

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            | United Kingdom (UK)  |
| (b) Notification number                     | B/GB/13/R46/01/S   |
| (c) Date of acknowledgement of notification | 18/01/2013   |
| (d) Title of the project                    | A Phase 2b, Double-Blind,<br>Placebo-Controlled, Multinational, Multicenter, Randomized Study Evaluating<br>the Safety and Efficacy of Intracoronary Administration of MYDICAR®<br>(AAV1/SERCA2a) in Subjects with Heart Failure and Investigation of the<br>safety and feasibility of AAV1/SERCA2a gene transfer in patients with chronic<br>heart failure and a left ventricular assist device |

Proposed period of release From 01/01/2013 until 31/12/2015

2. Notifier

Name of institution or company: Celladon Corporation

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 ...

(b) Identity of the GMO (genus and species)

Genus: *Dependovirus*

Species: Adeno-associated virus (vector). The AAV1/SERCA2a vector is a recombinant adeno-associated virus (rAAV) vector. It is a pseudotype of AAV serotype 1(AAV1) and is denoted as rAAV1/2. As such, the capsid proteins are from wt AAV1 and the AAV DNA (two 145 base inverted terminal repeats) is from wtAAV2.

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

The vector is stable by design in that the vector DNA is approximately the same size as the viral genome and it contains no viral genes. The investigational product's stability is assured by a well-characterized manufacturing process, in-process testing and batch release testing. AAV1/SERCA2a is manufactured using a recombinant HeLa-derived cell line (clone 2B12) and wild-type adenovirus type 5 as a helper virus to induce production. The producer cell clone contains stably integrated copies of a plasmid with expression cassettes for the vector genome and other necessary components for vector packaging. A rigorous purification process is then used to produce drug substance, including a flow-through anion exchange filter as an initial adenovirus removal step, an adenovirus heat inactivation step, three bind-and-elute chromatography steps and a viral filtration step. The process was designed for and has been validated for viral clearance based on the ICH Q5A guidance for viral safety. Vector quantitation assays include qPCR for DNase resistant particles and a cell based infectivity assay. Vector potency is measured in a 293-cell based relative potency assay, comparing dose titration curves of a test article to a qualified reference standard using a quantitative readout of the transgene protein. Helper virus DNA is assayed throughout manufacturing by qPCR. Tests required for lot release include qPCR for host cell DNA, residual infectious adenovirus (a cell based assay to detect infectious units) and adenovirus proteins, replication competent AAV (two rounds of cell based amplification followed by a Southern blot), host cell protein (ELISA) general purity by SDS PAGE, Benzoylase nuclease and endotoxin, bioburden. Tests for mycoplasma and an in vitro assay for viral contaminants are also required for lot release, but are performed on in-process samples.

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, BE, NL, UK, SE, DK and PL

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 BE, SE, DE, NL
- Notification number B/BE/12 BVW2  
B/SE/12/EU-2012-001700-37  
(DE & NL Notification numbers pending)

**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 placing on the market outside the Community by the same or other notifier?

Yes  No

If yes:

- Member State of notification ...
- Notification number B/././...

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

AAV1/SERCA2a is derived from the non-pathogenic helper virus replication-dependent AAV1. AAV1 infects humans and primates, but no other known environmental organisms, and the investigational product vector is expected to behave similarly. In addition, the vector is completely replication incompetent even in the presence of helper virus. The drug substance for the investigational product has been tested for helper virus (adenovirus) and replication-competent AAV virus by sensitive assays and none have been detected at the limits of detection. The SERCA2a protein is a fully human, intracellular, endoplasmic protein that is naturally expressed in cardiac/slow-twitch skeletal muscle and endothelial cells and does not represent a foreign antigen. Stable SERCA2a protein expression is limited to tissues in which the biochemical pathways are present and permissive for its membrane incorporation *in vivo*. Approximately 90% of humans have been exposed to wild-type AAV in adolescence, and the presence of even low levels of AAV neutralizing antibodies can block vector transduction of permissive cells.

In addition, AAV1/SERCA2a has no viral genes and a limited capsid packaging capacity making routes to mobilization highly improbable. The only route of release that could lead to a significant dose would be a direct injection of a substantial amount of the investigational product. Neither the non-clinical nor clinical studies have demonstrated adverse effects associated with relevant doses of AAV1/SERCA2a. Because each dose is administered in 50 mL of saline, any reasonably conceived environmental exposure would be orders of magnitude less than that received by study subjects. The SERCA2a gene is nontoxic and not oncogenic. The persistence of vector DNA is limited to the injection/infusion site (the heart) and the presence in other highly perfused tissues decreases with dose administered and time. Environmental impacts from release are negligible.

**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) *Parvoviridae*
- (ii) genus *Dependovirus*
- (iii) species adeno-associated virus

- (iv) subspecies ...
- (v) strain serotype 1
- (vi) pathovar (biotype, ecotype, race, etc.) ...
- (vii) common name AAV1

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

(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) If the organism is a microorganism

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

(b) If the organism is an animal: natural habitat or usual agroecosystem:

...

5. (a) Detection techniques

- qPCR with primers specific for the vector DNA.

(b) Identification techniques

- qPCR with primers specific for the vector DNA.
- ELISA for conformational intact AAV1 capsid proteins (viral particles).

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

Yes (X) No (.)

If yes, specify

DE, SE and GB classify AAV vectors as Risk Group 1 or activity classification 1 (GB).

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 (.)

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

AAV1 is not pathogenic or allergenic. It is considered a defective virus and requires the presence of a helper virus (adenovirus or herpes virus) for replication. It is known only to infect humans and other primates in the environment.

8. Information concerning reproduction

(a) Generation time in natural ecosystems:

AAV1 requires the co-infection of a helper virus so replication in an infected host can take from 24 to 48 hrs, but may never occur in the absence of an appropriate helper virus. Note that the vector is "gutless" and cannot replicate under any conditions.

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

*See (a).*

(c) Way of reproduction: Sexual .. Asexual X

(c) Factors affecting reproduction:

The parent virus requires the presence of a helper virus.

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:  
AAV is a non-enveloped virus that is relatively stable in the environment and stable to desiccation. However it is destroyed by 0.5% sodium hypochlorite and cleaning agents and hand-sanitizers that are effective against non-enveloped viruses.

10. (a) Ways of dissemination  
Dissemination may occur by inhalation, contact with mucus membranes (eyes, nose and mouth), faecal-oral transmission and occasionally waterborne transmission. The parent AAV virus is disseminated primarily by contact of mucus membranes. Direct contact of surfaces, exposure to aerosols and abrasions (sharps) can facilitate infection.

(b) Factors affecting dissemination  
Factors that may affect dissemination include aerosol generation, sharps injury and co-infection by helper 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)  
B/SE/08/EU-2007-006721-27(Sweden) and B/NL/05/001(Netherlands)

**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

SERCA2a is a "guttled" AAV viral vector with an inserted transgene SERCA2a. As such, all of the viral gene-encoding sequences have been removed leaving only the two small (145 bases) AAV serotype 2 inverted terminal repeat sequences at the 3' and 5' ends flanking the CMV hSERCA2a-polyA expression cassette.

The intention of the modifications was to render the virus completely replication-incompetent and to make mobilization negligible if not impossible, to maintain the skeletal muscle tropism of AAV1 and to allow expression of SERCA2a. To maintain the desired tropism the vector, AAV1/SERCA2a, is an AAV2/1 pseudotype containing the capsid proteins of adeno-associated virus serotype 1 (AAV1) and the sequence for AAV2 inverted terminal repeats

SERCA2a is an intracellular Ca<sup>2+</sup> pump located in the sarcoplasmic reticula of cardiac muscle cells. This enzyme catalyzes the hydrolysis of ATP, coupled with the translocation of calcium from the cytosol into the lumen of the sarcoplasmic reticulum, and is involved in regulation of the heart contraction/relaxation cycle. It is not a toxic or oncogenic protein. The primary objective is to up-regulate SERCA2a in patients with ischemic or dilated cardiomyopathy and NYHA class III/IV symptoms of heart failure (HF). SERCA2a genetic enzyme replacement in advanced HF patients may correct imbalances in Ca<sup>2+</sup> cardiac

metabolism, resulting in enhanced cardiac function and energetics, which will in turn translate to improved signs and symptoms of HF, quality of life and clinical outcomes.

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  
pTPK-ABG12

- (c) Host range of the vector  
Bacterial and mammalian cells

- (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

Kanamycin and Puromycin resistance genes are inserted. Transfected cells were selected for puromycin. Note that neither resistance gene is present in the GMO.

- (e) Constituent fragments of the vector

- the vector genome for AAV1/SERCA2a (two complete AAV2 ITRs flanking a CMV/SERCA2a/BGH polyA expression cassette)
- an AAV rep2/cap1 packaging cassette
- an expression cassette for the puromycin resistance gene
- Kanamycin resistance gene
- col E1 bacterial origin of replication for plasmid propagation in bacteria.

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

- (i) transformation (.)
- (ii) electroporation (.)
- (iii) macroinjection (.)
- (iv) microinjection (.)
- (v) infection (.)
- (vi) other, specify transfection

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 AAV1/SERCA2a DNA contains the following components: AAV serotype 2 based ITRs at the 3' and 5' ends, flanking the CMV hSERCA2a-polyA expression cassette. The expression cassette contains the cytomegalovirus immediate early enhancer/promoter (CMVie) driving transcription of sequences including a hybrid intron from the commercial plasmid pCI (Promega - GenBank Accession Number U47119), the hSERCA2a cDNA (coding sequence identical to GenBank Accession Number NM-001681), and a bovine growth hormone polyadenylation signal (BGHpA [GenBank Accession Number M57764]). The hybrid intron was designed using the 5'-donor site from the first intron of the human beta-globin and the branch and 3'-acceptor site from the intron located between the leader and body of an immunoglobulin gene heavy chain variable region.

(c) Source of each constituent part of the insert

Inverted terminal repeats: adeno-associated virus serotype 2  
 Transgene: SERCA2a is human  
 Enhancer/promoter: cytomegalovirus  
 Polyadenylation signal: bovine  
 Hybrid intron: human

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

The inverted terminal repeats are for capsid-packing. The transgene SERCA2a is for returning cardiac muscle SERCA2a protein expression levels to normal in heart failure subjects. The enhancer/promoter is for transcription of the transgene. The polyadenylation signal is for effective translation of the transgene mRNA. The intron is for proper maturation of the transgene mRNA for transcription.

(d) Location of the insert in the host organism

- on a free plasmid (.)
- integrated in the chromosome (.)
- other, specify The insert described is the entire vector

“genome.”



- (e) 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) Primate  
 (ii) family name for plants ...  
 (iii) genus *Homo*  
 (iv) species *sapiens*  
 (v) subspecies ...  
 (vi) strain ...  
 (vii) cultivar/breeding line ...  
 (viii) pathovar ...  
 (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:

- (b) 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):

...

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 (.) Not known (.)

Not applicable as the transfer is human to human.

#### **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 The capsid proteins are wtAAV1

- (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 AAV1/SERCA2a has all viral a gene sequences removed and is completely replication-incompetent even in the presence of helper virus.

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

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

Specify The viral capsid proteins (no genes present) have the same dissemination as the nonpathogenic parent virus.

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

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

Specify Like the recipient, AAV1/SERCA2a is non-pathogenic. The capsid proteins are wtAAV1. The transgene is fully human, nontoxic and not oncogenic.

2. Genetic stability of the genetically modified organism

AAV1/SERCA2a drug substance was produced under cGMP and has been tested for replication-competent AAV, helper adenovirus and other adventitious viruses by sensitive cellular, *in-vitro* and qPCR assays and none have been detected. In addition, AAV1/SERCA2a drug substance is tested for identity, potency and other impurities. All tests for identity, purity and quality have confirmed the stability of AAV1/SERCA2a. In addition, if transduced into a host that is co-infected with either wtAAV or helper virus, the small amount of AAV DNA makes homologous recombination very unlikely and the limited capsid packing capacity (5 kb) compared to the transgene (37 kb) restricts any

recombination events that could lead to a replication-competent vector carrying the transgene.

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)

With regard to point II(A)(11)(d), the three modified aspects of the vector are the removal of almost all of the viral DNA, the maintenance of the same tropism by maintaining a wt capsid and the insertion of the human gene SERCA2a. The removal of viral DNA eliminates any viral virulence. The maintenance of the wt capsid maintains wtAAV host range, tropism and absence of adverse immune responses due to AAV. The fully human protein SERCA2a expressed from the vector has been found to be well-tolerated in non-clinical and clinical (no adverse events associated with the vector) studies. With regard to allergic reactions to the viral capsid, a sensitive ELISpot assay conducted on all previous trial subjects showed no responses associated with the treatment. However, subjects did seroconvert to AAV1 positive, as expected. About 50% of the human population in Europe is already seropositive for AAV1 and 90% positive for AAV. Like the parent organism AAV1, the vector is nonpathogenic.

With regard to II(C)(2)(i) and consequences to human, animal and plant health; like the parent organism the vector is not pathogenic. In addition, it is limited to infection of humans and primates. In non-clinical work supporting the study, high dose intracoronary infusion of AAV1/SERCA2a demonstrated that transgene overexpression in minipigs was limited to tissues in the cardiac region. The human subjects in the proposed study will receive a systemic dose by intracoronary infusion of 50 mL of investigational product. At the much lower doses possible in the environment and due to the non-pathogenicity and replication incompetence of the vector, the environmental impact is thought to be negligible.

4. Description of identification and detection methods

- (a) Techniques used to detect the GMO in the environment  
- qPCR with primers specific for the vector DNA could be used to detect the organism in the environment.
- (b) Techniques used to identify the GMO  
- qPCR with primers specific for the vector DNA.  
- ELISA for conformational intact AAV1 capsid proteins (viral particles). This method is not suitable for environmental monitoring due to lack of sensitivity and specificity.

## 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 is a clinical trial to investigate the safety and feasibility of AAV1/SERCA2a in human subjects with advanced heart failure. There are no environmental benefits.

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 (.) No (X)

If yes, specify ...

3. Information concerning the release and the surrounding area

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

The Royal Brompton Hospital (Borough of London): Grid Ref – TQ27117 78312

Golden Jubilee National Hospital (Strathclyde Region): Grid Ref- NS48396 70822

Harefield Hospital: Grid Ref – TQ 05166 90857

Papworth Hospital: Grid Ref – TL 28746 62837

- (b) Size of the site (m<sup>2</sup>): ... m<sup>2</sup>  
(i) actual release site (m<sup>2</sup>): ... m<sup>2</sup>  
(ii) wider release site (m<sup>2</sup>): ... m<sup>2</sup>

Administration by intracoronary infusion of the investigational product will take place under containment in the catheterization (cath) laboratory of a hospital. The subjects will be monitored clinically following the procedure and either released or observed for a longer period within the hospital.

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

Not applicable

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

Not applicable

4. Method and amount of release

- (a) Quantities of GMOs to be released:

The subjects will be administered a single onetime dose of  $1 \times 10^{13}$  DNase resistant particles (DRP). Quantities released to the environment by shedding are expected to be considerably reduced due to processing by the subjects mononuclear phagocyte system.

- (b) Duration of the operation:

Less than 1 hour, followed by a subject observation period.

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

The investigational product will be contained during administration. The primary mode of containment during the catheterization procedure is application of

Standard (Universal) Precautions for infectious materials. Personnel performing the procedure in the cath lab will wear goggles, scrub suit, shoe covers, cap and mask, and gloves while support personnel will wear safety glasses, gown, shoe covers, cap and mask, and gloves. All personnel will be trained on aseptic technique and the containment and handling of blood borne pathogens.

All personnel involved in the direct use of the syringe pump for administration of investigational product must attend an in-service training on the proper use of the syringe pump and participate in a dry run of its setup and operation prior to infusing the first subject.

5. Short description of average environmental conditions (weather, temperature, etc.)  
Hospital catheterization laboratory
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 with AAV1/SERCA2a include 37 treated subjects totalling over 800 cumulative months of monitoring in the previous US phase 1 and phase 2 trials with no adverse events attributed to the investigational product. In addition, there have been over 700 subjects treated with AAV vectors (including 130 healthy adults who received a rAAV2 vector containing genes from HIV-1 in preventive vaccine studies sponsored by the International AIDS Vaccine Initiative) with an excellent safety profile and up to 10 years of follow-up in some subjects.

**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)	Primate
(ii) family name for plants	...
(iii) genus	<i>Homo</i>
(iv) species	<i>sapiens</i>
(v) subspecies	...
(vi) strain	...
(vii) cultivar/breeding line	...
(viii) pathovar	...
(ix) common name	human

2. Anticipated mechanism and result of interaction between the released GMOs and the target organism (if applicable)  
At the cellular level, HF is characterized by a number of abnormalities in the various steps of excitation-contraction coupling. In the mammalian heart, intracellular  $Ca^{2+}$  movements are tightly regulated at various levels within the cardiac cell. The sarcoplasmic reticulum (SR) plays an important role in orchestrating the movement of calcium ( $Ca^{2+}$ ) during each contraction and relaxation. Excitation leads to  $Ca^{2+}$  release from the SR, activating the myofilaments leading to contraction. During relaxation, the majority of  $Ca^{2+}$  is sequestered back into the SR by the SR  $Ca^{2+}$  ATPase pump (SERCA2a).

SERCA2a not only determines the amount of  $Ca^{2+}$  in the SR available to activate the next contraction, but also controls to a large extent the rate of cardiac relaxation. The expression level of SERCA2a significantly affects the overall cardiac contractile properties and ability of the heart to supply adequate amounts of oxygenated blood to the body. If the cardiac muscle becomes stressed from overwork or damaged from conditions such as chronic hypertension, myocardial infarction, or idiopathic causes, a foetal gene survival

program is initiated in cardiomyocytes, causing decreased expression of SERCA2a. This leads to a poorly contracting myocardium and subsequent poor blood flow and reduced oxygen supply to the body. Increasing the levels of SERCA2a protein in cardiomyocytes has been shown to normalize the abnormally high diastolic levels of cytosolic Ca<sup>2+</sup> typical of HF and improve clinical outcomes.

Intracoronary infusion of heart arteries with AAV1/SERCA2a results in stable transduction of heart muscle cells (cardiomyocytes), removal of the capsid proteins, transport of the single stranded vector DNA (ssDNA) to the nucleus, formation of double stranded vector DNA, formation of vector DNA episomes (non-integrating DNA structures) and expression of SERCA2a protein. Following translation, SERCA2a protein fully inserts into the sarcoplasmic reticulum.

When normal levels of SERCA2a are obtained, sufficient Ca<sup>2+</sup> is pumped from the cytosol allowing full relaxation of the heart muscle and increased volumes of blood pumped from the heart. The principal aim of the study is to evaluate and confirm the clinical safety and efficacy of MYDICAR<sup>®</sup> versus placebo added to an optimal HF regimen in the treatment of subjects with NYHA class III/IV symptoms of systolic HF. Celladon believes that targeted SERCA2a enzyme replacement in advanced HF patients will correct imbalances in Ca<sup>2+</sup> cardiac metabolism, resulting in enhanced cardiac function and energetics, which will in turn translate to improved clinical outcomes.

Most conventional medical strategies for the treatment of HF do not correct the underlying cause. MYDICAR<sup>®</sup> provides continuous delivery of therapeutic proteins locally at the site of disease after a single administration, targets the fundamental biochemical physiology of myocardial contractility, and can potentially lead to reversal of the pathophysiology associated with HF. The benefits of MYDICAR<sup>®</sup> may include prolonged survival, improvement or stabilization of myocardial function, and/or reduced frequency or duration of hospital stays. The anticipated result is increased time to, or avoidance of, clinical endpoints including hospitalization, transplant, ventricular assist implantation and death.

3. Any other potentially significant interactions with other organisms in the environment  
None
  
4. Is post-release selection such as increased competitiveness, increased invasiveness for the GMO likely to occur?  
 Yes (.)                      No (X)                      Not known (.)  
 Give details  
 The AAV1/AERCA2a vector lacks all of the viral gene coding sequences rendering it replication incompetent even in the presence of helper virus. However the viral capsid is wild-type so the host range and tropism are not altered. Because the vector cannot replicate and because of the limited capsid packaging capacity, there are no plausible routes for increased competitiveness or invasiveness.
  
5. Types of ecosystems to which the GMO could be disseminated from the site of release and in which it could become established  
 wtAAV1 is not known to infect any other organisms in the environment except primates. There is a chance that other humans could be infected with the vector, however because the amount would be so low and the vector is replication incompetent (even in the presence of helper virus) the result would be negligible.
  
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 name	...
		None

7. Likelihood of genetic exchange in vivo

- (a) from the GMO to other organisms in the release ecosystem:  
Negligible
- (b) from other organisms to the GMO:  
Negligible
- (c) likely consequences of gene transfer:  
*Increase in the expression of the SERCA2a protein to normal levels in the heart of failure subjects with advanced heart failure.*

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.):

Celladon is investigating MYDICAR<sup>®</sup> (AAV1/SERCA2a) gene therapy in patients with advanced NYHA class III/IV HF due to systolic dysfunction as a method to enhance cardiac function and improve patient outcomes by reducing the frequency and/or delaying HF-related hospitalizations compared to placebo-treated patients. Preliminary safety and efficacy of  $1 \times 10^{13}$  DRP MYDICAR<sup>®</sup> have been demonstrated in the CUPID phase 2 study, with MYDICAR<sup>®</sup>-treated subjects demonstrating a significant decrease in the frequency of pre-defined adjudicated cardiovascular (CV)-related adverse events per subject, including reductions in both number and duration of CV-related hospitalizations compared to placebo-treated subjects.<sup>1</sup>

To date, MYDICAR<sup>®</sup> has been studied in 51 subjects in a phase 1/2, multicenter trial of a single intracoronary administration of AAV1/SERCA2a (Protocol No. CELL-001, CUPID Trial).<sup>1-3</sup> Of the 51 subjects, 37 subjects received AAV1/SERCA2a, with a total of over 800 cumulative months of monitoring with no increases in adverse events, disease-related events, laboratory abnormalities, or arrhythmias observed in any of the treated subjects compared to those receiving placebo.<sup>1, 4, 5</sup>

In addition, there have been over 700 subjects treated with other AAV vectors (including 130 healthy adults who received a rAAV2 vector containing genes from HIV-1 in preventive vaccine studies sponsored by the International AIDS Vaccine Initiative) with an excellent safety profile and up to 10 years of follow-up in some subjects.

Routine testing for presence of the SERCA2a transgene in the cardiomyocyte target tissue following AAV1/SERCA2a has not been performed in clinical studies since this would require an invasive cardiac biopsy that has inherent risks in patients with advanced HF. Since SERCA2a is an integral membrane protein, there is no known surrogate that might indicate vector persistence. Therefore, opportunities to collect tissue for testing are unpredictable, such as cases of death followed by swift notification and rapid mobilization, orthotopic heart transplant where the native treated heart becomes available, implantation

of ventricular assist device where core tissue becomes available, and certain other cardiac procedures where biopsy is practical under the circumstances.

Quantitative PCR (qPCR) for the AAV/SERCA2a DNA was performed demonstrated persistence out to Month 31 in the target tissue of 1 subject, to Month 23 in another and to Month 22 in a third subject; all 3 subjects with qPCR positive vector DNA results were in the high-dose MYDICAR<sup>®</sup> group in the CUPID study.

1. Jessup M, Greenberg B, Mancini D, Cappola T, Pauly D, Jaski B, Yaroshinsky A, Zsebo K, Dittrich H, Hajjar R. Calcium Upregulation by Percutaneous Administration of Gene Therapy in Cardiac Disease (CUPID): A Phase 2 Trial of Intracoronary Gene Therapy of Sarcoplasmic Reticulum Ca<sup>2+</sup>-ATPase in Patients With Advanced Heart Failure. *Circulation* 2011;**124**(3):304-313.
  2. Hajjar R, Zsebo K, Deckelbaum L, Thompson C, Rudy J, Yaroshinsky A, Ly H, Kawase Y, Wagner K, Borow K, Jaski B, London B, Greenberg B, Pauly D, Patten R, Starling R, Mancini D, Jessup M. Design of a Phase 1/2 Trial of Intracoronary Administration of AAV1/SERCA2a in Patients With Heart Failure. *J Card Fail* 2008;**14**(5):355-367.
  3. Jaski B, Jessup M, Mancini D, Cappola T, Pauly D, Greenberg B, Borow K, Dittrich H, Zsebo K, Hajjar R. Calcium upregulation by percutaneous administration of gene therapy in cardiac disease (CUPID Trial), a first-in-human phase 1/2 clinical trial. *J Card Fail* 2009;**15**(3):171-81.
  4. Gwathmey JK, Yerevanian AI, Hajjar RJ. Cardiac gene therapy with SERCA2a: From bench to bedside. *Journal of Molecular and Cellular Cardiology* 2011;**50**(5):803-812.
  5. Kawase Y, Ladage D, Hajjar R. Rescuing the Failing Heart by Targeted Gene Transfer. *J Am Coll Cardiol* 2011;**57**(10):1169–80.
9. Possible environmentally significant interactions with biogeochemical processes (if different from the recipient or parental organism)  
Not applicable

## **H. Information relating to monitoring**

1. Methods for monitoring the GMOs  
AAV1/SERCA2a could be monitored by detection of the vector DNA with qPCR. No monitoring is planned for this study.
2. Methods for monitoring ecosystem effects  
The chance of ecosystem effects is negligible and monitoring is not planned.
3. Methods for detecting transfer of the donated genetic material from the GMO to other organisms  
The likelihood of transfer of AAV1/SERCA2a to other organisms is negligible and no monitoring is planned. The existing qPCR assay used in the non-clinical and clinical studies could be used.
4. Size of the monitoring area (m<sup>2</sup>)  
... m<sup>2</sup>  
Not applicable
5. Duration of the monitoring  
Not applicable
6. Frequency of the monitoring  
Not applicable



## **I. Information on post-release and waste treatment**

1. Post-release treatment of the site  
The procedure room will be cleaned according to standard institutional procedures for cleaning up blood borne pathogens but with fresh 0.5% sodium hypochlorite (10% solution of household bleach) left wet for 10 minutes.
2. Post-release treatment of the GMOs  
The unused investigational product concentrate (2 mL) will be destroyed by treatment with 5000 ppm sodium hypochlorite (10% solution of household bleach) for 10 minutes. The destroyed investigational product will be poured down a sink with running water, or otherwise in compliance with local and institutional disposal and cleaning procedures. Alternatively the unused investigational product could be autoclaved or incinerated according to each institution's policies and practice.
3. (a) Type and amount of waste generated  
Miscellaneous catheterization laboratory consumables including surgical instruments, gloves, masks, syringes, needles catheter, tubing and absorbent materials. The unused investigational product vial, stopper and crimp seal. The amount of waste is typical of a cardiac catheterization procedure were the waste is considered bio-hazardous material contaminated with blood borne pathogens.
3. (b) Treatment of waste  
Treatment with 5000 ppm sodium hypochlorite (10% solution of household bleach) for 10 minutes for all surfaces and non-disposable instruments (that cannot be autoclaved) which were potentially contaminated with the investigational product. All disposable materials that come into contact with the investigational product will be disposed of as hazardous biological materials according to individual institutional practices and policies. In general the disposable materials will be disposed in sharps containers or biohazard bags and decontaminated by autoclave or incineration, or both.

## **J. Information on emergency response plans**

1. Methods and procedures for controlling the dissemination of the GMO(s) in case of unexpected spread  
The chance of dissemination of the vector is negligible outside of the contained catheterization laboratory in the hospital.  
  
Accidental spills will be cleaned up as follows.
  - Notify others and isolate the area.
  - If not already wearing, put on appropriate personal protective equipment: gown or lab coat, gloves, surgical or procedure mask and safety glasses, shield or goggles.
  - Remove any broken glass or sharps with forceps or applicable tool and place into a sharps container.
  - Decontaminate the area of the spill.
  - Place absorbent material over the spill.
  - Working from the outside to the centre saturate the absorbent material with 10% beach solution (5000 ppm sodium hypochlorite).
  - Allow to stand for at least 10 minutes.
  - Place the absorbent material in an appropriate biohazard waster container and dispose as a biohazard material.

2. Methods for removal of the GMO(s) of the areas potentially affected  
Not applicable
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  
Not applicable