APPLICATION FOR CONSENT TO RELEASE A GMO – ORGANISMS OTHER THAN HIGHER PLANTS

PART A1: INFORMATION REQUIRED UNDER SCHEDULE 2 OF THE GENETICALLY MODIFIED ORGANISMS (DELIBERATE RELEASE) REGULATIONS 2002.

Part I
GENERAL INFORMATION

1. The name and address of the applicant and the name, qualifications and experience of the scientist and of every other person who will be responsible for planning and carrying out the release of the organisms and for the supervision, monitoring and safety of the release

The name and address of the applicant

For the first Study: "A Phase 2b, Double-Blind, Placebo-Controlled, Multinational, Multicenter, Randomized Study Evaluating the Safety and Efficacy of Intracoronary Administration of MYDICAR® (AAV1/SERCA2a) in Subjects with Heart Failure"

Celladon Corporation, 12760 High Bluff Drive, Suite 240, San Diego, CA 92130-2019
United States

.

For the second Study: "Investigation of the safety and feasibility of AAV1/SERCA2a gene transfer in patients with chronic heart failure and a left ventricular assist device"

Dr. Alexander Lyon – National Coordinating Investigator CUPID 2 Study and Lead Clinical Investigator for the SERCA-LVAD Trial, Senior Lecturer in Cardiology, Imperial College London, and Consultant Cardiologist, Royal Brompton Hospital, London.

2. The title of the project

"A Phase 2b, Double-Blind, Placebo-Controlled, Multinational, Multicenter, Randomized Study Evaluating the Safety and Efficacy of Intracoronary Administration of MYDICAR® (AAV1/SERCA2a) in Subjects with Heart Failure" and "Investigation of the safety and efficacy of AAV1/SERCA2a gene transfer in patients with chronic heart failure and a left ventricular assist device".

Part II
INFORMATION RELATING TO THE GMO

Characteristics of the Donor, Parental and Recipient Organisms

3. Scientific name and taxonomy

Donor

Human. SERCA2a is a human protein.

Recipient

Parvoviridae/Parvovirinae/dependovirus/ Adeno-associated virus serotype 1 (AAV1).

4. Usual strain, cultivar or other name

Donor

Not applicable.

Recipient

Wild type AAV1

5. Phenotypic and genetic markers

Donor

Not applicable

Recipient

Wild type virus, AAV1 is a small nonenveloped ssDNA nonpathogenic virus that is not known to be associated with any human diseases although over 90% of people are seropositive to AAV before entering adulthood. [1] It is only replication competent in the presence of a helper virus like adenovirus or herpes virus.

6. Degree of relatedness between donor and recipient or between parental organisms

None

7. Description of identification and detection techniques

The Progen AAV1 titration ELISA kit is utilized to determine the amount of intact AAV1 capsid viral proteins present. Samples, standards and controls are added to microtitration wells pre-coated with an anti-AAV1 capsid monoclonal antibody. After incubation, unbound antigen is removed by washing, and a biotin-conjugated anti-AAV1 capsid detection antibody is added. After incubation, unbound antibody is removed by washing, and a streptavidin-peroxidase conjugate is added. After incubation and an additional wash step, tetramethylbenzidine (TMB), the peroxidase substrate, is added to the wells. The reaction is stopped by the addition of an acidic stop solution, and the amount of oxidized substrate is determined by measuring the absorbance with a microplate reader.

8. Sensitivity, reliability (in quantitative terms) and specificity of detection and identification techniques

Specificity

During method development studies, specificity was demonstrated for the Capsid ELISA. The method was able to differentiate between AAV1, AAV2, and formulation buffer. AAV2 and formulation buffer gave a response that was at least 9X less than the lower limit of detection of the method.

Reliability and Sensitivity

Summaries of the method qualification results for the Capsid ELISA method is provided in Table 1.

Table 1. Method Qualification Summary for Capsid ELISA

Determination		Results
Accuracy	n=3 runs, 2 operators, 3 dilutions	87-112%
Linearity	n=3 runs, dilutions of 1 x 10^8 – 1 x 10^{10} cp/mL	1.0000
Intraassay Repeatability	n=2 replicates, 3 runs, 2 operators	0.2-5.1%
Intermediate precision	n=3 samples, 3 runs, 2 operators	2.3-14.6%
Sensitivity – LOQ	Reference standard dilutions	0.04-8.0% 1.1 x 10^7
Range	Undiluted samples, n=3 runs, 2 operators	1.1 x 10^7 – 4.5 x 10^{10}

9. Description of the geographic distribution and of the natural habitat of the organism including information on natural predators, preys, parasites and competitors, symbionts and hosts

The known environmental hosts are humans and primates. Over 90% of the human population in Europe and the US has been exposed to AAV. [1] AAV1 host cell tropism includes cardiac/slow-twitch skeletal muscle and endothelial cells. AAV replication is dependent on helper virus (adenovirus or herpes virus) for replication.

10. Organisms with which transfer of genetic material is known to occur under natural conditions

The known hosts for AAV1 are humans and primates. The primary modes of transmission are inhalation of aerosols and contact with mucus membranes.

11. Verification of the genetic stability of the organisms and factors affecting that stability

AAV1/SERCA2a drug substance was produced under cGMP and has been tested for replication-competent AAV and helper adenovirus by sensitive cellular assays, 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 present in the AAV1/SERCA2A vector GMO makes homologous recombination very unlikely and the limited capsid packing capacity (5 kb) compared to the transgene (4.2 kb) restricts any recombination events that could lead to a replication-competent vector carrying the transgene.

12. The following pathological, ecological and physiological traits:

a. Classification of hazard according to existing Community rules concerning the protection of human health and/or the environment

The parental virus AAV is not pathogenic. The vector has had all viral genes removed and is assumed to also be nonpathogenic. The vector has fully wild-type capsid proteins and the immune response is not expected to be different from that of the parental virus. Exposed individuals could seroconvert to an AAV1 positive titer. There has been no indication from any of the previous AAV human trials, including the CUPID phase 1/2 study that this humoral response (neutralizing antibodies) against the capsid proteins has any apparent clinical sequelae other than as it relates to potential ineffectiveness of a future AAV gene therapy administration.

AAV is classified as Risk Group 1 by the US NIH. [2] The UK ACDP has not categorised AAV, consequently the UK Scientific Advisory Committee on Genetic Modification states that Containment Level 1 is sufficient. (see Section 2.6.5 of the SACGM Compendium of Guidance [3]).The Belgium Scientific Institute of Public Health assigns a Risk 2 for immunocompetent humans as a maximum risk. The Germany Central Biosafety Commission classifies AAV2 as Risk Group 1 and AAV1 as Risk Group 2. [4] The Swiss Expert Committee for Biosafety considers both AAV1 and AAV2 as class 2. [3]

AAV vectors are classified as Risk Group 1 by the US NIH. [2] The UK Scientific Advisory Committee on Genetic Modification considers most recombinant AAV vectors to be low risk Activity Class 1 and they can be handled at Containment Level 1. (see Section 2.6.22 of the SACGM Compendium of Guidance [3]). The Germany Central Biosafety Commission classifies AAV vectors like AAV1/SERCA2a (no nucleic acid fragment except the ITRs and no potentially hazardous nucleic acid fragment) as Risk Group 1 and Containment Level 1. [4] The Swiss Expert Committee for Biosafety considers AAV vectors as Class 1 when produced in the absence of helper viruses. AAV1/SERCA2a was produced with assistance of adenovirus helper. [5] However, the manufacturing process was designed to remove any residual adenovirus and the investigational product intended for use in this study tested negative for the presence of adenovirus.

AAV vectors in the US are considered to be in Risk Group 2 if they are produced in the presence of Adenovirus as a helper virus. In the US, Celladon has conducted previous phase 1 and phase 2 studies under both Risk Group 1 & 2 and Biosafety Level 1 & 2

classifications depending on the individual site Institutional Biosafety Committee classifications. However, AAV1/SERCA2a drug substance, tested with a sensitive amplified (2 rounds) cellular assay, has demonstrated the absence of adenovirus in production batches of the drug product intended for all studies, including this phase 2b study. Celladon believes that based on the design of the manufacturing process (over 20 logs of adenovirus removal) and results of drug substance testing that MYDICAR is in Risk Group 1 and Biosafety Level 1 in the US. As with the previous studies, the US Appendix M submission to the NIH Recombinant DNA Advisory Committee (RAC) for the current phase 2b study was submitted as Risk Group 1 and was accepted.

AAV does not have a hazard classification under the European Economic Community (EEC) classification for the protection of workers with biological agents [Directive 2000/54/EC].

b. Generation time in natural ecosystems, sexual and asexual reproductive cycle

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 AAV1/SERCA2a vector is “gutless” and cannot replicate under any conditions.

c. Information on survivability, including seasonability and the ability to form survival structures, including seeds, spores and sclerotia

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

d. Pathogenicity, including infectivity, toxigenicity, virulence, allergenicity, carrier (vector) of pathogen, possible vectors, host range including non-target organisms and possible activation of latent viruses (proviruses) and ability to colonise other organisms

There are three modified aspects of the vector that address pathogenicity; the removal of nearly all of the viral DNA (94%), the maintenance of the native 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, based on results from the AAV1 Neutralizing Antibody assay. About 50% of the human population in Europe is already seropositive for AAV1 and over 90% positive for AAV. [1]

AAV1/SERCA2a belongs to the type of AAV vector commonly referred to a “gutted AAV vector”. As such, all of the viral gene coding sequences have been removed. The only remaining sequences are two small inverted terminal repeats (ITRs) that flank the

transgene expression cassette and comprise a combined total of only 290 bases (<6% of the viral genome). The ITRs allow the vector genome to be packaged into vector capsids. Unlike the parent virus, the vector is not replication competent even in the presence of helper virus. The elimination of 94% of the viral DNA reduces the probability of homologous recombination with related viruses that could lead to variants of the GMO.

Like the parent organism, AAV1, the vector is nonpathogenic. The vector has had all viral genes removed and is assumed to also be nonpathogenic. The vector has fully wild-type capsid proteins and the immune response is not expected to be different from that of the parental virus. Exposed individuals could seroconvert to an AAV1 positive titer. There has been no indication from any of the previous AAV human trials, including the CUPID phase 1/2 study that this humoral response (neutralizing antibodies) against the capsid proteins has any apparent clinical sequelae other than as it relates to potential ineffectiveness of a future AAV gene therapy administration. Over 90% of the population is already seropositive for AAV. [1]

e. Antibiotic resistance, and potential use of these antibiotics in humans and domestic organisms for prophylaxis and therapy

There are no antibiotic resistance genes in either AAV1 or the AAV1/SERCA2A construct GMO. However Kanamycin and Puromycin resistance genes are present in the manufacturing process constructs. A qPCR assay is used to monitor *Kan*^{R} and *Puro*^{R} DNA in the drug substance as part of manufacturing process characterization. A panel of specific qPCR assays is used to monitor co-packaging of these residual production-system-related non-transgene sequences as part of process characterization and routine lot release testing. Testing has been performed on multiple batches representative of the current manufacturing process. *Puro*^{R} sequences are 3 logs lower than the SERCA2a transgene, while *Kan*^{R} sequences are approximately 4 logs lower.

While not present in the GMO construct, but present as a minor contaminate the *Kan*^{R} sequence poses low environmental risk for human therapeutics, as it has been assessed for plant-based GMOs [6] with much broader potential distribution. It is a risk Group 1 antibiotic resistance marker which: (a) is widely distributed among soil and enteric bacteria and (b) confers resistance to antibiotics which have no or only minor therapeutic relevance in human medicine and have only restricted use in defined areas of veterinary medicine. The *Kan*^{R} resistance marker has a 13-year history of safe use in food crops. No restrictions are required with this class of marker genes either for field experimentation or for placing on the market. Therefore, inadvertent exposure to the non-transgene gene is unlikely to cause adverse effects and the risk is determined to be negligible.

Likewise, while not present in the GMO construct, but present as a minor contaminate, the *Puro*^{R} resistance sequence is not likely to have any impact as puromycin is an antibiotic that is not used in humans. Therefore, inadvertent exposure to the non-transgene gene is unlikely to cause adverse effects.

f. Involvement in environmental processes including primary production, nutrient turnover, decomposition of organic matter and respiration

Not applicable. Neither the vector nor the parent/recipient organism is involved in any environmental processes.

13. The sequence, frequency of mobilisation and specificity of indigenous vectors and the presence in those vectors of genes which confer resistance to environmental stresses.

AAV does not have the ability to replicate on its own. AAV1 is mobilized in hosts (human and primate) that are co-infected with a helper virus (e.g. herpes and adenovirus). In the laboratory AAV1 can infect other animals (e.g. rodents, pigs and sheep) but it has not been detected in the environment from animals other than humans and other primates. The environmental constraints limiting a broader distribution are not known.

AAV is a very small simple virus that only contains two open reading frames. There are 3 capsid proteins produced and 4 replication related genes produced from overlapping sequences. It is not clear how any of these proteins could confer resistance to environmental stresses.

14. The history of previous genetic modifications

The parent AAV1 is wildtype. The transgene has is an unmodified human gene, SERCA2a.

Characteristics of the vector

15. The nature and source of the vector.

The vector in this context is the plasmid pTPK-ABG12. The plasmid is the source of the entire AAV1 vector (GMO) genome insert and contains the viral *rep* and *cap* genes required for AAV1/SERCA2A production. As such the plasmid contains all of the sequences required to produce the AA1/SERCA2A vector. The plasmid has a bacterial cell host range. It was grown in *E. coli* for DNA construction and cloning purposes and stably transfected into a HeLa S3 derived clone to generate producer cell line 2B12, used for AAV1/SERCA2A vector (GMO) generation. When producer cells are infected with helper adenovirus (Ad) they make AAV1/SERCA2A. The approach for producing the AAV1/SERCA2a GMO is to infect 2B12 cells with Ad serotype 5.

The Ad5 helper virus is produced in a serum-free cell line (HeLa SF2) adapted to serum-free suspension conditions from the parental HeLaS3 cells. Banking systems for cell lines (2B12 producer cell line and HeLa SF2) and Ad5 seed have been established according to the ICH guideline Q5D, “Derivation and characterization of cell substrates used for production of biological products”. The cell substrates and adenovirus seed banks have been tested according to the ICH guideline Q5A, “Viral safety evaluation of biotechnology products derived from cell lines of human or animal origin”.

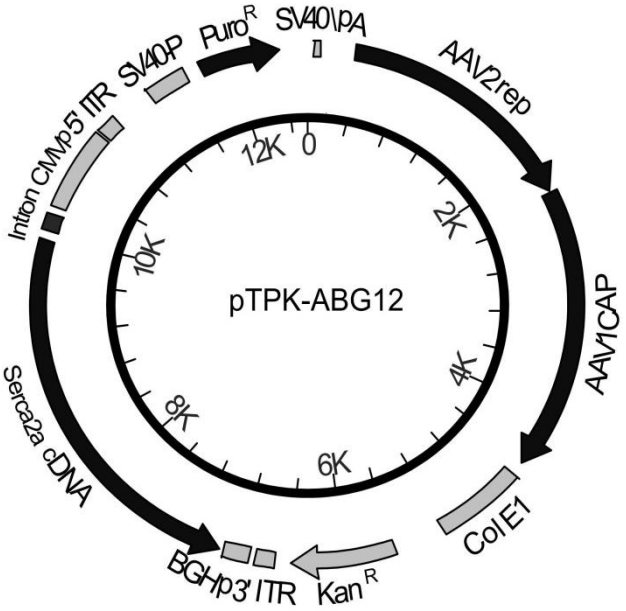


Figure 1. Schematic representation of the pTPK-ABG12 plasmid coding for the AAV1/SERCA2A genome

16. Sequence of transposons, vectors and other non-coding genetic segments used to construct the GMO and to make the introduced vector and insert function in the GMO

The AAV1/SERCA2a producer line (2B12) is a HeLa S3 derived clone designed for producing SERCA2a vector DNA packaged in AAV1 capsids. To generate the producer clone, cells expanded from the parental HeLa S3 were transfected with the triple play plasmid pTPK-ABG12. The pTPK-ABG12 plasmid contains the vector genome for AAV1/SERCA2a (two complete AAV2 ITRs flanking a CMV/SERCA2a/BGH polyA expression cassette); an AAV *rep*2/*cap*1 packaging cassette; an expression cassette for the puromycin resistance gene; Kanamycin resistance gene; and, ColE1 bacterial origin of replication for plasmid propagation in bacteria.

17. The frequency of mobilisation, genetic transfer capabilities and/or methods of determination of the inserted vector

The plasmid with an ColE1 bacterial origin of replication replicates in E. coli. The plasmid is stably transfected into the HeLa S3 cells. The plasmid is mobilized when the cells are infected with helper virus. The resultant product is rAAV vector.

18. The degree to which the vector is limited to the DNA required to perform the intended function

All of the plasmid DNA is required to perform AAV1/SERCA2a generation except the ColE1 and Kan^R sequences required for propagation in *E. coli* K12 that was used for original plasmid cloning and the $Puro^R$ that is used for generate a stable producer line.

Characteristics of the Modified Organism

19. Methods used for the modification

20. Methods used--

a. to construct inserts and introduce them into the recipient organism

The AAV1/SERCA2a producer line (2B12) is a HeLa S3 derived clone designed for producing SERCA2a vector DNA packaged in AAV1 capsids. In order to generate the producer clone, cells expanded from the parental HeLa S3 were transfected with the plasmid pTPK-ABG12. Transfected cells were selected for puromycin resistance in 96 well plates at densities where most wells contained fewer than two colonies (the culture from each well is defined as a masterwell). Plates were screened for production, and the cell line from the selected masterwell was subcloned by limiting dilution to produce clone 2B12. The 2B12 cells were expanded in in disposable shake flasks. Cells were centrifuged and resuspended in freezing medium to produce a pre-bank of the 2B12 cells.

The prebank was tested for sterility and Mycoplasma before thawing for expansion to produce the cGMP Master Cell Bank lot GAT-0001. The MCB lot GAT-0001, was produced under cGMP conditions. The MCB met all acceptance criteria in line with ICH guidelines Q5A-D.

Sequence of the AAV1 capsid was confirmed by isolating total genomic DNA from cells expanded from the MCB (Lot# GAT-0001), amplifying the capsid gene in a nested PCR-based approach, and direct primer extension sequencing of the PCR product.

When the producer cell line 2B12 is infected with adenovirus it produces AAV1 capsids filled with the GMO genome. This approach avoids the need to manufacture DNA. Residual adenovirus is removed by downstream purification.

b. to delete a sequence

The AAV packaging of the GMO genome into the AAV capsid proteins is performed by the AAV *rep* genes present in the plasmid vector. The ITR regions bracketing are GMO genome are recognized by the AAV Rep proteins and cleaved. The GMO genome is inserted into the capsid.

21. The description of any insert and/or vector construction

The insert is the entire GMO genome. It is fully described below in Item 24.

22. The purity of the insert from any unknown sequence and information on the degree to which the inserted sequence is limited to the DNA required to perform the intended function

Quality control assays are performed on the AAV1/SERCA2a vector purified drug substance including, but not limited to, those designed to detect infectious and non-infectious adenovirus, phenotypic wild-type replication competent AAV (rcAAV) variants (packaged particles that can replicate in the presence of helper virus), host cell DNA, host cell protein and total protein purity.

The AAV1/SERCA2a manufacturing process was designed to eliminate the adenovirus serotype 5 (Ad5) helper virus from the vector product, and well-defined segregation procedures are designed to prevent its re-introduction. [7] Testing for Ad5 is shown to be, at or below, detection limits in the elution from the capture column using a sensitive limit assay. Following this testing point are two additional robust clearance steps, for which the mechanism of clearance is well understood and for which the relevant operating parameters are monitored for every lot. Residual adenovirus DNA is monitored by qPCR using the Ad E2A gene as a marker.

The qPCR assay is used until the Ad clearance is at the background of non-transgene packaged Ad DNA. Combined, five orthogonal purification steps are conservatively capable of clearing >22 logs of Ad5, which provides more than an 8-log safety margin per dose of AAV1/SERCA2a.

As batch acceptance criteria the final drug substance is assayed for residual infectious adenovirus by a sensitive cell-based assay and for adenovirus proteins by western blot. The infectious adenovirus assay is based on two rounds of amplification on QBI-293A cells. With the second round of amplification cells are scored as positive or negative for adenovirus by visual inspection for cytopathic effect. The limit of detection for the assay is 10 i.u. Ad/sample tested. For the batch intended for use in the clinical study (EIX-0001) the results were negative at ≤10 i.u. Ad/1 x 10^{12} DRP.

As mentioned above the plasmid vector contains *rep*, *cap*, *Kan*^{R}, *Puro*^{R} genes. In addition, as mentioned above Ad5 is used in the manufacturing process. A qPCR assay is used to monitor *rep*, *cap*, *Kan*^{R}, *Puro*^{R} and Ad5 E2A DNA as part of manufacturing process characterization. A panel of specific qPCR assays is used to monitor co-packaging of these residual production-system-related non-transgene sequences as part of process characterization and routine lot release testing. Testing has been performed on multiple batches representative of the current manufacturing process. Sequences from the expression cassettes of the packaging plasmid (*rep*, *cap*, *Puro*^{R}) are all approximately 3 logs lower than the SERCA2a transgene, while *Kan*^{R} is approximately 4 logs lower.

Samples from AAV1/SERCA2a batches that were produced using the current manufacturing process were tested in a real-time quantitative polymerase chain reaction (qPCR) analytical procedure targeting E2A as a marker for Ad5 helper virus DNA. The results are shown in the table below and demonstrate lot to lot consistency. Other Ad sequences have not been tested.

E2a DNA Results

Date of Manufacture **Disposition**	**07:052** **30Nov07** **Development**	**08:055** **07Aug08** **Development**	**Engineering** **EIX-120508** **05Dec08**	**Clinical** **EIX-0001** **30Apr09**
DNase resistant copies E2a/mL	1.6 x 10^8	2.3 x 10^8	1.2 x 10^8	1.3 x $10^{8\,\dagger}$
%E2a in relation to SERCA2a	0.0004%	0.0012%	0.0008%	0.0006% †

† Preliminary results: extrapolated from below the lowest standard in the standard curve

With regards to inflammation, there have been no episodes of inflammatory response in animals and humans treated with AAV1/SERCA2a. Inflammatory response would have been evident by an increase in clinical chemistries such as cardiac or liver enzymes (drug and dose dependent) and histopathology. No such findings have been noted.

The entire AA1/SERCA2a genome sequence is required for the intended function.

23. Methods and criteria used for selection

There is no selection for the GMO genome in the GMO. There are no selection markers in AAV1/SERCA2a.

24. The sequence, functional identity and location of the altered, inserted or deleted nucleic acid segments in question and, in particular, any known harmful sequence

All sequences in AAV1/SERCA2A are of known function. There is one gene present (ATP2A2) for the SERCA2a protein. SERCA2a is a fully human nontoxic protein. There are no harmful sequences present.

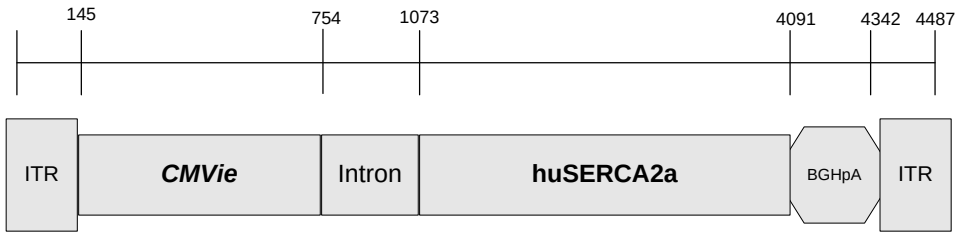


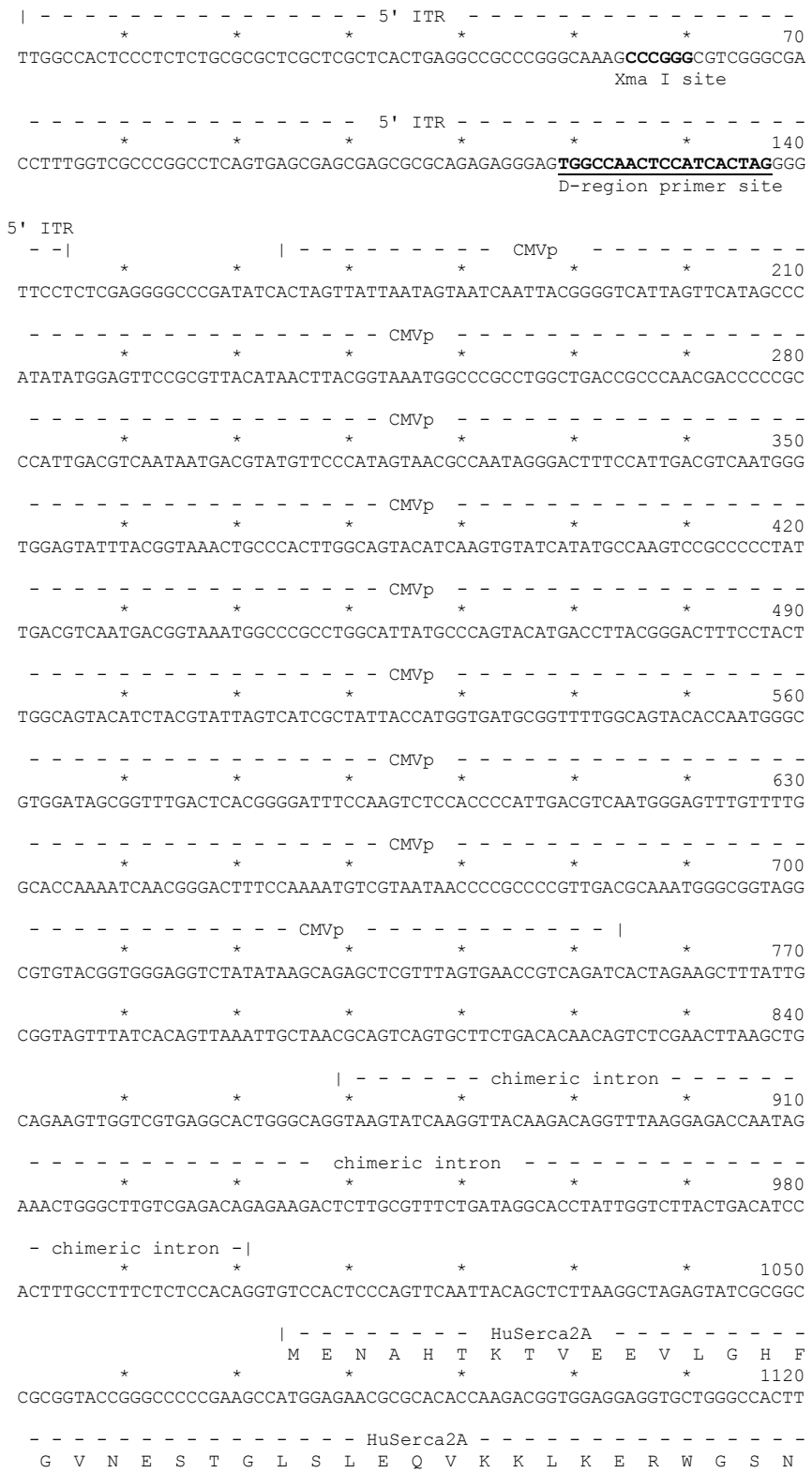
Figure 2. DNA of AAV1/SERCA2a

The vector genome in its entirety (4,487 bases) consists of:

1) The Cytomegalovirus immediate early enhancer/promoter (CMVie) (609 bases) and hybrid intron (319 bases) taken from pCI (Promega), GenBank accession number U47119, are included for transcription of the transgene and proper maturation of the transgene mRNA.
2) The hybrid intron (319 bases) uses the 5´-donor site from the first intron of 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;
3) The transgene hSERCA2a cDNA (3,018 bases) coding sequence, identical to GenBank accession number NM-001681, is included to study the return of cardiac muscle SERCA2a protein expression levels to normal in moderate to advanced heart failure subjects;
4) A bovine growth hormone polyadenylation signal (BGHpA) (251 bases) GenBank accession number M57764 is included for effective translation of the transgene mRNA; and
5) Two adeno-associated virus serotype 2 inverted terminal repeats, each 145 bases, flanking the expression cassette are included for capsid

packing.

The nucleotide sequence is shown in Figure 3 below, including translation of SERCA2a coding region. Regulatory regions (AAV ITR, CMV promoter, chimeric intron, and BGH poly adenylation signal) are indicated above the sequence.



```
        *       *       *       *       *       *     1190
CGGCGTCAACGAGAGTACGGGGCTGAGCCTGGAACAGGTCAAGAAGCTTAAGGAGAGATGGGGCTCCAAC

 - - - - - - - - - - - - - - - - HuSerca2A - - - - - - - - - - - - - - - -
 E  L  P  A  E  E  G  K  T  L  L  E  L  V  I  E  Q  F  E  D  L  L  V
        *       *       *       *       *       *     1260
GAGTTACCGGCTGAAGAAGGAAAAACCTTGCTGGAACTTGTGATTGAGCAGTTTGAAGACTTGCTAGTTA

 - - - - - - - - - - - - - - - - HuSerca2A - - - - - - - - - - - - - - - -
R  I  L  L  L  A  A  C  I  S  F  V  L  A  W  F  E  E  G  E  E  T  I  T
        *       *       *       *       *       *     1330
GGATTTTATTACTGGCAGCATGTATATCTTTTGTTTTGGCTTGGTTTGAAGAAGGTGAAGAAACAATTAC

 - - - - - - - - - - - - - - - - HuSerca2A - - - - - - - - - - - - - - - -
  A  F  V  E  P  F  V  I  L  L  I  L  V  A  N  A  I  V  G  V  W  Q  E
        *       *       *       *       *       *     1400
AGCCTTTGTAGAACCTTTTGTAATTTTACTCATATTAGTAGCCAATGCAATTGTGGGTGTATGGCAGGAA

 - - - - - - - - - - - - - - - - HuSerca2A - - - - - - - - - - - - - - - -
 R  N  A  E  N  A  I  E  A  L  K  E  Y  E  P  E  M  G  K  V  Y  R  Q
        *       *       *       *       *       *     1470
AGAAATGCTGAAAATGCCATCGAAGCCCTTAAGGAATATGAGCCTGAAATGGGCAAAGTGTATCGACAGG

 - - - - - - - - - - - - - - - - HuSerca2A - - - - - - - - - - - - - - - -
D  R  K  S  V  Q  R  I  K  A  K  D  I  V  P  G  D  I  V  E  I  A  V  G
        *       *       *       *       *       *     1540
ACAGAAAGAGTGTGCAGCGGATTAAAGCTAAAGACATAGTTCCTGGTGATATTGTAGAAATTGCTGTTGG

 - - - - - - - - - - - - - - - - HuSerca2A - - - - - - - - - - - - - - - -
  D  K  V  P  A  D  I  R  L  T  S  I  K  S  T  T  L  R  V  D  Q  S  I
        *       *       *       *       *       *     1610
TGACAAAGTTCCTGCTGATATAAGGTTAACTTCCATCAAATCTACCACACTAAGAGTTGACCAGTCAATT

 - - - - - - - - - - - - - - - - HuSerca2A - - - - - - - - - - - - - - - -
 L  T  G  E  S  V  S  V  I  K  H  T  D  P  V  P  D  P  R  A  V  N  Q
        *       *       *       *       *       *     1680
CTCACAGGTGAATCTGTCTCTGTCATCAAGCACACTGATCCCGTCCCTGACCCACGAGCTGTCAACCAAG

 - - - - - - - - - - - - - - - - HuSerca2A - - - - - - - - - - - - - - - -
D  K  K  N  M  L  F  S  G  T  N  I  A  A  G  K  A  M  G  V  V  V  A  T
        *       *       *       *       *       *     1750
ATAAAAAGAACATGCTGTTTTCTGGTACAAACATTGCTGCTGGGAAAGCTATGGGAGTGGTGGTAGCAAC

 - - - - - - - - - - - - - - - - HuSerca2A - - - - - - - - - - - - - - - -
  G  V  N  T  E  I  G  K  I  R  D  E  M  V  A  T  E  Q  E  R  T  P  L
        *       *       *       *       *       *     1820
TGGAGTTAACACCGAAATTGGCAAGATCCGGGATGAAATGGTGGCAACAGAACAGGAGAGAACACCCCTT

 - - - - - - - - - - - - - - - - HuSerca2A - - - - - - - - - - - - - - - -
 Q  Q  K  L  D  E  F  G  E  Q  L  S  K  V  I  S  L  I  C  I  A  V  W
        *       *       *       *       *       *     1890
CAGCAAAAACTAGATGAATTTGGGGAACAGCTTTCCAAAGTCATCTCCCTTATTTGCATTGCAGTCTGGA

 - - - - - - - - - - - - - - - - HuSerca2A - - - - - - - - - - - - - - - -
I  I  N  I  G  H  F  N  D  P  V  H  G  G  S  W  I  R  G  A  I  Y  Y  F
        *       *       *       *       *       *     1960
TCATAAATATTGGGCACTTCAATGACCCGGTTCATGGAGGGTCCTGGATCAGAGGTGCTATTTACTACTT

 - - - - - - - - - - - - - - - - HuSerca2A - - - - - - - - - - - - - - - -
  K  I  A  V  A  L  A  V  A  A  I  P  E  G  L  P  A  V  I  T  T  C  L
        *       *       *       *       *       *     2030
TAAAATTGCAGTGGCCCTGGCTGTAGCAGCCATTCCTGAAGGTCTGCCTGCAGTCATCACCACCTGCCTG

 - - - - - - - - - - - - - - - - HuSerca2A - - - - - - - - - - - - - - - -
 A  L  G  T  R  R  M  A  K  K  N  A  I  V  R  S  L  P  S  V  E  T  L
        *       *       *       *       *       *     2100
GCTCTTGGAACTCGCAGAATGGCAAAGAAAAATGCCATTGTTCGAAGCCTCCCGTCTGTGGAAACCCTTG

 - - - - - - - - - - - - - - - - HuSerca2A - - - - - - - - - - - - - - - -
G  C  T  S  V  I  C  S  D  K  T  G  T  L  T  T  N  Q  M  S  V  C  R  M
        *       *       *       *       *       *     2170
GTTGTACTTCTGTTATCTGCTCAGACAAGACTGGTACACTTACAACAAACCAGATGTCAGTCTGCAGGAT

 - - - - - - - - - - - - - - - - HuSerca2A - - - - - - - - - - - - - - - -
  F  I  L  D  R  V  E  G  D  T  C  S  L  N  E  F  T  I  T  G  S  T  Y
        *       *       *       *       *       *     2240
GTTCATTCTGGACAGAGTGGAAGGTGATACTTGTTCCCTTAATGAGTTTACCATAACTGGATCAACTTAT

 - - - - - - - - - - - - - - - - HuSerca2A - - - - - - - - - - - - - - - -
 A  P  I  G  E  V  H  K  D  D  K  P  V  N  C  H  Q  Y  D  G  L  V  E
        *       *       *       *       *       *     2310
```

```
GCACCTATTGGAGAAGTGCATAAAGATGATAAACCAGTGAATTGTCACCAGTATGATGGTCTGGTAGAAT

 - - - - - - - - - - - - - - - - HuSerca2A - - - - - - - - - - - - - - - -
L  A  T  I  C  A  L  C  N  D  S  A  L  D  Y  N  E  A  K  G  V  Y  E  K
         *        *        *        *        *        *     2380
TAGCAACAATTTGTGCTCTTTGTAATGACTCTGCTTTGGATTACAATGAGGCAAAGGGTGTGTATGAAAA

 - - - - - - - - - - - - - - - - HuSerca2A - - - - - - - - - - - - - - - -
 V  G  E  A  T  E  T  A  L  T  C  L  V  E  K  M  N  V  F  D  T  E  L
         *        *        *        *        *        *     2450
AGTTGGAGAAGCTACAGAGACTGCTCTCACTTGCCTAGTAGAGAAGATGAATGTATTTGATACCGAATTG

 - - - - - - - - - - - - - - - - HuSerca2A - - - - - - - - - - - - - - - -
K  G  L  S  K  I  E  R  A  N  A  C  N  S  V  I  K  Q  L  M  K  K  E
         *        *        *        *        *        *     2520
AAGGGTCTTTCTAAAATAGAACGTGCAAATGCCTGCAACTCAGTCATTAAACAGCTGATGAAAAAGGAAT

 - - - - - - - - - - - - - - - - HuSerca2A - - - - - - - - - - - - - - - -
F  T  L  E  F  S  R  D  R  K  S  M  S  V  Y  C  T  P  N  K  P  S  R  T
         *        *        *        *        *        *     2590
TCACTCTAGAGTTTTCACGTGACAGAAAGTCAATGTCGGTTTACTGTACACCAAATAAACCAAGCAGGAC

 - - - - - - - - - - - - - - - - HuSerca2A - - - - - - - - - - - - - - - -
 S  M  S  K  M  F  V  K  G  A  P  E  G  V  I  D  R  C  T  H  I  R  V
         *        *        *        *        *        *     2660
ATCAATGAGCAAGATGTTTGTGAAGGGTGCTCCTGAAGGTGTCATTGACAGGTGCACCCACATTCGAGTT

 - - - - - - - - - - - - - - - - HuSerca2A - - - - - - - - - - - - - - - -
G  S  T  K  V  P  M  T  S  G  V  K  Q  K  I  M  S  V  I  R  E  W  G
         *        *        *        *        *        *     2730
GGAAGTACTAAGGTTCCTATGACCTCTGGAGTCAAACAGAAGATCATGTCTGTCATTCGAGAGTGGGGTA

 - - - - - - - - - - - - - - - - HuSerca2A - - - - - - - - - - - - - - - -
S  G  S  D  T  L  R  C  L  A  L  A  T  H  D  N  P  L  R  R  E  E  M  H
         *        *        *        *        *        *     2800
GTGGCAGCGACACACTGCGATGCCTGGCCCTGGCCACTCATGACAACCCACTGAGAAGAGAAGAAATGCA

 - - - - - - - - - - - - - - - - HuSerca2A - - - - - - - - - - - - - - - -
 L  E  D  S  A  N  F  I  K  Y  E  T  N  L  T  F  V  G  C  V  G  M  L
         *        *        *        *        *        *     2870
CCTTGAGGACTCTGCCAACTTTATTAAATATGAGACCAATCTGACCTTCGTTGGCTGCGTGGGCATGCTG

 - - - - - - - - - - - - - - - - HuSerca2A - - - - - - - - - - - - - - - -
D  P  P  R  I  E  V  A  S  S  V  K  L  C  R  Q  A  G  I  R  V  I  M
         *        *        *        *        *        *     2940
GATCCTCCGAGAATCGAGGTGGCCTCCTCCGTGAAGCTGTGCCGGCAAGCAGGCATCCGGGTCATCATGA

 - - - - - - - - - - - - - - - - HuSerca2A - - - - - - - - - - - - - - - -
I  T  G  D  N  K  G  T  A  V  A  I  C  R  R  I  G  I  F  G  Q  D  E  D
         *        *        *        *        *        *     3010
TCACTGGGGACAACAAGGGCACTGCTGTGGCCATCTGTCGCCGCATCGGCATCTTCGGGCAGGATGAGGA

 - - - - - - - - - - - - - - - - HuSerca2A - - - - - - - - - - - - - - - -
 V  T  S  K  A  F  T  G  R  E  F  D  E  L  N  P  S  A  Q  R  D  A  C
         *        *        *        *        *        *     3080
CGTGACGTCAAAAGCTTTCACAGGCCGGGAGTTTGATGAACTCAACCCCTCCGCCCAGCGAGACGCCTGC

 - - - - - - - - - - - - - - - - HuSerca2A - - - - - - - - - - - - - - - -
L  N  A  R  C  F  A  R  V  E  P  S  H  K  S  K  I  V  E  F  L  Q  S
         *        *        *        *        *        *     3150
CTGAACGCCCGCTGTTTTGCTCGAGTTGAACCCTCCCACAAGTCTAAAATCGTAGAATTTCTTCAGTCTT

 - - - - - - - - - - - - - - - - HuSerca2A - - - - - - - - - - - - - - - -
F  D  E  I  T  A  M  T  G  D  G  V  N  D  A  P  A  L  K  K  A  E  I  G
         *        *        *        *        *        *     3220
TTGATGAGATTACAGCTATGACTGGCGATGGCGTGAACGATGCTCCTGCTCTGAAGAAAGCCGAGATTGG

 - - - - - - - - - - - - - - - - HuSerca2A - - - - - - - - - - - - - - - -
 I  A  M  G  S  G  T  A  V  A  K  T  A  S  E  M  V  L  A  D  D  N  F
         *        *        *        *        *        *     3290
CATTGCTATGGGCTCTGGCACTGCGGTGGCTAAAACCGCCTCTGAGATGGTCCTGGCGGATGACAACTTC

 - - - - - - - - - - - - - - - - HuSerca2A - - - - - - - - - - - - - - - -
S  T  I  V  A  A  V  E  E  G  R  A  I  Y  N  N  M  K  Q  F  I  R  Y
         *        *        *        *        *        *     3360
TCCACCATTGTGGCTGCCGTTGAGGAGGGGCGGGCAATCTACAACAACATGAAACAGTTCATCCGCTACC

 - - - - - - - - - - - - - - - - HuSerca2A - - - - - - - - - - - - - - - -
L  I  S  S  N  V  G  E  V  V  C  I  F  L  T  A  A  L  G  F  P  E  A  L
         *        *        *        *        *        *     3430
TCATCTCGTCCAACGTCGGGGAAGTTGTCTGTATTTTCCTGACAGCAGCCCTTGGATTTCCCGAGGCTTT
```

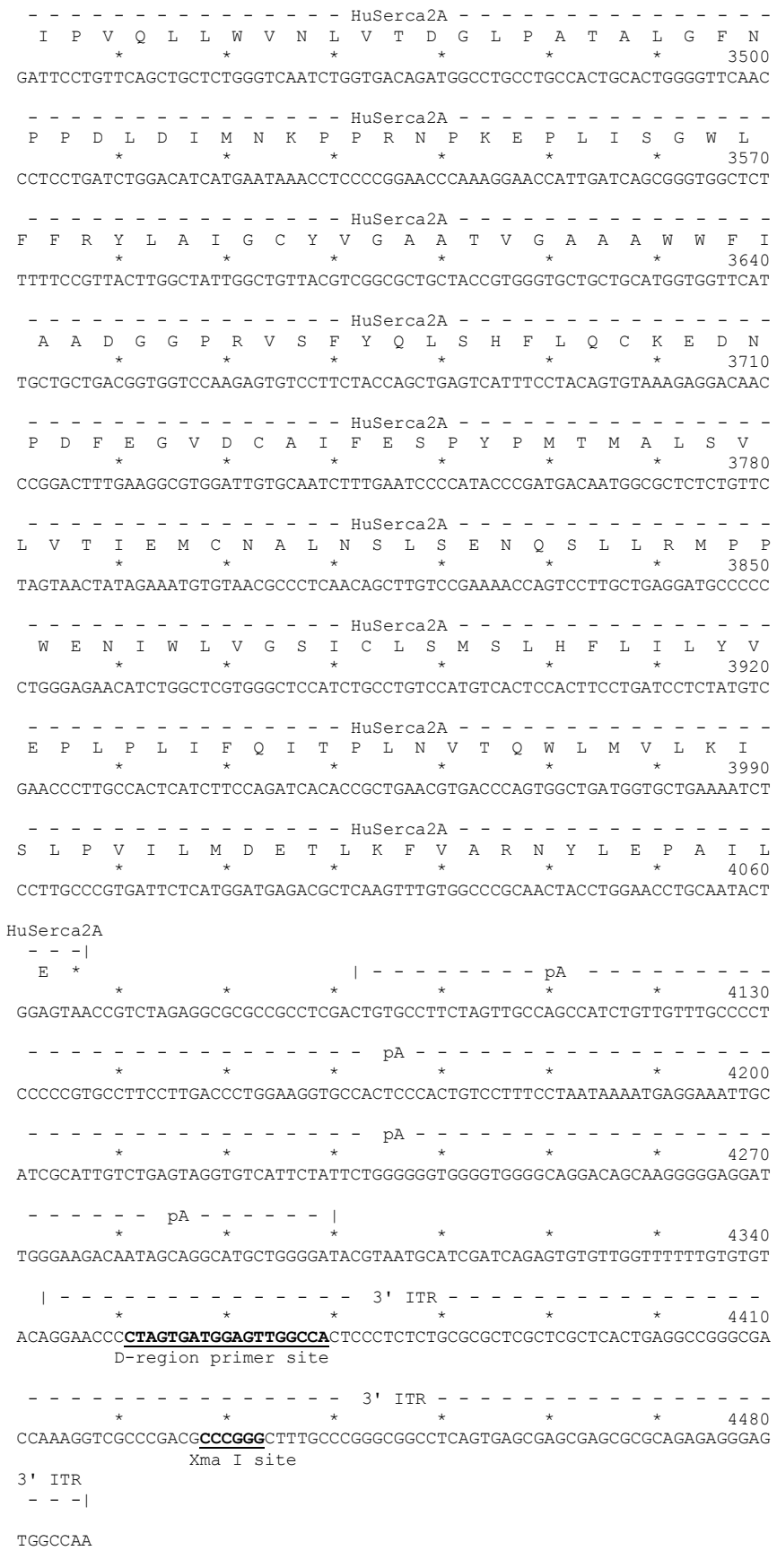


Figure 3. Nucleotide Sequence of AAV1/hSERCA2a Vector Genome

Characteristics of the Genetically Modified Organisms in their Final Form

25. The description of genetic traits or phenotypic characteristics and in particular any new traits and characteristics which may be expressed or no longer expressed

AAV1/SERCA2A survivability is expected to be similar to AAV1 because the structure of the viral particle has not changed. That is, the capsid proteins are wildtype AAV1 proteins and the vector genome is of similar size to the AAV1 genome. The entire viral genome has been removed except for the two small inverted terminal repeats (ITRs) comprising a total of only 290 bases and replaced with the SERCA2a expression cassette. A new characteristic is that the AAV1/SERCA2A cannot replicate even in the presence of helper virus because 94% of the viral genome is absent.

Another new characteristic is that if AAV1/SERCA2a enters a cell it cannot integrate into the genome because sequences responsible for integration have been removed. Integration of the AAV1/SERCA2a genome has not yet been evaluated experimentally, although based on a thorough literature review of similar vectors with AAV2 ITR, risk of and from integration is considered to be low.[8-15] To date, >140 animals have received AAV1/SERCA2a in various pharmacology and toxicology studies with follow-up times up to 1 year, and no tumours have been observed, although specific carcinogenicity studies have not been performed.

AAV1/SERCA2a was designed with the AAV1 capsid proteins to keep the same skeletal muscle tropism as AAV1. Among the various AAV serotypes, AAV1, 6 and 9 have differential tropism for the heart.[16, 17]

26. The structure and amount of any vector or donor nucleic acid remaining in the final construction of the modified organisms

The plasmid vector DNA present in AA1/SERCA2a is limited to only the intended SERCA2a transgene expression cassette and the two small viral inverted terminal repeats. The terminal repeats are 145 bases each.

27. The stability of the organism in terms of genetic traits

AAV1/SERCA2a drug substance intended for use in the clinical study was produced under cGMP and has been tested for host cell DNA, 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 expression cassette (4.2 kb) restricts any recombination events that could lead to a replication-competent vector carrying the transgene.

28. The rate and level of expression of the new genetic material in the organisms and the method and sensitivity of measurement of that rate and level

Constitutive SERCA2a protein expression is mostly found in muscle tissue (cardiac, diaphragm and skeletal), yet there is a small percentage of SERCA2a isoform in normal vascular smooth muscle cells [18] and endothelial cells. [19]

Despite broad distribution of vector DNA, vector derived SERCA2 protein has limited overexpression after IC infusion, and is found mainly in the heart, aorta, diaphragm, and coronary and pulmonary arteries, most likely reflecting the presence of endothelial cells and vascular smooth muscle cells in these tissues (in the Celladon Non-clinical Study 007, a toxicology and biodistribution study in minipigs). [20] Up-regulation is limited due to 90% occupancy of the SERCA2a protein of the sarcoplasmic reticulum in normal cardiomyocytes resulting in only a modest increase in expression of 10-20% in those cells (shown in the Celladon Non-clinical Study 003, a swine mitral valve regurgitation model of heart failure). [21]

29. The activity of the gene product

SERCA2a is an intracellular Ca^{2+} pump located in the sarcoplasmic reticula of cardiac muscle cells. This enzyme catalysis 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.

30. The description of identification and detection techniques, including techniques for the identification and detection of the inserted sequence and vector

A qPCR assay is designed and used to quantify SERCA2a DNA in AAV1/SERCA2a against a linearized plasmid DNA standard curve using real-time-PCR. The results are reported in DNase resistant particles (DRP) for AAV1/SERCA2a. This nomenclature distinguishes encapsidated, therefore DNase resistant, DNA from unencapsidated or “free” DNA. The samples are first treated with DNase to remove any unencapsidated DNA in the sample matrix followed by deactivation of the DNase and release of the vector genomes with Proteinase K. After the Proteinase K treatment the samples and controls are diluted appropriately and amplified by PCR using primers that target the SERCA2a transgene. The samples and positive control are processed in triplicate. The results for vector strength are interpolated from the plasmid DNA standard curve, which is calibrated as single-copy equivalents, and reported in DRP/mL. Strength in DRP/mL is reported on the average of three independent assays.

31. The sensitivity, reliability (in quantitative terms) and specificity of detection and identification techniques

The AAV1/SERCA2a qPCR detects a unique 106 base fragment of the human SERCA2a gene. The primers and probe used for this assay are located in the middle of the SERCA2a vector construct and span a ≈1.1 kb intron (between exons 14 and 15) within the

endogenous SERCA2a gene. The AAV1/SERCA2a DNA PCR amplification was designed so that the primers only detect vector specific SERCA2a.

Reliability and Sensitivity

Summaries of the method qualification results for the AAV1/SERCA2a DNA PCR and Capsid ELISA methods are provided in Table 2.

Table 2. Method Qualification Summary for AAV1/SERCA2a DNA PCR

Determination		Results
Intra-assay precision	n=3 replicates of plasmid standards and positive controls, and unknown sample	4-19% CV
Inter-assay precision	n=3 replicates of plasmid standards and positive controls, 3 assays, 2 operators	8-18% CV
Sensitivity – Limit of Quantitation (LOQ)	n=3 replicates of standards	% CV ≤3% 30 ss copies
Range	Based on upper and lower limit of standards	30 – 3 x 10^6 ss copies

32. The history of previous releases or uses of the organisms

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 effects attributed to the investigational product. [22] 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. [23, 24] In the current phase 2b study, which began enrolling in August 2012, 22 subjects (50% placebo) have been treated in the US or Europe at the time of this writing.

33. In relation to human health, animal health and plant health

Like the parent organism the GMO vector is not pathogenic. In addition, in a natural setting it is limited to gene delivery in 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 from distribution in the environment and due to the non-pathogenicity and replication incompetence of the vector, the environmental impact is thought to be negligible.

The question can be raised about gene expression in non-target tissue that could lead to an abnormal balance of protein isoforms producing undesired effects, including local autoimmune reactions.

We conducted extensive toxicology and biodistribution studies, as well as examined the level of SERCA protein in minipig tissues in the Celladon 007 study [20]. There was no dose-dependent, treatment-related evidence of inflammation, and increased SERCA2 protein expression was only found proximal to the infusion site. We did not observe increased SERCA2 expression in distal skeletal muscle. The results are as follows:

The pivotal safety and biodistribution study, Celladon 007, assessed AAV1/SERCA2a persistence in cardiac tissue and determined the biodistribution of AAV1/SERCA2a at 5, 30 and 90 days after exposure.

SERCA2 protein was detected in control (untreated) animals in the following tissues: all sections of the heart, aorta, diaphragm, skeletal muscle (gastrocnemius, biceps femoris and sartorius), coronary artery (main descending). After gene transfer, the expression of SERCA2 increased by 10-20% over control values in the heart when normalized to actin. The expression of SERCA2 in non-cardiac tissues was also assessed. The expression pattern in biceps femoris, sartorius, and gastrocnemius muscles was unchanged. The diaphragm showed a small (10-20%) increase in expression of SERCA2 90 days following gene transfer of high dose AAV1/SERCA2a. The lung, coronary and pulmonary arteries and the aorta also had mildly increased SERCA2 protein expression, most likely reflecting the presence of vascular smooth muscle cells in these tissues, possibly due to concentration by the isolation methodology of microsomal fraction (i.e., expression in VSMC from pulmonary arteries).

The expression of SERCA2 protein was investigated from an expanded number of tissues. These tissues were selected based on the expression pattern of total SERCA proteins in humans. [25] These data are summarized below.

Table 3. SERCA2a Protein Expression (Celladon 007)

Tissue	SERCA2 Protein Normally Expressed	SERCA2 Protein Over-Expressed after MYDICAR®
Cardiac Muscle	Yes	Yes
Diaphragm	Yes	Yes
Coronary Artery	Yes	Yes
Aorta	Yes	Yes
Pulmonary Artery	Yes	Yes
Skeletal Muscle	Yes	No
VSMC	Yes	?
Lung	No	No
Kidney	No	No
Liver	No	No
Spleen	No	No
Parathyroid	Yes	No
Pituitary	Yes	No
Photoreceptor cells of	Yes	No

the retina

VSMC, vascular smooth muscle cell

In the study described above, AAV1/SERCA2a administration was not associated with any pathological changes or changes in clinical chemistry panels.

a. Toxic or allergenic effects of the non-viable organisms and/or their metabolic products

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.

IV dosing was investigated in a nonclinical study where we evaluated the safety of AAV1/SERCA2a at approximately three times the clinical dose after intravenous administration in minipigs with and without pre-treatment with immunosuppressive therapy. There were no treatment-related changes in body weights, clinical observations, haematology, clinical chemistry, cardiac enzymes, coagulation parameters, electrocardiography parameters, gross pathology, or histopathology in animals treated with AAV1/SERCA2a with or without immunosuppressive therapy. Any inflammatory immune response would have been detected in clinical chemistries and/or abnormal histopathology, neither of which was observed. No airway administration studies have been performed with AAV1/SERCA2a.

With regard to allergic reactions to the viral capsid in clinical studies, a sensitive ELISpot assay conducted on all previous trial subjects to detect a cellular immune response showed none associated with the treatment. However, subjects did seroconvert to AAV1 positive, based on results from the AAV1 Neutralizing Antibody (NAb) assay, as expected. About 50% of the human population in Europe is already seropositive for AAV1 and 90% positive for AAV. [1] Like the parent organism AAV1, the vector is nonpathogenic. AAV1/SERCA2a does not have any metabolic proteins or processes.

b. the comparison of the organisms to the donor, recipient or (where appropriate) parental organism regarding pathogenicity

AAV1 is not pathogenic and AAV1/SERCA2a is not expected to be, based on its lack of viral genome, subsequent inability to replicate and the benign nature of the expressed human transgene SERCA2A. The transgene is fully human, nontoxic and not oncogenic.

Because the ATP2A gene (SERCA2) is a human gene there is homology to the endogenous gene and related variants that could lead to insertional mutagenesis. The target cells, cardiomyocytes, do not replicate at appreciable levels, and any theoretical risk of recombination between the vector and the ATP2A gene would be limited to dividing cells due to accepted mechanisms of homologous recombination during DNA replication. The actual risk of vector insertion into the genome of any cell is not known.

c. The capacity of the organisms for colonization

AAV1/SERCA2A is not capable of reproduction even in the presence of helper virus.

d. If the organism is pathogenic to humans who are immunocompetent

The parent AAV1 is not known to be pathogenic to immunocompetent humans. Toxicology studies have been conducted after intravenous infusion in immunosuppressed animals, with no apparent adverse consequences, indicating that the risk of adverse effect is very low even in immunocompromised individuals (Celladon 021: A 4-Month Non-GLP Single Dose Safety Study of Intravenous Infusion of AAV1/SERCA2a in Göttingen Minipigs with Anti-AAV1 Antibody Titers of <1:2 Receiving Immunosuppressive Therapy [26]). We do not expect a higher potential risk for immunocompromised people who come in contact with patients where virus shedding is highest based on the results of a nonclinical study where we evaluated the levels and safety of AAV1/SERCA2a at approximately three times clinical dose after intravenous administration in minipigs with and without pre-treatment with immunosuppressive therapy. There were no treatment-related changes in body weights, clinical observations, haematology, clinical chemistry, cardiac enzymes, coagulation parameters, electrocardiography parameters, gross pathology, or histopathology in animals treated with AAV1/SERCA2a with or without immunosuppressive therapy.

i. Diseases caused and mechanisms of pathogenicity including invasiveness and virulence

Not applicable. The parent organism is non-pathogenic and there are no viral genes. The transgene is a fully human protein.

ii. Communicability

Not applicable. The GMO contains no viral genes and is consequently totally incapable of reproduction.

iii. Infective dose

Not applicable, the GMO is not infective.

iv. Host range and possibility of alteration

The parent organism is limited in the environment to humans and other primates. The GMO capsids are wildtype AAV1 proteins so the host range would be the same. However as the GMO is completely incapable of replication there are really no hosts.

v. Possibility of survival outside of human host

The parent organism AAV is capable of survival for extended periods when dehydrated. The parent organism is also capable of survival for extended periods in aqueous solutions. However, considering that the GMO is non-enveloped and the surface is protein, its stability in the environment is considered to be very limited.

vi. Presence of vectors or means of dissemination

Not applicable. The vectors (plasmids) used to construct the GMO have ColE1 bacterial origin of replication and limited ability to disseminate.

vii. Biological stability

The biological stability is limited for non-enveloped protein particles in the environment.

viii. Antibiotic-resistance patterns

No antibiotic resistance genes present.

ix. Allergenicity

The GMO proteins are all wildtype AAV proteins that over 90% of the population has been exposed to with no known allergenic/ pathogenic consequences

x. Availability of appropriate therapies

There are no treatments developed for AAV1 as it is not pathogenic. The GMO has no viral genes and only a fully human gene whose protein product should not produce an allergic reaction. If there were an allergic reaction to the AAV capsid proteins it would be temporal as there are no genes present for continued production of the proteins. There is limited published information on experience with immunosuppression therapy in conjunction with AAV trials. However, in a recent study with AAV2 for gene transfer in subjects with hemophilia B, at a dose of 2×10^{11} vp/kg, a transient AAV specific cytotoxic T lymphocyte response was eliminated with a course of prednisolone (60mg/day and tapered off over 4 weeks).[27]

e. Other product hazards

None known.

Part III
INFORMATION RELATING TO THE CONDITIONS OF RELEASE

Information on the Release

34. The description of the proposed deliberate release, including the initial purpose or purposes of the release and any intention to use the genetically modified organisms as or in a product in the future

The release of the GMO will be made in the context of two trials, 1) clinical trial entitled: "A Phase 2b, Double-Blind, Placebo-Controlled, Multinational, Multicenter, Randomized Study Evaluating the Safety and Efficacy of Intracoronary Administration of MYDICAR® (AAV1/SERCA2a) in Subjects with Heart Failure" and 2) "Investigation of the safety and efficacy of AAV1/SERCA2a gene transfer in patients with chronic heart failure and a left ventricular assist device".

The principal aim of the Phase 2b study is to evaluate and confirm the clinical safety and efficacy of a single intracoronary infusion of 1×10^{13} DNase Resistant Particles (DRP) MYDICAR® (AAV1/SERCA2a) 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^{2+} cardiac metabolism, resulting in enhanced cardiac function and energetics, which will in turn translate to improved clinical outcomes. The principle aim of the LVAD study is to determine 1) the safety and efficacy of SERCA2a gene transfer in

patients with advanced chronic heart failure and LVAD support, 2) the magnitude of viral gene transfer to the human failing myocardium and 3) the influence of circulating neutralising antibodies to AAV1 upon myocardial gene transfer.

For the Phase 2b trial a total of about 200 heart failure patients will be enrolled in the trial in the U.S. and in Europe. The sponsor plans to conduct the study at up to 4 centres in the UK—1 centres in London, 1 in Hull and 2 in Glasgow—with about 16 to 20 UK patients to be enrolled, beginning in 1Q2013. However infusion of the investigational product will only take place at two of the sites. Enrolment will take 16 months from the start of the study. Subjects will be randomly assigned in a ratio of 1:1 to either MYDICAR® or placebo, therefore approximately 8 to 10 patients will receive MYDICAR® in this study.

For the LVAD study trial a total of about 24 patients will be enrolled at two sites in the UK, the Harefield Hospital in Middlesex and the Papworth Hospital in Cambridge. Infusion will take place at those sites. Enrolment will take 24 months from the start of the study. Subjects will be randomly assigned a ratio of 2:1 to either MYDICAR® or placebo; therefore approximately 16 patients will receive MYDICAR® in this study.

The total at all sites will be 24-26 patients receiving MYDICAR® in both studies in the UK.

35. The intended dates of the release and time planning of the experiment including frequency and duration of releases

The start date for both studies in the UK is planned for 1Q2013. For the Phase 2b study enrolment is planned for 16 months following start of the study. Up to 5 patients are planned to enrol at each of the 4 centres, for a total of 20 patients, total. Patients will be randomized in parallel in a ratio of 1:1, MYDICAR® to matching placebo (buffer without the active ingredient). For the LVAD study enrolment is planned for 24 months following start of the study. Up to a total of 24 patients are planned to enrol at the two centres. Patients will be randomized in parallel in a ratio of 2:1, MYDICAR® to matching placebo (buffer without the active ingredient). For both studies administration consists of a single intracoronary infusion on Day 0 for each patient.

36. The preparation of the site before the release

Study sites will be evaluated and trained on drug assignment, receipt, dispensing, storage and accountability procedures. In addition to receiving a site initiation visit by the sponsor that reviews investigational product storage, handling, dilution and administration according to the Study Pharmacy and Interventionalist manuals the sites will complete an in service training on use of the administration syringe pump and complete an administration dry run. Preparation and administration of the investigational product in an investigational pharmacy and cardiac catheterization laboratory will take place as "contained use".

37. Size of the site

The release will be performed at the investigator's centres, in a hospital catheterization laboratory. Subjects will be observed for a recovery period either in a room near the catheterization lab or in a normal hospital room.

38. The method or methods to be used for the release

Release will be a single intracoronary infusion of each study subject in the hospital setting of a cardiac catheterization laboratory. After administration the puncture wound created for arterial access for the administration of investigational product will be monitored in the cardiac catheterization laboratory, during the overnight hospitalization, and then just before discharge from the hospital. Use of an Angio-Seal vascular closure or similar medical device may be used to aid in rapid closure and sealing of the puncture site; the protocol allows radial, brachial or femoral arterial access as determined by the treating interventionist. After closure and sealing of the puncture site the site will be bandaged accordingly.

39. Quantities of GMOs to be released

Approximately 16-20 patients in the UK will undergo a single intracoronary infusion of 1×10^{13} DRP AAV1/SERCA2a Investigational product diluted in 50 mL of normal saline. Subjects will be randomized in a ratio of 1:1; therefore, 8-10 patients are expected to be randomised to receive the GMO over the study period in the CUPID2 trial.

In the LVAD trial, 16 of the 24 patients will be randomised to receive the GMO over the duration of the trial.

The total amount of investigational product to be utilized in the study in UK is approximately 24-26 vials containing 6 mL each of a solution of 3×10^{12} DRP/mL of investigational product.

The vector is replication incompetent even in the presence of a helper virus, so release of the vector following administration is limited to environmental release of the vector by subject shedding during a limited time period following administration.

40. The disturbance of the site, including the type and method of cultivation, mining, irrigation, or other activities

Not applicable.

41. Worker protection measures taken during the release

There is some risk that hospital staff could be exposed to AAV1/SERCA2a during dose preparation and administration so various precautions will be taken. Onsite preparation of investigational product consists of a dilution of approximately 4 mL to 60 mL dilution in saline. The dilution will be done using microbiological aseptic technique and in accordance with the biosafety level containment determined by the institution. The dilution, by design and best practice, minimizes the opportunity for aerosol generation and needle sticks. For example, mixing takes place in a closed setting between two syringes connected by Luer lock fittings through a stopcock. The primary mode of containment

during the catheterization procedure is application of Standard/Universal Precautions for blood borne infectious materials. In the cath lab, personnel performing the procedure 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.

42. The post-release treatment of the site

All disposable materials (including but not limited to gloves, masks, syringes, needles, catheter and tubing) that come into contact with the investigational product will be disposed of as biohazardous materials according to individual institutional practices and policies. In general the materials will be disposed of in sharps containers or biohazard bags and decontaminated by autoclave or incineration, or both.

The unused investigational product and vial, stopper and crimp seal can be decontaminated with a 10% aqueous solution of household bleach (5000 ppm sodium hypochlorite), autoclaved or incinerated according to institutional practice. Following decontamination, materials will be disposed of as biohazardous waste. If excess investigational product is destroyed by bleach it can be poured down a sink with running water or otherwise in compliance with local and institutional disposal and cleaning procedures.

Non-disposable materials, equipment and surfaces will be decontaminated with a 10% solution of household bleach. Some non-disposables may be autoclaved.

43. The techniques foreseen for elimination or inactivation of the organisms at the end of the experiment or other purposes of the release

Instructions for, and worksheets documenting (contained in the Study Pharmacy Manual) the destruction of unused undiluted and diluted Investigational product, along with associated generated waste will be followed and documented by the hospital staff in the investigational pharmacy and in the cardiac catheterization lab. In general, treatment with a fresh 10% solution of household bleach (5000 ppm sodium hypochlorite), autoclaving and/or incineration will be used for destruction of the GMO.

44. Information on, and the results of, previous releases of the organisms and in particular, releases on a different scale or into different ecosystems

Viral vectors, including vectors derived from adeno-associated virus (AAV), are frequently used in gene therapy. The consequences of the release in the environment are not entirely known yet, however some data are available in the literature. [28]

Preclinical data indicate that the biodistribution and persistence of AAV1/SERCA2a is similar to other AAV1- and AAV2-based vectors. The persistence of vector DNA is limited to the injection/infusion site (the heart) and highly perfused tissues and decreases with dose administered and time. AAV1/SERCA2a is expected to spread to other parts of the body before it is cleared. After IC delivery of AAV1/SERCA2a, rAAV particles which are not taken up in the heart are first passed through the lung via the coronary sinus, where they are thought to be cleared by the reticuloendothelial system (RES). Based on animal studies and clinical studies of other AAV gene therapy agents, it is expected that rAAV

concentrations will decrease quickly over time. The 2007 joint EMEA and ICH workshop on viral/vector shedding determined that while rAAV is extensively biodistributed and shedding is known, the virus is nonpathogenic and risks are estimated to be very low. [29] In studies of AAV2 vectors in cystic fibrosis [30] and HIV vaccines [31] administered via aerosol or intramuscularly, respectively, at doses as high as 1 x 10^{13} DRP, most samples were negative and those that were positive were at less than 1/1,000,000 of the dose administered even at 2 hours after dosing. Stool and urine samples were negative for all samples. In studies of AAV vectors for haemophilia B patients that were administered doses as high as 1 x 10^{14} DRP, persistence in saliva, urine and semen was 1 week or less. [32] Since rAAV is non-replicating, even in the presence of helper virus, there is no reason to believe that the added DNA will spread from the human subject to other persons or to the environment.

The Environment (Both On the Site and In the Wider Environment)

45. The geographical location and national grid reference of the site or sites onto which the release will be made, or the foreseen areas of use of the product

The GMO will be infused at up to 2 clinical trial centres in the UK. The UK national (OS) grid reference of the proposed sites of release provided in the table below.

Site	Principal Investigator	Address	National Grid Reference
1	Alexander Richard Lyon, M.D., MA, BM BCh, MRCP, Ph.D.	Royal Brompton and Harefield NHS Foundation Trust London SW3 6NP Tel: 020 7351 8827 Fax: 020 7351 8829	**TQ 26977 78395**
2	Dr. Mark Petrie, M.D., MBChB	Golden Jubilee National Hospital Cardiology Dept. Beardmore Street Clydebank G81 4HX Glasgow	Papworth Hospital Papworth Everard Cambridge CB23 3RE
3	John J V McMurray, M.D., FRCP, FESC, FACC, FAHA, FRSE	British Heart Foundation Cardiovascular Research Centre University of Glasgow 126 University Place G12 8QQ Glasgow	Patients to be dosed at Site #2, above, **NS 4126 6673**
4	Prof. John G. F. Cleland, M.D., FACC	Cardiovascular & Respiratory Studies, University of Hull Castle Hill Hospital Cottingham, HU165JQ Kingston-Upon-Hull	Patients to be dosed at Site #1, above, **TQ 26977 78395**

5	Dr Nick Banner MD, FRCP, FESC	Cardiology Department Harefield Hospital Hill End Road, Harefield Middlesex UB9 6JH	**TQ 05166 90857**
6	Mr Steven Tsui MD, FRCS	Dept of Cardiothoracic Surgery Papworth Hospital Papworth Everard Cambridge CB23 3RE	**TL 28746 62837**

46. The physical or biological proximity of the site to humans and other significant biota

Investigational product administration will be performed in a hospital cardiac catheterisation laboratory. Subjects will recover either in a recovery area off the catheterisation lab or in a conventional hospital room. All clinical site staff will be following Universal/Standard precautions as normally practiced in clinical hospital settings for the containment of potentially biohazardous materials.

47. The proximity to significant biotopes, protected areas or drinking water supplies

Not applicable. The release will be in a hospital setting as part of a clinical study. Investigational product administration will be performed in a hospital cardiac catheterisation laboratory. Subjects will recover either in a recovery area off the catheterisation lab or in a conventional hospital room. All clinical site staff will be following Universal/Standard precautions as normally practiced in clinical hospital settings for the containment of potentially biohazardous materials.

48. The climatic characteristics of the region or regions likely to be affected

Not applicable. The release will be in a hospital setting as part of a clinical study. Investigational product administration will be performed in a hospital cardiac catheterisation laboratory. Subjects will recover either in a recovery area off the catheterisation lab or in a conventional hospital room. All clinical site staff will be following Universal/Standard precautions as normally practiced in clinical hospital settings for the containment of potentially biohazardous materials.

49. The geographical, geological and pedological characteristics

Not applicable. The release will be in a hospital setting as part of a clinical study. Investigational product administration will be performed in a hospital cardiac catheterisation laboratory. Subjects will recover either in a recovery area off the catheterisation lab or in a conventional hospital room. All clinical site staff will be following Universal/Standard precautions as normally practiced in clinical hospital settings for the containment of potentially biohazardous materials.

50. Flora and fauna, including crops, livestock and migratory species

Not applicable. The release will be in a hospital setting as part of a clinical study. Investigational product administration will be performed in a hospital cardiac catheterisation laboratory. Subjects will recover either in a recovery area off the catheterisation lab or in a conventional hospital room. All clinical site staff will be following Universal/Standard precautions as normally practiced in clinical hospital settings for the containment of potentially biohazardous materials.

51. The description of target and non-target ecosystems likely to be affected

Not applicable. The release will be in a hospital setting as part of a clinical study. Investigational product administration will be performed in a hospital cardiac catheterisation laboratory. Subjects will recover either in a recovery area off the catheterisation lab or in a conventional hospital room. All clinical site staff will be following Universal/Standard precautions as normally practiced in clinical hospital settings for the containment of potentially biohazardous materials.

52. The comparison of the natural habitat of the recipient organisms with the proposed site or sites of release

The natural habitat of the recipient organism is the human host. The release will be in a hospital setting as part of a clinical study. Investigational product administration will be performed in a hospital cardiac catheterisation laboratory. Subjects will recover either in a recovery area off the catheterisation lab or in a conventional hospital room. All clinical site staff will be following Universal/Standard precautions as normally practiced in clinical hospital settings for the containment of potentially biohazardous materials.

53. Any known planned developments or changes in land use in the region which could influence the environmental impact of the release.

Changes in land use are not applicable to activities in a clinical hospital setting.

Part IV
INFORMATION RELATING TO THE INTERACTIONS BETWEEN THE ORGANISMS AND THE ENVIRONMENT

The Characteristics Affecting Survival, Multiplication and Dispersal

54. The biological features which affect survival, multiplication and dispersal

AAV1/SERCA2a vector cannot replicate or disseminate do to the absence of a viral genome or any viral genes. The vector is a non-enveloped protein particle containing DNA that can remain intact for extended periods of time (months to years) if dehydrated, cool and in the dark.

55. The known or predicted environmental conditions which may affect survival, multiplication and dissemination, including wind, water, soil, temperature and pH

In typical environmental conditions (for example in environmentally favourable conditions like fairly neutral pH) integrity of the vector is mostly affected by sunlight and temperature. Moisture is also a significant factor with dehydrated particles maintaining integrity longer.

56. The sensitivity to specific agents

AAV1/SERCA2a is destroyed by 5000 ppm sodium hypochlorite (10% solution of household bleach), and cleaning agents or hand-sanitizers that are effective against non-enveloped viruses. In addition, it can be destroyed by high pH, UV light, ionizing radiation, autoclaving or by incineration.

Interactions with the Environment

57. The predicted habitat of the organism

Not applicable as the investigational product is completely replication incompetent due the absence of any viral genes and 94% of the viral genome.

58. The studies of the behaviour and characteristics of the organisms and their ecological impact carried out in simulated natural environments, such as microcosms growth rooms and greenhouses

Celladon is investigating MYDICAR® (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 x 10^{13} DRP MYDICAR® have been demonstrated in the CUPID phase 2 study, with MYDICAR®-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. [22]

To date, MYDICAR® 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). [22, 33, 34] 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. [35, 36]

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. This is the scientific rationale for the SERCA-LVAD trial, where cardiac biopsies will be performed pre and post AAV1/SERCA2a delivery, to clarify the degree of SERCA2a transgene expression in the human heart.

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® group in the CUPID study.

59. The capability of post-release transfer of genetic material

a. from the genetically modified organisms into organisms in affected ecosystems

Exposure of humans to the investigational product could result in expression of the investigational transgene SERCA2a in unintended human recipients. There are no diseases associated with gain of function of SERCA2a. In animal studies, it was shown that after IC infusion, despite broad distribution of vector DNA, vector-derived SERCA2a protein overexpression was limited to the heart, aorta, diaphragm, and coronary and pulmonary arteries, (most likely reflecting the presence of endothelial cells and vascular smooth muscle cells in these tissues). In addition, up-regulation was limited to a modest increase in expression of 10-20% in those cells. Because the transgene is fully human, can only be expressed intracellularly and is then transported to its native intracellular compartment, no immune responses are expected. Toxicology studies have been conducted after intravenous infusion in immunosuppressed animals, with no apparent adverse consequences, indicating that the risk of adverse effect is very low even in immunocompromised individuals. [26]

b. from indigenous organisms to the genetically modified organisms

The small amount of AAV DNA (295 bases or 6% of the AAV viral genome) makes homologous recombination with indigenous viruses very unlikely. Also the limited capsid packing capacity (5 kb) of AAV compared to the transgene (4.2 kb) restricts any recombination events that could lead to a replication-competent vector carrying the transgene.

The main homology is between the fully human SERCA2a transgene, humans and other animals. Any productive outcome of the GMO gaining other human genes would be highly speculative.

60. The likelihood of post-release selection leading to the expression of unexpected or undesirable traits in the genetically modified organisms

There is only one gene in the AAV1/SERCA2a vector coding the human SERCA2a protein so there is no possible genetic selection for other traits.

61. The measures employed to ensure and to verify genetic stability, the description of genetic traits which may prevent or minimise dispersal of genetic material and methods to verify genetic stability.

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, a cell based assay to detect infectious adenovirus units and assays to detect adenovirus proteins (western blot) replication competent AAV (two rounds of cell based amplification followed by PCR), general purity by SDS PAGE, Benzonase nuclease and endotoxin. Tests for mycoplasma and an in vitro assay for viral contaminants are also required for lot release, but are performed on in-process samples.

62. The routes of biological dispersal, known or potential modes of interaction with the disseminating agent, including inhalation, ingestion, surface contact and burrowing.

There will be a single intracoronary infusion of each study subject in the hospital setting of a cardiac catheterization laboratory.

Other routes of exposure 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 with surfaces, exposure to aerosols and abrasions (sharps) can facilitate transmission.

An accidental spill of the investigational product in the investigational pharmacy, in the catheterization laboratory, or by shedding of vector from subjects could lead to environmental contamination theoretically resulting in unintended transfer to humans or

animals. wtAAV1 infects humans and primates, but no other known environmental organisms, and the vector would be expected to behave similarly.

Preclinical data indicate that the biodistribution and persistence of AAV1/SERCA2a is similar to other AAV1- and AAV2-based vectors. The persistence of vector DNA is limited to the injection/infusion site (the heart) and highly perfused tissues and decreases with dose administered and time.

Due to low risk of horizontal transmission, viral shedding studies have not been performed for AAV1/SERCA2a in humans or animals to date but a viral shedding study is planned prior to seeking marketing approval. The AAV1/SERCA2A vector system uses a replication defective AAV1 vector, making horizontal dissemination unlikely. Furthermore, after IC delivery of AAV1/SERCA2a, rAAV particles which are not taken up in the heart are first passed through the lung via the coronary sinus, where they are thought to be cleared by the reticuloendothelial system. Based on other rAAV trials in cystic fibrosis, lipoprotein lipase deficiency and haemophilia B patients with doses as high as 1×10^{14} DRP, viral particle shedding in urine, saliva, blood and semen is expected to last for about 1 week. As levels of shed vector are expected to be very low, and since AAV1/SERCA2a has a steep dose response curve, the risk of functional transduction during any period of shedding is also expected to be very low. The 2007 joint EMEA and ICH workshop on viral/vector shedding determined that while rAAV is extensively biodistributed and shedding is known, the virus is nonpathogenic and risks are estimated to be very low.

63. The description of ecosystems to which the organisms could be disseminated

Not applicable. AA1/SERCA2a vector is “gutless” and cannot replicate under any conditions.

64. The potential for excessive population increase of the organisms in the environment

Not applicable. AA1/SERCA2a vector is “gutless” and cannot replicate under any conditions.

65. The competitive advantage of the organisms in relation to the unmodified recipient or parental organism or organisms

Because 94% of the viral genome is absent and there are no viral genes the GMO has no competitive advantage. The fully human SERCA2a transgene is a calcium ion pump and confers no competitive advantage.

66. The identification and description of the target organisms if applicable

Eligible study subjects with moderate to advanced heart failure that meet all the study inclusion and exclusion criteria.

67. The anticipated mechanism and result of interaction between the released organisms and the target organisms 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^{2+} 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 nuclease, 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^{2+} 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 Phase 2b study is to evaluate and confirm the clinical safety and efficacy of MYDICAR® 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^{2+} cardiac metabolism, resulting in enhanced cardiac function and energetics, which will in turn translate to improved clinical outcomes.

The LVAD study will address whether an increase in SERCA2a levels and restoration of SR calcium, during LVAD support could assist in improving myocardial function and increase the number of patients who could be weaned off the devices. In other words, increasing SERCA2a levels with LVAD support could increase the probability of a ‘bridge to recovery’ programme rather than the LVAD being a ‘bridge to transplantation’ in extremely ill patients.

Most conventional medical strategies for the treatment of HF do not correct the underlying cause. MYDICAR® 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® 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.

68. The identification and description of non-target organisms which may be adversely affected by the release of the genetically modified organisms, and the anticipated mechanisms of any identified adverse reaction

wtAAV1 is not known to infect any organisms in the environment except primates. There is a chance that gene transfer could be made to other humans, however because the amount would be so low and the GMO is replication incompetent (even in the presence of helper virus) the result would be negligible.

69. The likelihood of post-release shifts in biological interactions or in the host range

Not applicable. The GMO has no viral genes and is completely replication incompetent.

70. The known or predicted interactions with non-target organisms in the environment, including competitors, prey, hosts, symbionts, predators, parasites and pathogens

None known or predicted.

71. The known or predicted involvement in biogeochemical processes

The parent AAV1 organism has no involvement in biogeochemical processes.

72. Any other potentially significant interactions with the environment

None known or anticipated.

Part V
INFORMATION ON MONITORING, CONTROL, WASTE TREATMENT AND EMERGENCY RESPONSE PLANS

Monitoring techniques

73. Methods for tracing the organisms and for monitoring their effects

Prior to submission of the Marketing Approval Application for MYDICAR Celladon will conduct a vector shedding study to monitor vector shedding in an open label study. qPCR assay for vector DNA in saliva, buccal swab, urine, and faeces Day 1, Day 3, Day 7; followed by weekly for 1 month, and then monthly for 3 months until there are two consecutive negative results. The AAV1/SERCA2a qPCR assay uses primers specific for a unique 106 base fragment of the human SERCA2a gene present in the vector.

74. Specificity (to identify the organisms and to distinguish them from the donor, recipient or, where appropriate, the parental organisms), sensitivity and reliability of the monitoring techniques

The GMO can be identified by qPCR. The PCR primers only detect the transgene sequence within the GMO. The primers are designed to detect a 106 bp fragment in the SERCA2a transgene in the GMO. The donor (human) DNA in humans is not detected because there is an intervening 1.1 kb intron. The primers do not hybridise to the recipient DNA. The assay has been shown to detect less than 30 DNA copies in blood and tissue. The sensitivity and reliability of the assay in other matrices should be similar.

75. Techniques for detecting transfer of the donated genetic material to other organisms

Celladon has validated a qPCR assay for detection of donated transgene DNA in human tissue. The assay was used in the previous phase 1/2 study and will be used in this study to test tissue samples taken from subjects in the cases of heart transplant, ventricular assist device insertion or death. That assay could be applied to monitor the transfer of the donated genetic material to other organisms. There is no plan to do so as transfer and impact to other organisms is negligible.

76. Duration and frequency of the monitoring

The health of patients enrolled in the study will be monitored for two years, or longer, over the course of the study. On Day 0, subjects will undergo cardiac catheterization and angiography, followed by infusion of investigational product. In the Phase 2b study at Months 1, 3, 6, 9 and 12 (12-Month Active Observation Period), subjects will undergo a battery of safety, efficacy and economic assessments, followed by quarterly visits (Months 15, 18, 21, 24, etc.) in the Long-Term Follow-Up for collection of information on clinical events and resource utilization until the last enrolled subject completes 12 months of observation and at least 180 adjudicated HF-related hospitalizations have occurred, whichever comes later. All subjects will be observed and followed for a minimum of 24 cumulative months. The 24 cumulative months includes the amount of time in the 12-Month Active Observation Period plus the amount of time in Long-Term Follow-Up.

In the LVAD study subjects will be monitored weekly including a clinical evaluation, record of all medications and blood tests. Then subjects will be monitored monthly to month 6 including a clinical evaluation, record of all medications and blood tests followed by an annual follow up including a clinical evaluation and record of all medications for 10 years. While some viral shedding by subjects following administration is expected, there is negligible risk from shedding and exposure of family members or other casual contacts from infectious AAV1/SERCA2a so shedding and effects will not be monitored in the present study. There is negligible risk from shedding of MYDICAR® into the ecosystem and therefore effects will not be monitored. There is negligible risk for transfer of donated genetic material from the patient to other organisms. Therefore, the transfer of donated genetic material will not be investigated. There is negligible risk from the transfer of donated genetic material from the patient to other people.

Control of the Release

77. Methods and procedures to avoid and/or minimise the spread of the organisms beyond the site of release or the designated area for use

Methods and Procedures at the Site of Release

Preparation of the investigational product will take place in an approved hospital pharmacy. Onsite preparation of investigational product consists of a dilution from approximately 4 mL to 60 mL dilution in saline. The dilution will be done using microbiological aseptic technique and in accordance with the biosafety level containment determined by the institution. The dilution by design, and best practice, minimizes the opportunity for aerosol generation and needle sticks. For example mixing takes place in a closed setting between two syringes connected together by Luer lock fittings through a stopcock.

Administration of the investigational product will be by authorized trained personnel in a hospital catheterization lab according to good clinical practice and the study protocol. The primary mode of containment during the catheterization procedure is application of Standard/Universal Precautions for infectious materials. In the cath lab personnel performing the procedure will wear goggles, scrub suit, shoe covers, cap and mask, and gloves while support personnel will wear support personnel wear safety glasses, gown, shoe covers, cap and mask, and gloves.

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. The investigational sites abide by all EU, country and self-imposed guidelines regarding the conduct of clinical trials, as well as the appropriate biosafety regulations required by the EMA for gene therapy medicinal research. We believe that research conducted within this framework adequately mitigates the risks of such research to the public health and therefore no additional measures will be undertaken. Only qualified personnel who are familiar with procedures that minimize undue exposure to themselves and to the environment will undertake the preparation, handling and safe disposal of AAV1/SERCA2a.

Destruction of unused IP and destruction or decontamination of all materials that may have been contaminated by IP is discussed in Item 80, Waste treatment, below.

Because the potential for germ-line transmission cannot be entirely ruled out, subjects will be advised to use effective birth control for 3 months following administration of investigational product (see CELL-004 Study Protocol, Section 4.3 Inclusion and Exclusion criteria). For the LVAD study subjects will be required to be using an effective method of contraception. We do not believe these recommendations need to be universally applied to family members or to the health care personnel caring for patients in this protocol.

Methods and Procedures Beyond the Site of Release

78. Methods and procedures to protect the site from intrusion by unauthorised individuals

The investigational sites, hospitals, have restricted access. The investigational product is stored in a refrigerator or freezer in a secured and limited-access area.

79. Methods and procedures to prevent other organisms from entering the site

Preparation and administration in the clinical setting are performed using aseptic technique.

Waste Treatment

80. Type of waste generated

Pharmacy 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 where the waste is considered biohazardous material potentially contaminated with blood borne pathogens.

81. Expected amount of waste

For each of the up to 4 UK sites the amount of liquid waste will be 2 mL of undiluted investigational product at 3×10^{12} DRP/mL, and 10 mL of 1:15 diluted investigational product per each subjects one time infusion. During the dilution one needle and a three syringes will come into contact with the investigational product. During the catheterization procedure a high pressure tubing line and a catheter will contact the investigational product. The remaining waste generated will be consistent with a typical catheterization procedure resulting in the generation of a few biohazard waste bags of potentially contaminated materials (for example, disposable gloves, miscellaneous laboratory absorbent material, and other routine pharmacy and cath lab disposables).

82. Description of treatment envisaged

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. Unused portions of the undiluted and diluted investigational product will be destroyed by, for example treatment with bleach, autoclaving and/or incineration, but in accordance with each institution's applicable practices and policies.

Emergency response plans

83. Methods and procedures for controlling the GMOs in case of unexpected spread

Personal protective equipment including safety glasses, gloves, and gown or lab coat should be worn when working with the GMO. If skin or eyes are exposed they should be rinsed with copious amounts of water. Needle sticks should be rinsed with water. In the case of a needle stick or ingestion a physician should be consulted.

84. Methods, such as eradication of the organisms, for decontamination of the areas affected

The chance of dissemination of the vector is negligible outside of the contained hospital pharmacy or catheterization lab. Should the investigational product be spilled or otherwise dispersed during the preparation or administration the procedures in the Study Pharmacy Manual or the Interventionalist Manual, distributed to all investigator's sites should be performed in accordance with standard practices for cleaning up biohazard waste spills, like those for treating potential blood borne pathogens.

Accidental spills will be cleaned up according to standard local practice. For example as follows from the Pharmacy Manual:

- 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.
- Allow to stand for at least 10 minutes.
- Place the absorbent material in an appropriate biohazard waster container and dispose as a biohazard material.

85. Methods for disposal or sanitation of plants, animals, soils and any other thing exposed during or after the spread

Not applicable

86. Methods for the isolation of the areas affected by the spread

Not applicable

87. Plans for protecting human health and the environment in case of the occurrence of an undesirable effect

Monitoring of the patients and of potential adverse events will be performed according to the study protocol.

For the Phase 2b study responsibility for the decision to halt study enrolment for safety lies with the sponsor, the Data Management Committee (DMC) and the study Medical Monitor. Events that would trigger a safety review are detailed in the DMC Charter. The DMC can assess the potential impact of the event(s) and suggest appropriate interventions.

All serious adverse events, adverse reactions, suspected adverse reactions, suspected unexpected serious adverse reactions and other unanticipated problems will be reviewed by the study sponsor, Medical Monitor and the DMC. The sponsor may ask the DMC to review any individual event(s) thought to be of major significance, including but not limited to an unexpected incidence or type of event or a death or other serious outcome for which a causal connection with the intervention is plausible. If the incidence of any of these events exceeds expectations or if causality can clearly be ascribed to the investigational product, the DMC may consider stopping, suspending or modifying the trial.

Review of serious, unexpected and related adverse events by the Medical Monitor, DMC, Institutional Review Boards/Institutional Ethics Committee, the sponsor, or relevant regulatory authorities may result in suspension of the study at an individual site, or at all study sites, as applicable.

Additional study risk mitigation includes the following:

- Ongoing data review by an independent unblinded Data Monitoring Committee (DMC), operating under a written charter, responsible for safeguarding the interests of trial subjects, assessing the safety of the interventions during the trial and monitoring the overall conduct of the clinical trial (see CELL-004-A1 Clinical Protocol dated 30 May 2012, Version 2, Section 11.17 “Data Monitoring Committee”)
- Medical Monitor: review of all serious adverse events and overseeing subject safety by a qualified medical expert during the course of the trial (see CELL-004-A1 Clinical Protocol dated 30 May 2012, Version 2, Section 8 “Assessment of Safety”)
- Comprehensive medical monitoring of subjects during the conduct of the trial (see CELL-004-A1 Clinical Protocol dated 30 May 2012, Version 2, Section 11.8 “Monitoring of the Study”)
- Study stopping rules under the authority of the independent DMC and Medical Monitor (see CELL-004-A1 Clinical Protocol dated 30 May 2012, Version 2, Section 8.9 “Study Stopping Rules”)
- Judicious inclusion/exclusion criteria (see CELL-004-A1 Clinical Protocol dated 30 May 2012, Version 2, Section 4 “Subject Selection and Withdrawal”)

For the LVAD study the study will be co-ordinated and managed by the Clinical Trials and Evaluation Unit (CTEU) a dedicated clinical trials department within the Royal Brompton Hospital. The CTEU will also assist in the training of investigators and co-ordinators at the start-up of the study and for performing monitoring procedures

throughout. A smaller trial management team consisting of lead investigators at each site and CTEU will hold regular meetings to review the progress of the study.

Part VI
A Description of the Methods

The nature and source of the GMO vector are given in Item 15 above. The methods used to construct inserts and introduce them into the recipient organism are given in Item 20. The purity and information on the identity of the inserted sequence is given in Item 22. Studies in relation to human health including toxicology, biodistribution and allergenic effects are given in Item 33.

References

1. Tenenbaum, L., E. Lehtonen, and P. Monahan, *Evaluation of risks related to the use of adeno-associated virus-based vectors.* Curr Gene Ther, 2003. **3**(6): p. 545-65.
2. *NIH Guidelines for Research Involving Recombinant DNA Molecules*. 2011. Available from: http://oba.od.nih.gov/oba/rac/Guidelines/NIH_Guidelines_prn.pdf.
3. *The SACGM Compendium of guidance, Part 2: Risk assessment of genetically modified microorganisms (other than those associated with plants)*. 2007. Health and Safety Executive, Suffolk, U.K. www.hse.gov.uk/biosafety/gmo/acgm/acgmcomp.
4. *Position statement of the Central Biosafety Commission on the risk assessment of human adeno-associated viruses and AAV-derived vectors, Ref. No. 6790-10-73*. 2005. Bundesamt für Verbraucherschutz und Lebensmittelsicherheit.
5. *Recommendation of the SECB on the classification of work with genetically modified viral vectors*. 2009. Swiss Expert Committee for Biosafety SECB, Federal Office for the Environment, Berne. www.efbs.admin.ch.
6. *Opinion of the Scientific Panel on Genetically Modified Organisms on the use of antibiotic resistance genes as marker genes in genetically modified plants, Question N° EFSA-Q-2003-109*. 2004. The EFSA Journal.
7. Thorne, B.A., et al., *Characterizing clearance of helper adenovirus by a clinical rAAV1 manufacturing process.* Biologicals, 2008. **36**(1): p. 7-18.
8. Snyder, R., et al., *Correction of hemophilia B in canine and murine models using recombinant adeno-associated viral vectors.* Nat Med., 1999. **5**(1): p. 64-70.
9. Mount, J., et al., *Sustained phenotypic correction of hemophilia B dogs with a factor IX null mutation by liver-directed gene therapy.* Blood, 2002. **99**(8): p. 2670-6.
10. Nathwani, A.C., et al., *Sustained high-level expression of human factor IX (hFIX) after liver-targeted delivery of recombinant adeno-associated virus encoding the hFIX gene in rhesus macaques.* Blood, 2002. **100**(5): p. 1662-1669.
11. Nakai, H., et al., *AAV serotype 2 vectors preferentially integrate into active genes in mice.* Nat Med, 2003. **34**(3): p. 297-302.
12. Bell, P., et al., *No evidence for tumorigenesis of AAV vectors in a large-scale study in mice.* Mol Ther, 2005. **12**(2): p. 299-306.

13. Wang, L., et al., *Sustained correction of disease in naive and AAV2-pretreated hemophilia B dogs: AAV2/8-mediated, liver-directed gene therapy.* Blood, 2005. **105**(8): p. 3079-3086.

14. Nichols, T., et al., *Protein replacement therapy and gene transfer in canine models of hemophilia A, hemophilia B, von willebrand disease, and factor VII deficiency.* ILAR J., 2009. **50**(2): p. 144-67.

15. Li, H., et al., *Assessing the potential for AAV vector genotoxicity in a murine model.* Blood, 2011. **117**(12): p. 3311-9.

16. Palomeque, J., et al., *Efficiency of eight different AAV serotypes in transducing rat myocardium in vivo.* Gene Ther, 2007. **14**(13): p. 1055.

17. Asokan, A., D.V. Schaffer, and R. Jude Samulski, *The AAV Vector Toolkit: Poised at the Clinical Crossroads.* Mol Ther, 2012.

18. Wu, K., D. Bungard, and J. Lytton, *Regulation of SERCA Ca^{2+} pump expression by cytoplasmic Ca^{2+} in vascular smooth muscle cells.* Am J Physiol Cell Physiol., 2001. **280**(4): p. C843-51.

19. Hadri, L., et al., *SERCA2a Gene Transfer Enhances eNOS Expression and Activity in Endothelial Cells.* Mol Ther, 2010. **18**(7): p. 1284-1292.

20. Yi, G., *"Celladon 007: A 3-Month, Single Dose Safety and Biodistribution Study of Direct Intracoronary Infusion of AAV1/SERCA2a in Normal Göttingen Minipigs."*, 2006, Skirball Center of Cardiovascular Research, Cardiovascular Research Foundation, 8 Corporate Drive, Orangeburg, NY 10962: Orangeburg.

21. Kawase, Y., *"Celladon 003: A 16-Week, Single Dose, Pharmacology Study of AAV1/SERCA2a Delivered via Antegrade Epicardial Coronary Artery Infusion (AECAI) Compared to Vehicle Control in a Swine Mitral Regurgitation Model of Heart Failure."*, 2006, Massachusetts General Hospital, Cardiology Laboratory of Integrative Physiology and Imaging, 55 Fruit Street, Boston, MA 02114.

22. Jessup, M., et al., *Calcium Upregulation by Percutaneous Administration of Gene Therapy in Cardiac Disease (CUPID): A Phase 2 Trial of Intracoronary Gene Therapy of Sarcoplasmic Reticulum Ca^{2+}-ATPase in Patients With Advanced Heart Failure.* Circulation, 2011. **124**(3): p. 304-313.

23. Carter, B., H. Burstein, and R. Peluso, *Adeno-Associated Virus and AAV Vectors for Gene Delivery*, in *Gene and Cell Therapy: Therapeutic Mechanisms and Strategies, Third Edition*, N. Templeton and D. Lasic, Editors. 2008, CRC Press LLC, Taylor & Francis Group Boca Raton, FL. p. 115-157.

24. Buchlis, G., et al., *Factor IX expression in skeletal muscle of a severe hemophilia B patient 10 years after AAV-mediated gene transfer.* Blood, 2012. **119**(13): p. 3038-41.

25. Kuroda, M., et al., *Immunohistochemical study on the distribution of sarcoplasmic reticulum calcium ATPase in various human tissues using novel monoclonal antibodies.* Virchows Archiv A Path Anat, 1992. **421**: p. 527-532.

26. Damaj, B., *"Celladon 021: A 4-Month Non-GLP Single Dose Safety Study of Intravenous Infusion of AAV1/SERCA2a in Göttingen Minipigs with Anti-AAV1 Antibody Titers of <1:2 Receiving Immunosuppressive Therapy."*, 2009, Bio-Quant, Inc., 10635 Roselle Street, Suite H, San Diego, CA 92121.

27. Nathwani, A.C., et al., *Adenovirus-Associated Virus Vector–Mediated Gene Transfer in Hemophilia B.* N Engl J Med, 2011. **365**(25): p. 2357-2365.

28. Brandon, E.F.A., et al., *Effect of Administration Route on the Biodistribution and Shedding of Replication-Deficient AAV2: A Qualitative Modelling Approach.* Current Gene Therapy, 2010. **10**(2): p. 91-106.

29. *EMEA/ICH Workshop on Viral/Vector Shedding*. in *The XVth Annual Congress of the European Society of Gene and Cell Therapy*. 2007. Rotterdam, The Netherlands.

30. Moss, R., et al., *Repeated Adeno-Associated Virus Serotype 2 Aerosol-Mediated Cystic Fibrosis Transmembrane Regulator Gene Transfer to the Lungs of Patients With Cystic Fibrosis.* Chest, 2004. **125**: p. 509-521.

31. Vardas, E., et al., *A Phase 2 Study to Evaluate the Safety and Immunogenicity of a Recombinant HIV Type 1 Vaccine Based on Adeno-Associated Virus.* AIDS Res Hum Retroviruses., 2010. **26**(8): p. 933-42.

32. Manno, C., et al., *AAV-mediated factor IX gene transfer to skeletal muscle in patients with severe hemophilia B.* Blood, 2003. **101**: p. 2963-2972.

33. Hajjar, R., et al., *Design of a Phase 1/2 Trial of Intracoronary Administration of AAV1/SERCA2a in Patients With Heart Failure.* J Card Fail, 2008. **14**(5): p. 355-367.

34. Jaski, B., et al., *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): p. 171-81.

35. Gwathmey, J.K., A.I. Yerevanian, and R.J. Hajjar, *Cardiac gene therapy with SERCA2a: From bench to bedside.* Journal of Molecular and Cellular Cardiology, 2011. **50**(5): p. 803-812.

36. Kawase, Y., D. Ladage, and R. Hajjar, *Rescuing the Failing Heart by Targeted Gene Transfer.* J Am Coll Cardiol, 2011. **57**(10): p. 1169–80.