Adviesraad voor Bioveiligheid
Conseil consultatif de Biosécurité

Advice of the Belgian Biosafety Advisory Council on the notification B/BE/20/BVW3 of the company Transgene for deliberate release in the environment of genetically modified organisms other than higher plants for research and development

20/10/2020
Ref. SC/1510/BAC/2020_1011

Context

The notification B/BE/20/BVW3 has been submitted by Transgene to the Belgian Competent Authority in August 2020 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 I/IIa study of intra-tumoral BT-001 (TG6030) administered alone and in combination with pembrolizumab in patients with cutaneous or subcutaneous lesions or easily injectable lymph nodes of metastatic/advanced solid tumors"***. The purpose of this first-in-human clinical trial is to study the safety and tolerability of BT-001 repeated intra-tumoral administrations alone (Phase I, Part A) and in combination with pembrolizumab (Phase I, Part B and Phase IIa).
TG6030 is an investigational medicinal product (IMP) developed for oncolytic antitumoral activity combined with targeted chemotherapy. It is a recombinant Vaccinia Virus (VV) with restricted replication to highly dividing cells due to the functional deletion of the thymidine kinase (TK) and ribonucleotide reductase (RR) of the viral genome and genetically modified to express the the human Granulocyte-Macrophage Colony stimulating factor (hGM-CSF) and 4-E03, a human monoclonal antibody of the IgG1 isotype targeting the human Cytotoxic T-Lymphocyte-Antigen 4 (anti-hCTLA-4 mAb). Pembrolizumab is a humanized antibody working as a immunotherapy.
An investigational medicinal product with another insert but with the same Vaccinia Virus vector, has been evaluated by the Council in the context of application B/BE/18/BVW1 (ref SC/1510/BAC/2018_0472).
The intra-tumoral administrations of BT-001, in one or several lesions or injectable lymph nodes, will in total not exceed 4ml for each of the different dose levels investigated (10^6, 10^7or 10^8 PFU/ml).

The parental strain of BT-001 is the Copenhagen strain, a VV that was used for smallpox vaccination in Denmark and the Netherlands in the 1950s. VV are lipid enveloped viruses that are sensitive to inactivation by both physical inactivation (e.g. heat) and disinfectants (lipid solvents and mild detergents). VV is considered as group 2 biological agent.

It is planned to conduct the trial in a clinical site located in Brussels. However, Wallonia and the Flemish Region are also considered as potential receiving environments. In Belgium it is estimated that up to 10 patients will be included.

The dossier has been officially acknowledged by the Competent Authority on 13 August 2020 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 SBB also took part in the evaluation of the dossier. 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 would not raise any problems for the environment, animal health or human health (people coming in contact with the treated patient and/or with the GMO) in the context of its intended use. See Annex I for an overview of all the comments from the experts.

The scientific evaluation has been performed considering following legislation:
- Annex II (principles for the risk assessment) and annex III (information required in notifications) of the Royal Decree of 21 February 2005.
- Commission Decision 2002/623/EC of 24 July 2002 establishing guidance notes supplementing Annex II to Directive 2001/18/EC.
The pure medical aspects concerning the efficacy of the medicinal product and its safety for the treated patient, as well as aspects related to social, economic or ethical considerations, are outside the scope of this evaluation.

On 24 September 2020, 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 several revised documents in response to the changes requested by the BAC, were received by the Competent Authority on 10 October 2020 and transmitted to the secretariat of the BAC on 12 October 2020. This complementary information was reviewed by the coordinator and the experts.

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 received no reaction from the public.

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

No detailed information demonstrating the integrity of the expression cassette of the primary research COPTG19384 was included in the biosafety dossier. However it was verified with and confirmed by the quality assessors of the Federal Agency of medicinal products and health products that the information provided by the applicant in the context of the clinical trial application was deemed acceptable. No further remarks were raised concerning the information provided in the dossier.

3. The conditions of the release

Given that transient shedding of BT-001 after administration would most likely occur during the hospitalization of the patient, the BAC remarked that more stringent risk management measures should be implemented during the hospitalization of the patients so as to make hospitalization with private bathroom and toilet mandatory.

The BAC had also some remarks with respect to the precautions to follow for the patient when returning home. For example, the applicant was asked to further detail the information provided in the informed consent and the patient information to ascertain that patients would avoid contact with their pets or any other animal during the study period. These precautions should be applicable up to seven days after the last BT-001 injection or, in case of occurrence of pustules, until the scab has fallen off (approximately 3 weeks after the pustule appearance). Also, when patients/personnel remove dressings and place them in a waterproof plastic bag to bring it back to the hospital for destruction, the applicant was asked to specify that the bag should be stored in a safe place so as to avoid any unwanted contact by individuals other than the patient or animals.

In its list of questions addressed to the notifier, the BAC also advised the notifier to further adapt the technical sheet with respect to the precautions for individuals that may be pregnant.

The notifier adequately implemented the remarks and requests addressed by the BAC in a revised version of several documents making part of the biosafety dossier including the revised patient information note and informed consent, as well as a patient emergency card with access to public web-based information.

4. The risks for the environment or human health

The BAC took note that the applicant gave consideration to the possibility of recombination with other poxviruses and the environmental exposure experience with another TK-inactivated VV of the Copenhagen strain. This information is in line with the information the notifier has provided previously in the context of a request of additional information on the recombination of the same recombinant vaccinia virus with other poxviruses (SC/1510/BAC/2018_0472).

Furthermore, the notifier adequately implemented the remarks of the BAC in the revised documents including the patient information note and informed consent with regards potential contact with animals (e.g. pets or other animals).

Referring to its evaluation of application B/BE/18/BVW1 (ref SC/1510/BAC/2018_0472), during which the BAC noticed that an investigation of shedding of TG6002 in saliva, faeces and urine in patients with

or without ibuprofen premedication was planned for the TG6002.02 clinical trial, the BAC remarks that shedding analysis planned in the TG6002.02 clinical trial has not been performed yet. Given that no relevant data are available, shedding will be assumed, thereby triggering notification requirement according to Directive 2001/18/EC for any forthcoming clinical trial using the same Vaccinia viral vector. The BAC recommends the notifier to take due account of the possibility to collect shedding data in forthcoming clinical trials using the recombinant Vaccinia virus.

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

Even if the risks are low, the BAC stressed the importance of biosafety precautions to avoid unintended dissemination of the GMO. Upon detailed advice of the BAC the notifier amended the technical sheet so as to render the instructions for handling and accidental spill even more clear.

In its dossier the notifier presented a method for the detection of the GMO with information on a primer set targeting the left arm of the deleted TK region of VVCopenhagen strain and the adjacent genetic insert sequence of COPTG19384 as well as information on the length of the anticipated generated amplicon. The proposed detection method for COPTG19384, a PCR-method, is deemed acceptable for detection of the GMO in the context of this first in human clinical study, which is an early stage in the drug development program. However, in view of potential future applications as a next stage in the drug development program, the BAC recommended the notifier to deliver the information on the sensitivity of the qPCR assay and the plaque assay as described in V.A.2 of the 2020-000505-80-BT-001.01-Annex_IIIA-final-12August2020-CONFIDENTIAL. In its answer to the list of questions, the notifier confirmed to be attentive to present this information in future BT-001.01 applications.

Conclusion

Based on the scientific assessment of the notification made by the Belgian experts, the Biosafety Advisory Council concludes that it is unlikely that BT-001 developed for oncolytic immunotherapy, 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 revised documents implementing the remarks addressed by the Biosafety Advisory Council.

- The notifier takes due account of the possibility to collect shedding data in forthcoming clinical trials using the recombinant Vaccinia virus vector.

- 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 two weeks when the first patient starts the treatment and the last patient receives the last treatment.

- At the latest six months after the last visit of the last patient included in the trial, the notifier must send to the competent authority at the attention of the Biosafety 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 BT-001 .

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/20/BVW3 (ref. SC/1510/BAC/20_0835)

Adviesraad voor Bioveiligheid
Conseil consultatif de Biosécurité

Compilation of comments of experts in charge of evaluating the dossier B/BE/20/BVW3
And comments submitted to the notifier

24 September 2020
Ref. SC/1510/BAC/2020_0835

Mandate for the Group of Experts: Mandate of the Biosafety Advisory Council (BAC) of 13 August 2020.
Coordinator: Jozef Anné (KUL)
Experts: Rik Gijsbers (KUL), Willy Zorzi (ULiège), Karen Willard-Gallo (Jules Bordet Institute, ULB), Aline Baldo (WIV-ISP, SBB)
SBB: Katia Pauwels

INTRODUCTION

Dossier **B/BE/20/BVW3** concerns a notification from Transgene 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 13 August 2020 and concerns a clinical trial entitled "A Phase I/IIa study of intra-tumoral BT-001 (TG6030) administered alone and in combination with pembrolizumab in patients with cutaneous or subcutaneous lesions or easily injectable lymph nodes of metastatic/advanced solid tumors".The investigational medicinal product is a recombinant, conditionally replicative Vaccinia virus of Copenhagen strain , genetically modified to encode the human Granulocyte-Macrophage Colony stimulating factor (hGM-CSF) and 4-E03, a human monoclonal antibody of the IgG1 isotype targeting the human Cytotoxic T-Lymphocyte-Antigen 4 (anti-hCTLA-4 mAb).
An investigational medicinal product with another insert but with the same Vaccinia Virus vector, has been evaluated by the Council in the context of application B/BE/18/BVW1.

♦ 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 24-09-2020 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

1. INFORMATION RELATED TO THE CHARACTERISTICS OF THE DONOR, THE RECIPIENT OR PARENTAL ORGANISM

(e.g. possibility of natural transfer of genetic material to other organisms, pathological, ecological and physiological characteristics, indigenous vectors ...)

Comment 1

Has evaluated this item and has no questions/comments.

Comment 2

Has evaluated this item and has no questions/comments.

Comment 3

Vaccinia virus (VV), considered to be a minor human pathogen, has been used to safely vaccinate millions of humans against smallpox. This virus replicates exclusively in the cytoplasm thereby eliminating risk from viral DNA integrating in the host genome and as it does not latently infect cells it is rapidly cleared from the host. The risks associated with this vector are limited with most serious adverse events detected in patients at risk, including children (<12 months old), immunocompromised individuals, patients with inflammatory skin conditions and pregnant or breast feeding women due to the risk for the foetus/infant.

Comment 4

Has evaluated this item and has no questions/comments.

2. INFORMATION RELATED TO THE VECTOR

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

Comment 1

In 2020-000505-80-BT-001.01-Annex_II-final-12August2020.pdf the applicant indicates that the final VV vector product BT-001 preferentially replicates in tumor cells, to induce cell death and inflammation. What is known of the other cells that may support replication?

Copenhagen VV is immunogenic on its own. Upon vaccination, the immune response induced against vaccinia virus cross-reacts and neutralizes smallpox. Furthermore, orthopoxviruses are immunologically

cross-reactive and cross-protective, so that after infection with any member of this genus a protection against an infection with any other member of the genus is obtained (Essbauer S. *et al.*, 2010). Thus, I presume that patients that have been vaccinated with a vaccine earlier, carry neutralizing Ab (especially older people that have been taking part in the large scale small pox vaccination program). Will patients be screened for pre-existing titers of neutralizing Ab against the VV? Is their cross reactivity with Ab raised against other VV vaccines, such as MVA (modified vaccinia Ankara)? This is not mentioned.

Comment SBB :

No specific information is provided on the type of cells that could support replication of BT-001.01. However related information to replication capacity of BT-001.01 can be derived from Section 4.3.1 of the investigator's brochure summarizing the biodistribution data that were obtained for two animal models (tumor-bearing mice) upon intratumoral administration of BT-001. Tumor and blood samples revealed that the virus and the transgenes were detected mainly in tumors and not detected or at low level in bloodstream. Section 4.3.2 summarizes the biodistribution profile of TG6002, a recombinant vaccinia virus utilizing the same vector backbone as BT-001.01, upon intravenous administration in rabbits. Transient and extremely low viral shedding was observed through urine and TG6002 was not found to be shed through the feces under the study conditions. It is further specified that because BT-001 will be administrated intratumorally, the spreading of the virus is expected to be lower than after intravenous administration.
With regards the parental Vaccinia virus Copenhagen strain (VV-COP), the applicant also mentions that the parental strain is not found in natural ecosystems and that avian cells and BHK cells are permissive but VV-COP is unable to propagate in normal human and most other mammalian cells tested (Annex IIIA-final- 12 August 2020). No literature reference has been provided.

It is agreed that no information is provided on cross reactivity of Ab raised during smallpox vaccination or any other MVA-derived vaccine. It can reasonably be assumed that the presence of pre-existing titers of neutralizing Ab would mainly be a concern with respect to the efficacy of the investigational medicinal product for the patient and that it would not raise any additional environmental risk to the human health or the environment.

Comment coordinator :

The presence of neutralizing Ab is an interesting question but not of importance here, since as also mentioned by SBB, the application of the vector is an intratumoral injection. For a study about cross reactivity see e.g. Altenburg *et al.*, 2018.

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 not evaluated this item.

3. INFORMATION RELATED TO THE CHARACTERISTICS OF THE GMO

3.1. Information related to the genetic modification

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

Comment 1

Has evaluated this item and has no questions/comments.

Comment 2

Has evaluated this item and has no questions/comments.

Comment 3

Anti-CTLA-4 antibodies are currently used to treat cancer patients and although there can be severe adverse side effects associated with this treatment and do not constitute a serious problem for the environment. The treatment in this trial is an effort to reduce this toxicity by directly targeting the tumor rather than systemically treating the patient, which can have larger effects on the immune system as a whole. The only problem might be a transient adverse effect in the case of a needle stick or other exposure of a health care worker or shedding and infection of family members or other associates.

Comment 4

Has evaluated this item and has no questions/comments.

3.2. Information on the molecular characteristics of the final GMO

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

Comment 1

a.Judging from the fig1 information provided about the vector (p10 and 30/110 - 2020-000505-80-BT-001-impd-quality-july2020.pdf), the RVV carries two p7.5K promoter sequences. Identical DNA sequences increase the risk of recombination and then elimination of transgenes (as mentioned by the applicant). HDR could result in elimination of the sequence in between both promoters (size in NCBI db indicates 480bp) and judging from the form this is being tested for by PCR. However, at p34/110 it is mentioned that the integrity of the expression cassette is verified and detailed in DP0619 provided in attachments, however, I could not find this document.
Are there clones selected that have an insert deleted? Were the primers used designed to pick such an event?

b. At p53/110 the applicant mentions that in-house testing is performed, to ensure vector identity by PCR: *"Vector identity by PCR (ABL) on purified MVS* A specific pair of primers is used to amplify a DNA

fragment, covering a portion of the expression cassette and the adjacent viral region, by PCR." This description is not useful, since no info is provided on the primer set used, and the amplicon generated. Same goes for p83/110: 2.1.P.5.2.7 Vector identity.

c.The biological stability of the product is considered 94% (see p34/59 of 2020-000505-80-BT-001.01-Annex_IIIA-final-12August2020-CONFIDENTIAL.pdf) after 5 passages. Is this normal? How does this relate to other products?

d. At p4/26 Table1 in 2020-000505-80-protocol-synopsis-v1.0-BE-17july2020.pdf shows the respective doses that will be tested (10e6-10e8 PFU/ml). It is not clear whether the doses will be adapted to the body weight of the patients.

Comment SBB and coordinator :

a. Agreed that attachment DP0619 on the generation, production and characterization of gene stability of DP0619 was not provided along with the IMPD. The SBB would like to remark that the inclusion of the IMPD into the biosafety dossier is not mandatory, nor are the attachments associated to the IMPD. The coordinator also refers to page 5/33 of 2020-000505-80-BT-001.01-SNIF-BE-final-12August2020 stating that 'the genetic stability study demonstrated 94 % stability of BT-001 final research virus stock after 5 passages on CEF. Sequencing of the entire genome of BT-001 drug substance demonstrated that vector genomic integrity was maintained at a passage level comparable to production batch'.
b. The coordinator notes that in ' 2020-000505-80-BT-001-detection-method-july2020.pdf ' information on a primer set targeting the left arm of the deleted TK region of VVCopenhagen strain and the adjacent genetic insert sequence of COPTG19384 as well as information on the length of the generated amplicon has been provided.
c. For information, in the IMPB it is also specified that a total of 94% of correct clones was identified after 5 passages.

The SBB remarks that a, b and c mainly relate to quality assessment of the proposed IMPD and may go beyond the environmental risk assessment. The coordinator agrees that those remarks go beyond environmental risk assessment and that those can be mentioned to FAMHP.
The quality assessors of the federal agency of medicinal products have been asked on 22/09 whether
i) the applicant provided the PCR-study supporting the integrity of the expression cassette of COPTG19384 , referred as study DP0619 in the IMPD in the framework of the clinical trial application
ii) this information has been considered adequate by (quality) assessors of FAMHP.
The quality assessors provided the following feedback : *The DP0619 report was provided in the IMPD. This study presents generation and characterisation of the primary research stock of COPTG19384 vector. It includes indeed a PCR analysis of the recombinant vector with primers complementary to sequences flanking the I4L locus, flanking the TK locus and primers complementary to sequences to the light and heavy chain of the IgG1 antibody. The primary stock was also analysed by sequencing of both expression cassettes with results showing 100% homology with the expected sequence. Moreover, the expression of the encoded proteins in the supernatants of the infected cells was analysed by Western Blotting and ELISA. The presented results are deemed acceptable to confirm the integrity of the expression cassette.*
From this primary research stock a MVS has been generated. The genomic integrity of this MVS was also confirmed by: sequencing of the entire genome (sequencing report was provided), restriction enzyme mapping and expression of the encoded proteins.

d. There is no indication that the dose will be adapted to the body weight of the patients. However, given the intra-tumoral mode of administration, particular attention is given to the calculation of the injection volume in function of the size of the target lesions. The total volume of BT-001, to be allocated to one to five syringes depending on the number of lesions to be treated (one syringe per lesion), cannot exceed 4 ml (cfr 2020-000505-80-BT-001_administration procedure and 2020-000505-80-BT-001_preparation procedure). This applies to the three different dose levels investigated (10e6, 10e7 and 10e8 PFU/ml).

Comment 2

In the document of this dossier : 2020-000505-80-BT-001.01-Annex_IIIA-final-12August2020-CONFIDENTIAL : Concerning the point : II. A. b) 7. Sensitivity, reliability (in quantitative terms) and specificity of detection and identification techniques and V. A. 2. Specificity (to identify the GMOs, and to distinguish them from the donor, recipient or, where appropriate, the parental organism), sensitivity and reliability of the monitoring techniques :

General comment : There is no information about LOD data in the test used to detect or monitor GMOs. (see also in this dossier, the document 2020-000505-80-BT-001-detection-method-july2020.pdf)

The here-developed PCR tests are not raised to detect GMOs, only to analyse the « integrity and the identity » of the recombinant construction of the GMOs.

« identity test used has been developed to be specific by the design of its primers (Table 2). Its validation is ongoing according to International Conference on Harmonization (ICH) Q2 (R1) guideline. There will be no lower limit of quantification or precision for this assay as it is a qualitative test. »

The PCR test concerning the final Drug GMO(s) detection must be provided with a LOD value : see in attachment,

- the first document from JRC Scientific and Technical Reports (European Commission): Guidance Document on Measurement Uncertainly for GMO Testing Labories (eur2275en.pdf) and

-the second one from www.fda.gov : Guidelines for the Validation of Analytical Methods for Nucleic Acid Sequence-Based Analysis of Food, Feed, Cosmetics and Veterinary Products - 1st Edition - U.S. Food and Drug Administration Foods Program September 2019 (ValidationNucleicAcidSequenceBasedAnalysisFoodFeedCosmeticsVeterinary.pdf)

For qualitative PCR methods, the basic performance characteristics are:
• Extraction Efficiency
• Sensitivity (Limit of Detection-LOD)
• Specificity (Selectivity)
• False Negative and Positive Rates
• Robustness/Ruggedness
Sensitivity-Data obtained from testing the method at different concentrations of the target sequence in order to determine the sensitivity of the method should be provided. Limits of detection (LOD) should be defined using samples comprised of single ingredients only. The LOD is usually understood as the concentration of the target DNA at which an amplification product is detected with a probability of at least 0.95 (LOD95%). The LOD should be determined by means of a dilution series of the target DNA. For each dilution level, 12 PCR replicate measurements are performed. The dilution level with the lowest

number of copies for which all 12 replicates are positive is considered to be an approximate value for LOD95%. This data may be represented as DNA weight/reaction (ng or pg etc.) or the target copy number/reaction. After the LOD of the assay is determined using a dilution series of the target DNA, the originating lab should perform experiments to estimate the LOD of the assay in various food matrices.

Comment SBB :

The expert refers to guidelines which relate to the monitoring of GMOs in the context of food/feed applications for the placing on the market. It should be remarked that requirements for detection and identification are more stringent in the context of marketing authorization application as compared to those in the context of deliberate releases for any other purposes than for placing on the market. In the first case, validated methods for quantification are required.

Focus on LOD in the context of clinical trials is often required when the applicant presents **data** on the presence of the GMO, like for example shedding data. Such data usually becomes available in later phases of a drug development program. In order to be able to assess shedding data, it is agreed that data should be accompanied with information of the LOD in order to assess the sensitivity of the method and the reliability of the presented data and the conclusions thereof.

The current clinical trial application concerns a first in human study, which is an early stage in the drug development program. The information that is required under II.A.b)7 and V.A.2 in the context of the current clinical trial application should be considered in the framework of presenting a method that is reliable, sensitive and specific.
In accordance with art 13§2h of the Royal Decree of 21 February 2005 on the deliberate release of GMOs, the applicant provided a statement that he would agree to deliver to the SBB a control sample of the GMO and the related scientific documentation (method for detection of the construct) at the latest 15 days after the start of the trial. In this context, the applicant also provided in '2020-000505-80-BT-001-detection-method-july2020.pdf ' information on a primer set targeting the left arm of the deleted TK region of VVCopenhagen strain and the adjacent genetic insert sequence of COPTG19384 as well as information on the length of the anticipated generated amplicon. The proposed detection method for COPTG19384, a PCR-method, is deemed acceptable for detection of the GMO in the context of this first in human clinical trial application. In view of future applications during the possible further drug development program, the applicant is encouraged to deliver the information on the sensitivity of the qPCR assay and the plaque assay as described in V.A.2 of the 2020-000505-80-BT-001.01-Annex_IIIA-final-12August2020-CONFIDENTIAL, as this will be requested in future applications.

Comment 3

Has not evaluated this item.

Comment 4

Has not evaluated this item.

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

This comment relates more to patient safety, and less to biosafety aspects of the product. See also p11/59 in 2020-000505-80-BT-001.01-Annex_IIIA-final-12August2020-CONFIDENTIAL.pdf.
Expression of the genes of interest (GOI) hs GM-CSF and 4-E03 IgG1. Even when the proteins are well tolerated upon injection of pure protein (p14/110 - 2020-000505-80-BT-001-impd-quality-july2020.pdf) have these proteins been tested to be immunogenic in RVV context? Is the specific expression in the tumor cells sufficient to exclude immune reaction against the respective hs GM-CSF and 4-E03 IgG1proteins when expressed *in vivo*?

Comment SBB :

Agreed that these questions go beyond the environmental risk assessment of the proposed use of the recombinant Vaccinia virus BT.001-01.

Comment 2

Has evaluated this item and has no questions/comments.

Comment 3

See comments in 3.1

Comment 4

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

In 2020-000505-80-BT-001.01-Annex_II-final-12August2020.pdf at p8/17 clinical staff and household personnel receives training. The product will be provided to patients, carrying cutaneous or subcutaneous tumors or readily injectable lymph nodes of metastasized or developed solid tumors. Since these patients are out of treatment, and will be very sick, I reckon it would be best/helpful that other members of the family (and not solely the patient), get trained and informed about the possible contamination and side-effects of the treatment.
See also p35/59 section III.A.3 in 2020-000505-80-BT-001.01-Annex_IIIA-final-12August2020-CONFIDENTIAL.pdf.

Comment 2

Has evaluated this item and has no questions/comments.

Comment 3

Has evaluated this item and has no questions/comments.

Comment 4

Concerning workers protection measures:
In the dossier, the applicant says that the workers should wear waterproof gloves. Workers should wear protective gloves against micro-organisms conform to the ISO norm 374-5:2016.
A chirurgical mask is considered as a medical device rather than personal protective equipment.
Workers should wear a mask conform with the norm NBN EN 529, a FFP2 type (EN149:2001) with a P2 filter (EN 143:2000) for the administration of BT-001. They should wear closed and resistant shoes in order to be protected against sharp and syringes that fall.

***Comment SBB**:*
It is remarked that the following information has been included at p21 of the Summary Notification Information Format as well as in the Technical sheet :

*During BT-001 handling, a **gown**, **gloves** (conform EN374, EN420, EN455 and ISO 16604 with an AQL of 0.65 or lower), a **surgical mask** (conform with the norm NBN EN 529, a FFP2 type (EN149:2001) and with a P2 filter (EN 143:2000), **safety goggles with side shields** (or a mask with eye protection) and **needle stick resistant shoes** must be worn. Wearing a hygiene cap and overshoes is not mandatory.*

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

Section ‘Prevention of potential dissemination from the injection site’: to me it is not logic that the patient/personnel should remove dressings and place them in a waterproof plastic bag to bring it back to the hospital for destruction. The chance is high that these bags get lost, or that animals, children or partner may get in contact with the content. It would be more logic to soak the dressing in bleach or EtOH to inactivate the possible minute amounts of vector. To be sure and in order to keep track of material, this ‘inactivated’ dressing can then in turn be collected in a dedicated plastic to be brought back to the hospital.

In the Risk Mitigation protective measure for public health, it should also be mentioned that close contact with animals and not only pets (not only when pustules are observed) should also be avoided during the study period to prevent dissemination of the vector.

Comment Coordinator

Personally I don't think this will be the case that the bag get lost but it might be stressed that the bags should be stored in a safe place

With respect to close contacts with animals, the following comment was also made for B/BE/18/BVW1: "Regarding the possible transfer of genetic material, it was noticed that proposed measures by the notifier were focusing on avoiding transmission to humans, without clearly addressing potential spreading to animals (e.g. pets)." Can be highlighted, but "In the unlikely event of inadvertent administration to non-target organisms, further spread would be unlikely as there were only rare cases of secondary transmissions during the smallpox vaccination campaign with vaccinia virus and the pathogenicity of BT-001 is reduced compared to vaccinia virus."

Proposal of question to be addressed to the applicant :

The applicant is requested to provide the patient information note and informed consent and to ascertain that the text relative to the precautions to follow for the patient when turning back home contain clear information so that

i) patients avoid contact with their pets or any other animal during the study period. These precautions should be applicable up to seven days after the last BT-001 injection or, in case of occurrence of pustules, until the scab has fallen off

ii) when patients/personnel remove dressings and place them in a waterproof plastic bag to bring it back to the hospital for destruction, the bag is stored in a safe place so as to avoid any unwanted contact by individuals other than the patient or animals.

The applicant is also requested to adapt the section 2020-000505-80-BT-001.01- Annex II-final-12 august2020 accordingly.

Comment 2

General comment : There is no information about LOD data in the test used to detect or monitor GMOs. In case of shedding, there is no improved method to detect or monitor the problem (see point 3.2 of this report).

Comment SBB

See previous comment of the SBB under 3.2 comment 2 of this document.

Comment 3

In Annex II, Page 10 / 17 it is mentioned : "*The patient will be hospitalized during those observation periods in a private hospital room with ~~as far as possible~~ dedicated bathroom and toilet.".* It must be with a private bathroom and toilet or a common bathroom/toilet that is exclusively reserved for the patient when a room with private bathroom/toilet is not available.

Comment SBB

See also comment 1 under section 6.2.

Comment 4

Could the applicant consider the risk of recombination of BT-001 with other poxviruses even if the probability of occurrence is very low and even if preventive measures are in place in the proposed clinical trial to minimize dissemination and inadvertent transmission.
In Annex II Environmental risk assessment, the applicant concludes that with preventive measures which will be applied in the proposed clinical trial, the BT-001 GMO is not considered to represent a risk for the public health and for the environment.
The risk is a combination of the hazard (the potential adverse effect of the use of the vector and the consequences of each adverse effects identified) and the likelihood of occurrence and is expressed in qualitative terms from negligible – low – moderate to high. The risk cannot be considered as zero.

***Comment SBB**:*
It is remarked that consideration has been given to the possibility of recombination with other poxviruses at p27 of the Summary Notification Information Format under section 'likelihood of genetic exchange *in vivo'.* The applicant also refers to environmental exposure experience with another TK-inactivated VV of the Copenhagen strain at p 13 of Annex II Environmental risk assessment. This information is in line with the information the company has provided previously in the context of a request of additional information on the recombination of the same recombinant vaccinia virus with other poxviruses (cfr advice of the Biosafety advisory Council on dossier B/BE/18/BVW1- ref BAC_2018_0472).

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

At p1 of 2020-000505-80-BT-001-technical-sheet-BE-EN-v1.0-10june2020.pdf under precautions it is mentioned that pregnant individuals must not handle, prepare or administer BT-001. Does this imply that women cannot perform these handlings, since there is always a theoretical possibility that they are pregnant. Wouldn't it be more logic to redefine this to "individuals that may be pregnant" or provide a rationale?
This is more clearly indicated later in document 2020-000505-80-protocol-synopsis-v1.0-BE-17july2020.pdf at p10/26: E15 Are pregnant or a breastfeeding woman, where pregnancy is defined as the state of a female after conception and until the termination of gestation, confirmed by a positive hCG laboratory test (> 10 mIU/mL).

Comment 2

See the comment in points 3.2 and 5.2

Comment 3

As stated in multiple places, immunocompromised people, pregnant women and babies and individuals with inflammatory skin lesions must not come into contact with the patient.

Comment 4

Immunocompromised individuals will not be allowed to enter in the patient's room and to come into contact with the patient. However, the applicant does not explain the consequences if an immune-compromised individual enters into contact with BT-001.

Comment coordinator
See p7/17 2020-000505-80-BT-001.01-Annex_II-final-12August2020 where the applicant describes observations made for VV use in the worldwide smallpox eradication program :

It was clearly shown that the great majority of the serious adverse events occurred in defined subsets of what are referred to as "at risk" groups including:
• Children <12 months of age
• Severely immunocompromised individuals (e.g. organ transplant recipients, HIV-positive individuals, or those receiving chronic immunosuppressive medication)
• Patients with inflammatory skin conditions (e.g. eczema requiring previous treatment, atopic dermatitis, etc.).

In addition, vaccination was not recommended during pregnancy (due to the exceedingly rare risk of fetal vaccinia) or for breastfeeding women (because of the theoretical risk of transmission to the nursing infant).

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

Patients should avoid contact with pets, however, in the dossier the applicant does not consider the risk for animals if they come into contact with BT-001. Any potential adverse effect should be considered in the ERA even if the probability of occurrence is very low.

Comment coordinator :
See above 5.1, comment 1

5.4. Information on selective advantages or disadvantages conferred to the GMO compared to the parental organism.

Comment 1

Has evaluated this item and has no questions/comments.

Comment 2

Has evaluated this item and has no questions/comments.

Comment 3

Has evaluated this item and has no questions/comments.

Comment 4

Has evaluated this item and has no questions/comments.

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

Comment 1

Has evaluated this item and has no questions/comments.

Comment 2

Has evaluated this item and has no questions/comments.

Comment 3

Has not evaluated this item.

Comment 4

Has evaluated this item and has no questions/comments.

5.6. Information on the possibility of the GMO to exchange genetic material with other micro-organisms and possible consequences for human health or the environment.

Comment 1

Has evaluated this item and has no questions/comments.

Comment 2

Has evaluated this item and has no questions/comments.

Comment 3

Has not evaluated this item.

Comment 4

Could the applicant consider the risk of recombination of BT-001 and other poxviruses even if the probability of occurrence is very low.

Comment SBB
See previous comment of the SBB under section 5.1.

5.7. Information on the possibility of gene transfer to other organisms and about the selective advantages or disadvantages conferred to those resulting organisms (possible consequences for human health or the environment).

Comment 1

Has not evaluated

Comment 2

Has evaluated this item and has no questions/comments.

Comment 3

Has not evaluated this item.

Comment 4

Has evaluated this item and has no questions/comments.

6. INFORMATION RELATED TO THE MONITORING, SURVEILLANCE AND CONTROL, WASTE TREATMENT AND EMERGENCY PLANS PROPOSED BY THE APPLICANT

6.1. Monitoring plan proposed by the notifier and possibility to identify the occurrence of non-anticipated adverse effects.

(adequation between the monitoring plan and risks identified during the risk assessment, when appropriate measures to minimize the potential risks to offspring ...)

Comment 1

Has evaluated this item and has no questions/comments.

Comment 2

See the comment in points 3.2 and 5.2

Comment 3

The instructions for handling an accidental spill detailed in the technical sheet need to be modified as indicated in red below.

TECHNICAL SHEET

In case of incident while handling BT-001, please act as recommended below:
- **Accidental spillage**:

Cleaning process is the following ~~(2 possible methods)~~:
~~Method 1:~~
1. Wait for 30 minutes in order to settle down the aerosols.
2. After aerosols have settled, cover the spill area with paper towels and other absorbent material starting from the outer edge to the center. These steps need to be reversed – the spill should not be left for 30 min as a liquid that can create new aerosols through possible air currents. The first step is to cover the spill area from (outer perimeter to the center) with absorbent material until there is no unabsorbed liquid remaining.
3. Then add a layer of dry paper towels to cover the spill absorbed towels and apply the solution described below directly to these dry towels and leave for 30 minutes before removing for disposal.
3. ~~Moisten a compress with the solution described below:~~

Use a solution of **bleach at 0.6% of active chlorine:**
o 4 tablets [if 1.5 g/tablet of active chlorine] per litre of water, or
o 1 volume of bleach at 2.6%Cl [i.e. 26 g/l of active chlorine] for 4 volumes of water, or
o 1 volume of bleach at 9.6%Cl [i.e. 96 g/l of active chlorine] for 17 volumes of water.

4. Wipe the spill area (the application should start from the outer perimeter and work inwards).
5. Leave in contact for **30 minutes**.
5b. Remove the paper towels and broken vials with tongs or forceps and discard in a biohazard waste bag.
6. Wipe the area with paper towels to remove bleach (that can corrode surfaces), then rinse with tap water.
7. Go through steps 1 to 5 again but in step 4 leave in contact for **at least 10 minutes**.
8. Leave to dry.

Not a very good idea to give two different approaches – there need to be clear instructions and in general Method 1 is more likely to be more protective of the worker and the environment.
~~Method 2 (alternative):~~
~~Use a **standard disinfectant active on vaccinia virus-based products** containing the chemical substance(s) indicated below, respecting the manufacturer's instructions to ensure adequate contact time and to confirm the ability of the equipment to withstand the disinfectant used.~~
~~Follow step 1. and 2. from the preceding Method 1.~~
~~Then, pour carefully the appropriate disinfectant over the absorbent material starting from the edge to the centre and allow sufficient contact time for the disinfectant to inactivate the GMO.~~
~~Remove the paper towels and broken vials with tongs or forceps and discard in a biohazard waste bag.~~

Comment 4

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

At p13/17 of 2020-000505-80-BT-001.01-Annex_II-final-12August2020.pdf the risk assessment for the environment is discussed. Theoretically the virus can shed in body fluids, which will be monitored and assessed in the BT-001.01 trial. Here virus will be shed to the environment via the bathroom or toilet. Are special measures to be considered to prevent this potential shedding? Further on in the same section, when a spill occurs, it is indicated that all waste generated should be autoclaved, incinerated, or treated with sodium hypochlorite solution by personnel trained to dispose of biohazard waste. This seems disproportionate.
(see also at p49/59 of 2020-000505-80-BT-001.01-Annex_IIIA-final-12August2020-CONFIDENTIAL.pdf).

Comment SBB

Potential exposure to particles via the use of the patients bathroom or toilet has not been specifically addressed by the applicant except for the fact that in Annex II, Page 10 / 17 it is mentioned that : "*The patient will be hospitalized during those observation periods in a private hospital room with as far as possible dedicated bathroom and toilet."* As proposed in comment 3 under section 5.1., the BAC could consider to request the applicant to make a private bathroom and toilet mandatory or a common bathroom/toilet that is exclusively reserved for the patient when a room with private bathroom/toilet is not available.

The proposed measures in case of spill for this clinical trial are commonly recommended for accidental spills of GMOs. In its advice for an investigational medicinal product with another insert but with the same Vaccinia Virus vector, the Council gave particular importance to the description of these risk management measures.

Comment 2

See the comment in points 3.2 and 5.2

Comment 3

In Annex II, Page 10 / 17 it is mentioned that "After a BT-001 IT administration, patients will not be immediately discharged home."

What is the minimum observation period to decrease the potential risk of dissemination to the environment and the patient's household contacts? This should be stated.

Comment coordinator :

In 2020-000505-80-BT-001.01-SNIF-BE-final-12August2020.pdf p23/33 “Specific precautions for pustule management and hygiene kit provision” These measures are applicable up to seven days after the last BT-001 injection or, in case of the occurrence of pustules, up to the scab has fallen off, i.e. about 3 weeks after the pustule appearance:”

Comment 4

Has evaluated this item and has no questions/comments.

6.3. Information on the waste generated by the activity and its treatment.
(e.g. type of waste, amount ...)

Comment 1

Has evaluated this item and has no questions/comments.

Comment 2

Has evaluated this item and has no questions/comments.

Comment 3

See comments in 6.1.

Comment 4

Has evaluated this item and has no questions/comments.

6.4. If applicable, information on the emergency plan(s) proposed by the notifier.

Comment 1

Has evaluated this item and has no questions/comments.

Comment 2

Has evaluated this item and has no questions/comments.

Comment 3

Has evaluated this item and has no questions/comments.

Comment 4

In case of accidental spills or breakage of a vial containing the GMO, the medical staff should alert people in the area of the spill, remove contaminated clothes and leave the area for 30 min. He should close the area and post “DO NOT ENTER”. After 30 min, he must wear a clean lab coat and wear gloves, glasses and a mask and clean up the spill.

Comment SBB :
These measures are described in the technical sheet (2020-000505-80-BT-001-technical sheet- BE-EN-v.0-10june2020.pdf).

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

See the comment in points 3.2 and 5.2

Comment 3

Has evaluated this item and has no questions/comments.

Comment 4

Has not evaluated this item.

7. OTHER INFORMATION

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

Comment 1

None

Comment 2

None

Comment 3

None.

Comment 4

None

References

Altenburg *et al.* (2008) Effects of pre-existing orthopoxvirus-specific immunity on the performance of Modified Vaccinia virus Ankara-based influenza vaccines. Sci Rep. 8(1):6474. doi: 10.1038/s41598-018-24820-2. https://doi.org/10.1038/s41598-018-24820-2

JRC Scientific and Technical Reports (European Commission): Guidance Document on Measurement Uncertainly for GMO Testing Labories (eur2275en.pdf)

Guidelines for the Validation of Analytical Methods for Nucleic Acid Sequence-Based Analysis of Food, Feed, Cosmetics and Veterinary Products - 1st Edition - U.S. Food and Drug Administration Foods Program September 2019 (ValidationNucleicAcidSequenceBasedAnalysisFoodFeedCosmeticsVeterinary.pdf)