Bioveiligheidsraad
Conseil de Biosécurité

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Secretariaat
Secrétariat

O./ref.: WIV-ISP/BAC/2009_889

Title: Advice of the Belgian Biosafety Advisory Council on the notification B/BE/08/BVW1 of the company MedImmune for deliberate release in the environment of genetically modified organisms other than higher plants for research and development

Context

The notification B/BE/08/BVW1 has been submitted by MedImmune to the Belgian Competent Authority in October 2008 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 title of the notification is: **"A Phase 1/2a, Randomized, Double-Blind, Placebo-Controlled, Dose-Escalation Study to Evaluate the Safety, Tolerability, Immunogenicity and Vaccine-like Viral Shedding of MEDI-534, a Live, Attenuated Intranasal Vaccine Against Respiratory Syncytial Virus (RSV) and Parainfluenza Virus Type 3 (PIV3), in Healthy 6 to < 24 Month-Old Children and in 2 Month-Old Infants".** The planned activity concerns a clinical trial where the live, attenuated GM Bovine Parainfluenza virus (MEDI-534) will be administered as intranasal vaccine to healthy young infants for the prevention of lower respiratory tract illness caused by respiratory syncytial virus (RSV) and parainfluenza virus type 3 (PIV3). The purpose of the study is to evaluate the safety and immune response generated by administration of multiple doses of this vaccine in 2 groups of healthy infants : 6 to <24 month-old children and 2 month-old infants.

Each subject in the study will receive three doses of either MEDI-534 or matched placebo, at intervals of approximately two months. Given the dose schedule it is not possible for the subjects to remain within contained facilities for the duration of the study. As the trial centres are located in Brussels and in Flanders, as the patients will be treated ambulatory and are expected to shed vaccine virus through nasal secretions, the national territory is considered as the wider potential release area of the GM Bovine Parainfluenza virus.

The dossier has been officially acknowledged by the Competent Authority on 29 October 2008 and forwarded to the Biosafety Advisory Council for advice.
Within the framework of the evaluation procedure, the Biosafety Advisory Council, under the supervision of a coordinator and with the assistance of its Secretariat, contacted experts to evaluate the dossier. Two experts from the common list of experts drawn up by the Biosafety Advisory Council and the Division of Biosafety and Biotechnology (SBB) 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 for its intended use, would not raise any problems for the environment, animal health or human health (people coming in contact with the treated patient and/or with the GMO).

On 18 December 2008, based on a list of questions prepared by the Biosafety Advisory Council, the Competent Authority requested the notifier to provide additional information about the notification. The answers to these questions were received from the notifier on 26 January 2009 and reviewed by the coordinator and the experts.

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

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

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

Summary of the Scientific evaluation

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

It has been demonstrated that MEDI-534 seems to adapt *in vitro* to cells in which it replicates by accumulation of specific mutations, provoking an increase in MEDI-534 level of replication. This was observed in MRC-5 cells but no data is available yet about the possibility of a similar genetic adaptation in the infant respiratory system.
This possibility of adaptation resulting in higher level of replication (higher titer of viruses) may actually increase the vaccine shedding. The monitoring foreseen by the notifier should bring important information about this.

2. Information related to the vector

The Biosafety Council requested more information concerning the plasmids used for the vaccine strain production;

From the received answer the removal of the helper viruses is still not clearly described. The notifier considers that serial passages are sufficient, it is not clear how it was checked.

3. Information related to the characteristics of the GMO

No major risks were identified.

The question of the Biosafety Advisory Council about the sensitivity to detect virus variants or wild-type virus in the virus culture supernatant was adequately answered by the notifier.

The question of the Biosafety Advisory Council asking why the virulence of MEDI-534 in bovines has not been studied was adequately answered by the notifier.

4. The condition of release

The question of the Biosafety Advisory Council asking if the vaccine is easily administered to small children and if there any risk of loss of product (sneezing, cough,....) was adequately answered by the notifier.

The Biosafety Advisory Council asked that the fact that pregnant women and immunocompromised people could potentially be at risk should be correctly explained to the mothers of the treated children and to the carekeepers. This was satisfactory addressed in the "Patient Information and Informed Consent Form" meant for the parents(s) or legal representative(s) of the infant.
The informed consent that will be given to the parents is long and well detailed. However It is important that the parents understand the importance of strict adherence to the conditions stipulated in the informed consent, in particular the measures designed to limit exposure and virus spread, which effectively only they can control. A lack of understanding and compliance could lead to unanticipated and unwanted exposures to the MEDI-534 vaccine during this study.

5. The risks for the environment and human health

5.1 There is a risk of transmission of the GM bovine parainfluenza virus to bovines and family pets like hamsters and ferrets but the Biosafety Council hardly accepts the argument of the company when it says that the vast majority of cattle would be seropostive as a result of exposurure to the commonly circulating bPIV3 or as a result of vaccination. This argument is based on an assumption and no data are provided in the dossier. Moreover the Biosafety Council is not aware of any surveillance of bovine parainfluenza disease in Belgium.

5.2 It seems clear that some amount of viruses will be transmitted by the study subjects to others. With the exception of pregnant women and people with immunodeficiencies (cancer, AIDS, chronic illness) this should be relatively limited and benign.
The potential risk for pregnant women and people with immunodeficiencies is adequately explained to the parents in the informed consent document but as said above there is, on the side of the parents, a risk of lack of understanding and compliance.
Moreover, it is a total unknown what levels of replication will be attained in this young population – the available data from older children and adults indicates poor replication because of preexisting immunity to RSV and PIV3, but infants (<6 months old) are unlikely to have any significant neutralizing immunity. In this regard, some very useful information can be expected from this planned trial.
Ideally, the vaccinated infant would be kept away from any pregnant or potentially pregnant women and immunodeficient individuals for 28 days after each vaccine dose. In the real world there will be close contacts mainly with parents but also with similarly aged children and women of child bearing age.

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

The limited survival of the GMO in the field is well documented

However, since the MEDI-534 level of replication (and therefore the shedding) is still not known in very young seronegative children (who maybe have not already been exposed to wild-type PIV3), evaluation of the shedding and potential transmission to close households should be performed more thoroughly. Generally speaking, parents could be considered as the major household individuals who will have contact with the vaccinee. The notifier should perform a serological test of the parents before and after the study and this should be included in the consent form to be signed by the legal representative.

7. Additional points considered by the experts of the Belgian Biosafety Advisory Council

Although out of the scope of the Directive 2001/18, the Biosafety Advisory Council drew the attention of the notifier on some points concerning the efficacy and safety of the product.
The questions of the Council and the answers received from the notifier are given in annex II.

Conclusion

Based on the scientific assessment of the notification made by the Belgian experts, the Biosafety Advisory Council concludes that even if some amount of the genetically modified Bovine Parainfluenza virus Type 3 engineered to produce a life, attenuated intranasal vaccine against Respiratory syncitial virus (RSV) and Parainfluenza virus type 3 (PIV3) in healthy children and infants will be transmitted by the study subjects to others, the risk is relatively limited and benign for the general population. The risk is however higher for immunodeficient individuals and pregnant women. Concerning the risk for domestic animals, specially bovines, some important information lacks in the dossier and at this point the Biosafety Council can hardly state that the risk is benign for bovines coming into contact with the recombinant virus.

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

- The notifier should perform a serological test of the parents before and after the study and this should be included in the consent form to be signed by the legal representative.
- In absence of data concerning the risk for bovines individuals with close contacts with bovines should be excluded.
- The notifier and the investigators must strictly apply the protocol, the biosafety monitoring and, if necessary, the emergency measures as described in the dossier.
- Any protocol amendment has to be previously approved by the Competent Authority.
- The notifier is responsible to verify that each 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 90/219/EEC on Contained use of genetically modified organisms.
- The Biosafety Advisory Council should be informed within 2 weeks when the first patient starts the treatment and the last subject 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 the final study report including a report with details concerning the biosafety aspects of the project. This report will at least contain:
    - the number of patients included in the trial
    - the results of the virological surveillance tests including the serological tests performed in the parents
    - the list of all adverse events
    - a report on the accidental releases, if any, of the recombinant Bovine Parainfluenza virus

p.o.

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

Annex 1: Compilation of comments of experts in charge of assessing the dossier B/BE/08/BVW1 (ref: BAC_2008_855)
Annex 2: Additional comments related to the safety and efficacy of the product and answers given by the applicant.

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Compilation of Comments of Experts in charge of assessing the dossier B/BE/08/BVW1

Mandate for the Group of Experts: mandate of the Biosafety Advisory Council (BAC) of 31 October 2008
Coordinator: Dr. P. Hermans
Experts: Frank Koenen (CODA-CERVA), Céline Verheust (SBB), Karen Willard-Gallo (ULB-Bordet)
Domains of expertise of experts involved: Molecular genetics, design of vectors, virology (human virus and animal virus), human medicine, veterinary medicine, infectious diseases, wildlife disease, vaccination, zoonoses, biosafety, risk assessment
Secretariat (SBB): Didier Breyer, Martine Goossens, Philippe Herman

INTRODUCTION

Dossier **B/BE/08/BVW1** concerns a notification of the company MedImmune for deliberate release in the environment of genetically modified organisms other than higher plants according to Chapter II of the Royal Decree of 21 February 2005.
The notification has been officially acknowledged on 29 October 2008 and concerns a clinical trial with a recombinant Bovine Parainfluenza virus Type 3 which has been genetically modified to produce a life, attenuated intranasal vaccine against Respiratory syncitial virus (RSV) and Parainfluenza virus type 3 (PIV3) in healthy children and infants.

♦ 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

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

- For RNA viruses, spontaneous mutations are expected (misincorporated nucleotides by the RNA polymerase). At which frequency do these mutations occur? Have these mutations been identified during growth of the vaccine strain MEDI-534? What could be the effects of these mutations on the vaccine strain (stability, efficacy, virulence)?
- Could the MEDI-534 RNA genome be converted into DNA in the presence of a retroviral polymerase of an endogenous virus? If yes, what are the risks associated with this conversion?
- Could the notifiers give more information concerning the mechanism of attenuation of bPIV in humans?

Comment 2

Intra-nasally administered bovine PIV3 (bPIV3) has been evaluated as a vaccine throughout the pediatric age spectrum. These studies have shown that bPIV3 vaccination is efficient and safe in infants and children; however, although the seroconversion rate is high (>50%), vaccination with bPIV3 does not protect against infection with human PIV3 (hPIV3). MEDI-534 uses bPIV3 as an attenuated backbone for insertion of the hPIV3 hemagglutinin-neuraminidase (HN) and fusion (F) proteins and the RSV (respiratory syncytial virus) surface protein (F). The effectiveness of this vaccine against both PIV3 and RSV challenge has been demonstrated in African green monkeys. MEDI-534 is capable of infecting humans, non-human primates, bovines, hamsters and neonatal ferrets; however, because bPIV3 is attenuated in humans and non-human primates, the MEDI-534 vaccine, containing the bPIV3 backbone with two hPIV3 and one RSV surface protein genes, is equally attenuated in humans. Microorganisms and plants lacking receptors for hPIV3 attachment and entry (F and HN) cannot be infected with MEDI-534.

Vaccine virus shedding is clearly a property of the MEDI-534 vaccine since viral replication is necessary to obtain immune protection. A previous study showed that nasal shedding can occur days and weeks after vaccination with 10^5 $TCID_{50}$. One study in a daycare environment did not detect transfer of bPIV3 virus from vaccinated children to other children or adults in the center or as in the home. The proposed study does take extra precautions by excluding subjects that are in close daily contact with other infants < 6 months old, pregnant women and immunocompromised individuals either at home or in the daycare environment.

Comment 3

The sequence stability was not demonstrated completely by sequencing. Limiting to 20% the detection of possible mutated nucleotides has consequences for the interpretation of the cell and animal passages. In addition we could not find the percentage of homology. We would like to see the

sensitivity limits to detect a mutation only higher than 20 % included in the final conclusions where it is stated that de relative changes in the protein expression is not caused by changes in the genetic sequence of RSV F, and RSV F gene was stably maintained. (p19 specific questionnaire).

2. INFORMATION RELATED TO THE VECTOR

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

Comment 1

- The notifiers should have integrated more information concerning the plasmids used for the vaccine strain production. They are not described in the Notification Form but only in the Medicinal Product Dossier.
- The vaccine strain is rescued by reverse genetics, by co-transfection of 5 plasmids into VERO cells. Could genetic transfer occur between these plasmids, generating different vaccine strains? This is not discussed in the dossier.
- On page 38 of the Medicinal Product Dossier, under the subheading 2.1.S.3.1 “Elucidation of structure and other characteristics”, it is stated that “a” MEDI-534 vaccine preparation obtained by plasmid rescue was characterized (including sequencing). How many preparations have been created and used? Have they all been sequenced? It is not clear if this preparation corresponds to the master virus seed (MVS) which will be used to produce the final drug substance)?

Comment 2

Studies have shown that bPIV3 is an attenuated and immunogenic vaccine and demonstrated that it can be an effective vector for expressing foreign viral antigens. MEDI-534 is a recombinant vaccine that was produced by inserting the hPIV3 F and HN genes in place of the bPIV3 F and HN genes with an additional insertion of the RSV F protein at position 2 of the genome. The final MEDI-534 vaccine expresses immunodominant surface glycoproteins from both RSV and hPIV3.

Comment 3

The removal of the helper viruses is not clearly described. The authors consider that serial passages are sufficient, but we could not find how it was checked.

Comment from SBB: This remark is not pertinent since no helper viruses were used for the preparation of MEDI-534 (see p. 23 of 2.1.S Drug substance).

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

Standard and well controlled molecular biology techniques were used to design and insert specific sequences in the vector.

Comment 3

For the methods the authors refer to a P-reviewed paper by Haler. What is suggested by sequence analysis of the inserts demonstrated that no mutations are "accumulated"…, (p.44 2.1.S. Drug substance)?

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

Is the drug substance MEDI-534 entirely sequenced at the final stage in order to be sure that no genetic modification occurred? (This question may have been answered in the dossier but the dossier is not so well structured).

Comment 2

Various *in vitro* and *in vivo* studies were performed to investigate genotypic and phenotypic stability in preparation for this trial.

Because the hPIV3 HN and F proteins, but not the RSV F protein, are necessary for virus propagation and spread, only expression of RSV F was observed to diminish over time. None of the viral proteins were found to have altered amino acid sequences in rescued virus from serial passages *in vitro* (Vero cells) and in the various animal models studied.

The MEDI-534 genome remains as RNA because the virus does not contain a viral RNA-directed DNA polymerase. Thus, it is unlikely that it will integrate into the host genome. There is minor potential for recombination between wild-type and MEDI-534 viruses in vaccine subjects that are also infected (clinically or sub-clinically) with wild-type hPIV3.

The trial includes studies to determine the types of respiratory viruses recovered in the nasal washings and to investigate the persistence of MEDI-534 by determining whether detectable PIV3 is wild-type hPIV3 or MEDI-534 virus. Any vaccine virus isolated will be checked for genetic stability, and this should be considered as an absolute must for each and every case of recovered virus.

Comment 3

The stability of the final GMO was analysed with a sensitivity detecting only a nucleotide mutation higher than 20%. How it was obtained by spiking (single, multiple) is not clear. This is important since

the detection limits can change from genomic region to genomic region or by single nucleotide polymorfisme (SNP). This is even more important since different protein processing was seen in Western Blot (pag42 of IMPD - 2.1.S. Drug substance) and a change in cell plaques. These findings need an in depth discussion.

3.3. Considerations for human, animal or plant health

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

Comment 1

Has evaluated this item and has no questions/comments.

Comment 2

Vaccination of infants (up to 3 years of age) against influenza has been associated with symptomatic asthma, and the effects include acute and delayed adverse reactions. In addition, there are reasonable scientific discussions that modulation of the immune system by early life vaccination potentially promotes allergies and/or autoimmune reactions in infants (in particular, those with allergic or specific HLA genetic backgrounds) although there is currently no direct evidence for this association. Short term adverse events, including adverse immune reactions, will be assessed during the trial, but long-term adverse reactions will be largely ignored (anything beyond 6 months or the end of RSV season). At the very least, it seems reasonable to request that any acute adverse immune responses triggered by exposure to the viral, residual cellular or chemical components of MEDI-534 vaccine and/or its replication in host cells be followed up by long term assessment (up to 5 years) of allergic and autoimmune disease in these particular children.

Comment 3

The invasiveness and virulence, the toxic effects in humans and the survival outside of the receiving host (humans) is well described. The bPIV3 Kansas/5626/15626/84 strain that serves as a backbone for MEDI-534 vaccine naturally occurs in cows. A bPIV3 infection on its own is usually uncomplicated and sub clinical or with mild clinical illness only (Vangeel 2007). A bPIV3 infection can predispose secondary infections in the respiratory tract of bovines. The virulence of MEDI-534 in bovines has not been studied. It also replicates in the respiratory tract of hamsters, ferrets and monkeys. The authors minimise the risk indicating the sharing of nasal secretions from vaccinated infants with animals is required (RA p13) and several other places in the documents (Notification form pages 41 & 42). We would recommend studying the possible infectivity and virulence at least in bovines, the natural host of the strain that served as backbone.

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

Is the vaccine easily administered to small children? Is there any risk of wrong-administration, of over-dosage or under-dosage?

Comment 2

The final vaccine preparation, the producer Vero (monkey) cells and reagents used in vaccine preparation have been extensively checked for microbial contamination and other adventitious agents throughout the production protocol and shown to be negative at each step in the process. This appears to be well controlled by current state-of-the-art detection techniques.

MEDI-534 has restricted replication in seropositive (RSV and hPIV3) individuals.

The potential risk associated with host cell contaminants in the final vaccine preparation is also important to consider and our current knowledge of their potential long term consequences is unknown. MedImmune's studies show that a 10^6 $TCID_{50}$ dose of MEDI-534 contains 0.08 pg of Vero cell DNA (majority in 500 bp fragments) and 42 ng of Vero cell protein. There is also some residual Benzonase and BSA. There could be unknown effects of one or more of these contaminants on the immature immune responses of the study subjects, especially in the cohort of 2 month old infants. In particular, some unwanted effects could come from protein contaminants and include exacerbated development of asthma or autoimmune diseases. Furthermore, these undesirable clinical effects may not appear during the trial period but emerge later as the infant's immune system matures. According to the clinical trial protocols they do not foresee any follow up beyond 6 months or the end of RSV season (April). Since this is a real concern that is the object of paper in the medical literature, I am wondering why, with the detailed information MedImmune has provided for everything else, why they have not addressed this issue.

Comment 3

Has evaluated this item and has no questions/comments.

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

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

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

Comment 1

Has evaluated this item and has no questions/comments.

Comment 2

MEDI-534 must replicate in the nasal mucosa to provoke an immune response and vaccination with MEDI-534 in RSV and PIV3 seronegative subjects leads to vaccine virus shedding, thereby releasing the GMO. The duration of viral shedding after vaccine administration in infants is unknown, but because bPIV3 is attenuated in humans it has been suggested that replication levels will be lower than with wild-type hPIV3. Nevertheless, there is a very real possibility of secondary transmission, in particular to other infants, pregnant women and immunocompromised individuals. The exclusion of infants, who during the trial period are in daily contact with people in the above groups, seems a reasonable means of preventing potentially deleterious virus transmission. However, for the 6-24 month cohort there is a relatively high probability that mothers of these infants could become pregnant during the trial period and be inadvertently exposed to MEDI-534 during the initial stages of an undetected pregnancy. What is the proposed follow up should this occur?

Comment 3

Well described. Shedding is possible in naïve recipients but very limited in seropositive individuals. This is supported by clinical studies for seropositive individuals but as well the magnitude as the duration of shedding in seronegative children is still unknown. Shedding leading to secondary transmission to vulnerable population is possible (several times mentioned, best summarised in 3RA and a statement on RE, p 2, part RA p6-7) and should be considered more carefully in the RA.

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

As pregnant women and immunocompromised people could potentially be at risk, this needs to be correctly explained to the mothers of the treated children and to the carekeepers. Could the notifier provide a copy of the information leaflet foreseen for the parents of the children and for the clinical staff with the responsibility of administering MEDI-534 or collecting nasal swabs ?

Comment 2

Virus shedding is a necessary characteristic of the MEDI-534 vaccine; however, the duration and extend of viral production and shedding in children is unknown. The risk of transmission from vaccinated subjects to other children as wells as pregnant women and immunocompromised individuals is unknown. See above 5.1.

Comment 3

The possible risk is mentioned but not clinically evaluated and quantified.

5.3. Information on possible effects on animal health or on the environment.

Comment 1

Since virulence of MEDI-534 has not been studied in bovines, the notifiers should insist on the fact that any contact between the patient or any person closed to the patient and bovines should be avoided.

Comment 2

Has evaluated this item and has no questions/comments.

Comment 3

The possible risk of transmission is mentioned but not clinically evaluated. The virulence of MEDI-534 in bovines has not been studied. It also replicates in the respiratory tract of hamsters, ferrets and monkeys. The authors minimise the risk indicating the sharing of nasal secretions from vaccinated infants with animals is required (RA p13) and several other places in the documents (Notification form pages 41 & 42).

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

There do not appear to be any selective advantages. bPIV3 is attenuated in humans and the inclusion of hPIV3 and RSV surface proteins does not confer a selective advantage to bPIV3 for replication, only for infection in humans.

Comment 3

Has evaluated this item and has no questions/comments - The tested cell types are limited to Vero cells

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

Reverting to the wild-type virus would mean reverting to bPIV3 – i.e. exchanging the hPIV3 HN and F proteins for the bovine genes and losing expression of RSV F. Although mutations could occur to cause reversion of the hPIV3 proteins to the bovine amino acid sequence and the RSV F gene could be deleted, it is unlikely that a sufficient number of replications will occur for all of these changes to occur in the same virus isolate. Furthermore, the consequences of this genetic reversion would seem to be negligible since the virus would revert to wild-type bPIV3, which is already present in the environment.

A theoretical effect that could have important consequences on human health and the environment would arise if recombination with hPIV3 occurred in an infected individual and the resulting virus acquired the full capacity for replication and transmission that characterizes hPIV3. This new strain of hPIV3 might be more infectious or acquire other characteristics due to the presence of the RSV F protein and would be disseminated in the environment via human replication and transmission in a manner similar to wild-type virus.

Comment 3

Has not evaluated this item.

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

See above 5.5

Comment 3

I could not find conclusive information. The number of experiments is rather limited and the experiments were often stopped rather early.

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

Comment 1

Has evaluated this item and has no questions/comments.

Comment 2

See above 5.5

Comment 3

I could not find conclusive information. The number of experiments is rather limited and the experiments were often stopped rather early.

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

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

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

Comment 1

Has evaluated this item and has no questions/comments.

Comment 2

Has not evaluated this item.

Comment 3

A clear monitoring plan and risk identification is described. In the study exclusion criteria are outlined to limit the contact between treated and possible susceptible individuals (RA p 9; MI-CP78 study investigator's Brochure). However the authors seem too confident concerning the follow up and minimise the risks of transmission by seronegative vaccinees. The duration of shedding of seronegative infants is still under investigation and is partially deduced from experiments with the

bPIV3 strain. The monitoring plan for shedding (RA p 9) is rather limited. Since the authors foresee only shedding during the first days we would recommend intensifying the monitoring during the first two weeks. This has the advantages if an adverse effect occurs this can be traced easier.

6.2. Surveillance and control of the release

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

Comment 1

Has evaluated this item and has no questions/comments.

Comment 2

Their protocols and precautions seem adequate.

Comment 3

In the study the procedure to avoid or minimise the spread is laid down in exclusion criteria to limit the contact between treated and possible susceptible individuals (RA p 9; MI-CP78 study investigator's Brochure). However the authors seem too confident concerning the follow up and minimise the risks of transmission by seronegative vaccines. The duration of shedding of seronegative infants is still under investigation and is partially deduced from experiments with the bPIV3 strain.

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

After vaccine administration, the single use syringe will either be placed in a locked container or sealed bag and after trial accounting will be disposed of onsite using standard biohazard disposal mechanisms employed at the center. This approach follows standard procedures and guidelines.

Comment 3

The normal waste treatment is well described, however the treatment of possible waste generated due to more abundant nasal secretion of the vaccinees at home is not. Although the limited survival of the GMO in the field is well documented, the treatment of this "domestic" waste needs to be considered.

Comment SBB: The above proposal is not realistic in the context of this clinical trial.

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

Comment 1

Has evaluated this item and has no questions/comments.

Comment 2

Has not evaluated this item.

Comment 3

Has not evaluated this item.

6.5 Information related to the identification of the GMO and the detection techniques
(e.g. identification methods and detection techniques, sensitivity, reliability and specificity of the proposed tests ..)

Comment 1

Has evaluated this item and has no questions/comments.

Comment 2

Has evaluated this item and has no questions/comments.

Comment 3

Has evaluated this item and has no questions/comments.

7. OTHER INFORMATION

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

Comment 1

None

Comment 2

This study principally addresses the short term effects of the MEDI-534 vaccine in infants (the trial will follow the infants for 6 months or through the RSV season). There is no mention of the long term effects of exposing infants, with immature immune systems, to the various components present in the vaccine preparation, which is somewhat disconcerting since the vaccine subjects are 2 month and 6-24 months old. In particular infants with adverse reactions involving acute or delayed hypersensitivity

reactions or other immune based problems should be followed over a longer period of time (5 years) to determine whether this group had a higher incidence of allergic or autoimmune diseases than normal.

Note: there are theories suggesting an increased prevalence of autoimmune diseases results from a decreased incidence of childhood infections due to vaccination.

Comment 3

This notification is well documented however important information is missing concerning the possible spread of MEDI-534 in seronegative infants. Some experiments are ongoing and we would recommend sharing this information before starting the present trial. In addition some experiments are stopped rather early. With a longer duration more information could have been gained concerning stability. In addition since the excretion and spread is expected during the first weeks a more frequent sampling during this period can be recommended.
The risk for immunocompromised people is underestimated and not documented.
The risk for bovines and other animal species should be studied.

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Additional comments related to the safety and efficacy of the product and answers given by the applicant.

Although out of the scope of the Directive 2001/18, the Biosafety Advisory Council would like to draw the attention of the notifier on the following points.

Vaccination of infants (up to 3 years of age) against influenza has been associated with symptomatic asthma, and the effects include acute and delayed adverse reactions. In addition, there are reasonable scientific discussions that modulation of the immune system by early life vaccination potentially promotes allergies and/or autoimmune reactions in infants (in particular, those with allergic or specific HLA genetic backgrounds) although there is currently no direct evidence for this association. Short term adverse events, including adverse immune reactions, will be assessed during the trial, but long-term adverse reactions will be largely ignored (anything beyond 6 months or the end of RSV season). At the very least, it seems reasonable to request that any acute adverse immune responses triggered by exposure to the viral, residual cellular or chemical components of MEDI-534 vaccine and/or its replication in host cells be followed up by long term assessment (up to 5 years) of allergic and autoimmune disease in these particular children.

The potential risk associated with host cell contaminants in the final vaccine preparation is also important to consider and our current knowledge of their potential long term consequences is unknown. MedImmune's studies show that a 10^6 $TCID_{50}$ dose of MEDI-534 contains 0.08 pg of Vero cell DNA (majority in 500 bp fragments) and 42 ng of Vero cell protein. There is also some residual Benzonase and BSA. There could be unknown effects of one or more of these contaminants on the immature immune responses of the study subjects, especially in the cohort of 2 month old infants. In particular, some unwanted effects could come from protein contaminants and include exacerbated development of asthma or autoimmune diseases. Furthermore, these undesirable clinical effects may not appear during the trial period but emerge later as the infant's immune system matures. According to the clinical trial protocols they do not foresee any follow up beyond 6 months or the end of RSV season (April). Since this is a real concern that is the object of paper in the medical literature, Why, with the detailed information MedImmune has provided for everything else, they have not addressed this issue.

<u>MedImmune Response</u>
Long-term, multi-season safety studies are typically not performed as part of early-phase vaccine clinical development. Such studies are only performed after the primary safety profile and indicated population of the vaccine for commercialization have been clearly established. Designing a long-term safety study during late-stage clinical development allows study entry criteria to be less restrictive; thus, permitting enrolment of a larger and more representative population of vaccine recipients for a meaningful or targeted analysis of rare events. Therefore, many long-term safety studies with vaccines are best accomplished in a post-marketing environment where retrospective cohort analysis can be performed on a large scale through governmental or HMO (healthcare management organizations) systems.
MI-CP178 is a Phase 1/2a study designed to identify important safety signals that are most likely to occur in a close temporal relationship with administration of vaccine that could limit further clinical development of the vaccine candidate. The biologic mechanism for MEDI- 534 to induce an immune response requires that the vaccine virus replicate in the nasal mucosa. Commonly observed adverse events would be related to local reactogenicity in the nose (rhinorrhea, nasal congestion) or resulting from virus replication causing symptomatology similar to wild-type infection from RSV or PIV3 causing upper respiratory illness-like

symptoms. The study has been designed to identify safety signals that may limit further clinical development of this vaccine candidate. The trial is not adequately powered for the detection of rare events, which would more likely be captured as part of Phase 3 pivotal studies or in post-marketing safety studies as described above. MedImmune has designed this trial to protect subjects by monitoring for local and systemic toxicity that have been identified through the preclinical studies performed using MEDI-534 and bPIV3 vaccines, from clinical studies with bPIV3 (the MEDI-534 backbone) in adults, children and young infants, from the clinical data from MEDI-534 studies in adults, seropositive children and from the ongoing MI-CP149 US study in RSV/PIV3 seronegative 6 to < 24 month olds.
Theoretical toxicities related to the vaccine candidate have been described in the Investigator's Brochure and the vast majority of these outcomes would be expected to occur in a temporally close association with vaccination related to vaccine virus replication.
Additionally, there is no evidence from previous preclinical and clinical studies for MEDI- 534 to remain viable within the host to result in latent effects. It is important to note that there is no evidence that live attenuated RSV or PIV3 vaccines cause wheezing and no wheezing signal was identified in studies with the bPIV3 viral backbone in infants (Greenberg 2005). Any adverse events that are determined to be vaccine-related are subject to care as described in the study's informed consent form.

As described fully in the study protocol and Investigator's Brochure, subjects will be followed through the subsequent RSV season after vaccination to survey for enhanced RSV disease. The MI-CP178 study is intended to enroll infants and children globally over a period of approximately 2 years. Therefore, exposure to vaccine will be very limited at any one time. Safety data will be collected in real time to ensure no protocol stopping criteria have been met and will therefore not put additional infants at unnecessary risk. This approach reflects current best practices in adaptive clinical trial design.

As part of the preclinical safety assessment of MEDI-534, a multiple-dose GLP toxicity study in Sprague-Dawley rats was performed to assess potential toxicity of residual BenzonaseTM present in the final MEDI-534 product. The study was conducted using MEDI-534 in formulation buffer (219 mM sucrose, 11 mM potassium phosphate, pH 7.0) and in formulation buffer with 0.8 units of BenzonaseTM added. Because BenzonaseTM is a residual in the formulation process, MEDI-534 with BenzonaseTM added to a four-fold excess over the highest level observed in development lots was tested. Results from this study demonstrated that intranasal administration of MEDI-534 with or without BenzonaseTM was well tolerated.
Further details on the study can be found in the Investigator's Brochure.

The specifications for residual amounts of BenzonaseTM, BSA, host cell DNA and host cell protein implemented during manufacture of MEDI-534 and established as batch release criteria, were selected in accordance with the most current guidance available from both the United States Food and Drug Administration (Characterization and Qualification of Cell Substrates and Other Biological Starting Materials Used in the Production of Viral Vaccines for the Prevention and Treatment of Infectious Diseases, 2006) and the International Conference on Harmonisation (ICH) documents Q5A and Q5D. The theoretical risks from the presence of residual cellular DNA or protein include oncogenic, infectivity or tumorigenic potential. Therefore, mitigation of these risks is accomplished by minimizing biological activity by decreasing the amounts of these residuals and additionally for host cell DNA to below the size of a functional gene. Based on the US FDA guidance referenced above, for continuous non-tumorigenic cell lines such as Vero cells, which are widely used and well characterized, it is recommended that residual DNA levels be less than 10 ng/dose for parenteral inoculation. Additionally, these residuals are widely found in other live, attenuated commercially available vaccines which have established long-term safety profiles in children and infants.