TG1050

SUMMARY NOTIFICATION INFORMATION FORMAT FOR THE RELEASE OF GENETICALLY MODIFIED ORGANISMS OTHER THAN HIGHER PLANTS IN ACCORDANCE WITH ARTICLE 11 OF DIRECTIVE 2001/18/EC

April 2014

TABLE OF CONTENT

LIST OF ABBREVIATIONS

Ad5	Adenovirus serotype 5
aa	Amino acids
ALAT	Alanine aminotransférase
ASAT	Aspartate aminotransférase
CMV	Cytomegalovirus
DNA	Desoxynucleic acid
DP	Drug product
DS	Drug substance
Env	Enveloppe
GMO	Gene modified organism
HBsAg	Hepatitis B surface antigen
HBV	Hepatitis B virus
HIV	Human immunodeficiency virus
HPLC	High-performance liquid chromatography
ICH	International Conference on Harmonisation
IFN	Interferon
IMP	Investigational medicinal product
MHC	Major histocompatibility complex
MOI	Multiplicity of infection
MVS	Master virus seed
PCR	Polymerase chain reaction
PGK	Phospho glycerate kinase
Pol	Polymerase
Pre-MVS	Pre-master virus seed
QC	Quality control
Q-PCR	Quantitative Polymerase chain reaction
RCA	Replication competent adenovirus
RNA	Ribonucleic acid
RS	Reference standard
SC	Subcutaneous
TS	Test sample
vp	Viral particles

A. GENERAL INFORMATION

1. Details of notification

a) Member State of notification France

b) Notification number B/FR/15/GT02

c) Date of acknowledgement of notification 31/12/2014

d) Title of the project A phase I/IB randomized, double-blind, placebo controlled dose finding study to evaluate the safety, tolerability, and immune response of single and multiple dose of the hepatitis B specific replication-defective adenovirus serotype 5 (Ad5) vector based vaccine, TG1050, and an initial evaluation of immunologic and antiviral activity in the treatment of patients with chronic hepatitis B infection

e) Proposed period of release From Q4 2014 until Q4 2017 (date of study completion)

2. Notifier

Name of institution or company **Transgene SA**, France

3. GMOs characterization

The GMO is the active ingredient (ADTG18201) of an investigational medicinal product named **TG1050.**

TG1050 contains a non-replicative recombinant Adenovirus of type 5 (Ad5) encoding the fusion protein Core-Polymerase-Env of Hepatitis B virus (HBV). The GMO is also named **ADTG18201**.

a) Indicate whether the GMO is a:

viroid ☐
RNA virus ☐
DNA virus ☒
bacterium ☐
fungus ☐
animal ☐
- mammals ☐
- insect ☐
- fish ☐
- other animal ☐ specify phylum, class

other, specify (kingdom, phylum and class)

b) Identity of the GMO (genus and species)

Genus: Mastadenovirus
Species: human adenovirus serotype 5 (Ad5)

c) Genetic stability – according to Annex IIIa, II, A (10)

At the research scale, a genetic stability study of the ADTG18201 construct to a passage level at least comparable to a production batch was performed. The main steps were 1) the selection of an initial clone of AdTG18201 by limited clonal dilution, 2) the 9 passages of this clone on the production cell line HER96 mimicking a larger production scale, 3) the isolation and characterization at protein expression and sequence levels of at least 100 clones. The study demonstrated the genetic stability of AdTG18201, with almost 100%, of the clones presenting the expected HBV nucleotidic sequences and the correct expression of the HBV fusion protein.

At the production level, the genetic stability of the ADTG18201 is controlled through several tests:

- Genomic integrity by restriction mapping
- Proportion of viruses expressing HBV antigen by Western blot on isolated sub-clones
- Vector sequence by sequencing of the whole genome

The test by restriction mapping is performed on the Pre-Master Virus Seed (Pre-MVS), the Master virus Seed (MVS) and on each clinical lot (at the step purified bulk).

The proportion of viruses expressing HBV antigen is performed on 100 sub-clones isolated from the MVS.

The sequencing of the whole genome of ADTG18201 is controlled on a lot issued from the MVS.

4. Is the same GMO release planned elsewhere in the Community (in conformity with article 6 (1)), by the same notifier?

Yes ☒ No ☐

If yes, insert the country code(s): DE

Please use the following country codes:
Austria AT; Belgium BE; Bulgaria BG; Cyprus CY; Czech Republic CZ; Denmark DK; Estonia EE; Finland FI; France FR; Germany DE; Greece GR; Hungary HU; Ireland IE; Italy IT; Latvia LV; Lithuania LT; Luxembourg LU; Malta MT; Netherlands NL; Poland PL; Portugal PT; Romania RO; Slovak Republic SK; Slovenia SI; Spain ES; Sweden SE; United Kingdom GB.

5. Has the same GMO been notified for release elsewhere in the Community by the same notifier?

Yes ☐ No ☒

If yes:
- Member State of notification
- Notification number

6. Has the same GMO been notified for release or placing on the market outside the Community by the same or other notifier?

Yes ☒ No ☐

If yes:

- Member State of notification New Zealand, Canada

7. Summary of the potential environmental impact of the release of the GMOs

The GMO TG1050 is a recombinant virus manufactured in pharmaceutical facilities and thus do not exist in nature. Considerable care will be taken on clinical sites to ensure that the GMO is contained and that areas/personnel are not exposed to the GMO. All wastes resulting from the GMO handling are to be treated according to regular hospital procedure for infectious wastes. In case of accidental spillage, specific recommendation for decontamination and destruction are to be followed to avoid any risk of dispersion to the environment, contaminated work area must be secured and access should be limited only to people authorized to handle the GMO.

Given its genetic modification (deletion of E1 and E3 regions), the GMO can only replicate in cells which carry complementing regions of the E1 genes. The probability that the missing E1 function is complemented is extremely low in natural ecosystems. Furthermore since the modified virus is replication deficient, it is less pathogenic than the wild-type Ad5 and there is minimal capacity for colonization. If it is exposed to the environment, it is unlikely to survive for extended periods. Finally, the SC route is also considered to limit the risk of shedding to the environment.

Taking this information into consideration, a biological dispersion of the GMO to the environment is unlikely and its potential environmental impact is considered negligible.

B. INFORMATION RELATING TO THE RECIPIENT OR PARENTAL ORGANISMS FROM WHICH THE GMO IS DERIVED

- The **parental organism** is the wild-type Ad5.
- The **recipient** is the recombinant Ad5 deleted in its E1 (completely) and E3 (partially) regions (Ad5 $E1^-$ $E3^-$).

1. Parental organism characterization:

a) Indicate whether the recipient or parental organism is a:

viroid ☐
RNA virus ☐
DNA virus ☒
bacterium ☐
fungus ☐
animal ☐
- mammals ☐
- insect ☐
- fish ☐

- other animal ☐ specify phylum, class other, specify

2. Name

(i)	Order and/or higher taxon (for animals)	
(ii)	Genus	Mastadenovirus
(iii)	Species	human adenovirus
(iv)	Subspecies	subgroup C
(v)	Strain	human serotype 5 (Ad5)
(vi)	Pathovar (biotype, ecotype, race, etc.)	
(vii)	Common name	human adenovirus serotype 5 (Ad5)

3. Geographical distribution of the organism

a) Indigenous to, or otherwise established in the country where the notification is made:

Yes ☒ No ☐ Not known ☐

b) Indigenous to, or otherwise established in other EC countries:

(i) Yes ☒

If yes, indicate the type of ecosystem in which it is found:

Atlantic	☒
Mediterranean	☒
Boreal	☒
Alpine	☒
Continental	☒
Macaronesian	☒

(ii) No ☐

(iii) Not known ☐

The parental organism is ubiquitous in the environment.

c) Is it frequently used in the country where the notification is made?

Yes ☐ No ☒

d) Is it frequently kept in the country where the notification is made?

Yes ☐ No ☒

4. Natural habitat of the organism

a) If the organism is a microorganism

Water	☐
Soil, free-living	☐
Soil in association with plant-root systems	☐
In association with plant leaf/stem systems	☐
In association with animal	☐

other, specifiy humans

If the organism is an animal: natural habitat or usual agroecosystem: Not applicable.

5. (a) Detection techniques

Human adenovirus is detected by cell culture and/or PCR.

5. (b) Identification techniques

Human adenovirus is identified by restriction mapping and PCR.

6. Is the parental organism classified under existing Community rules to the protection of human health and/or the environment?

Yes ☒ No ☐

If yes, specify

Group/Class 2 under Directive 90/679/EEC (Protection of workers from risks related to exposure to biological agents at work).

7. Is the parental organism significantly pathogenic or harmful in any other way (including its extracellular products), either living or dead?

Yes ☒ No ☐ Not known ☐

If yes:

a) to which of the following organisms:

Humans	☒
Animals	☐
Plants	☐
Other	☐

b) give the relevant information specified under Annex IIIA, point II. (A)(11)(d) of Directive 2001/18/EC

Over 50 distinct human Ad serotypes have been isolated, which vary in their pathogenicity. The subgroup C Ad (which includes serotype 5) usually cause mild upper respiratory,

gastrointestinal and ocular infections in young children. Shedding via the gut following acute infection can occur, and latent infection of the adenoids and tonsils is also recognized. However, life-long immunity to the specific serotype is thought to be the usual outcome of primary infection. Exposure to subgroup C adenoviruses is widespread in the population; the majority of adults are seropositive for this type of adenovirus. In immune-competent adults, infections with Ad5 are mostly asymptomatic and usually self-limiting.

8. Information concerning reproduction

a) Generation time in natural ecosystems:

Wild type Ad5 replicates in approximately 24 hours.

b) Generation time in the ecosystem where the release will take place:

The reproduction of the recipient is restricted compared to the parental organism (i.e. the wild-type Ad5) due to the modification in E1 gene, which abrogates the capacity of replication. If it is exposed to the environment, it is unlikely to survive for extended periods.

c) Way of reproduction: Sexual ☐ Asexual ☒

d) Factors affecting reproduction:

As mentioned above, the reproduction of the recipient is restricted compared to wild-type Ad5 due to the deletion of the E1 region, which abrogates the capacity of replication. If it is exposed to the environment, it is unlikely to survive for extended periods.

9. Survivability

a) ability to form structures enhancing survival or dormancy:

(i)	endospores	☐
(ii)	cysts	☐
(iii)	sclerotia	☐
(iv)	asexual spores (fungi)	☐
(v)	sexual spores (fungi)	☐
(vi)	eggs	☐
(vii)	pupae	☐
(viii)	larvae	☐
(ix)	other, specify…	

b) Relevant factors affecting survivability:

Wild-type Ad5 is resistant to dehydration and is able to persist in aerosols and water. In frozen condition, the virus can be kept for years.
The recipient Ad5 $E1^-$ $E3^-$ is unlikely to survive for extended periods.
Adenoviruses are destroyed with bleach at 0.5% of active chlorine (i.e. 5 g/l of active chlorine) or autoclaving at 121°C for 20 minutes.

10.(a) Ways of dissemination

The common route of wild-type Ad5 transmission is via inhalation or uptake in the eye of aerosols produced through couching/sneezing by infected individuals. Adenovirus

transmission can also occur via the fecal-oral route, but this transmission route requires intimate contact and is therefore unusual.
The proposed clinical study will be conducted at standard healthcare facilities. The dissemination of the GMO in ecosystems is limited to humans (healthcare staff and any other person in close contact with the patient) and to the surrounding areas (healthcare facilities), because dissemination would require either close contact with the injection site or with the needle stick, or indirect contact with contaminated surfaces or objects or with contaminated biological fluids (blood, urine, feces).

10. (b) Factors affecting dissemination

Dissemination of wild-type Ad5 is affected by the dose shed, the production of aerosols and the intimacy of contact.
In the proposed clinical study, specific recommendation for decontamination and destruction will be followed by the healthcare staff to avoid any risk of dispersion to the environment.

11. Previous genetic modifications of the recipient already notified for release in the country where the notification is made (give notification numbers)

No.

C. INFORMATION RELATING TO THE GENETIC MODIFICATION

1. Type of the genetic modification

i. Insertion of genetic material ☒
ii. Deletion of genetic material ☒
iii. Base substitution ☐
iv. Cell fusion ☐
v. Other, specify ☐

2. Intended outcome of the genetic modification

The following genetic modifications were performed on the Ad5 backbone:

- The E1 region, required for viral replication, was deleted and replaced by the expression cassette containing the CMV immediate early enhancer/promoter, a chimeric human β globin/IgG intron, the HBV fusion protein Core-Pol-Env and the SV40 late polyadenylation signal. The advantage of this deletion is to inhibit the spread of the virus and to provide space for the insertion of foreign genes.
- The E3 region was partially deleted. Proteins encoded by the E3 region counteract the host immune response.

The intended outcome of the genetic modifications is a therapeutic purpose. The GMO, TG1050, is a targeted active immunotherapy product designed for the treatment of patients with chronic hepatitis B. Its mechanism of action is based on the capacity of the GMO to induce an active specific immune response against HBV antigens: innate immune response and cytolytic T cells immune response. The innate immune response to TG1050 could lead to the clearance of HBV infected hepatocytes while CD8+ T cell specific of encoded HBV

antigens (core, pol and env) could eliminate HBV infected hepatocytes through the cytolytic T cells immune response.

3. (a) Has a vector been used in the process of modification

Yes ☒ No ☐

If no, go straight to question 5.

3. (b) If yes, is the vector wholly or partially present in the modified organism?

Yes ☒ No ☐

If no, go straight to question 5.

4. If the answer to 3(b) is yes, supply the following information

a) Type of vector

Plasmid	☒
Bacteriophage	☐
Virus	☐
Cosmid	☐
Transposable element	☐

Other, specify

b) Identity of the vector

The GMO is made from the plasmid vector pTG18201.

c) Host range of the vector

The plasmid vector replicates in *Escherichia coli* bacteria.

d) Presence in the vector of sequences giving a selectable or identifiable phenotype

Yes ☒ No ☐

Antibiotic resistance ☒
Other, specify

Indication of which antibiotic resistance gene is inserted

Ampicillin.

e) Constituent fragments of the vector

pTG18201 contains the full length Ad5 genome with deletions in E3 and E1 regions, the E1 region being replaced by the expression cassette containing the CMV immediate early enhancer/promoter, a chimeric human β globin/IgG intron, the HBV fusion protein Core-Pol-Env and the SV40 late polyadenylation signal.

f) Method for introducing the vector into the recipient organism

i. transformation ☐
ii. electroporation ☐
iii. macroinjection ☐
iv. microinjection ☐
v. infection ☐
vi. other, specify The AdTG18201 vector was generated by a transfection step of the linearized plasmid pTG18201 DNA in HER96 complemented cell line: the digestion of pTG18201 by PacI restriction enzyme gave a DNA fragment containing all the viral genome part and eliminated the bacterial ORI as well as the ampicillin resistance gene. This DNA fragment was then transfected in HER96.

5. If the answer to B.3 (a) and (b) is no, what was the method used in the process of modification?

i. transformation ☐
ii. microinjection ☐
iii. microencapsulation ☐
iv. macroinjection ☐
v. other, specify

6. Composition of the insert

a) Composition of the insert

Synthetic HBV fusion protein Core-Polymerase-Env.

b) Source of each constituent part of the insert

Synthetic synthesis for the HBV fusion protein Core-Polymerase-Env

c) Intended function of each constituent part of the insert in the GMO

The intended function of the insert is to induce strong and multi-specific T cell responses to treat chronic Hepatitis B infection.

- The Core protein has been demonstrated to be the target of T cell responses in patients resolving HBV infection spontaneously or upon treatment (Ferrari C. *et al.*, 1990) (Penna A. *et al.*, 1997). This antigen carries 2 epitopes described to be associated with resolution of infection (Tsai S.L. *et al.*, 1992) (Rossol S. *et al.*, 1997) (Webster G.J. *et al.*, 2004) (Bertoni R. *et al.*, 1997). The Core protein is considered as an essential component of the vaccine.
- The Polymerase protein has been demonstrated to be rich in T cell epitopes (even if less extensively studied than the Core protein). It includes one epitope described to be associated with resolution of HBV infection (Webster G.J. *et al.*, 2004).
- The HBsAg protein or Envelope protein has also been demonstrated to be rich in T cell epitopes, including two epitopes described to be associated with resolution of HBV infection (Webster G.J. *et al.*, 2004).

d) Location of the insert in the host organism

- on a free plasmid ☐
- integrated in the chromosome ☐
- other, specify

The insert is integrated in the E1 region of the Ad5 genome.

e) Does the insert contain parts whose product or function are not known?

Yes ☐ No ☒

If yes, specify

D. INFORMATION ON THE ORGANISM(S) FROM WHICH THE INSERT IS DERIVED

1. Indicate whether it is a:

Viroid ☐
RNA virus ☐
DNA virus ☐
bacterium ☐
fungus ☐
animal ☐
- mammals ☐
- insect ☐
- fish ☐
- other animal ☐ specify phylum, class

other, specifiy HBV fusion protein Core-Polymerase-Env obtained from synthetic DNA (**D 2 – D 5 are not applicable**)

2. Complete name

i. Order and/or higher taxon (for animals)
ii. Family name (for plants)
iii. Genus
iv. Species
v. Subspecies
vi. Strain
vii. Cultivar/breeding line
viii. Pathovar
ix. Common name

3. Is the organism significantly pathogenic or harmful in any other way (including its extracellular products) either living or dead?

Yes ☐ No ☐ Not known ☐

If yes, specify the following

a) To which of the following organisms?

Humans	☐
Animals	☐
Plants	☐
Other	☐

b) Are the donated sequences involved in any way to the pathogenic or harmful properties of the organism?

Yes ☐ No ☐ Not known ☐

If yes, give the relevant information under Annex III A, point II(A)(11)(d):

4. Is the donor organism classified under existing Community rules relating to the protection of human health and the environment, such as Directive 90/679/EEC On the protection of workers from risks related to exposure to biological agents at work?

Yes ☐ No ☐

If yes, specify

5. Do the donor and recipient organism exchange genetic material naturally?

Yes ☐ No ☒ Not known ☐

E. INFORMATION RELATING TO THE GENETICALLY MODIFIED ORGANISM

1. Genetic traits and phenotypic characteristics of the recipient which have been changed as a result of the genetic modification

The GMO TG1050 is considered in all aspects similar to the recipient Ad5 $E1^-$ $E3^-$.

(a) is the GMO different from the recipient as far as survivability is concerned?

Yes ☐ No ☒ Not known ☐

Specify

(b) is the GMO in any way different from the recipient as far as mode and/or rate of reproduction is concerned?

Yes ☐ No ☒ Unknown ☐

Specify

(c) is the GMO in any way different from the recipient as far as dissemination is concerned?

Yes ☐ No ☒ Not known ☐

Specify

(d) is the GMO in any way different from the recipient as far as pathogenicity is concerned?

Yes ☐ No ☒ Not known ☐

Specify

d) Genetic stability of the genetically modified organism

At the research scale, a genetic stability study of the ADTG18201 construct to a passage level at least comparable to a production batch was performed. The main steps were 1) the selection of an initial clone of AdTG18201 by limited clonal dilution, 2) the 9 passages of this clone on the production cell line HER96 mimicking a larger production scale, 3) the isolation and characterization at protein expression and sequence levels of at least 100 clones. The study demonstrated the genetic stability of AdTG18201, with almost 100%, of the clones presenting the expected HBV nucleotidic sequences and the correct expression of the HBV fusion protein.

At the production level, the genetic stability of the ADTG18201 is controlled through several tests:

- Genomic integrity by restriction mapping
- Proportion of viruses expressing HBV antigen by Western blot on isolated sub-clones
- Vector sequence by sequencing of the whole genome

The test by restriction mapping is performed on the Pre-Master Virus Seed (Pre-MVS), the Master virus Seed (MVS) and on each clinical lot (at the step purified bulk).

The proportion of viruses expressing HBV antigen is performed on 100 sub-clones isolated from the MVS.

The sequencing of the whole genome of ADTG18201 is controlled on a lot issued from the MVS.

e) Is the GMO significantly pathogenic or harmful in any way (including its extracellular products), either living or dead?

Yes ☐ No ☒ Unknown ☐

(a) to which of the following organisms?

Humans ☐
Animals ☐
Plants ☐
Other ☐

(b) give the relevant information specified under Annex III (A) point II (A)(11)(d) and II (C)(2)(i)

- ***Risks associated with the donor genes***

The fusion protein inserted in the Ad5 vector is constituted of synthetized HBV proteins that have been truncated and mutated. The known enzymatic activities of these "wild-type" antigens Core, Pol and Env should therefore be abolished.

- ***Risks associated with the AdTG18201 vector***

- Toxicity

Wild type human Ad5 is ubiquitous and causes self-limiting infections of the upper respiratory tract and the common cold. There may be a possibility that the recombinant AdTG18201 vector mimic some of the characteristics of the wild-type Ad5. Even if replication competent Ad5 were generated, the associated risk is low since human adenoviral infection is very common and the majority of adults have already been infected and developed neutralizing antibodies.

In human, ~10 000 volunteers have received Ad5-based vaccines, essentially healthy volunteers with at minima 23 phase 1 and 7 phase 2 trials reported (ClinicalTrials.gov). Recombinant Ad-based gene transfer vectors are currently under investigation in a variety of gene therapy and vaccine clinical trials (more than 400 clinical trials are already done or ongoing, see for a review: http://www.wiley.com//legacy/wileychi/genmed/clinical/.

- Integration

Upon cell infection, adenoviral vectors are known to be present in the nucleus of the infected cells as an independent DNA (episomal structure). However, the genome of Ad cannot integrate into the genome of the host cell.

- Propagation, recombination or rearrangements

The adenoviral vector constituting TG1050 is devoid of replication capacity due to the deletion of the E1 region of the viral genome. One cannot exclude that the missing E1 function, necessary for viral replication, could be complemented during co-infection of the vector with a wild-type Ad5. Such event is unlikely to cause any significant harm in patients for the following reasons:

- This complementation will not extend beyond the viremia of Ad5, which is known to be limited. The vector will be complemented only as long as the parental virus propagates.
- This complementation will be limited to areas where both types of viral particles are numerous enough to simultaneously infect the same cell. Consequently, only a fraction of the dose of vector administered will be amplified, and this amplification will be less efficient than the amplification of the wild-type parental virus (Ad5).
- The most likely recombination event which could occur is the homologous recombination, that could lead to the generation of either a recombinant vector $AdE1^{+}$ $E3^{-}$ competent for the replication but having lost the HBV transgene or the recombinant vector Ad-HBV $E1^{-}E3^{+}$, deficient for the replication. Thus, these recombinants do not present higher risk than the recombinant AdTG18201 vector. Much more unlikely, illegitimate recombination events (Ad-HBV $E1^{+}E3^{-}$) only remain theoretical and thus undetectable since there are so rare.

Furthermore, the AdTG18201 vector is propagated in HER96 complementation cell line able to complement the E1 missing functions. The absence of sequence homology between the vector and the complementation cell line genomes should eliminate the occurrence of RCA

during the manufacturing process. A control test for the detection of RCA is performed for each released clinical lot.

- Horizontal transmission

Dissemination of the GMO to healthcare staff and household members is a potential risk for this and all gene therapy vectors. Infection could occur orally from hand to mouth or by exposure to aerosols, or by needle stick accidents. The evaluation of the probability of spreading from the host to other persons or to the environment is based on biodistribution and viral shedding data.

The biodistribution of adenoviral vectors has been assessed in several animal studies (Huard J. *et al.*, 1995) (Toloza E.M. *et al.*, 1996) (Wildner O. and Morris J.C., 2002) (Wood M. *et al.*, 1999). Sheets et al. conducted biodistribution and toxicological studies with various Ad (type 5 and 35), expressing different gene inserts (genes from HIV-1, Ebola or Marburg) and with different genetic modifications (vectors deleted in E1, E1 & E3, or E1, partial E3, and E4 manufactured by either Crucell or GenVec). Interestingly, these data suggest that the biodistribution and toxicology profiles of adenovector vaccines are determined largely by the platform and not by the expressed gene-insert or by the manufacturer's construct. Those studies were performed in rabbits injected intramuscularly with one of the above adenovector vaccine at ~10^{11} vp. The Ad5 vaccines biodistributed only to spleen and liver and otherwise remain in the site of injection muscle and overlying subcutis. By 3 months, the adenovector was cleared with, at most, a few animals retaining a small number of copies in the injection site and spleen. Repeated dose toxicology studies identified treatment-related toxicities confined primarily to the sites of injection, in certain clinical pathology parameters, and in body temperatures and food consumption immediately post-inoculation. Systemic reactogenicity at the sites of injection were reversible.

As regards viral shedding data, only few shedding studies in human had been published and no report of either biodistribution or viral shedding data of a non-replicative Ad5 administered by SC route is available in the literature (Tiesjema B. *et al.*, 2010). Albeit not recent, human biodistribution data obtained from the analysis of post mortem samples from 9 patients with head and neck cancer treated by intratumoral injection of a recombinant replication-impaired Ad5 CMV-p53 at $2x10^{10}$ vp [or 10.3 Log vp] showed the presence of DNA vector in some but not all tissues analyzed. For example, at 4 days and at 32-42 days post injection, DNA vector was found in liver, lung, lymph node and injected tumor but not gonads. DNA vector was not detected in samples collected at 229 days post treatment. Besides, samples were collected from household members and no clear and direct evidence of horizontal transmission was obtained from PCR and serological studies (Group C.G.T.E., 2002).

In the inventory of shedding data from clinical gene therapy trials published by Schenk-Braat et al., shedding of replication-deficient adenoviral vectors was not observed after intratumoral, intranasal or inhalation, intracoronary, intramyocardial, intravitreous, intraarterial, intrapleural or intramuscular administration in 21 out of 50 publications (Schenk-Braat E.A. *et al.*, 2007). The remaining 29 publications reported on shedding of vector DNA or infectious particles in various excreta, primarily depending on the route and site of administration and the time of analysis. In general, shedding in blood was short lasting, peaking during the first hours and disappearing a few days after administration. Interestingly, in none of the individuals studied could the presence of the vector or RCA be demonstrated.

In a phase 1 clinical study conducted with a replication-deficient Ad5-delivered fibroblast growth factor-4 in patients with unreconstructable critical limb ischemia, no evidence of viral

spreading to blood was observed in 2 days up to 12 weeks following injection of up to $2.9x10^{10}$ vp [or 10.5 Log vp] in muscles of the leg. In addition, viral DNA was not detected in urine, feces and semen samples or throat swabs of human subjects injected in leg muscles up to 12 weeks after viral delivery (Barouch D.H. *et al.*, 2003).

The route of administration (i.e. SC route) which has been selected for the proposed clinical trial should minimize the possibility of vector leakage.

To conclude, the exposure of healthcare staff or household members to the GMO could lead to a transient infection, but infected cells will be rapidly degraded by the immune system.

- Vertical transmission

The probability of germ line transmission is negligible considering the absence of integration of adenoviral vectors and given that there are no specific circumstances showing persistence of a similar replication-deficient Ad5 vector in the gonads.

f) Description of identification and detection methods

a) Techniques used to detect the GMO in the environment

The PCR technique performed with adequate specific primers allows the detection of the Ad (wild-type, deleted or recombinant). A culture technique with appropriate cell lines can distinguish the different viral vectors: wild-type replicative Ad5, recipient Ad5 $E1^-$ $E3^-$ or the GMO AdTG18201.

b) Techniques used to identify the GMO

The identity of the GMO can be confirmed by controlling the genomic integrity by restriction enzyme mapping or by PCR as described above.

F. INFORMATION RELATING TO THE RELEASE

1. Purpose of the release (including any significant potential environmental benefits that may be expected)

The release will be the administration of the GMO investigational medicinal product in a hospital or clinic setting to patients suffering from chronic hepatitis B, as a part of a multinational, multicenter clinical trial protocol. There are no foreseen problems with this release.

g) Is the site of the release different from the natural habitat or from the ecosystem in which the recipient or parental organism is regularly used, kept or found?

Yes ☐ No ☐

If yes, specify

Not applicable. The GMO is not naturally found in the environment.

h) Information concerning the release and the surrounding area

a) Geographical location (administrative region and where appropriate grid reference):

The GMO will be administered in various hospitals/clinics in France, Germany, New Zealand, Canada.

b) Size of the site (m^2):

i. Actual release site (m^2):

See below.

ii. Wider release area (m^2):

No specific size is required for the release area. The room where the patients will be treated is a conventional hospital room.

c) Proximity to internationally recognized biotopes or protected areas (including drinking water reservoirs), which could be affected:

Not applicable.

d) Flora and fauna including crops, livestock and migratory species which may potentially interact with the GMO

Not applicable.

i) Method and amount of release

a) Quantities of GMOs to be released

Considering:

- the maximal dose administered per patient, i.e. 10^{11} vp [or 11.0 Log vp] per injection,
- the maximum total number of patients planned to be treated with TG1050 in the whole phase 1 study, i.e. 68 patients (Among 96 patients randomized in the study, 68 patients will receive TG1050 and 28 patients will receive matching placebo) ,
- the maximal number of TG1050 injections per patients, i.e. 3 for the 36 part A patients receiving TG1050 and 6 for the 32 part B patients receiving TG1050,

the maximum quantity of GMO expected to be released during the whole study is $3x10^{13}$vp [or 13.5 Log vp].

Note: All these TG1050 doses will not be released in European clinical sites (approximately 2/3 of clinical sites in Europe).

b) Duration of the operation

The maximum duration of the operation for a given patient will be 9 weeks for the patients receiving a single injection of the GMO followed by an 8 week period without product and then 2 once-weekly injections at Weeks 8 and 9.

It will be 3 weeks for those patients receiving 3 once-weekly injections of the GMO,

c) Methods and procedures to avoid and/or minimize the spread of the GMOs beyond the site of the release

The GMO is released for clinical use only, supplied in closed vials and labeled appropriately. The administration is under the responsibility of the investigator, according to the clinical protocol and in respect of the Good Clinical Practice. The product must be prepared in aseptic conditions compliant with injectable preparations. The work area where TG1050 will be

prepared for injection will be cleaned before and after manipulation with a standard disinfectant active on Adenovirus-based products.

During all GMO manipulations lab coat, gloves and mask must be worn; goggles are recommended. All transfers of TG1050 (in vial or syringe containing the dose to be injected) must be done using leak-proof container/bag. Prior to the administration, the product must be prepared under conditions compliant with injectable preparations. Furthermore, the staff will follow the standard hospital policy recommended for the manipulation of live virus vaccines.

All personnel involved in handling the product is also informed that in case of skin contamination, the skin must be wash thoroughly with water and soap, rinse abundantly and disinfect with iodine solution at 4% or bleach solution at 0.45%. In case of eyes contamination, it is recommended to immediately rinse thoroughly with water only or physiological saline solution (NaCl 0.9%), and an examination by an ophthalmologist must take place as soon as possible.

j) Short description of average environmental conditions (wheather, temperature, etc.)

Not applicable.

k) Relevant data regarding previous releases carried out with the same GMO, if any, specially related to the potential environmental and human health impacts from the release

The proposed clinical study is the first-in-man use of the GMO TG1050.

G. INTERACTIONS OF THE GMO WITH THE ENVIRONMENT AND POTENTIAL IMPACT ON THE ENVIRONMENT, IF SIGNIFICANTLY DIFFERENT FROM THE RECIPIENT OR PARENT ORGANISM

1. Name of target organisms (if applicable)

(a) Order and/or higher taxon (for animals)
(b) Family name (for plants)
(c) Genus Homo
(d) Species Sapiens
(e) Subspecies
(f) Strain
(g) Cultivar/breeding line
(h) Pathovar
(i) Common name Human

2. Anticipated mechanism and result of interaction between the released GMOs and the target organism (if applicable)

The anticipated mechanism of action of the GMO is to induce an active specific immune response against HBV antigens: innate immune response and cytolytic T cells immune response. The innate immune response to TG1050 could lead to the clearance of HBV

infected hepatocytes while CD8+ T cell specific of encoded HBV antigens (core, pol and env) could eliminate HBV infected hepatocytes through the cytolytic T cells immune response.

3. Any other potentially significant interactions with other organisms in the environment

There is minimal potential for gene transfer to other species under the proposed release of the GMO. As mentioned above, the GMO will be released in a conventional clinic or hospital room and is unlikely to come in contact with other animal species. There is a theoretical, but highly unlikely, risk that the GMO and a wild-type Ad5 infect the same human cell and recombine to form a new GMO with wild-type replication properties. Subsequent spreading of such newly formed GMO in the community and infection of other individuals would be negligible considering the high prevalence of subjects with Ad5 pre-existing immunity.

4. Is post-release selection such as increased competitiveness, increased invasiveness for the GMO likely to occur?

Yes ☐ No ☒ Not known ☐

Give details

The GMO has strongly decreased competitiveness compared to wild-type Ad5 due to the deletion on the E1 region that abrogates its replication. However, in the unlikely recombination event described under G3, the newly formed GMO with natural replication properties is unlikely to exhibit increased competitiveness compared to wild-type Ad5.

5. Types of ecosystems to which the GMO could be disseminated from the site of release and in which it could become established

The dissemination of the GMO in ecosystems would be limited to humans (healthcare staff and any other person in close contact with the patient) and to the surrounding areas (healthcare facilities), because dissemination would require either close contact with the injection site or with the needle stick, or indirect contact with contaminated surfaces or objects or with contaminated biological fluids (blood, urine, feces). However, due to its genetic modifications, the GMO is less pathogenic than the wild-type Ad5. Besides, infection of other individuals would be negligible considering the high prevalence of subjects with Ad5 pre-existing immunity.

6. Complete name of non-target organisms which (taking into account the nature of the receiving environment) may be unintentionally significantly harmed by the release of the GMO

(i) Order and/or higher taxon (for animals)
(ii) Family name (for plants
(iii) Genus
(iv) Species
(v) Subspecies
(vi) Strain
(vii) Cultivar/breeding line
(viii) Pathovar

(ix) Common nature

Not applicable.

7. Likelihood of genetic exchange in vivo

(a) from the GMO to other organisms in the release ecosystem:

Exchange between the GMO and other organisms is unlikely (see section G.3).

(b) from other organisms to the GMO:

Exchange between other organisms and the GMO is unlikely (see section G.3).

(c) Likely consequences of gene transfer:

As mentioned in section E.1, the most likely gene transfer event which could occur is an homologous recombination, that could lead to the generation of either a recombinant vector $AdE1^{+}$ $E3^{-}$ competent for the replication but having lost the HBV transgene or the recombinant vector Ad-HBV $E1^{-}E3^{+}$, deficient for the replication. Thus, these recombinants do not present higher risk than the recombinant AdTG18201 vector. Much more unlikely, illegitimate recombination events (Ad-HBV $E1^{+}E3^{-}$) only remain theoretical and thus undetectable since there are so rare.

8. Give references to relevant results (if available) from studies of the behaviour and characteristics of the GMO and its ecological impact carried out in simulated natural environments (e.g. microcosms, etc.):

No data are available.

9. Possible environmentally significant interactions with biogeochemical process (if different from the recipient or parental organism)

Not applicable.

H. INFORMATION RELATING TO MONITORING

1. Methods for monitoring the GMOs

Monitoring of the direct and indirect effects of the GMO on patients will be achieved using the following clinical assessments described in the clinical protocol: physical examinations, vital signs, adverse event reporting, assessment of injection site reactions, complete blood cells count, biochemistry analyses, etc.

2. Methods for monitoring ecosystem effects

There is no risk for shedding of virus into the ecosystem. Therefore, ecosystem effects will not be monitored.

3. Methods for detecting transfer of the donated genetic material from the GMO to other organisms

Not applicable as TG1050 is not predicted to interact with non-target organisms because of its replication-defective property, the manner of its proposed release and the expected transient nature of its gene expression

4. Site of the monitoring area (m2)

Not applicable: the GMO will be administered to patients by SC injections in conventional hospital or clinic rooms.

5. Duration of the monitoring

According to the clinical protocol, patients administered with the GMO will be followed for more than one year.

6. Frequency of the monitoring

According to the clinical protocol, monitoring visits are planned on a regular basis up to the end of the follow up more than one year after enrollment.

I. INFORMATION ON POST-RELEASE AND WASTE TREATMENT

1. Post-release treatment of the site

The work area where TG1050 will be prepared for injection will be cleaned before and after manipulation with a standard disinfectant active on Adenovirus-based products.
Following the patient's discharge home, the room and the toilets will be cleaned in a standard way using a standard disinfectant based solution for cleaning the surfaces and floor.

2. Post-release treatment of the GMOs

Material in contact with the GMO must be considered as contaminated by infectious material..The disposable material contaminated by TG1050, such as:

- material for preparation/administration [injected syringes, Luer-Lock stoppers, needles],
- used and partially used vials,
- partially injected and non-injected syringes,
- other used material [gauze, dressings, gloves]
- unused vials

must be discarded according to regular hospital procedure for infectious waste (e.g. autoclaving, incineration, or treatment with sodium hypochlorite solution).

3. (a) Type and amount of waste generated

A maximum of 96 subjects will be recruited in the study. Each dose of TG1050 will be supplied in Type I 2-mL glass vials as well as its placebo and the ARM formulation buffer to be used for some dilutions. Based on the current protocol with a maximum of 3 injections for the 36 part A patients receiving TG1050 and 6 for the 32 part B patients receiving TG1050 and taking into account resupply hypothesis, 500 vials of TG1050 are planned for the present study and could generate a waste material. All these TG1050 doses will not be supplied to European clinical sites (2/3 of clinical sites in Europe approximately). –All other non-disposable material used for TG1050 preparation, such as: labcoat, goggles, patient gown, bedding, linens, towels, etc., must be cleaned/treated according to regular hospital procedure for infectious material (e.g. hot water 71°C washing with detergent and hot air drying).

3. (b) Treatment of waste

See I.2.

J. INFORMATION ON EMERGENCY RESPONSE PLAN

1. Methods and procedures for controlling the dissemination of the GMO(s) in case of unexpected spread

It will be recommended to personnel involved in TG1050 handling to act as recommended below in case of incident with its use.

- **Accidental spillage**:

Contaminated work area must be secured and access should be limited only to people authorised to handle TG1050. Cleaning process is the following:

• bleach at 0.6% of active chlorine (4 tablets [if 1.5 g/tablet of active chlorine] per litre of water or 1 volume of bleach at 2.6%Cl [i.e. 26 g/l of active chlorine] for 4 volumes of water or 1 volume of bleach at 9.6%Cl [i.e. 96 g/l of active chlorine] for 17 volumes of water).

The work area should be cleaned with active chlorine following these steps: 1. moisten a pad with above solution. 2. leave in contact for **30 minutes**. 3. rinse area with tap water. 4. go through steps 1 to 3 again but in step 2 leave in contact for at least **10 minutes**. 5. leave to dry.

or:

• a disinfectant active on Adenovirus-based products containing: halogens, phenols, alcohols, glutaraldehyde or formaldehyde (e.g Aniopray SF IP, Anioxy-spray IP) used according to Manufacturers' instructions to ensure adequate contact time and to confirm the ability of the equipment to withstand the disinfectant used.

- **Skin contamination with / without injury**:

An absorbent tissue should be placed immediately on the affected area, having removed contaminated clothes which should be treated as infectious material. After removing this tissue, wash the skin with mild soap thoroughly. Rinse abundantly with tap water. Removed absorbent tissue should be disposed as infectious material according regular hospital procedure. Then treat the skin area as follows:

- Treat with a solution of bleach at 0.45% of active chlorine (3 tablets [if 1.5 g/tablet of active chlorine] per litter of water or 1 volume of bleach at 2.6%Cl [i.e. 26 g/l of chlorine] for 5 volumes of water or 1 volume of bleach at 9.6%Cl [i.e. 96 g/l of chlorine] for 20 volumes of water).

  Treat following these steps: 1. moisten a pad with above solution. 2. leave in contact with the contaminated area for at least **5 minutes**. 3. rinse skin area abundantly with tap water.

or:

- Treat with a solution of 4% iodine for **5 minutes**, using a pad. Rinse abundantly with tap water. Then treat the area with a solution of 10% iodine for **5 minutes**. Rinse abundantly with tap water.

In addition, in case of injury, cover with a sterile gauze dressing, which should be appropriately discarded according to regular hospital procedure when removed. The

injured person should receive counselling from the investigator and should then be closely followed for a period of at least 2 weeks.

- **Eyes contamination**:

Rinse immediately and for **3 minutes** the affected eye(s) with tap water or ideally physiological saline solution (NaCl 0.9%) making the liquid flow laterally into the affected eye(s). If a single eye is affected, avoid contaminating the other eye (the affected eye must be below the other eye while rinsing). Keep the eyelids open and move the eye in all directions. The injured person should receive counselling from an ophthalmologist as soon as possible.

- **Ingestion**:

Do not induce vomiting and call the investigator or a doctor immediately. The person should be closely followed for a period of at least 2 weeks.

2. Methods for removal of the GMO(s) of the areas potentially affected

See J.1.

3. Methods for disposal or sanitation of plants, animals, soils, etc. that could be exposed during or after the spread

Not applicable.

4. Plans for protecting human health and the environment in the event of an undesirable effect

Patients will be monitored for the occurrence of adverse events and serious adverse events according to the clinical protocol. Each serious adverse event will be recorded and assessed by the hospital staff and the study sponsor, and Health Authorities will be notified when applicable.

Specific plans for protecting the environment are not considered necessary, for the reasons stated above.

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