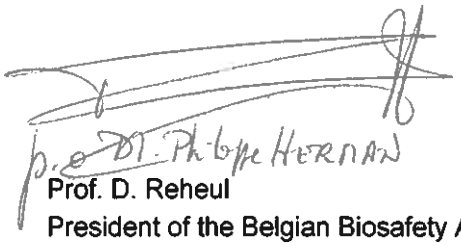
- Any protocol amendment has to be previously approved by the Competent Authority.

- 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.

- 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 AAV .



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

Annex 1: Suggestions for improvement of the Personnel instructions
Annex 2: Compilation of comments of experts in charge of assessing the dossier B/BE/12/BVW2 (ref: BAC_2012_0977)

Annex 1
Suggestions for improvement of the Personnel instructions and its Synopsis

1. Waste handling (point 4, page 3 of the instructions)
To minimize the risk of creating an aerosol by aspirating the concentrated product, the BAC proposes to suppress in the text the proposal to empty the vial by aspiration. Direct disposal of the residual material as biohazard waste should be recommended instead.

2. In the event of injury or exposure (point 7.C., page 4 of the instructions)
Concerning oral and eye exposure, the spit or eye washings must first go into a container containing bleach (final concentration 10%) and only be rinsed down the sink with water after 10 min of disinfection. It would be inappropriate to directly wash virus from the accidental exposure directly into the water supply.

3. Synopsis
The Synopsis should be amended according to the above remarks
In order to clearly indicate the purpose of this document its title should be changed in 'Synopsys of Personnel Instructions for working with MYDICAR'
In this document item 5 should read better 5A to 5F (no subitems of 5D).

Bioveiligheidsraad
Conseil de Biosécurité

22 November 2012

Secretariaat
Secrétariat

O./ref.: WIV-ISP/41/BAC_2012_0977
Email: BAC@wiv-isp.be

Compilation of Comments of Experts in charge of assessing the dossier B/BE/12/BVW2

Mandate for the Group of Experts: mandate of the Biosafety Advisory Council (BAC) of 15 August 2012
Coordinator: Prof. Philippe Hermans
Experts: Aline Baldo (WIV-ISP), Anton Roebroek (KUL), Liliane Tenenbaum (CHUV-Lausanne), Karen Willard-Gallo (ULB), Nicolas Willemarck (WIV-ISP), Willy Zorzi (ULg)
Domains of expertise of experts involved: Virology, AAV, molecular genetics, genetic engineering, design of vectors, in vivo/in vitro experiments, biosafety, contained use, workers protection
Secretariat (SBB): Didier Breyer, Martine Goossens, Philippe Herman, Katia Pauwels

INTRODUCTION

Dossier **B/BE/12/BVW2** concerns a notification of the company CELLADON 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 16 October 2012 and concerns a clinical trial with MYDICAR® a recombinant AAV vector genetically modified to express the protein SERCA2a. This GM-medication is intended for intracoronary administration and is developed for the treatment of patients suffering severe heart failure.

♦ 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 22-11-2012 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

Adeno Associated Viruses (AAV) are not pathogenic, have low immunogenicity and good stability. For this reason they are of wide interest as gene therapy vectors with many currently being used in approved clinical trials. Numerous studies have shown that for stable cardiac gene transfer AAV is a safe and clinically relevant delivery system.

Comment 2

Has evaluated this item and has no questions/comments.

Comment 3

Has evaluated this item and has no questions/comments.

Comment 4

Recombinant Adeno-associated virus (AAV) vectors have been commonly used in human clinical trial. Wild-type AAV is a non-pathogenic virus for humans. Despite the high seroprevalence of AAV in the human population, the virus has not been linked to any human illness.

Comment 5

Has evaluated this item and has no questions/comments.

Comment 6

As mentioned by the applicants (page 10 of « Environmental Risdk Assessment for Mydicar…. »), DNA sequences from helper plasmids or adenovirus can be encapsidated during the rAAV production (Chadeuf G, Mol Ther. 2005 Oct;12(4):744-53). The applicant reports a low but nevertheless significant presence of rep cap and antibiotic-resistance sequences (3 to 4 logs lower than than the SERCA2a sequence).
-The presence of capsid sequences (cap gene or fragments of cap gene) even in a low percentage of transduced cells may elicit a cytotoxic lymphocyte response against the capsid (Li et al., J.Virol. 2007, vol. 81p. 7540–7547). The previous clinical trials with AAV2/1-SERCA2a however suggest that anti-capsid CD8+ lymphocytes appeared only in some patients and were asymptomatic. Therefore it is

very unlikely that the low doses of virus to which the hospital personal will be exposed directly or after shedding could present a risk. Strategies to eliminate capsid sequences have been proposed but were not used in this application (Halbert CL et al., Gene Therapy (2011) 18, 411–417).

-The presence of adenoviral sequences, even at low frequency could, if expressed, elicit an immune response. The applicants mention that they monitored the presence of adenoviral E2a DNA by qPCR but do not provide the results (page 10). Page 8, the applicants mention that the batch was clear of adenoviral sequence based on qPCR detection of E2A. Since wild-type adenovirus was used, it would be worth monitoring more than one sequence. Did the applicant test adenoviral sequences other than E2A, e.g. structural genes which are likely to be strongly inflammatory in human ?

Since no major immune or inflammatory reaction was reported in patients infused with 10^{13} viral particles, it is unlikely that hospital personal exposed directly or after shedding will be at risk of a significant immune response in normal conditions. However, in case of accidental exposure during the dilution or infusion of the virus by spilling or use of sharp instruments, these risks might be worth evaluating. In these cases, the route of accidental administration would either be intravenous or intranasal. Was the rAAV2/1-SERCA2a clinical batch or an equivalent rAAV2/1 batch containing similar amounts of contaminating sequences evaluated in preclinical studies after intravenous or airway administration ?

2. INFORMATION RELATED TO THE VECTOR

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

Comment 1

The vector contains a capsid gene from AAV serotype 1, 3' and 5' cis-acting inverted terminal repeats from AAV serotype 2 and an expression cassette for the SERCA2A gene that includes the cytomegalovirus immediate early enhancer/promoter (CMVie) and a bovine growth hormone polyadenylation signal (BGHpA). Recombinant AAV (rAAV) vectors have been shown to persist largely (but not entirely) in an episomal form, which contrasts with integration in the host genome characteristic of wild-type AAV (wtAAV). Studies found that the highest levels of transgene expression in the heart are achieved with rAAV2/1, although rAAB2/9 is thought to be the most naturally cardiotropic.

The use of wtAAV1 capsid proteins and a CMV promoter (not cardiac specific nor regulated) in MYDICAR may not provide the best means of containing vector delivery to the heart (newer approaches have demonstrated better targeting and control) and should be taken into consideration in terms of the GMO's ability to spread to multiple tissues in vaccinated patients as well its potential widespread dissemination in association with an accidental exposure.

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 vector consists of an AAV serotype 1 capsid and inverted terminal repeats (ITR) from AAV serotype 2. 94% of the wt AAV genome has been deleted. The vector contains no viral coding sequence and cannot replicate under any conditions.

Comment 5

ERA, page 5, top : indicate clearly, that the 6 % is relative to the AAV2 genome and the 0.3 % relative to the CMV genome. Not to be confused with the viral vector genome.

ERA, page 7, 2.2.3. : the non-coding ITRs have a size of 145 bp, as stated earlier.

Annex IIIA, page 13, II.B.3. : the significance as explanation for the non-occurrence of mobilization of 'as there is no homology between the vector and the cell line' is not clear.

Comment 6

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

The characteristics of the CMVie enhancer/promoter should be better characterized in relation to its potential to activate sequences (upstream or downstream) of rAAV randomly integrated in the host genome (while the frequency of integration is thought to be low it is nonetheless present and as such potential effects should be taken into consideration).

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.

Comment 5

Has evaluated this item and has no questions/comments.

3.2. Information on the molecular characteristics of the final GMO

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

Comment 1

Has evaluated this item and has no questions/comments.

Comment 2

The IMP consists of a AAV serotype I capsid, which targets cardiac muscle cells but also skeletal muscle and endothelial cells. The overexpression of the SERCA2 protein in not-heart cells is irrelevant for this study and the effects are not predictable. Nevertheless the notifier preferred to use an ubiquitous CMV promoter instead of a heart-specific promoter? Can the notifier comment this choice of promoter?

Comment 3

Has evaluated this item and has no questions/comments.

Comment 4

Has evaluated this item and has no questions/comments.

Comment 5

Has evaluated this item and has no questions/comments.

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

Again the potential for insertional mutagenesis and/or inadvertent effects by the CMVie on upstream or downstream host genes in patients exposed to rAAV needs to be clearly indicated in the informed consent. As currently stated, it is presented as the SERCA2a gene only that potentially could be inserted. This is misleading since the risk from the CMV promoter may actually be greater than the transgene itself both in patients and healthy individuals inadvertently exposed. Additionally, it is not known which isoforms will be produced either in the target tissue (heart) or secondarily infected organs (lung, liver, skeletal muscle, etc.) in patients or the numerous other organs potentially targeted in healthy individuals depending upon the entry route. Because expression of the human SERCA2a gene is controlled by the CMVie promoter there could potentially be significant differences in its expression (potentially different in various tissues) compared to the endogenous gene. For example,

an abnormal balance in the isoforms expressed in a given tissue could produce undesired effects, including local autoimmune reactions. This issue has not been addressed but should be considered as potentially possible – just because pigs and sheep did not have antibodies to SERCA2a at 90 days (3.1.5.3) does not preclude potential deleterious immune responses in humans.

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 insert is the gene coding for the human SERCA2a protein. The SERCA2a protein may have potential pro-arrhythmic effects (Chen et al, 2004, Circulation; Del Monte, 2004, PNAS). Does the investigator monitor patients for arrhythmia?

Additional comment SBB:
The aspects concerning the safety of the medicinal product for the treated patient are outside the scope of this evaluation but the above remark is pertinent for the safety of healthy individuals inadvertently exposed to the product.

The AAV particles are stable outside the host; the particles are stable in a wide pH range between 3 and 9 and are resistant to heating at 56°C for at least 1h. Given the high stability of AAV particles outside the host and the high concentration of the vector, the presence of even very small amounts of rAAV should be identified and adequate decontamination should be undertaken. The decontamination procedures must specify that bleach must be freshly prepared and it must specify the contact time. It is specified in the pharmacy manual but not in all documents.

Comment 5

Has evaluated this item and has no questions/comments.

Comment 6

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

AAV (wt and rAAV) are resistant to some disinfectants, dehydration and are known to persist in aerosols or aqueous solutions. For this reason it is CRITICAL that clear information be provided to the health care workers involved in preparing and administering MYDICAR detailing the correct

procedures to follow after accidental exposure (see below). In its current form, detailed information is provided for how to disinfect and clean a spill and dispose of any residual virus but there is nothing other than a general First Aid statement for accidental contamination (aerosol, liquid contamination via mucus membranes and the eyes or needle stick injury). There are no personnel instructions other than the pharmacy manual which focuses on preparation and recording and not biosafety.

Comment 2

Has evaluated this item and has no questions/comments.

Comment 3

In the point 5.2, concerning the Investigational Product Preparation, in the CUPID Phase 2b Pharmacy Manual 30 June 2012, all the pictures describing the handling show that handling is performed without gloves. Is it normal?

Comment 4

Personal protective equipment include glasses, gloves, lab coat, shoe cover but should also include a face protection (mask) to protect the mucosa in case of projection because the primary modes of transmission of the AAV1 are inhalation of aerosol and contact with mucous membrane.

Emergency procedures in case of accident (inhalation of aerosols, contact with mucus membranes, injection) must be completed. The workers that have skin, mucous membrane or percutaneous contact with the investigational product must wash the affected areas as soon as possible.
For intact skin: washing with antiseptic soap active towards non-enveloped viruses and water for 15 minutes.
For non-intact skin and needlestick cuts: washing with antiseptic soap active towards non-enveloped viruses and water for 15 minutes.
In case of intra-oral exposure: the worker should spit and rinse the mouth with water, mucous membrane should be flushed with water.
In case of eye projection: washing the eyes abundantly with physiological liquid for 15 minutes using eyewash, remove contact lenses first.

Is the investigational product prepared in the room of the patient? If the IP is prepared outside of the room of the patient, how is the drug transferred from one local to another?

Comment 5

ERA, page 21, 5.4. Post administration : State clearly that the excess investigational product can be poured down a sink only after decontamination.

ERA, page 21, 5.5. Emergence response and **Annex IIIA, page 34 to 36, V.C. Waste treatment and V.D. Emergency response plans :** The Pharmacy Manual describes in detail how to prepare the investigational product and how to deal with and record the destruction of the investigational product. Similarly a Waste Treatment and Emergency Response Procedure Manual and a one-page synopsis of it should be prepared in order to inform all people involved how to deal with contaminated waste, accidental spills and needle stick accidents etc.

Comment 6

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

The patient will be monitored following administration of MYDICAR for discharge of fluid from the catheterization site. However, there is no mention of how long this monitoring will take place and whether it should be extended beyond the specified period if leakage is detected. It also seems reasonable that if there is leakage and adsorbent bandages are used to cover the site when the patient is eventually sent home, that she/he be provided with an appropriate container for disposing of this material as biohazard waste. This container could be sealed and returned to the hospital site for controlled disposal once bandages are no longer required. This would reduce the possibility of inadvertent exposure to concentrated virus by family members or to the environment.

Comment 2

During at least 1 week there will be possible shedding via secretions such as saliva, urine, faeces,… (based on other rAAV trials in cystic fibrosis, lipoprotein lipase deficiency and haemophilia B patients). Although it is clarified that risk of infection via shedding of viral particles is low, it cannot be excluded. In the dossier it is not clear which measures are taken to minimize this risk of infection. Is there a quarantine period in the hospital foreseen? There are procedures to avoid infections via the infected toilet, used kitchen material (plates, glasses, forks, knives, spoons,…), via contaminated bedding and clothes,… ?

Comment 3

In the document Celladon CUPID Phase 2b CELL-004-A1 Clinical Protocol, Final 30 May 2012. About the potential risks of Treatment with MYDICAR®, part 1.3.1.6 Re-Activation of Productive Infection:

« In general, given the advanced patient population, the risks of re-activation of productive infection and adverse events associated with such an event if the subject is infected with both wild-type AAV and a helper virus such as Adenovirus (Ad), herpes simplex virus (HSV), pseudorabies virus (PrV) and human papilloma virus (HPV), is very low. »

Questions :

1) What is the likelihood of the occurrence of such a kind of situation of co-infection by both wild-type AAV and a helper virus ? The herpes simplex virus are for example ones of the most common viruses in humans.

2) Even if rAAV is considered as non-replicating, in the presence of adenoviral helper virus what could be the theoretical likelihood that such an event appears nevertheless ?

In the ERA document : concerning the point 4. Step 4. Estimation of the risk for humans and the environment in the case of a possible release of the investigational product.

The likelihood of the various possible harmful occurring effects is assigned a value (high, moderate, low or negligible). What is the mathematical range of these values of likelihood (for high, moderate, low and negligible) ?

Comment from SBB:
Quantitative evaluation is unlikely to be possible. See COMMISSION DECISION 2002/623/EC of 24 July 2002 establishing guidance notes supplementing Annex II to Directive 2001/18/EC of the European Parliament and of the Council on the deliberate release into the environment of genetically modified organisms and repealing Council Directive 90/220/EEC (OJ L 200, 30.7.2002, p. 22) (pdf)

Comment 4

The viral shedding should be controlled and adequate procedures should be taken during the shedding. Based on other AAV trials in haemophilia B, lipoprotein lipase deficiency and cystic fibrosis patients, viral particle shedding in urine, saliva, blood and semen is expected to at last for about 1 week.
The risk of horizontal dissemination of rAAV appears to be quite low but worth close monitoring in human trials (Tenenbaum et al., 2003, Current Gene Therapy). Virus vector shedding could lead to horizontal transmission to hospital staff, the family,...

Delivery of AAV vectors through the systemic circulation in humans has resulted in contamination of semen DNA with vector sequences. The risk in this setting is that, if the vector integrates into germ cells, the integration event may disrupt the highly ordered sequence of gene expression and repression events that characterize normal embryonic and fetal development, resulting in fetal malformation or death (High and Aubourg, 2011, rAAV human trial experience). The authors suggest that to ensure that men who wish father children after the gene transfer procedure have an uncontaminated source of semen for fertilization; individuals are encouraged to bank sperm prior to vector administration.

Comment from SBB:
Men included into the study are invited to use condom and spermicide at least during the 3 months following the administration of the study drug. This should be long enough to avoid risks related to potential contaminated semen.

Comment 5

Annex III, page 29, bottom : a viral shedding study is planned prior to seeking approval. Please specify further. Is marketing approval meant here? It is indeed important that shedding of AAV1/SERCA2a by treated patients is studied in more detail.

Note from SBB:
YES marketing approval is meant here by the notifier.

Comment 6

The effects of SERCA2a expression in offspring are mentioned to be potentially serious. However, the monitoring that should be made in case of inadvertent pregnancy is not described (to my knowledge). What could be the consequences of ectopic expression of SERCA2A in organs other than the heart and during development?

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

Again a real and unknown danger here is the effects of MYDICAR in healthy individuals (principally health care workers or family members are at risk of exposure). Clearly it must be stated that patients should be sequestered from immunocompromised individuals at the treatment site as well as maintaining a distance from friends and family members in this clinical category for an initial period where virus shedding is highest. This should be highlighted in a Technical Sheet (see below 6.1). The notifier has classified a number of risks as negligible when in reality they are unknown – this should be corrected.

Comment 2

In the MYDICAR_safety_data_sheet are the spill and incident procedures not described into details and sometimes it missing some crucial points, such as contact time when using chemical inactivation. It should also be emphasized if you want to use bleach as disinfectant that it has to be prepared freshly (bleach solutions are not stable). Also the spill procedure (6. accidental release measures) is not complete. It should detail that besides wearing gloves and eye protection also a lab gown and mouth mask is required during the spill procedure. It is recommended to use an effective disinfectant during the absorptions of the spill.

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 detailed 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 GMOs 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 addition, because the product preparation is complicated (see Pharmacy manual) with risk of a splash and needle incidents it is required to wear gloves, lab gown, mouth and eye protection during preparation.

Comment 3

Has evaluated this item and has no questions/comments.

Comment 4

No informations are available for immunocompromised people who come into contact with the patients during shedding? If immunocompromised people have an overexpression of the protein SERCA2a, do they risk arrhythmia?

Comment 5

Has evaluated this item and has no questions/comments.

Comment 6

Page 14 of « Environmental Risk Assessment for Mydicar…. », the applicant mentions that, after intracoronary infusion the "SERCA2a protein is mostly found in muscle tissue (cardiac, diaphragm and skeletal) and is not expressed in organs such as lung or liver".
However, this strong muscle specificity is more related to the route of administration rather than to the tropism of AAV1 serotype. Since the applicant uses a constitutive promoter rather than a cardiac specific promoter (Müller et al., Cardiovascular Res. 73 (2007) 453-462), it is likely that different routes of administration will result in gene transfer to other organs. Indeed, pulmonary gene transfer can be achieved after intranasal delivery of rAAV2/1 (Liqun Wang et al., Mol Ther. 2009 J;17(1):81-7).
Although it is very unlikely that hospital personal exposed directly or after shedding will be at risk of a significant exposure, in case of accidental direct exposure to the viral batch, the risks related to expression of SERCA2A in the airway epithelium should be documented.
Could the applicant document the risks of modifying of calcium-signalling in the lungs?

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.

Comment 5

Has evaluated this item and has no questions/comments.

Comment 6

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

We do not really know what will happen if MYDICAR inadvertently infects a healthy individual through accidental exposure. It seems less likely that the virus will create problems since a multitude of serotypes are present in nature; however, there are potentially adverse effects due to expression of the SERCA2a gene under control of the CMVie promoter. This promoter would not be subject to normal cellular controls or tissue specific microenvironmental factors. This aspect should not be dismissed as negligible, because again it is in reality unknown.

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.

Comment 5

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

See comment under point point 5.1.

Comment 4

Has evaluated this item and has no questions/comments.

Comment 5

Has evaluated this item and has no questions/comments.

Comment 6

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

The notifier informs that insertion is not possible and that there is a negligible risk of recombination with wt virus because the loss of 94% viral genome. What about the homology with the endogenous ATP2A gene (SERCA2) in the genome? Is there an increased risk of recombination with a possible co-insertion of the ITRs and the ubiquitous promoter in the genome?

Comment 3

Has evaluated this item and has no questions/comments.

Comment 4

Has evaluated this item and has no questions/comments.

Comment 5

Has evaluated this item and has no questions/comments.

Comment 6

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

Again, to reiterate, there are potentially adverse effects due to expression of the SERCA2a gene under control of the CMVie promoter where normal cellular controls or tissue specific regulatory factors are different or absent.

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.

Comment 5

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

This is a big shortfall in the notifier's proposal. There is a Pharmacy Manual with detailed instructions for the preparation of MYDICAR prior to administration but there is no Biosafety Manual or Personnel Instructions describing procedures that reduce accidental exposure through spills or needle stick injuries, and what the specific steps are that should be taken in the event of an accident. First Aid is mentioned in the material safety data sheet with a recommendation to wash immediately with plenty of water (WRONG – everywhere else in the dossier the instructions are that residual virus or spills must be carefully decontaminated before disposal). In addition, the emergency response (5.5 in Environmental Risk Assessment) addresses cleaning up accidental spills only. The notifier must provide precise instructions in a separate appendix that details a clear and concise plan of action to be

followed in the event of a serious accidental exposure, including necessary medical follow up. This in particularly important because the effects of expressing the SERCA2a gene (under CMV control) in a healthy individual are not known.

Additionally, a 1-2 pg plasticized Technical Sheet that summarizes precautions, biosafety procedures, decontamination of spills, disposal of contaminated material, and accidental exposure for use in the pharmacy and treatment room is indispensible for the health care workers to have on hand.

Comment 2

Has evaluated this item and has no questions/comments.

Comment 3

Has evaluated this item and has no questions/comments.

Comment 4

High and Aubourg (2011, rAAV human trial experience) suggest that to ensure that men who wish father children after the gene transfer procedure have an uncontaminated source of semen for fertilization; individuals are encouraged to bank sperm prior to vector administration.
Comment from SBB:
Men included into the study are invited to use condom and spermicide at least during the 3 months following the administration of the study drug. This should be long enough to avoid risks related to potential contaminated semen.

Comment 5

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

The notifier has classified a number of risks as negligible when in reality they are unknown – this should be corrected. And it should be clearly indicated throughout that the biosafety precautions are critical to avoid dissemination of the GMO that could lead to unknown adverse effects.

Comment 2

Here is a gap (hiatus) in the study. There is a need to study this aspect more in detail (before marketing can be allowed?) (I think the notifier has planned such a study, isn't it?)

Additional comment SBB:
In its notification dossier the applicant states the following:

"Prior to submission of the Marketing Approval Application for MYDICAR Celladon will conduct a vector shedding study to monitor vector shedding in an open label study. qPCR assay for vector DNA in saliva, buccal swab, urine, and faeces Day 1, Day 3, Day 7; followed by weekly for 1 month, and then monthly for 3 months until there are two consecutive negative results."

Comment 3

Has evaluated this item and has no questions/comments.

Comment 4

Has evaluated this item and has no questions/comments.

Comment 5

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

I found the instructions for decontamination and disposal to be relatively thorough.

Comment 2

What about the shedding waste, see 5.1.

Comment 3

Has evaluated this item and has no questions/comments.

Comment 4

What happens with the shedding waste? Will the shedding waste be decontaminated?

Comment 5

As discussed under item 4.

Comment 6

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

There really are none but they are needed (see above 6.1). Note that the first step in the event of skin contamination is to soak up all liquid with an absorbent paper or other material which is subsequently appropriately decontaminated, then decontaminate the skin (indicate the product and concentration) and only then rinse with water. This is critical for minimizing further exposure to other individuals and release of the GMO into the environment (i.e. through rinsing it down the sink as currently instructed). Eye washing liquids (if eyes are contaminated) should be collected and inactivated before disposal. In the event of a needle stick or sharps accident (including broken vials), specific procedures must be detailed that help to limit dissemination of the virus in the infected individual as well as monitor long term health effects.

Comment 2

In the ERA document : 5.5. Emergency Response
Accidental spills will be cleaned up as follows.
1. Notify others and isolate the area.

We propose this modification: Notify others, exit and isolate the room by prohibiting access to it during 15-30 min, time necessary to eliminate aerosol (by sedimentation or by capture through HEPA filtration if possible).

Comment 3

They should have a spill kit in case of accidental release of the investigational product. The spill kit should contain appropriate disinfectant, personal protective equipments, waste containers, absorbent material (such as paper towels) and forceps (to remove broken glass or sharps).

The procedure concerning the decontamination in case of spill should be completed:

- The doors should be closed
- The spill material must be cover with freshly prepared 10% beach solution
- The employee should avoid splashing
- The broken glass or sharps must be pick up with mechanical means. The debris should be deposited into a sharps disposal container. Employee must never pick up sharps directly by hand.
- The disinfectant must be in contact with the investigational product for at least 10 minutes.
- Working from the edges to the centre.
- The area must be re-clean with fresh paper towels soaked with disinfectant.
- Disposable PPE should be discarded in waste containers and non-disposable PPE should be decontaminated.
- The employee should wash hands with soap and water.
- The incident should be reported to the investigator.

Emergency procedures in case of accident (inhalation of aerosols, contact with mucus membranes, injection) should be completed (see Q4).

Comment 4

As discussed under item 4.

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

Has evaluated this item and has no questions/comments.

Comment 6

Has evaluated this item and has no questions/comments.

7. OTHER INFORMATION

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

Comment 1

Overall, the dossier is well presented and supported except for the issues detailed above that need to be specifically addressed by the notifier.

Comment 2

ERA, page 26 : references 42 and 43 are identical.

References

Chadeuf G et al. Evidence for Encapsidation of Prokaryotic Sequences during Recombinant Adeno-Associated Virus Production and Their *in Vivo* Persistence after Vector Delivery. *Molecular Therapy (2005) 12, 744–753*

Chen Y, Escoubet B, Prunier F, et al. Constitutive cardiac overexpression of sarcoplasmic/endoplasmic reticulum Ca2_-ATPase delays myocardial failure after myocardial infarction in rats at a cost of increased acute arrhythmias. *Circulation* 2004.109:1898–903.

Daya S, Berns K. Gene therapy using Adeno-Associated virus vectors. *Clinical Microbiology Reviews*. 2008. 21: 583-593.

del Monte F, Lebeche D, Guerrero JL, et al. Abrogation of ventricular arrhythmias in a model of ischemia and reperfusion by targeting myocardial calcium cycling. *Proc Natl Acad Sci U S A.* 2004; 101: 5622–7.

Halbert CL et al. Capsid-expressing DNA in AAV vectors and its elimination by use of an oversize capsid gene for vector production. *Gene therapy,* 2011 Apr;18(4):411-7.

High K.A., Aubourg P. rAAV human trial experience. Richard O. Snyder and Philippe Moullier (eds.). Adeno-Associated Virus: *Methods and Protocols, Methods in Molecular Biology*. 2011; 807: 429-457.

Kawase Y., Li H., Prunier F., Lebeche D., Shi Y., Jin H., Hadri L. Reversal of cardiac dysfunction after long-term expression of SERCA2a by gene transfer in a pre-clinical model of heart failure. *Journal of the American College of Cardiology*. 2008; 51: 1112-1119.

Li C et al. Adeno-Associated Virus Type 2 (AAV2) Capsid-Specific Cytotoxic T Lymphocytes Eliminate Only Vector-Transduced Cells Coexpressing the AAV2 Capsid In Vivo. J. Virol. 2007, 81, 7540-7547

Liqun Wang R. et al. Recombinant AAV Serotype and Capsid Mutant Comparison for Pulmonary Gene Transfer of α-1-Antitrypsin Using Invasive and Noninvasive Delivery. *Molecular Therapy* vol. 17 no. 1, 81–87

Müller O J et al. Targeting the heart with gene therapy-optimized gene delivery methods. *Cardiovascular Research* 73 (2007) 453–462

Tenenbaum L., Lehtonen E., Monahan P.E. Evaluation of risks related to the use of adeno-associated virus-based vectors. *Current Gene therapy*. 2003, 3: 545-565.