PART 1 (COUNCIL DECISION 2002/813/EC)

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

In order to tick one or several possibilities, please use crosses (meaning x or X) into the space provided as (.)

A. General information

1. Details of notification

(a)	Member State of notification	Belgium
(b)	Notification number	B/BE/25/BVW5
(c)	Date of acknowledgement of notification	TBC
(d)	Title of the project	A phase 2, adaptive, double-blinded, placebo controlled, randomized, multi-center trial to evaluate the efficacy, safety and tolerability of intracoronary infusion of AB-1002 in adult subjects with New York Heart Association (NYHA) Class III heart failure and non-ischemic cardiomyopathy
(e)	Proposed period of release	01.01.2024- 31.12.2030

2. Notifier

Name of institution or company:

AskBio, Inc.
20 T.W. Alexander Drive
Suite 110
Research Triangle Park
NC 27709, USA

3. GMO characterisation

(a) Indicate whether the GMO is a:

viroid	(.)
RNA virus	(.)
DNA virus	(X)
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: Dependoparvovirus

Species: Adeno-associated virus human serotype 2 and 8 (AAV2i8)

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

AAV is a single stranded DNA virus that demonstrates a high degree of genetic stability as evidenced by the close relationship of the rep and cap genes from multiple AAV serotypes and genomovars. In support of these sequence homology data is the fact that AAV uses a host DNA polymerase for viral replication which is not error prone when compared to RNA polymerases used by RNA viruses. In support of genetic stability is the observation that AAV proviral DNA episomes isolated from multiple human tissue samples consistently have the expected canonical AAV2 rep and cap sequence.

Based on these characteristics of wild-type AAV, AB-1002 is also expected to be highly genetically stable. The AB-1002 vector genome sequence is verified by direct sequencing.

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

Yes (X) No (.)

If yes, insert the country code(s) BG, PL, RO

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

Yes (X) No (.)

If yes:

- Member State of notification AT
- Notification numbers B/AT/03/24
- Member State of notification DE
- Notification number B/DE/24/PEIP02025

- Member State of notification ES
- Notification number B/ES/24/15

- Member State of notification NL
- Notification numbers B/NL/24/015
- Member State of notification HU
- Notification number B/HU/24/03

Please use the following country codes:

Austria AT; Belgium BE; Germany DE; Denmark DK; Spain ES; Finland FI; France FR; United Kingdom GB; Greece GR; Ireland IE; Iceland IS; Italy IT; Luxembourg LU; Netherlands NL; Norway NO; Portugal PT; Sweden SE

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

Yes (X) No (.)

If yes:

- Member State of notification USA
- Notification number: Phase 1 study (NCT04179643)

- Member State of notification UK
- Notification number: Phase 2 study (NCT05598333)
- Member State of notification Canada
- Notification number: NSN-22040

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

This gene therapy product is utilizing a novel chimeric cardiotropic AAV2/AAV8 vector capsid (AAV2i8). AB-1002 consists of the AAV2i8 capsids that are packaged with the self-complementary AAV genome including a promoter the transgene encoding for I-1c and a synthetic polyadenylation sequence.

I-1c expression effectively reduces Protein phosphatase 1 (PP1) activity in heart failure and increases phospholamban (PLN) phosphorylation resulting in improved calcium handling and cardiac function.

The release of AB-1002 as described in this application, is not expected to result in adverse environmental impact, for the following reasons: Lack of pathogenicity of the parental virus; replication-incompetent GMO; Minimal risk of transmission by viral shedding; Minimal risk of insertional mutagenesis, Minimal risk of liver-specific transgene expression; Minimal risk associated with the transgene.

B. Information relating to the recipient or parental organism from which the GMO is derived

1. Recipient or parental organism characterisation:

(a) Indicate whether the recipient or parental organism is a:
(select one only)

viroid (.)
RNA virus (.)
DNA virus (X)
bacterium (.)
fungus (.)
animal
- mammals (.)
- insect (.)
- fish (.)
- other animal (.)
(specify phylum, class)

other, specify

2. Name

(i)	order and/or higher taxon (for animals)	ssDNA virus
(ii)	genus	Dependoparvovirus
(iii)	species	Adeno-associated virus
(iv)	subspecies	N/A
(v)	strain	serotype 2 and 8
(vi)	pathovar (biotype, ecotype, race, etc.)	N/A
(vii)	common name	N/A

3. Geographical distribution of the organism

(a) Indigenous to, or otherwise established in, the country where the notification is made:
Yes (X) No (.) Not known (.)

(b) Indigenous to, or otherwise established in, other EC countries:
(i) Yes (X)

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

Atlantic	X
Mediteranean	X
Boreal	X
Alpine	X
Continental	X
Macaronesian	X
Black sea	X
Pannonian	X
Steppic	X

(ii) No (.)
(iii) Not known (.)

(c) Is it frequently used in the country where the notification is made?
Yes () No (X)

(d) Is it frequently kept in the country where the notification is made?
Yes () No (X)

4. Natural habitat of the organism

(a) Is the organism a microorganism? **yes**/~~no~~

water	(.)
soil, free-living	(.)
soil in association with plant-root systems	(.)
in association with plant leaf/stem systems	(.)
other, specify	In association with animals (primate hosts)

(b) Is the organism an animal: No

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

5. (a) Detection techniques
AAV can be detected by quantitative polymerase chain reaction (qPCR) using primers specific for the viral genome.

(b) Identification techniques
AAV can be identified by quantitative polymerase chain reaction (qPCR) using primers specific for the viral genome.

6. Is the recipient organism classified under existing Community rules relating to the protection of human health and/or the environment?
Yes (.) No (X)
If yes, specify

7. Is the recipient organism significantly pathogenic or harmful in any other way (including its extracellular products), either living or dead?
Yes (.) No (X) Not known (.)

Additional information: Wildtype AAV is non-pathogenic and has not been classified under Directive 2000/54/EC of the European Parliament and of the Council of 18 September 2000 on the protection of workers from risks related to exposure to biological agents at work. AAV has no known pathogenic effects, even though the estimated seroprevalence of some common human serotypes is 90%. Consequently, AAV fulfils the definition of a group 1 biological agent according to the Directive 2000/54/EC (a biological agent that is unlikely to cause human disease).

If yes:

(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) of Directive 2001/18/EC

8. Information concerning reproduction

(a) Generation time in natural ecosystems: AAV is replication defective, thus the generation time is variable depending on the presence or absence of a helper virus.

(b) Generation time in the ecosystem where the release will take place: AAV is replication defective, thus the generation time is variable depending on the presence or absence of a helper virus.

(c) Way of reproduction: Sexual N/A Asexual yes

(d) Factors affecting reproduction:

The presence of a helper virus, such as adenovirus or herpes simplex virus, promotes AAV gene expression, genome replication and production of virions. In the absence of a helper virus, wild-type AAV is replication-incompetent. Please note that the final GMO, AB-1002, is replication-incompetent even in the presence of a helper virus due to the removal of the viral rep and cap genes.

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	AAV does not form survival structures

(b) Relevant factors affecting survivability:

Members of the parvovirus family such as AAV are stable viruses that can persist in the environment for extended periods of time (thought to be on the order of several weeks). AAV particles are resistant to a wide range of pH (pH 3-9) and can resist elevated temperatures (55°C for 1 hour). AAV does not form survival structures. However, as with all viruses, replication of AAV cannot occur outside of a host cell.

10. (a) Ways of dissemination

AAV may be transmitted through direct or indirect contact. AAV may be transmitted through inhalation, ingestion, and possibly sexual transmission.

(b) Factors affecting dissemination

Replication of the virus (wild-type AAV) is only possible in host cells that have been co-infected with a helper virus (e.g., adenovirus, herpes simplex virus).

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

The sponsor has not notified any previous genetic modifications of the parental virus AAV2 or AAV8 for release in the EU.

C. Information relating to the genetic modification

1. Type of the genetic modification

(i)	insertion of genetic material	(X)
(ii)	deletion of genetic material	(X)
(iii)	base substitution	(.)
(iv)	cell fusion	(.)

(v) others, specify

2. Intended outcome of the genetic modification

The intended outcome of the genetic modification was to generate a recombinant AAV vector containing a human I-1c expression cassette for the treatment of patients with New York Heart Association Class III Heart Failure. Preclinical studies have shown that administering constitutively activated inhibitor 1 (I-1c) of PP1 within the failing rat heart improves not only contractility, but also reverses adverse remodeling by directly decreasing fibrosis and cardiac hypertrophy. These results further support the development of I-1c gene transfer for HF in humans.

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

Yes (X) No (.)

If no, go straight to question 5.

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

Yes (X) 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	(X)
bacteriophage	(.)
virus	(.)
cosmid	(.)
transposable element	(.)
other, specify	

(b) Identity of the vector

Transgenic, auxiliary and packaging plasmids are used to manufacture the GMO, as listed below:

- Plasmid #1 – Transgene: Provides a constitutively active form of the Human Inhibitor-1 of protein phosphatase-1 gene
- Plasmid #2 – Rep/Cap: Contains the AAV2 rep genes and a modified AAV2 cap gene that serve to encapsidate the transgene.
- Plasmid #3 – Helper: Furnishes essential adenoviral helper genes required for rAAV replication

(c) Host range of the vector

The plasmids used to manufacture the final GMO have been propagated in bacteria (*E.coli*) and cannot replicate in mammalian cells.
The production cell line for the final GMO is a mammalian cell line.

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

Yes (X) No (.)

antibiotic resistance (x)
other, specify

Indication of which antibiotic resistance gene is inserted
Ampicillin

(d) Constituent fragments of the vector

AB-1002 is comprised of a synthetically designed expression cassette containing cDNA for the I-1c gene, CMV promoter sequences, and Poly A sequence for transcription termination. Short DNA flanking regions, ITRs from the AAV2 genome are maintained to direct packaging of the DNA into viral particles and stability of the transferred DNA after transduction of host cells.

Constituent fragments of the three vectors used in the manufacture of the GMO are described in 4 (b).

(f) Method for introducing the vector into the recipient organism

(i)	transformation	(.)
(ii)	electroporation	(.)
(iii)	macroinjection	(.)
(iv)	microinjection	(.)
(v)	infection	(.)
(vi)	other, specify	AB-1002 is manufactured by triple plasmid transfection in human HEK293 cells without the presence or creation of wildtype viruses.

5. If the answer to question 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

The insert consists of a CMV promoter, a constitutively active form of protein phosphatase 1 Inhibitor 1 (I-1c) and a polyadenylation signal, flanked by AAV inverted terminal repeats (ITRs).

(b) Source of each constituent part of the insert

- Human I-1c sequence: *homo sapiens*
- Polyadenylation signal: *SV40 virus*
- CMV promoter: *cytomegalovirus*

- ITRs: AAV

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

- CMV promoter: Intended to drive high-level I-1c expression.
- I-1c: Transfer of I-1c delivered intracoronary infusion and constitutive I-1c expression is expected to improve clinical signs and symptoms of CHF and reduce CV mortality.
- Polyadenylation signal: Intended to provide cis sequences for efficient polyadenylation of the I-1c mRNA. This element functions as a signal for a specific cleavage event at the 3' end of the nascent transcript and addition of a long polyadenyl tail.
- ITRs: Cis acting elements required for genome replication and packaging

(e) Location of the insert in the host organism

-	on a free plasmid	(.)
-	integrated in the chromosome	(.)
-	other, specify	ssDNA viral genome

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

Yes (.) No (X)

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	(X)
-	insect	(.)
-	fish	(.)
-	other animal	(.)
	(specify phylum, class)	
other, specify		

2. Complete name

(i)	order and/or higher taxon (for animals)	Primates
(ii)	family name for plants	N/A
(iii)	genus	Homo
(iv)	species	Homo sapiens
(v)	subspecies	N/A
(vi)	strain	N/A
(vii)	cultivar/breeding line	N/A
(viii)	pathovar	N/A
(ix)	common name	Human

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

Yes (.) No (X) 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 (X) Not known (.)

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

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 to exposure to biological agents at work?

Yes (.) No (X)

If yes, specify

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

Yes () No (X) Not known (.)

E. Information relating to the genetically modified organism

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

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

Yes (.) No (X) 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 (X) No (.) Unknown (.)

Specify

AB-1002 viral genome has been significantly modified compared to the parental virus in order to render it replication incompetent. All the viral gene coding sequences have been removed, including the rep and cap genes required for replication. The only remaining sequences are two inverted terminal repeats (ITRs), which are non-coding DNA sequences and flank the transgene expression cassette. The ITRs allow the vector genome to be packaged into vector capsids. The final GMO, AB-1002, is replication

incompetent. AB-1002 replication could only occur in the extremely unlikely event of a host cell being infected by three separate viruses (AB-1002, wild-type AAV and a helper virus such as human adenovirus or herpes simplex virus). The risk of this occurring is considered to be negligible.

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

Yes (X) No (.) Not known (.)

Specify

AB-1002 is replication-incompetent even in the presence of a helper virus due to the removal of the viral rep and cap genes. As AB-1002 replication could only occur in the extremely unlikely event of a host cell being infected by three separate viruses: AB-1002, wild-type AAV and a helper virus such as adenovirus or herpes simplex virus.

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

Yes (.) No (X) Not known (.)

Specify

No pathogenic effects of wild-type AAV in humans are known. The introduction of the I-1c expression cassette is not expected to result in development of pathogenicity. Thus, neither the wild-type AAV nor AB-1002 are known or expected to be pathogenic.

2. Genetic stability of the genetically modified organism

AAV is a single stranded DNA virus that demonstrates a high degree of genetic stability; based on this, AB-1002 is also expected to be genetically stable. The integrity of the I-1c expression cassette will be confirmed by direct sequencing.

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

Yes (.) No (X) 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)

4. Description of identification and detection methods

(a) Techniques used to detect the GMO in the environment

AB-1002 can be detected by quantitative polymerase chain reaction (qPCR).

(b) Techniques used to identify the GMO

AB-1002 can be identified by qPCR.

F. Information relating to the release

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

The purpose of the release of the GMO is the use in the following clinical trial: A phase 2, adaptive, double-blinded, placebo controlled, randomized, multi-center trial to evaluate the efficacy, safety and tolerability of intracoronary infusion of AB-1002 in adult subjects with New York Heart Association (NYHA) Class III heart failure and non-ischemic cardiomyopathy (protocol number: ASK-CHF2-CS201).

The primary objective is to evaluate the efficacy and safety of a single antegrade intracoronary artery infusion of the investigational medicinal product containing GMO compared to placebo in subjects with non-ischemic cardiomyopathy and NYHA Class III symptoms of heart failure.

2. 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 (X) No (.)

If yes, specify: The GMO is released in a hospital setting.

3. Information concerning the release and the surrounding area

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

Site 1:

AZORG	Moorselbaan 164	9300 Aalst	Belgium
Universitair Ziekenhuis Antwerpen	Drie Eikenstraat 655	2650 Edegem	Belgium
Universitair Ziekenhuis Gent	Corneel Heymanslaan 10	9000 Ghent	Belgium
AZ Delta	Deltalaan 1	8800 Roeselare	Belgium

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

(i) actual release site (m^2): Not applicable. A specific size for the site of release cannot be defined as AB-1002 will be administered to patients as part of a clinical trial.

(ii) wider release site (m^2): Not applicable. A specific size for the site of release cannot be defined as AB-1002 will be administered to patients as part of a clinical trial.

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

Not applicable. AB-1002 will be administered by a one-time intracoronary infusion in a hospital setting. Thus, it is not anticipated to come into contact with any recognized biotopes or protected areas.

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

Administration of AB-1002 will occur only within a controlled hospital setting; therefore, it is not anticipated that it will come into contact with plants, animals or soil.

4. Method and amount of release

(a) Quantities of GMOs to be released:

Patients in the first arm of this Phase 2 study will receive a dose of 7.15E13 vg per subject. For the second arm a dose of 1.43E14 vg per subject was chosen.

(b) Duration of the operation:

The database will be locked and the study unblinded after all subjects have completed the 52 week observation period. Patient will continue in a subsequent 4-year long-term follow-up phase.

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

AB-1002 will be administered to the patients in the clinical trial ASK-CHF2-CS201 by intracoronary infusion. Following cellular uptake and uncoating of the viral particle, the vector genome is expected to be maintained in host cells in episomal form (Nakai 2001, Duan 1998, Schnepp 2003). Recombinant AAV vectors are non-replicative and are not expected to pose a risk of transmission.

There are no known or expected immediate or delayed environmental impacts resulting from AB-1002. The drug product will be managed under controlled conditions reducing the risks for AB-1002 to interact with non-target organisms.

Disinfectable, appropriately labelled, leakproof, and unbreakable containers will be provided to the sites before the sites will be activated for patient enrollment. AB-1002 will be stored, prepared and administered by trained medical professionals, in a hospital setting only to patients that meet criteria for inclusion into clinical study ASK-CHF2-CS201. Staff will follow the waste and disposal policies as per local site requirement to dispose of consumables used in the preparation and administration of the GMO. The risk of accidental or inappropriate disposal of the product into the sewer or waste at site, is considered to be negligible. In the unlikely event that spillage would occur, the product is non-pathogenic and non-replicative, limiting the spread and risks to the environment or personnel.

5. Short description of average environmental conditions (weather, temperature, etc.)

Not applicable. Administration of AB-1002 will occur only within a controlled hospital setting.

6. 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.

Clinical studies using recombinant AAV vectors have been performed worldwide in more than 700 patients with an excellent safety profile (Maguire 2008, Jaski 2009, Jessup 2011, Nathwani 2011, Jacobson 2012, MacLaren 2014, Mendell 2015), and there have been no known immediate or delayed effects on human health due to direct and indirect interactions of the GMO and persons working with, coming into contact with, or in the vicinity of the GMO release(s). The product is non-pathogenic and non-replicative, limiting the spread and risks to the environment or personnel. There are no known or expected immediate or delayed environmental impacts resulting from AB-1002.

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 organism (if applicable)

(i)	order and/or higher taxon (for animals)	Primates
(ii)	family name for plants	N/A
(iii)	genus	Homo
(iv)	species	Homo Sapiens
(v)	subspecies	N/A
(vi)	strain	N/A
(vii)	cultivar/breeding line	N/A
(viii)	pathovar	N/A
(ix)	common name	Human

2. Anticipated mechanism and result of interaction between the released GMOs and the target organism (if applicable). **Yes**/~~no~~

specifiy

AAV2i8 shows high transduction in cardiac and skeletal muscle and low transduction in liver. It is thus expected that administration of AB-1002 will result in the expression of the I-1c gene in myocardial cells of study subjects.

3. Any other potentially significant interactions with other organisms in the environment.
No other organisms other than human subjects receiving the medicinal product will be exposed to levels of AB-1002 that could represent a potential hazard. Potential hazards of exposure to AB-1002 are only predicated upon administration of AB-1002. SinceAB-1002 is replication-incompetent, it is expected that the vector would be rapidly cleared from any non-target organism without causing any harmful effect. Minimal exposure, such as environmental exposure, to AB-1002 is not expected to affect any organisms, neither directly nor indirectly.

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

Yes (.) No (X) Not known (.)

Give details
Since AB-1002 is unable to replicate, post-release selection cannot occur.

5. Types of ecosystems to which the GMO could be disseminated from the site of release and in which it could become established.
Since AB-1002 is unable to replicate, it is not expected to spread to the environment to a significant degree and is not expected to become established in any ecosystems.

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

N/A

(i)	order and/or higher taxon (for animals)	N/A
(ii)	family name for plants	N/A

(iii)	genus	N/A
(iv)	species	N/A
(v)	subspecies	N/A
(vi)	strain	N/A
(vii)	cultivar/breeding line	N/A
(viii)	pathovar	N/A
(ix)	common name	N/A

7. Likelihood of genetic exchange in vivo

(a) from the GMO to other organisms in the release ecosystem:
In the proposed clinical study AB-1002 will be administered via antegrade intracoronary artery infusion. Following cellular uptake and uncoating of the viral particle, the AB-1002 vector genome is expected to be maintained in host cells in episomal form (Nakai 2001, Duan 1998, Schnepp 2003). Non-clinical studies showed high transduction within the cardiac and skeletal muscles, and low liver tropism. Taken together, the risks associated with gene transfer by AB-1002, and transgene expression in organisms other than the patients enrolled in the proposed clinical study ASK-CHF2-CS201, are considered to be negligible.

(b) from other organisms to the GMO:
The elimination of most of the viral DNA reduces the probability of homologous recombination with related viruses that could lead to variants of the GMO.

(c) likely consequences of gene transfer:
While recombination between AB-1002 and a wild-type AAV to generate a hybrid vector genome that contains both the I-1c expression cassette and the AAV rep and cap genes remains a theoretical possibility, such a molecule even if generated in a cell would not replicate unless a helper adenovirus/herpes virus was also present. Moreover, such a hybrid genome would be too large to package the hybrid DNA into an AAV particle. It is known that AAV possesses a packaging limit of approximately 5kb (Wu 2010), and a hybrid molecule of rep-cap genes plus the I-1c expression cassette would be predicted to be in excess of this limit. The risks associated with gene transfer from wild-type AAV to AB-1002 are thus considered to be negligible.

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 stimulated natural environments (e.g. microcosms, etc.)
No such studies have been conducted with AB-1002.

9. Possible environmentally significant interactions with biogeochemical processes (if different from the recipient or parental organism):
AB-1002 is not known or predicted to have an impact on biogeochemical processes.

H. Information relating to monitoring

1. Methods for monitoring the GMOs
Viral shedding will be assessed in the study. Samples including stool, semen, blood, urine, and saliva will be collected at the visits indicated below (see point H.5)

2. Methods for monitoring ecosystem effects

The presence of AB-1002 in bodily fluids following administration of AB-1002 will be determined by ddPCR.

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

Transfer of the I-1c expression cassette to study subjects will be detected by assessing I-1c activity using appropriate clinical read-outs.

4. Size of the monitoring area (m^2)

Not applicable

5. Duration of the monitoring

In the Phase 2 study, shedding assessments will be performed. Samples as described above (point 1) will be taken on day 1 following administration of AB-1002, day 4, then at weeks 1, 2, 4, 8, and 12, then months 6, 9 and 12.

6. Frequency of the monitoring

Samples as described above (point 1) will be taken on day 1 following administration of AB-1002, day 4, then at weeks 1, 2, 4, 8, and 12, then months 6, 9 and 12.

I. Information on post-release and waste treatment

1. Post-release treatment of the site

Any surfaces contaminated with AB-1002 will be disinfected in accordance with local guidelines and institutional procedures related to the management of biohazardous substances and using a disinfectant effective against AAV as per manufacturer´s instructions.

2. Post-release treatment of the GMOs

All disposable materials that come into contact with AB-1002 will be disposed of according to individual institutional practices and policies for the disposal and decontamination of biohazardous waste. In general, disposable materials will be disposed of in sharps containers or biohazard bags and decontaminated by autoclaving or incineration, or both. Non-disposable materials will be decontaminated according to institutional practices and procedures, e.g. by treatment with an appropriate disinfectant and/or autoclaving.

All unused vials need to be kept in the required storage conditions (≤ -60°C). The sponsor will be contacted regarding any unused vials of AB-1002. These will be held at the institution's investigational pharmacy and returned to the sponsor or designee according to sponsor's instruction.

3. (a) Type and amount of waste generated

The following types of waste are anticipated:

- Empty IMP vials; Glass vials
- Polypropylene syringes and needles
- Filters, 3-way stopcock, 4-way manifold
- Infusion line and catheter
- Gauzes, Gloves

(b) Treatment of waste

All disposable materials that come into contact with AB-1002 will be disposed according to hospital procedures for the disposal of biohazardous waste.

J. Information on emergency response plans

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

In the event that the contents of the AB-1002 vial/s or diluted product for infusion are accidentally released and come in contact with shipping materials, pharmacy/hospital surfaces, the spillage will be decontaminated and removed according to institutional/hospital practice.
AB-1002 is stored in containers at or below -60°C. On the day of surgery AB-1002 deep frozen stock vials will be thawed and diluted before preparing the dosing syringes (Luer-Lok syringes, labeled, placed in leak proof bag in insulated container containing chilled cold packs). Staff will be advised that care must be taken when manipulating vials and that the use of needles should be kept to a minimum. In the event of injury, staff will follow local institutional procedures.
In case of accidental contact of AB-1002 with skin, eyes or clothing, the affected area will be washed with copious amounts of water and staff will follow institutional procedures for the management of biohazardous material.

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

Any surface area exposed to the GMO will be disinfected using appropriate disinfectant as per local guidelines and institutional policies and procedures.

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

Administration of AB-1002 will occur only within a controlled hospital setting; therefore, it is not anticipated that it will come into contact with plants, animals or soil. Furthermore, AB-1002 is not capable of infecting plants or microbes.

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

Staff will follow local guidelines and institutional procedures for the handling and disposal of genetically modified organisms. Furthermore, safety recommendations and guidance on the management of incidents related to AB-1002 are provided in the safety instructions for investigators and staff included in this submission. All patients will be carefully monitored for any adverse reactions during this study. An independent data and safety monitoring board (DSMB) will be appointed to review the accumulated safety data at least quarterly, or more frequently as required. The DSMB will be notified of serious adverse events (SAEs) and/or triggering of stopping rules within 5 business days of the initial report to the Sponsor. The DSMB may, at any time, recommend modifying or stopping the study early (study hold or termination) due to safety concerns based on review of the data.

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