Adviesraad voor Bioveiligheid
Conseil consultatif de Biosécurité

Advice of the Belgian Biosafety Advisory Council on the notification B/BE/21/BVW8 of the company MeiraGTx UK II Limited for deliberate release in the environment of genetically modified organisms other than higher plants for research and development

19/04/2022
Ref. SC/1510/BAC/2022_0437

Context

The notification B/BE/21/BVW8 has been submitted by MeiraGTx UK II Limited to the Belgian Competent Authority in January 2022 for a request of deliberate release in the environment of genetically modified organisms (GMO) 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 entitled "*Phase 3 Randomized, Controlled Study of AAV5-hRKp.RPGR for the Treatment of X-linked Retinitis Pigmentosa Associated with Variants in the RPGR gene*". The investigational medicinal product (IMP) is being developed as a gene therapy product for individuals with X-linked retinitis pigmentosa (XLRP) caused by mutations in the human retinitis pigmentosa guanosinetriphosphatase regulator (*RPGR)* gene (*RPGR-XLRP*). The primary objective of the proposed study is to asses the effect of bilateral treatment of the IMP on retinal function in individuals of 3 years of age or older.

The prevalence of retinitis pigmentosa (RP) is estimated to be 1:3000, with 5-15% of the cases inherited as an X-linked trait. Approximately 75% of the subjects with X-linked RP harbour mutations in the *RPGR* gene. For those latter subjects the experimental gene therapy approach aims at an localized gene augmentation with human RPGR gene, of which it is hypothesized that it will result in the rescue of both rod and cone photoreceptor cells and slow or halt progressive retinal degeneration.

AAV5-hRKp.RPGR is a disabled version of a non-pathogenic wild-type adeno-associated virus (AAV), modified by deletion of the *rep* and *cap* genes rendering it unable to replicate, even in the presence of a helper virus. It is a recombinant AAV carrying the RPGR gene, flanked with AAV2 inverted terminal repeats and it is encapsidated within AAV5 capsid proteins.

Overall, approximately 60 patients will be included in this Phase III study, with 10 expected to be enrolled in Belgium. AAV5-hRKp.RPGR will be administered as a bilateral treatment, with the second eye treated 7 to 21 days after the first, either with a 2.0×10^{11} vector genomes/eye dose (low dose) or a 4.0×10^{11} vector genomes/eye dose (intermediate dose). The IMP will be injected into the subretinal space following a standard surgical vitrectomy. Vector shedding will be monitored at several time points after administration utilizing qPCR.

This study will be conducted at one clinical sites located in Flanders. The national territory is considered as the potential release area of AAV5-hRKp.RPGR.

The dossier has been officially acknowledged by the Competent Authority on 28 January 2022 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. Three 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. 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 (GMO) 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 the 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.
- Good practice document on the assessment of GMO related aspects in the context of clinical trials with AAV clinical vectors developed by the national competent authorities and the Commission services available at https://ec.europa.eu/health/sites/health/files/files/advtherapies/docs/aavs_gp_en.pdf

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

On 4 March 2022, based on a list of questions prepared by the BAC, the Competent Authority requested the notifier to provide additional information about the notification. The answers from the notifier to these questions were received by the Competent Authority on 28 March 2022 and transmitted to the secretariat of the BAC on the same day, after which the BAC was able to come to a conclusion with respect to the environmental aspects associated to the proposed clinical trial.

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 above-mentioned Royal Decree. The Competent Authority received no reactions from the public that are related to biosafety issues.

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

Upon assessment of the viral vector production system, the BAC asked the notifier to provide further clarification on the description of a polyadenylation signal. In its answer the notifier updated this information in the documents (SNIF, CAF documents) thereby improving consistency throughout the text. The BAC had no further remarks with respect to the molecular characteristics of the vector.
Other information regarding the molecular characteristics of AAV5-hRKp.RPGR was found to be adequately described in the dossier.

3. The conditions of the release

The current MGT-RPGR-021 study consists of four cohorts assigning participants either to an immediate bilateral treatment with 2.0 x 10^{11} vector genomes/eye dose, an immediate bilateral treatment with 4.0 x 10^{11} vector genomes/eye dose, or to a deferred bilateral treatment with the same low or high dose. Patients randomized in both immediate bilateral treatment cohorts, will receive AAV5-hRKp.RPGR treatment on Day 1, whereas patients randomized in both deferred bilateral treatment cohorts will receive the AAV5-hRKp.RPGR treatment after 12 months of participation in the study MGT-RPGR-021 (control group), as part of the long-term extension study MGT-RPGR-022. Pediatric participants (<18 years of age at screening) will be randomized in separate pediatric-only cohorts in the same ratio as adult participants. For all patients the bilateral treatment consists in a subretinal injection of AAV5-hRKp.RPGR with the second eye treated 7 to 21 days after the first.

Vector shedding will be monitored by qPCR AAV vector genome detection at several time points after administration. For the proposed study MGT-RPGR-021, shedding data will be collected from lacrimal fluid (tear) from both eyes, saliva, whole blood and serum samples on each day of surgery, post-op 1 Day 1, Day 4, W1 (optional), post-op 2 Day1 and Day 4.

According to the information provided by the notifier all involved personnel on the sites during preparation and administration are required to wear standard hospital protective equipment including coats and gloves. The preparation of the IMP for administration is recommended to be conducted in a biological safety cabinet. The BAC remarks that the use of goggles and mask are mandatory should the use of a biosafety cabinet not be possible (e.g. handling of spill incident, during product administration to the patient).

The notifier was also asked to improve the informed consent forms for patient, patient's family and caregivers in order to minimize potential transmission of the viral vector to other people or to the environment when patients are leaving the hospital setting. The notifier met the request by providing a take-home summary focusing on the handling of eye pads as outlined in document MGT-RPGR-021_22 ICF Addendum Take home summary_V1.0_18Mar2022 . The BAC remarks that patients should also be informed on the possibility that vector genomes may also be present in saliva of the patients after administration of the IMP.

4. The risks for the environment or human health

AAV5-hRKp.RPGR is a recombinant, replication deficient adeno-associated virus-based vector not harbouring any antibiotic or other resistance genes. Like its parental virus strain, it is not known to be

pathogenic. The genetic modification introduced in this AAV2/5-derived vector does not confer the GMO with properties that could confer risks to the human population or the environment.

There is only a remote possibility of homologous recombination between the ITR-sequences of AAV2 in the IMP wild-type AAV in case a triple infection by AAV5-hRKp.RPGR, wild type AAV (providing the rep and cap functions) and a helper virus occurs in exposed persons. 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 current hRKp.RPGR transgene. Moreover, the genetic material from *rep* and *cap* genes together with the hRKp.RPGR transgene would be too large in size to be packed in AAV capsid, making it impossible to form a replication competent viral particle that would contain the transgene and the *rep* and *cap* genes necessary for multiplication.

In the case of transfer of vector to an unintended immune-competent human recipient, the risks are expected to be considerably reduced as compared to any potential risk for the participant, since the vector is not able to replicate and the 'dose' that may conceivably be transferred (from e.g. aerosol, splashing or fomites) will be orders of magnitude lower than that received by patients. Worst case, the receiver will develop an immune respons to the AAV5 capsid.

Shedding data collected form the ongoing MGT009 study and the proposed MGT021-22 study will further contribute to a proper environmental risk evaluation. These shedding data will need to be evaluated in light of the observed quantity of shed viral material, and the period of time during which shedding is observed. Should qPCR analysis reveal detectable presence of vector genome, it will be important to answer the question whether the observed shed viral vector genome consists out of functional replication-deficient viral vector particles.
Upon BAC request and pending completion of a clinical study report from study MGT009, which will be available late Q2 2022, the notifier provided interim results of shedding data collected from lacrimal fluid, saliva and serum. On the basis of these interim results, the BAC considers the choice of time points and the type of samples for analysis of shedding during the proposed MGT-RPGR-021 study appropriate but remarks that it would be relevant to continue testing until two consecutive results are at or below the limit of detection as determined for each of the matrices.

The route of administration could theoretically lower the potential of germline transmission of AAV5-hRKp.RPGR, if any occurs. Given the advanced categorization of the proposed trial (phase III) and in view of potential marketing application, the notifier was requested to provide an assessment of germline transmission to be included in the CAF. Non-clinical reproductive toxicology data were conducted in mice and rabbits, of which the BAC is of the opinion that results are not conclusive to assess germline transmission owing to the difference in dose and the fact that AAV are very species specific. It is noticed that, despite the difference in AAV capsid (this study AAV2/5), clinical data studying the presence of AAV vector genomes in semen in a human AAV2/2_hRPE65 study for the treatment of LCA2 (Bainbridge *et al.*, 2008) could not detect AAV particles in semen at D30 post treatment by qPCR. Taking into account the subretinal route of administration, the transient biodistribution, the non-integrating properties of the viral vector and the request to participants, who are fertile and sexually active, to use barrier and spermicide contraception for at least 6 months following treatment, the BAC can support the conclusion on AAV5-hRKp.RPGR low risk of germline transmission.

The BAC concludes that, based on the non-pathogenic and non-replicative nature of AAV5-hRKp.RPGR and the assumed lower amounts of shed and intact viral particles of AAV5-hRKp.RPGR

as compared to the therapic dose, the overall risk associated to exposure and transmission to other individuals can be considered negligible.

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

While non-replicating, it is anticipated that AAV5-hRKp.RPGR, like any other AAV, is stable in a wide pH range (3-9) and like other non-enveloped viruses, is quite resistant to alcohol disinfectants. Following a remark of the BAC with respect to the disinfection, decontamination, waste treatment and procedures for the management of accidental spills, the notifier provided an update of the document Safety handling statement with detailed instructions for clean-up procedures depending on the properties of the working surfaces.

Given the assessment of the likelihood of further propagation of AAV5-hRKp.RPGR, the BAC supports the view that, in terms of risk for the environment or human health, the proposed measures as described in the revised documents are proportionate and adequate in the context of the intended trial provided that the additional requests as outlined in the conditions here below are met.

Conclusion

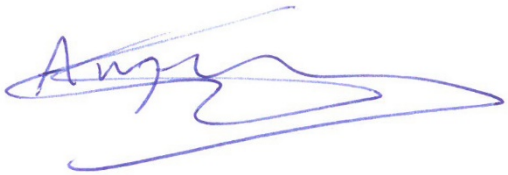
Based on the scientific assessment of the notification made by the Belgian experts, the Biosafety Advisory Council concludes that it is unlikely that AAV5-hRKp.RPGR developed as a gene therapy approach for the treatment of X-linked Retinitis Pigmentosa Associated with Variants in the *RPGR* gene, will have 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 as described in the following updated documents (and for some still to be adapted in accordance with the conditions stipulated below):

- 3.1.MGT-RPGR-021_GMO_AAV_CAF_clean_17Mar22
- 4.1.021_022_GMO_AAV_CAF confidential annex_clean_17Mar22
- 6.0 Safety statement AAV v1.4 BE-15Mar22 (Biosafety Instructions for handling AAV5-hRKp.RPGR for health care workers and staff) – *to be updated in accordance with condition 2*
- 2020-002873-88_MGT-RPGR-021_Protocol_amend 5_15JUL2021- *to be updated in accordance with condition 4 here below*
- MGT-RPGR-021_22 ICF Addendum Take home summary_V1.0_18Mar2022-version ENG_DUT_ and FRE - *to be updated in accordance with condition 5 here below*

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

1. The notifier and the investigators must strictly apply the clinical trial protocol and the safety instructions as described in the dossier and the updated and new documents listed here above.
2. With respect to the Biosafety Instructions for handling AAV5-hRKp.RPGR for health care workers and staff, it should be indicated that the preparation of the IMP for administration is recommended to be conducted in a biological safety cabinet. The use of goggles and mask are mandatory should the use of a biosafety cabinet not be possible (e.g. handling of spill incident and administration of the IMP to the patient).

3. The notifier takes due account of its commitment to report shedding data obtained from the ongoing (study MGT009) and planned clinical trials in view of any further step in the clinical development of AAV5-hRKp.RPGR.
4. The notifier should continue the collection of shedding data until two consecutive results are at or below the limit of detection as determined for each of the matrices. The notifier is required to update the protocol for study MGT-RPGR-021 accordingly. In addition, the notifier is requested to consider the collection of shedding data on lacrimal fluid (tear) from both eyes, saliva, whole blood and serum samples for study MGT-RPGR-022 as well.
5. The notifier should indicate in document MGT-RPGR-021_22 ICF Addendum Take home summary_V1.0_18Mar2022_version ENG_DUT_and FRE that the saliva from patients treated with AAV5-hRKp.RPGR may also contain viral vector genome.
6. Any protocol amendment has to be previously approved by the Competent Authority.
7. The notifier is responsible to verify that each study centre has qualified personnel experienced in handling infectious material and that the investigator has the required authorisations to perform the clinical trial activities inside the hospital (laboratory, pharmacy, hospital room, consultation room...) according to the Regional Decrees transposing Directive 2009/41/EC on the contained use of genetically modified micro-organisms.
8. The BAC should be informed within two weeks when the first patient starts the treatment and the last patient receives the last treatment.
9. At the latest six months after the last visit of the last patient included in the trial, the notifier must send the competent authority for the attention of the BAC a report with details concerning the biosafety aspects of the project. This report shall contain at least:
    a. The total number of patients included in the trial and the number of patients included in Belgium;
    b. A summary of all adverse events marked by the investigators as probably or definitely related to the study medication;
    c. A report on the accidental releases, if any, of AAV5-hRKp.RPGR.



Prof. Dr. ir. Geert Angenon
President of the Belgian Biosafety Advisory Council

Annex I: *Compilation of comments of experts in charge of evaluating the dossier B/BE/21/BVW8 (ref. SC/1510/BAC/2022_284 and SC/1510/BAC/2022_0421)*

Adviesraad voor Bioveiligheid
Conseil consultatif de Biosécurité

Compilation of comments of experts in charge of evaluating the dossier B/BE/21/BVW8 And comments submitted to the notifier

04 March 2022
Ref. SC/1510/BAC/2022_0284

Mandate for the Group of Experts: Mandate of the Biosafety Advisory Council (BAC) of 07 January 2022.
Coordinator: Rik Gijsbers (KULeuven)
Experts: Anton Roebroek (KULeuven), Liliane Tenenbaum (Lausanne University Hospital), Willy Zorzi (ULiège)
SBB: Katia Pauwels

INTRODUCTION

Dossier **B/BE/21/BVW8** concerns a notification from MeiraGTx UK II Limited for the 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 28 January 2022 and concerns a clinical trial entitled "*Phase 3 Randomized, Controlled Study of AAV5-hRKp.RPGR for the Treatment of X-linked Retinitis Pigmentosa Associated with Variants in the RPGR gene*". The investigational medicinal product is a AAV5- derived recombinant replication deficient vector carrying the human RPGR gene.

♦ 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 04-03-2022 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.

List of comments/questions received from the experts

2. INFORMATION RELATED TO THE INVESTIGATIONAL MEDICINAL PRODUCT

2.1. Description of the production system

(e.g. maps of the vectors used, characteristics of the cell lines used, possibility of complementation or recombination….)

Comment 1

Document CAF confidential annex (page 3/24) states mistakenly, that RNA transcription from the transgene plasmid is replicated and packaged: "RNA transcribed from the transgene plasmid, pAAV5-hRKp.RPGRk, is replicated and packaged into vector particles that are replication-incompetent." RNA transcription from the transgene plasmid **is not part** of the process generating vector genomes for packaging!

Document CAF confidential annex describes for plasmid pAAV5-hRKp.RPGRk details about a polyadenylation signal described (wtpA + MZ polyA, e.g. table 1, page 7/24). This polyadenylation signal will also be present in the clinical vector. The use of the addition '+MZ' is suggestive for addition of sequences to wtpA sequences. The (functional) significance of this '+MZ' should be explained.

SBB comment

For the sake of accuracy, the notifier could indeed be asked to correct the erroneous statement in the confidential annex about RNA transcription.
Of note, in the non-confidential CAF, on p8/28, as well as in the IB (p64/85), the notifier states that '*Production of the vector in the manufacturing process and second-strand synthesis of the vector genome rely on the host DNA polymerase, characterised by high fidelity DNA polymerisation and additional proofreading exonuclease activity, leading to very low error rate of DNA replication.*' No mention is made about RNA transcription in these documents.

A question on the polyadenylation signal has also been raised by a second expert (see comment 2 below). See also text proposal for a question to be addressed to the notifier under comment 2.

Coordinator comment

The expert is correct. This is an error in the text, and it would be best to correct this error. In the SNIF file p11/17 point2 the applicant does provide the correct description as also stated by SBB.

In the SNIF file and throughout the document, some essential wording is wrong. Also in the SNIF file it is stated that "between the ITRs an expression cassette to deliver a functional transgene encoding the human RPGR gene has been inserted". This should be "transgene (cDNA) encoding the human RPGR protein". Also later in SNIF point 6(a) p8/17 this error is included.

Comment 2

We are lacking information about the polyadenylation sequence present in the vector carrying the transgene.
Furthermore, the definition of the polyA sequence provided is sometimes wrong. Does it indicate that in this sequence, another element distinct from the polyA itself enhances transgene expression? If this is the case, this element should be described.
File 2.1 MGT.RPGR-021-GMO….:
Page 5: A polyadenylation signal does not "enhance gene expression". It is necessary for termination of messenger RNA transcription.
Page 7: What is "wtpA + Mz" ?
see also file 3.1… page 3 and file 11 page 2.
In File 11. P2 it is described as "a derivative of SV40 poly adenylation signal" . The fragment(s) of the SV40 genome which have been introduced in the vector should be described as well as their known function (if any) in addition to being a polyadenylation signal.

Coordinator comment

In the SNIF file the polyA signal in the AAV construct is indicated as originating from SV40, as is the intron (SNIF p8/17). The applicant indicates that gene transcription is enhanced by inclusion of pA, this is incorrect (agreed with expert's comment 2).
Even though this is not strictly ERA related to me, this information is scientifically not correct and gives a careless impression. I would suggest clarifying this point.

SBB comment

Referring to experts' comments 1 and 2 the following question could be addressed to the notifier :

The polyA sequences are necessary for termination of messenger RNA transcription and not to enhance transcription as indicated on p5 of the CAF, non-confidential part (document 2.1. MGT-RPGR-021_GMO_AAV CAF_11jan22_clean).
However, from the information provided in the non-confidential CAF at p5 '*A polyadenylation signal that enhances gene expression*'; p7 ' wtpA+Mz PolyA: Polyadenylation sequence' as well as information in Table 1 from the confidential part of the CAF as well as on p2 of the IMPD '*a derivative of SV40 poly adenylation signal'*, and the info in the SNIF file (p8/17), it is not clear which fragments of the SV40 genome have been introduced in the vector and what is their known function (if any) in addition to being a polyadenylation signal. The notifier is requested to provide further clarification.

Comment 3

Has evaluated this item and has no questions/comments.

2.2. Demonstration of absence of formation of replication-competent virus

(e.g. assessment of risk of generation of replication competent AAV, test methods and test data, ….)

Comment 1

Has evaluated this item and has no questions/comments.

Comment 2

The vector plasmid expressing the transgene has a polyadenylation signal that is not fully described, although its sequence is provided (see 2.1).

Furthermore, for the encapsidation plasmid described in File 3.1_AAV CAF page 4, no polyadenylation signal is mentioned for the termination of the Rep2 and Cap2 transcripts. Was the AAV5 polyA used? Or the AAV2 polyA? Or an exogenous polyA? If it is the case, is there a sequence homology between the SV40 polyA of the vector plasmid and the polyA of the encapsidation plasmid which could recombine and contribute to the generation of rc AAV?

The sensitivity threshold for the percentage of rcAAV seems high (this is always the case for this kind of tests; see (Allen JM, et al. 1997. Identification and elimination of replication-competent adeno-associated virus (AAV) that can arise by nonhomologous recombination during AAV vector production. J Virol 71:6816-22; see also: https://www.fda.gov/media/151599/download, pages 25-26). In the present application it has been evaluated as < 20 IU in 5.0 10e8 vg, thus approx. <2 10e4 per ml of the viral vector batches (page 17 of file 3.1).
Homologous regions between the 3 plasmids used for viral vector production should be avoided. Although it is still controversial, wild-type AAV has been related to hepatocarcinoma incidence (see: La Bella T, *et al*. 2020. Adeno-associated virus in the liver: natural history and consequences in tumour development. Gut 69:737-747; see also : Schäffer *et al*. 2021 Integration of adeno-associated virus (AAV) into the genomes of most Thai and Mongolian liver cancer patients does not induce oncogenesis. BMC Genomics 11;22(1):814).

SBB comment

The first remark has been implemented in the proposed question under section 2.1, comment 2.

Referring to the expert's second comment, the following question could be addressed to the notifier :

From the maps of the plasmids used for the clinical vector production AAV5-hRKp.RPGR, described in the confidential part of the CAF document, no polyadenylation signal is mentioned for the termination of the Rep2 and Cap5 transcripts. The notifier is requested to clarify which polyadenylation signal has been used. Furthermore, the notifier is asked to discuss any potential sequence homology between the SV40 polyA of the vector plasmid and the polyA of the encapsidation plasmid, both in the light of the relatively high sensitivity threshold for the percentage of rcAAV (p17 of confidential part of CAF document) and taking into account potential recombination events and possible generation of replication competent AAV.

Coordinator comment

I do not understand why this would be an issue. If recombination would occur, the construct would contain only a single ITR. We could ask for clarification of the pA signal in this construct, but it seems trivial to me that is the natural pA present in Cap5. As far as I know, we also did not inform for this detailed info in other dossiers (see for example B/BE/21/BVW3 in 2021 where this info is also not provided for the packaging plasmid).

I would not include the above mentioned comment.

Remark :
Part of the misconception originates from the fact that vector is used as 'plasmid' and 'viral vector'. For clarity, I would use plasmid when possible, and viral vector when addressing the rcAAV viral vectors

With respect to the expert's comment: encapsidation plasmid described in the document mentions Cap5 transcripts

SBB comment

With respect to the expert's third comment on possible relation between wild-type AAV and hepatocarcinoma incidence it is acknowledged that observations of tumor formation and the potential of AAV vectors to integrate into the host cell genome warrants further investigation. It can be considered that these events fall within the assessment of patient's safety considerations. From an environmental risk assessment point of view, possible hazards associated to relative high amounts of the drug product administered to patients should be balanced against the relative low likelihood of accidental exposure of non-target individuals to such high doses of the drug product (during preparation of an injection sample for example).

Coordinator comment

I agree with the SBB. The comment of the expert holds, indeed, recombination should be avoided, especially when retroviral vectors are concerned. In the case of rcAAV, the resulting virus will still be a dependo-virus, and will require additional helper virus infection to be taken place together with the recombination event, since recombination alone is not sufficient. In addition, seroepidemiology shows that infections with adeno-associated virus (AAV) are widespread (34% of healthy blood samples were AAV positive), but diverse AAV serotypes isolated from humans or nonhuman primates have so far not been proven to be causes of human disease (https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5286889/).

Comment 3

Has evaluated this item and has no questions/comments.

2.3. Diagram (map) of the clinical vector

Comment 1

Has evaluated this item and has no questions/comments.

Comment 2

See above: description of the polyA sequence.

Comment 3

Has evaluated this item and has no questions/comments.

2.4. Molecular characterisation of the clinical vector

(e.g. annotated sequence of the genome, genetic stability, ….)

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.

2.5. Description of the insert

(e.g. description of the expression cassette, potential harmful properties of the transgene, ….)

Comment 1

See 2.1. Remark about '+MZ'.

Comment 2

Has evaluated this item and has no questions/comments.

Comment 3

Has evaluated this item and has no questions/comments.

2.6. Biodistribution and shedding

(e.g. shedding data, administered dose, route of administration, biodistribution data, methods used for detection of viral shedding….)

Comment 1

The documents CAF (page 10-11/28) and CAF confidential annex (page 23/24, Annex 5) describe biodistribution and shedding of the viral vector in animal studies. Shedding to the environment (tear fluid) was not discussed in the CAFs, although relevant information on shedding into tear fluid is described in document '2020-002873-88_MGT-RPGR-02_IB_Version 5_18FEB2021'. According to the SNIF, shedding of AAV5-hRKp.RPGR from clinical trial participants to the environment is expected to be none to minimal based on the intraocular route of administration. All this information should ideally have been included in de CAF itself also. Anyhow, the clinical trial includes the analysis of shedding by the patient into lacrimal fluid (tear) from both eyes and saliva in order to confirm the assumption that based on the intraocular route of administration shedding of AAV5-hRKp.RPGR into the environment is indeed not an environmental safety issue.

SBB comment

Related to the expert's comment, p25 of the protocol describes timepoints at which qPCR for genome detection will be performed : post-op D1, D4, W1, US, post-op2 D1 and D4 , US and W4. The notifier could be requested to confirm this information by including it in the CAF as well.

Additional SBB comment

According to the document CAF (p12/18), data on biodistribution and viral shedding are currently being collected, as part of human study MGT009.

The collection of shedding data is encouraged as these contribute to a proper environment risk evaluation. Ideally, these data should be evaluated considering the observed quantity of shed viral vector material, the period during which shedding is observed. It will be important to answer the question whether the observed shed viral genome material is only vector DNA or a remnant thereof, whether or not present in shed cells, whether it reflects integrated vector DNA in shed cells or whether it consists of remaining or rescued replication-deficient viral vector particles.

Therefore, the notifier could be requested to detail the methodology that was used for collecting shedding data as part of the MGT009 study by indicating the number of samples taken, the nature of the samples analyzed, the test method (e.g. infectivity assay, nucleic acid detection), the limit of detection and the limit of quantification. Considering that shedding data from the human study MGT009 may have been collected since the time of notification of the current biosafety dossier, the notifier is encouraged to reveal any data in this regard, should these be available. In particular, the notifier could be asked to further confirm/justify the time points of sampling proposed for MGT021 considering shedding data that may be available for the human study MGT009.

Additional SBB comment

On p 67 of Investigator's Brochure (IB), section 6.4.6. it is stated that no studies of developmental or reproductive toxicity for AAV5-hRKp.RPGR have been conducted to date. It is also indicated that reproductive and developmental non-clinical toxicity studies are not planned owing to the nature of the gene therapy product under development. Indeed, the provided analysis of biodistribution data do not report on the absence/presence of vector genome in reproductive organs. In the same section of the IB, the notifier briefly assesses the risk of germline transmission by referring to other studies involving intra-ocular injection of rAAV vectors as compared to systemic delivery of higher dosis of rAAV. This is information that could have been included in the CAF document as well.

Although the route of administration could theoretically lower the potential of germline transmission, no assessment of germline transmission has been provided in the CAF document. Given the advanced categorization of the proposed trial (phase III) and in view of potential Marketing application, the notifier could be requested to provide an assessment of germline transmission to be included in the CAF and to further justify its approach of not pursuing studies of developmental or reproductive toxicity.

Coordinator comment

I agree that given the advanced nature of the trial, germline transmission assessment should be discussed, and the risk should be assessed in the CAF. On the other hand, the subretinal injection in the eye is not readily expected to result in significant doses of clinical AAV vector in the gonads. I presume information will be available that can justify their approach.

Comment 2

Has evaluated this item and has no questions/comments.

Comment 3

p58 of CLINICAL INVESTIGATOR'S BROCHURE (10. 2020-002873-88_MGT-RPGR-02_IB_Version 5_18FEB2021) it is written that: *Overall, safety data obtained to date (cutoff date of 2 November 2020) suggest that a single, unilateral subretinal injection of AAV5-hRKp.RPGR is generally well tolerated.*
Following the significant incidence of AE = adverse event and SAE = serious adverse event in the different groups of treated patients (reported in Table 13), is it pertinent to classify this treatment-emergent as « generally well tolerated » ?
Could the notifier clearly describe what is meant by « generally well tolerated ». Is it in relation (perhaps) with the fact that no patient died during the treatment (as reported at p60 of the document)?

SBB comment
p58 of the investigator's brochure details the incidence and the nature of the observed adverse events and serious adverse events. The expert's remarks relate to patient's safety considerations (note that some of the adverse events have been related to the surgical procedure) and go beyond the scope of the environmental risk assessment of AAV5-hRKp.RPGR.

Coordinator comment
I agree with the SBB that this is not ERA related.

3. INFORMATION RELATED TO THE CLINICAL TRIAL

3.3. Storage of the clinical vector at the clinical site

(e.g. storage location, conditions of storage, …)

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.

3.4. Logistics for on-site transportation of the clinical vector

(information on logistics of in-house transportation, characteristics of the container, disinfection procedures, labelling of the containers, ...)

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.

3.5. Reconstitution, finished medicinal product and administration to the patients

(e.g. mode of administration, information on dosing and administration schedule, information on concomitant medication,…)

Comment 1

The patient will be able to leave the hospital on the same day as surgery. However, he/she will not be able to drive / travel by him / herself as his / her eye will be covered by an eye pad overnight and the surgery is expected to cause temporary blurring of sight, and some discomfort (info in ICF). With respect to the eye pad, it is not clear whether the pad will be removed the day after at the follow-up visit (info about first follow-up visit from protocol) or already earlier by the patient at home. In case of the first option, the patient will not be able to drive by him / herself to the location of the follow-up location! This should be stated in the info for the patient in the ICF. In case of the second option, the patient should get clear instructions how to dispose properly of the pad.

SBB comment

The informed consent form could indeed benefit from sufficiently detailed instructions for the patient, patient's family and caregivers in order to avoid potential transmission of the viral vector to other people or to the environment, if any, when patients are leaving the hospital setting.

In order for patients to adhere to these instructions, it is important to explain why measures are taken and what are the likely sources of contaminated material. The notifier could provide a small take home summary (preferably one-page, plasticized document) to insure that the patients can easily consult the information in a understandable format whenever needed.

As part of the instructions given to patients, the notifier could be requested:

- To mention which bodily fluids are anticipated to contain viral vector genome
- To explain at which time points eye pads may be removed and how these eye pads should disposed of.
- To provide instructions aimed at limiting contact with materials or surfaces frequently contaminated with bodily fluids (e.g. handkerchiefs, used eye pads).
-

Comment coordinator

In addition:

- Provide detailed protocol(s) and suggest effective solutions to decontaminate possible contaminated areas, tissues, skin, …

(see also next expert's comment 1 under section 3.6)

Comment 2

Has evaluated this item and has no questions/comments.

Comment 3

Has evaluated this item and has no questions/comments

3.6. Measures to prevent dissemination into the environment

(e.g. control measures, PPE, decontamination/cleaning measures after administration or in the case of accidental spilling, waste treatment, recommendation given to clinical trial subjects, …)

Comment 1

Document Safety handling statement should describe in section **Disinfection, decontamination, waste treatment** (page 3/4) which virucidal agent next to 1% sodium hypochlorite could be used and state clearly that in case of AAV and AAV-derived vectors, like for other non-enveloped viruses and vectors, decontamination with ethanol is not effective.
In order to help health care personnel, the notifier is asked to provide the above-mentioned Safety handling statement as a plasticized document.

SBB comment

Referring to the expert's comment and taking into account one of the expert's comment 3 here below, the following question could be addressed to the notifier :

The Document Safety handling statement should detail in section **Disinfection, decontamination, waste treatment** (page 3/4) which virucidal agent next to 1% sodium hypochlorite could be used and state clearly that in case of AAV and AAV-derived vectors, like for other non-enveloped viruses and vectors, decontamination with ethanol is not effective.
Since contaminated work surfaces may have different properties: porous and nonporous materials, stainless steel, solid surface, floor, or table the notifier could be requested to identify the best disinfectant for each type of contaminated surface or solution. Clean-up procedures should specify minimum contact time for each of the proposed decontamination solution.
To help health care personnel, the notifier is asked to provide the above-mentioned Safety handling statement as a plasticized document.

Comment 2

Has evaluated this item and has no questions/comments.

Comment 3

Considering the 3 following points:

1) In p68, point 6.6.1 List of excipients of CLINICAL INVESTIGATOR'S BROCHURE (10. 2020-002873-88_MGT-RPGR-02_IB_Version 5_18FEB2021) :
-For Phase III development, AAV5-hRKp.RPGR gene therapy vector particles are suspended in buffer composed of sodium chloride, tromethamine, HCl, trehalose, ***Poloxamer 188****, and WFI, at pH 7.5.*

2) In p 15 – non confidential CAF document - section 3.6. :
-Appropriate validated detergent and methods, suitable for AAV and according to local legislation will be used for decontamination and disinfection measures after administration of AAV or in the case of accidental spilling.

-AAV is readily inactivated by several disinfectants such as 0.5% sodium hypochlorite, 0.45% potassium peroxymonosulfate (Korte et al., 2021), 0.5% peracetic acid, 10% bleach iodine and iodine (1%) (5- or 30-minute contact time; Howard and Harvey, 2017).

3) In p15 point I. Post-release treatment of the site of the 4. 2020-002873-88_GMO_SNIF_09Dec2021.pdf document : *Any surface contaminated with AAV5-hRKp.RPGR will be decontaminated according to applicable site-specific policies and procedures, using a disinfectant with validated efficacy against AAV*

and

the BASF MSD (here, in attachment) reporting that the **Poloxamer 188** must not be placed (incompatible !) into contact with strong bases, strong acids nor strong oxidants,

It could be proposed to the notifier:
- to revise the list of suggested disinfectants/decontaminants and to add, for each of them, a clear description of the application method.
- in case of spilling and/or surface decontamination, to choose the available disinfectants/decontaminants, please consider not only the AAV vectors themselves to be treated but also their status when admixed with the excipients, especially in the context of this present phase III development.

SBB comment :

Referring to the expert's comment on the stability/reactivity of poloxamer 188, and unlike the BASF material safety data sheet (MSDS) and the Thermofisher MSDS, it is noticed that MSDS of other manufacturers (Merck, Sigma-Aldrich) do not mention incompatibility issues with strong bases, strong acids and strong oxidizing agents. As an excipient, poloxamer 188 will only be present at a percentage of the total amount in case of spilled investigational medicinal product and it is not clear to what extent it may impact on the effectiveness of disinfectants.
It can be envisaged to address the following question to the notifier :

> Given that Poloxamer 188 is an excipient of the investigation medicinal product that will be administered during the proposed phase III clinical trial and considering that the concomitant use of Poloxamer 188 with strong bases, strong acids and strong oxidizing agents should be avoided (BASF Safety data sheet (ID no. 30631537/SDS_GEN_00/EN), the notifier is requested to comment on the appropriateness of the list of suggested disinfectants/decontaminants (*0.5% sodium hypochlorite, 0.45% potassium peroxymonosulfate, 0.5% peracetic acid, 10% bleach iodine and iodine (1%)* as indicated in the non-confidential CAF in case of spilling.

Coordinator comment

Poloxamer 188 surfactant is generally included in medium and buffers to prevent rAAV to bind to surfaces (and thus to ensure no substantial amounts of rAAV particles are lost). The product is therefore included throughout the procedure (see document 11). In the final formulation buffer the concentration is minimal (10 mM Tris, 180 mM NaCl, 0.001% (w/v) Poloxamer 188, 2% (w/v) Trehalose dihydrate, pH 7.5 – see p17/282 File11 ; and 0.0005 – 0.0015% in the final formulated bulk (p178/282 File11).
P188 has been reported earlier to be included in the AAV preparations from Pfizer in earlier dossier for example. I would consider this comment not related to ERA.

With respect to the expert's comment on detailing the application method, see proposed text under comment 1 of this section.

Additional SBB comment :
In the instructions for staff 'safety handling statement', p3/4, the procedures for the management of accidental spills should be improved to include a time period to vacate the affected for a length of time that allows aerosols to be carried away and heavier particles to settle (at least 30 min are generally required).. During that time, the area should be closed and a sign "DO NOT ENTER" should be posted.

5. ENVIRONMENTAL RISK ASSESSMENT

(applicability of the specific environmental risk assessment provided for in Section 2 of the '*Good Practice on the assessment of GMO related aspects in the context of clinical trials with AAV clinical'* taking into account the specific characteristics of the investigational medicinal product)

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. OTHER INFORMATION

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

Comment 1

Although the ICFs (in Dutch) were only superficially evaluated, it was noticed that the table of content (inhoudsopgave) of document ' MGT-RPGR-021_Belgium_Main ICF_v8.2.0_22Nov2021_DUT Clean' is in English instead of Dutch.

***SBB comment* :**
This could be added as an editorial remark.

Comment 2

Has no further questions/comments.

Comment 3

Has no further questions/comments.

References

Allen JM, *et al*. 1997. Identification and elimination of replication-competent adeno-associated virus (AAV) that can arise by nonhomologous recombination during AAV vector production. J Virol 71:6816-22.

Food and Drug Administration (FDA) 2021. Cellular, Tissue and Gene Therapies Advisory Committee meeting briefing document: Toxicity Risks of Adeno-associated Virus (AAV) Vectors for Gene Therapy. https://www.fda.gov/media/151599/download

La Bella T, *et al*. 2020. Adeno-associated virus in the liver: natural history and consequences in tumour development. Gut 69:737-747

Schäffer *et al*. 2021 Integration of adeno-associated virus (AAV) into the genomes of most Thai and Mongolian liver cancer patients does not induce oncogenesis. BMC Genomics 11;22(1):814.doi: 10.1186/s12864-021-08098-9

Adviesraad voor Bioveiligheid
Conseil consultatif de Biosécurité

Compilation of comments of experts in charge of evaluating the dossier B/BE/21/BVW8
Evaluation of the answers of the notifier

14 April 2022
Ref. SC/1510/BAC/2022_0421

Mandate for the Group of Experts: Mandate of the Biosafety Advisory Council (BAC) of 07 January 2022.
Coordinator: Rik Gijsbers (KULeuven)
Experts: Anton Roebroek (KULeuven), Liliane Tenenbaum (Lausanne University Hospital), Willy Zorzi (ULiège)
SBB: Katia Pauwels

INTRODUCTION

Dossier **B/BE/21/BVW8** concerns a notification from MeiraGTx UK II Limited for a clinical trial entitled "*Phase 3 Randomized, Controlled Study of AAV5-hRKp.RPGR for the Treatment of X-linked Retinitis Pigmentosa Associated with Variants in the RPGR gene*". The investigational medicinal product is a AAV5- derived recombinant replication deficient vector carrying the human RPGR gene.

On 4 March 2022, based on a list of questions prepared by the BAC (SC/1510/BAC/2022_0281), the Competent Authority requested the notifier to provide additional information about the notification. The answers from the notifier to these questions were received by the Competent Authority on 28 March 2022. This complementary information was reviewed by the coordinator and the experts in charge of the evaluation of this notification.

Evaluation coordinator

Q1-3 were answered adequately.

Q4 on germline transmission.
We must consider dose (if adults would receive much higher doses) and the fact that animal models (mice, rabbits) are not relevant to assess germ line transmission since AAV (and other viruses) are very species specific. Potentially, NHP studies may aid here.
The final statement is most relevant to me, 'no vector genomes were detected in semen of participants in a Phase I/II clinical study of AAV2/2hRPE65 for the treatment of LCA2 (Bainbridge *et al*., 2008). Factors such as the route of administration (ocular vs. parenteral), vector type (non-integrating), dose level, and transient biodistribution of the vector to reproductive organs support this product's low risk for germline transmission.'

Q5-8 were answered adequately.

Evaluation expert 1

Please consider that the response of the notifier to Question 7 is, in the present form, not available and must be modified:
In the Question 7, the SBB communicated to the notifier the following « commonly accepted by the scientific field » advice :

In the instructions for staff 'safety handling statement', p3/4, the procedures for the management of accidental spills should be improved by including a time period to vacate the affected area for a length of time that allows aerosols to be carried away and heavier particles to settle (at least 30 min are generally required). During that time, the area should be closed and a sign "DO NOT ENTER" should be posted.

In the Sponsor Response, it is reported that :
… « *Moreover, the vector is replication-incompetent and the parental AAV is not pathogenic to humans and not an intrinsic airborne pathogen. Based on the above, evacuation in case of a spill is not needed. However, as precaution, the sponsor proposes that staff wears a facemask in case of spill clean-up.*»

Please indicate that the reason mentioned above is not appropriate for declaring that the evacuation of the room is not necessary. <But>, as precaution, the sponsor proposes that staff wears a facemask in case of spill clean-up. It would be better to eliminate these sentences which bring contradiction between themselves (« not dangerous but, nevertheless the wearing of the mask is recommended ») and confusion in regard to the procedure proposed by the SBB, in the question 7.
In the beginning of the new proposed amendment to the safety handling statement (p7), please indicate that it is necessary to wait at least 30 min (allowing aerosols to be carried away and heavier particles to settle) before new entry into the area.
During this time, we invited the notifier to follow the advice of SBB proposing the evacuation of the room, without forgetting to alert others of the spill to avoid accidental exposure and to close the area with a sign "DO NOT ENTER" posted on the door.
During this awaiting time, it is not appropriate to entry and to begin the spill clean-up on surfaces and equipment, because the aerosols are continuing to be present and to settle on the surfaces.

Comment coordinator

The discussion is maybe too much in detail?
In the letter the BAC indicated that "the use of goggles and mask are mandatory should the use of a biosafety cabinet not be possible (e.g. handling of spill incident)". I would guess this also the case when the drug product (DP) is provided to the eye of the patient.
If we asked to specify 'a time period' earlier, it makes sense to be consequent and do this again here.
On the other hand, I'm afraid that, considering the low volumes injected (I would guess some 100µl max), spills would be 'drops' at worst, unless the whole drug product vial would be spilled. In addition, the DP should be aliquoted in syringes with the specific volume to be injected, and this will be done under a hood.
I agree that precautionary measures should be taken, but mainly to prevent the incident from happening.
The parental AAV5 virus is quite seroprevalent (anti-AAV5 neutralizing antibodies ~30%) (https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6629972/). If a spill, with the low volumes in mind, would occur, I reckon it is best to clean it as soon as possible.

Comment SBB

Precautionary measures including a time period to vacate the affected area in case of spill accident have been requested by the BAC for other clinical trials involving the use of AAV (see for example B/BE/18/BVW7 (*SC/1510/BAC/2018_1082*), B/BE/19/BVW2 (*SC/1510/BAC/2019_0491*) and B/BE/21/BVW3 (*SC/1510/BAC/2021_1036),* being the reason why the notifier was suggested to improve the document with the instructions for staff.

However, further comparison between the dossiers involving the use of AAV viral vectors reveals that the current proposed handling volumes of the drug product are approximately one order of magnitude less as compared with the volumes and amounts handled in the clinical trials B/BE/19/BVW2 (intravenous administration; each vial contains 8,5 ml and dose administered varies between 2 à 3 ml of product/kg weight, hence possibly 10 ml is being administered), B/BE/18/BVW7 (intravenous administration, at a dose 2x 10^{13} gene copies/kg weight, vials contain 10 ml, with 10x 10^{13} gene copies rug product (1x 10^{13} gene copies/ml), so 2 ml/ kg weight will be administered, order of volume administered could vary between 10 à 50 ml product), B/BE/21/BVW3 (intra cisterna magna injection, 1.4×10^{14} vg as a single dose administered , injectable volume range of 1.2 ml).
Indeed, the protocol of the current trial specifies that volume per injection should not exceed 0,8 ml (subretinal injection).

Given that BAC is asking that the use of goggles and mask is mandatory whenever the use of a biosafety cabinet is not possible, taking into account the non-pathogenic and replication-deficient nature of the IMP, the prevalence of wt AAV serotype 5 in the human population and the relative low amounts used, the position of the notifier could be accepted.

Evaluation expert 2

In my opinion the notifier addressed correctly and satisfactorily the comments/questions that we have raised in February 2022.

Evaluation expert 3

The expert is satisfied with the answers of the notifier