PART 1 (COUNCIL DECISION 2002/813/EC)

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

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

A. General information

1. Details of notification

(a)	Member State of notification	**Netherlands**
(b)	Notification number	**B/NL/07/011**
(c)	Date of acknowledgement of notification	**December 13, 2007**
(d)	Title of the project	

A Phase 3 Clinical Trial to Evaluate the Safety and Efficacy of Treatment with 2 mg Intralesional Allovectin-7® [bicistronic plasmid DNA vector VCL-1005 encoding Human Leukocyte Antigen (HLA)-B7 and β-2 microglobulin, formulated with the cationic lipid delivery system DMRIE-DOPE] Compared to Dacarbazine (DTIC) or Temozolomide (TMZ) in Subjects with Recurrent Metastatic Melanoma

(e) Proposed period of release **From June 1, 2008 until June 1, 2018**

2. Notifier

Name of institution or company:

University Medical Center Groningen
Hanzeplein 1
9713 GZ Groningen
PO Box 30.001
9700 RB Groningen
The Netherlands

Study sponsor:
Vical Incorporated
10390 Pacific Center Court
San Diego, CA, USA 92121-4340

3. GMO characterisation

(a) Indicate whether the GMO is a:

viroid	(.)
RNA virus	(.)
DNA virus	(.)
bacterium	(.)

fungus (.)
animal
- mammals (.)
- insect (.)
- fish (.)
- other animal (.)

specify phylum, class

The Allovectin-7® medicinal product is not a GMO per se. It is an investigational gene therapy medicinal product consisting of a purified plasmid DNA (pDNA) drug substance formulated with a synthetic cationic lipid/neutral colipid delivery system (DMRIE-DOPE) in an injection vehicle consisting of 0.9% sodium chloride supplemented with 10 µg/mL glycerin. The pDNA drug substance, designated VCL-1005, is derived from the commonly used laboratory cloning vector pBR322. The VCL-1005 pDNA contains an antibiotic resistance gene and a bacterial origin of DNA replication, and is manufactured at Vical's facility in the U.S. by growth in Escherichia coli (E. coli) cultures maintained under antibiotic selection with kanamycin. The final pDNA drug substance contained in Allovectin-7® is an acellular product free of contaminating bacteria or viruses.

In a prior correspondence from a representative of the EMEA it was confirmed that Allovectin-7® is not classified as a GMO, but falls under the GMO legislation as a gene therapy medicinal product administered to humans.

For the purposes of this notification, the VCL-1005 pDNA component of Allovectin-7® is considered to be the active component of the GMO.

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

Not relevant. The VCL-1005 pDNA does not have a taxonomic classification.

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

Annex IIIa, II, A(10): Verification of the genetic stability of the organisms and factors affecting it. **Available data indicates the prokaryotic and eukaryotic sequence elements of the VCL-1005 pDNA are genetically stable under the controlled conditions used for plasmid manufacture, and will not become unstable via germline integration of plasmid sequences in trial subjects treated with Allovectin-7® or via conjugative gene transfer between bacteria.**

The VCL-1005 pDNA does not encode a conjugative pilus or contain sequence elements such as *oriT* utilized in conjugative gene transfer between bacteria.[1] The results of initial experiments performed with pBR322 indicate it is not detectably mobilised under conditions permissive for conjugative gene transfer.[2] The inserted genes and noncoding regulatory elements of VCL-1005 are not known or predicted to alter the stability of the pBR322 vector in bacteria or to effect its mobilisation.

The kanamycin resistance gene of VCL-1005 originates from the naturally occurring bacterial transposon Tn903,[3] and includes the promoter and coding sequence for the 3´

aminoglycoside phosphotransferase type I (APH-3´ type I) enzyme. The encoded gene product is expressed in bacteria and confers resistance to kanamycin or similar aminoglycoside antibiotics (neomycin, paromomycin, ribostamycin, lividomycin, and gentamicin B).[4] The kanamycin resistance gene and flanking sequences of VCL-1005 do not contain any of the inverted repeat sequences necessary for classical transposition of the gene into bacterial chromosomes or other plasmids.[3]

Data from a nonclinical biodistribution study of Allovectin-7® performed in mice indicates the VCL-1005 pDNA is rapidly degraded in blood (half-life < 5 minutes) and does not persist in gonadal or other tissues following intravenous injection.[5] Intravenous injection was utilized in this study as a "worst-case" scenario relative to the intended clinical route of intralesional administration. Data from other nonclinical biodistribution studies of intramuscularly injected pDNA vaccines, including an anthrax pDNA vaccine formulated in DMRIE-DOPE, indicate that the injected pDNA is progressively cleared from tissues and does not integrate into host cell genomic DNA at the site of injection.[6,7,8,9]

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

   Yes (**x**) No (.)

   If yes, insert the country code(s) **FR**

   Release is also planned in PL, BE, DE, ES, and IT. The investigational product is not considered a GMO in these countries; therefore, notification for release does not apply and was not done for these countries.

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

   Yes (**x**) No (.)

   If yes:
   - Member State of notification **FR**
   - Notification number **Not known**

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

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

   Yes (.) No (**x**)

   If yes:
   - Member State of notification **CH**
   - Notification number **Not known**

   Allovectin-7® is currently in clinical testing in Switzerland (CH) as well as the U.S. and Canada. These countries are not member states of the EU and have not requested a Summary Notification of release.

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

The data and considerations provided below support the following conclusions regarding the potential environmental impact of the Allovectin-7® medicinal product:

- **The Allovectin-7® medicinal product is not infectious or capable of replication in humans or mammals.**
- **Based on the results of previous clinical and nonclinical studies, the Allovectin-7® medicinal product is not pathogenic to humans or animals.**
- **The risk of uptake and sustained replication of the VCL-1005 pDNA in resident human bacteria or bacteria present in the environment is remote.**
- **The risk of dissemination of the VCL-1005 pDNA via excretion from trial subjects or integration into human or mammalian germline DNA is negligible.**
- **The viral and bacterial DNA sequence elements of the VCL-1005 pDNA are essentially unchanged from their naturally occurring parental forms, and as such pose no risk for recombination events resulting in increased virulence or spread of the parental organisms.**

The potential environmental risk of Allovectin-7® is therefore considered to be minimal and acceptable in view of the potential clinical benefit to metastatic melanoma subjects.

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

1. Recipient or parental organism characterisation:

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

(select one only)

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

other, specify

VCL-1005 is a pDNA derived from the commonly used laboratory cloning vector pBR322,[2] an artificial bacterial plasmid which does not exist in the natural environment and does not have a taxonomic classification.

2. Name

(i)	order and/or higher taxon (for animals)	…
(ii)	genus	…
(iii)	species	…
(iv)	subspecies	…
(v)	strain	…
(vi)	pathovar (biotype, ecotype, race, etc.)	…
(vii)	common name	**pBR322**

3. Geographical distribution of the organism

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

The pBR322 cloning vector is used in research laboratories worldwide.

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

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

Atlantic ..
Mediteranean ..
Boreal ..
Alpine ..
Continental ..
Macaronesian ..

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

(Used in research laboratories.)

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

(Used in research laboratories.)

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

(Used in research laboratories.)

4. Natural habitat of the organism

(a) If the organism is a microorganism

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

The pBR322 pDNA is not known to exist in the natural environment, but in theory could reside in E. coli or other members of the family Enterobacteriaceae, which can be found in soils, wastewater, and human or mammalian gastrointestinal flora.

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

5. (a) Detection techniques

Polymerase Chain Reaction (PCR) assays.

(b) Identification techniques

Polymerase Chain Reaction (PCR) assays.

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

Yes (.) No (**x**)

If yes, specify

…

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

Yes (.) No (**x**) Not known (.)

If yes:

(a) to which of the following organisms:

humans (.)
animals (.)
plants (.)
other (.)

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

…

8. Information concerning reproduction

(a) Generation time in natural ecosystems:

The generation time of pBR322 in natural ecosystems is not known. The pBR322 plasmid is capable of replication in permissive species of bacteria such as E. coli. In laboratory cultures, doubling times of 24-71 minutes have been reported.[10]

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

Relevant nonclinical data suggest that plasmids such as pBR322 or VCL-1005 will not undergo episomal replication in mammalian cells.[11]

(c) Way of reproduction: Sexual .. Asexual (**x**)

(d) Factors affecting reproduction:

Both the recipient pBR322 pDNA and the VCL-1005 pDNA are capable of reproduction (i.e., replication) in bacteria such as E. coli that could be present on or in human recipients of Allovectin-7® or in the natural environment. In addition, the pBR322-derived sequences of VCL-1005 could hypothetically be replicated via integration into somatic cell or germline DNA in humans or animals. Factors affecting

the the potential for reproduction of pBR322 or VCL-1005 by each of these mechanisms are discussed below.

Replication of pDNA in bacteria present on or in human recipients of Allovectin-7® is not expected to occur based on the following data and considerations:

1. ***The pDNA does not disseminate to sites where bacteria are present.*** **Bacteria are typically present in anatomic sites such as the large and small intestine, skin, eyes, ears, nose, throat and mouth. With the exception of the skin, these sites are generally distinct anatomical compartments separated from the cutaneous, subcutaneous, or nodal lesions to be injected intralesionally with the Allovectin-7® product. Data from clinical studies indicate that the pDNA does not disseminate into the circulation following intralesional injection[12,13] and thus would not be expected to disseminate into these sites. However, rare instances of bacterial exposure to the VCL-1005 pDNA on the skin of trial subjects cannot be excluded (for example, within epidermal tissue directly in the path of the injection needle) and are addressed below.**

2. ***The pDNA cannot gain entry into bacteria.*** **It is estimated that 500 to 1000 different species of bacteria live in the human body. At least 40 bacterial species (about 1% of known bacterial species) are known to become naturally competent for receptor-mediated uptake of DNA under certain circumstances. Clinically pathogenic species known to become naturally competent include Campylobacter jejuni, Campylobacter coli, Haemophilus influenzae, Haemophilus parainfluenzae, Helicobacter pylori, Moraxella species, Neisseria gonorrhoeae, Neisseria meningitidis, Staphylococcus aureus, Streptococcus pneumoniae, Streptococcus sanguis, and Streptococcus mutans.[14] Expression of a specific set of genes is required for competence. Except in Neisseria gonorrhoeae, which has been reported to be constitutively competent, expression of competence genes is transient, and is tightly controlled by environmental signals including quorum-sensing and nutritional deprivation.[15] In some Gram-negative strains, such as Haemophilus influenzae[16] and Neisseria species,[17] efficient DNA uptake occurs only if a specific DNA sequence is present. The process of DNA uptake by competent bacteria is accompanied by endonuclease cleavage and degradation of one strand of the DNA. Incoming single-stranded DNA can be integrated into the bacterial chromosome by a process that requires sequence homology between the incoming DNA and the bacterial chromosome.[14] In specific circumstances, plasmids can be reconstituted and maintained as episomal elements.[15] However, the risk of pDNA encountering a pathogenic bacterial species in trial subjects in a competent state is low. In clinical isolates of the pathogenic strains listed above, transformation frequencies ranged from 10^{-2} to 10^{-6} in viable cells.[14] Bacterial cells that are not naturally competent for exogenous DNA uptake are otherwise impermeable to pDNA or pDNA/lipid complexes under normal physiologic conditions. For example, artificial transformation of the E. coli strains used in molecular biology laboratories is typically accomplished by the use of a procedure involving hypotonic swelling of the bacteria in an aqueous solution containing $CaCl_2$, followed by a brief heat shock at 42°C. These or other extreme physical conditions (for example, freezing and thawing) which may render bacteria permeable to pDNA are not expected to occur in trial subjects.**

3. ***Wild-type bacteria do not support transformation by the pDNA.*** **Strains of E. coli used for molecular cloning, including the DH10B strain currently used for manufacture of Allovectin-7®, are specifically maintained genotypes containing mutations that enhance transformation and plasmid replication.[18] In some cases, wild-type bacterial species may possess species-specific DNA restriction and modification systems capable of destroying foreign DNA that is not methylated in the same manner as their own.[14]**

4. ***Wild-type bacteria do not support replication of the pDNA.*** **Replication of the VCL-1005 pDNA in bacteria is controlled by the Col E1 origin of replication sequence present in the plasmid backbone. The parental Col E1 plasmid from which the origin of replication sequence of VCL-1005 is derived is a narrow host-range plasmid that generally does not transform or replicate in bacterial hosts outside of the family Enterobacteriaceae. In addition, Col E1-based replicons require several E. coli host factors to support replication.[19] These considerations suggest that, with the exception of E. coli and other Enterobacteriaceae, replication of the VCL-1005 pDNA is unlikely to occur in bacteria found in trial subjects, including the pathogenic bacteria listed above in point 2 that are known to acquire natural competence. The risk of transformation and replication in E. coli or other Enterobacteriaceae is addressed below.**

5. ***Replication of pDNA in bacteria would not occur in the absence of antibiotic.*** **In trial subjects or individuals inadvertently exposed to Allovectin-7®, selection and outgrowth of rare bacterial transformants carrying the aminoglycoside 3´ phosphotransferase gene would require the continued presence of aminoglycoside antibiotics (kanamycin, neomycin, paromomycin, ribostamycin, lividomycin, and gentamicin B) known to be inactivated by the expressed product of the VCL-1005 kanamycin resistance gene.[4] In the absence of continued exposure of trial subjects to these antibiotics, rare instances of pDNA uptake would not confer any selective advantage on the bacterial host, and would be expected to negatively impact bacterial growth and survival due to the reduced availability of host cell factors for bacterial DNA replication.[20] In the special case of bacteria transformed to kanamycin resistance through integration of the plasmid-derived kanamycin resistance gene fragment into their chromosomal DNA, the absence of antibiotic selection, the requirement for DNA sequence homology for integration into chromosomal DNA of the host, and the potential negative effects of integration on the host greatly reduce the risk of such transformants in trial subjects.**

6. ***Antibiotics such as kanamycin are no longer widely used.*** **Kanamycin or similar aminoglycoside antibiotics are no longer widely used for treatment of human infections due to their toxicity, the development of resistant bacterial strains, and the availability of newer antibiotics that are less toxic and/or more effective against drug-resistant strains. According to the Merck Manual for Healthcare Professionals, aminoglycosides are used for serious gram-negative infections, especially Pseudomonas aeruginosa. Aminoglycosides are used alone infrequently, typically for plague or tularemia. They can be used for treatment of severe infections due to suspected gram-negative bacteria in conjunction with**

a broad-spectrum β-lactam antibiotic. Because of increasing aminoglycoside resistance, a fluoroquinolone can be substituted for the aminoglycoside in initial empiric regimens, or the aminoglycoside can be stopped after 2 to 3 days unless an aminoglycoside-sensitive P. aeruginosa is identified.[21] While transformation of P. aeruginosa by the VCL-1005 pDNA in a trial subject could be of concern, it should be pointed out that this species is not among those known to support plasmid replication via the Col E1 origin of replication, or to develop natural competence.[14] In addition, the use of an aminoglycoside antibiotic in conjunction with a broad-spectrum β-lactam antibiotic would be expected to eliminate strains of P. aeruginosa harboring resistance to only the aminoglycoside.

These data and considerations support the conclusion that replication of the VCL-1005 pDNA will not occur in bacteria on or in trial subjects. While rare instances of bacterial uptake of plasmid cannot be ruled out, available evidence suggests that such rare instances would not result in pDNA replication or the creation and perpetuation of stably altered, antibiotic-resistant bacterial strains in the absence of antibiotic selection.

Replication of the VCL-1005 pDNA in bacteria present in the natural environment is not expected to occur based on the data and considerations given above, and the following additional data and considerations:

1. ***Intact pDNA will not gain entry into bacterial cells.*** **Bacteria in the natural environment can be rendered permeable to pDNA by extreme physical conditions such as freezing and thawing, exposure to an electrical charge, hypotonic swelling, or heating under conditions that result in a temporary loss of cell wall integrity without cell death. The possibility of replication of the VCL-1005 pDNA in these cases is considered remote due to many of the reasons listed above, including the narrow host range of the plasmid's Col E1-derived origin of replication element, the existence of bacterial host defense mechanisms such as restriction and modification systems, and the absence of antibiotic selection.**

2. ***Intact pDNA will not gain entry into the mammalian digestive tract.*** **In the case of ingestion by humans or mammals, the risk of transformation of resident E. coli or other bacteria in the gastrointestinal tract is increased. However, ingestion of significant amounts of intact VCL-1005 pDNA secondary to its inadvertent release into the environment must be viewed as a highly unlikely circumstance due to the nearly ubiquitous presence of nucleases in the natural environment. Moreover, the likelihood of ingestion resulting in successful transformation of gastrointestinal bacteria would be diminished significantly due to degradation of the ingested pDNA by digestive system nucleases, depurination of the pDNA in the highly acidic environment of the stomach and upper intestine, and other reasons listed above. Rare instances of transformation of intestinal bacteria would not be expected to lead to significant plasmid replication in the absence of antibiotic selection. Antibiotics are not effective for treatment of suspected cases of pathogenic E. coli infection in humans, and may increase the risk of hemolytic uremic syndrome.[22] In addition, as stated above, aminoglycoside antibiotics are infrequently used alone for treatment of human infections, and are not present in the natural environment of mammals or among the antibiotics used to**

promote the growth of livestock. For these reasons, the likely extent of replication of the VCL-1005 pDNA following ingestion by humans or mammals and transformation of resident bacteria is considered negligible.

Replication of pDNA sequences via integration into somatic cell or germline DNA is not expected to occur based on the following data and considerations:

1. ***Data from clinical studies indicating that pDNA does not disseminate into the circulation following intralesional injection.*** **Data from Phase 1 clinical studies of Allovectin-7® or a precursor product encoding HLA-B7 only have shown that the pDNA component was undetectable in blood samples obtained from subjects after administration by intralesional injection.[12,13] Blood samples obtained immediately before and at multiple times after injection were analyzed for the presence of pDNA by a PCR assay employing primers specific for the plasmid insert sequences (sensitivity < 2 pg/mL). Plasmid DNA was not detected at any time in the blood after gene transfer, even as early as 5 minutes after injection at doses of up to 300 µg.[13]**

2. ***Rapid degradation of pDNA in blood and tissues.*** **In the nonclinical biodistribution study of Allovectin-7®, the VCL-1005 pDNA was found to have a half-life of less than 5 minutes in blood.[5] In humans, the mean half-life of circulating fetal DNA in maternal plasma has been reported to be 16.3 minutes.[23] In early developmental studies in mice involving intramuscular injection of a reporter pDNA encoding firefly luciferase, over 95% of the residual plasmid DNA recovered from injected muscles was found to be degraded by 90 minutes after injection.[24]**

3. ***Lack of pDNA persistence in gonadal tissues.*** **The nonclinical biodistribution study of Allovectin-7® assessed distribution of the VCL-1005 pDNA into gonadal tissues following intravenous injection of mice with 50 µg Allovectin-7®. Analysis of tissue samples collected at later time points was performed using a 32-cycle, endpoint PCR assay followed by visualization of the PCR products by Southern blotting. Estimation of PCR product band intensities on Southern blot autoradiograms in comparison to pDNA standards indicated that ~125 plasmid copies per µg genomic DNA were present in gonadal tissues at 28 days after intravenous injection, with clearance to levels at or below the sensitivity of the assay (~50 plasmid copies per µg genomic DNA) by six months after injection.[5] In other studies involving comparable investigational products, the results of seven different biodistribution studies of pDNA vaccines administered by intramuscular injection have shown that pDNA was not quantifiable by real-time PCR in genomic DNA isolated from gonadal tissues by the final time points tested (no later than two months after injection). The lower limit of quantification of the PCR assays used in these studies was >10 plasmid copies (3 studies) or 50 plasmid copies (4 studies) per µg genomic DNA.[7] Similarly, a biodistribution study performed in support of a Vical pDNA vaccine for anthrax formulated with DMRIE-DOPE found no evidence for pDNA persistence in gonadal tissues after intramuscular injection.[6]**

4. ***Lack of pDNA persistence in a clinical subject.*** **Tissues were obtained at autopsy from a subject treated with Allovectin-7® that experienced a Serious Adverse**

Event (SAE) consisting of a buildup of ascites fluid following the second course of treatment. Tissues from the colon, aortic lymph node, and the left inguinal injection site were analyzed for the presence of the VCL-1005 pDNA using a highly sensitive PCR assay based on the method employed for the biodistribution study of Allovectin-7®. VCL-1005 pDNA sequences were not detectable in any of the patient samples. The PCR assay exhibited a sensitivity of one plasmid copy in control samples.

5. ***Lack of evidence for pDNA integration*. Multiple nonclinical studies of pDNA vaccines administered intramuscularly by needle and syringe have found no evidence for pDNA integration into injection site tissues. An integration study of a Vical pDNA vaccine for anthrax formulated in DMRIE-DOPE and administered by intramuscular injection to rabbits found no evidence for integration of pDNA into injection site muscle tissue DNA harvested 64 days after intramuscular injection of 1 mg VCL-AB01. The quantitative real-time PCR assay used for analysis had a lower limit of detection of 10 plasmid copies.[6] Other studies of pDNA vaccines administered by intramuscular injection have also found no evidence for integration of plasmid DNA in injection site tissues.[6,7,8] The potential for integration into germline tissues has not directly been evaluated for Allovectin-7® or other pDNA products due to the lack of pDNA persistence in gonadal tissues at later time points in biodistribution studies.[5,7,9]**

9. Survivability

(a) ability to form structures enhancing survival or dormancy:

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

The pBR322 or VCL-1005 pDNAs are not known to form any of the structures listed above. Instances of inadvertent environmental release would generally be expected to lead to rapid degradation of the pDNA due to the abundance of deoxyribonucleases in the natural environment. However, based on experimental findings related to the presence of free DNA in the environment,[14] trace amounts of pDNA could persist in soils or wastewater.

(b) relevant factors affecting survivability:

See B(8)(d) above.

10. (a) Ways of dissemination

See B(8)(d) above.

(b) Factors affecting dissemination

See B(8)(d) above.

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

None.

C. **Information relating to the genetic modification**

1. Type of the genetic modification

(i)	insertion of genetic material	(**x**)
(ii)	deletion of genetic material	(**x**)
(iii)	base substitution	(**x**)
(iv)	cell fusion	(.)
(v)	others, specify …	

2. Intended outcome of the genetic modification

The pBR322 vector has been modified to insert a eukaryotic expression cassette designed to express a messenger RNA (mRNA) encoding HLA-B7 and β-2 microglobulin in eukaryotic cells.

In addition, the prokaryotic backbone region of the pBR322 vector has been modified as follows:

- **The antibiotic resistance genes (encoding ampicillin and tetracycline resistance) have been deleted and replaced with the kanamycin resistance gene to allow for antibiotic selection with kanamycin during plasmid manufacture.**
- **The sequences controlling replication in bacteria were modified to increase pDNA yield during manufacture.**

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

Yes (.) No (**x**)

If no, go straight to question 5.

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

Yes (.) No (.)

If no, go straight to question 5.

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

(a) Type of vector

plasmid	(.)
bacteriophage	(.)
virus	(.)
cosmid	(.)
transposable element	(.)
other, specify …	

(b) Identity of the vector

…

(c) Host range of the vector

…

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

Yes (.) No (.)

antibiotic resistance (.)
other, specify …

Indication of which antibiotic resistance gene is inserted
…

(e) Constituent fragments of the vector
…

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

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

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

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

VCL-1005 was constructed using standard recombinant DNA cloning methods.

6. Composition of the insert

(a) Composition of the insert

The eukaryotic insert of VCL-1005 consists of the Rous Sarcoma Virus (RSV) promoter, HLA-B7 coding sequences, the murine encephalomyocarditis virus Cap Independent Translational Enhancer (CITE), the β-2 microglobulin coding sequence, and the Bovine Growth Hormone (BGH) transcriptional terminator.

A plasmid map of VCL-1005 is provided in Figure 1 below.

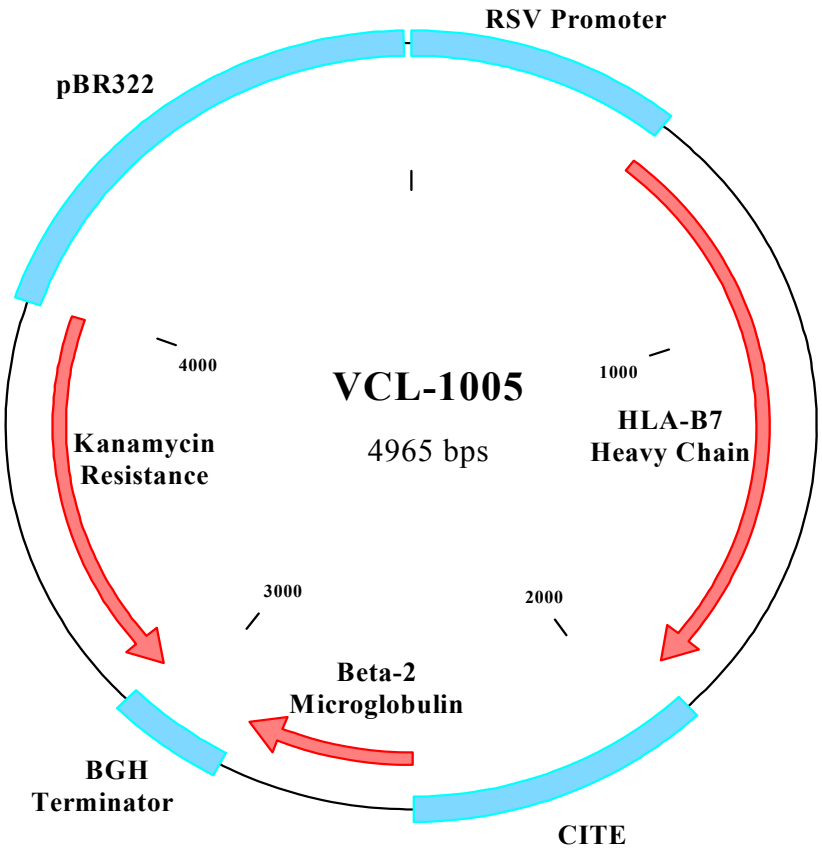

Figure 1. VCL-1005 plasmid map. Regions of the plasmid are depicted as boxes and coding sequences as arrows. The RSV promoter region controls expression of an mRNA encoding the HLA-B7 heavy chain and β-2 microglobulin proteins, which together form a class I Major Histocompatibility Complex (MHC). The two coding sequences are separated by the CITE sequence, an internal ribosomal entry site that permits co-expression of the HLA-B7 and β-2 microglobulin proteins from a single bicistronic mRNA, followed by the BGH transcriptional terminator. The kanamycin resistance gene is expressed in bacteria and is used as a selection marker for the VCL-1005 plasmid in the bacterial host. Replication in permissive bacterial hosts such as E. coli is regulated by the sequences contained in the pBR322-derived region of VCL-1005, which includes a bacterial origin of DNA replication.

(b) Source of each constituent part of the insert

The constituent parts of the insert (the RSV promoter, HLA-B7, CITE, β-2 microglobulin, and BGH terminator sequences) and their location in base pair numbers within the VCL-1005 pDNA are shown in Table 1 below. The sources shown represent the ultimate natural or synthetic origin of the constituent parts.

Table 1. VCL-1005 pDNA Constituent Parts and Their Origins

Fragment	Base pair numbers	Origin of Sequence
RSV promoter	**1 to 538**	**Rous Sarcoma Virus 3´ LTR**
HLA-B7	**539 to 1853**	**Human HLA-B7 cDNA**
Coding Region	**535 to 1621**	
Signal Peptide	**535 to 606**	
Mature protein	**607 to 1621**	
Cloning Site	**1854 to 1901**	**Synthetic DNA fragment**
CITE	**1902 to 2479**	**Murine encephalomyocarditis virus**
β-2 Microglobulin	**2480 to 2847**	**Chimpanzee β-2 Microglobulin cDNA[a]**
Coding Region	**2480 to 2837**	
Linker	**2848 to 2888**	**Synthetic DNA fragment**
BGH terminator	**2889 to 3112**	**Bovine Growth Hormone Gene**
Linker	**3113 to 3151**	**Synthetic DNA fragment**
Kanamycin resistance gene	**3152 to 4018**	**Bacterial transposon Tn903**
Coding Region	**3967 to 3154**	
pBR322 backbone and bacterial origin of replication	**4019 to 4965**	**Cloning vector pBR322**

[a] The mature β-2 microglobulin protein encoded by the chimpanzee cDNA is identical in amino acid sequence to the mature human β-2 microglobulin protein.

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

The Rous Sarcoma Virus 3´ LTR functions to promote high-level transcription of the HLA-B7 and β-2 microglobulin coding sequences into RNA.

The HLA-B7 sequence encodes the class I MHC heavy chain of the human B-7 allele.

The synthetic DNA fragments provided restriction endonuclease sites for subcloning of the various DNA fragments comprising the VCL-1005 plasmid.

The murine encephalomyocarditis virus CITE sequence functions to enhance translation of the β-2 microglobulin coding sequences in the absence of a proximal 5´ mRNA cap structure, enabling expression of both the HLA-B7 and β-2 microglobulin proteins from a single mRNA.

The chimpanzee β-2 microglobulin sequence encodes the class I MHC light chain, which together with HLA-B7 forms a class I MHC molecule on the surface of transfected cells. The intended function of class I MHC expression is to provide an immunotherapeutic effect in the treatment of metastatic melanoma.

The BGH terminator functions to terminate transcription of the primary RNA transcript encoding HLA-B7 and β-2 microglobulin, and directs cleavage and polyadenylation of the transcript to form the mRNA.

(e) Location of the insert in the host organism

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

The eukaryotic insert of VCL-1005 is subcloned into the pBR322 vector.

(f) Does the insert contain parts whose product or function are not known?
Yes (.) No (**x**)
If yes, specify …

D. Information on the organism(s) from which the insert is derived

1. Indicate whether it is a:

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

2. Complete name

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

Rous Sarcoma Virus (RSV promoter sequence within base pairs 1-538):

- **Family: Retroviridae**
- **Subfamily: Orthoretrovirinae**
- **Genus: Alpharetrovirus**
- **Species: Rous sarcoma virus**
- **Strain : Schmidt-Ruppin**

Encephalomyocarditis virus (CITE sequence within base pairs 1902-2479):

- **Family: Picornaviridae**
- **Genus: Cardiovirus**
- **Species: Encephalomyocarditis virus**

Human (HLA-B7 cDNA sequence within base pairs 539-1853):

- **Kingdom: Animalia**
- **Phylum: Chordata**
- **Class: Mammalia**
- **Order: Primates**
- **Family: Hominidae**

- **Genus: Homo**
- **Species: H. sapiens**

Chimpanzee (β-2 microglobulin cDNA sequence within base pairs 2480-2847):

- **Kingdom: Animalia**
- **Phylum: Chordata**
- **Class: Mammalia**
- **Order: Primates**
- **Family: Hominidae**
- **Subfamily: Homininae**
- **Genus: Pan**
- **Species: P. troglodytes**

Bovine (BGH terminator sequence within base pairs 2889-3112):

- **Kingdom: Animalia**
- **Phylum: Chordata**
- **Class: Mammalia**
- **Order: Artiodactyla**
- **Family: Bovidae**
- **Subfamily: Bovinae**
- **Genus: Bos**
- **Species: B. taurus**

3. Is the organism significantly pathogenic or harmful in any other way (including its extracellular products), either living or dead?
Yes (**x**)**(viruses)** No (.) Not known (.)
If yes, specify the following:

(b) to which of the following organisms:

humans (.)
animals (**x**)
plants (.)
other ..

(b) are the donated sequences involved in any way to the pathogenic or harmful properties of the organism
Yes (**x**) No (.) Not known (.)

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

Annex III A, point II(A)(11)(d): Pathogenicity: infectivity, toxigenicity, virulence, allergenicity, carrier (vector) of pathogen, possible vectors, host range including non-target organism. Possible activation of latent viruses (proviruses). Ability to colonise other organisms.

Based on the clinical and nonclinical safety experience with Allovectin-7® described under E(1)(d) below, the RSV promoter and encephalomyocarditis CITE sequences of VCL-1005 are non-pathogenic in humans or animals.

The Rous Sarcoma Virus 3´ LTR promoter functions in the transcription of proviral RSV sequences integrated into genomes of avian hosts. This property may contribute to the infectivity, toxicity, and virulence of the wide-type virus and the ability of the virus to transform cells as a carrier vector for oncogenes. [25]

The encephalomyocarditis virus CITE sequence enables efficient cap-independent translation of viral mRNAs, and thereby facilitates replication of the virus in infected cells when cap-dependent translation of cellular mRNAs is otherwise inhibited by the virus. This property may contribute to the infectivity, toxicity, and virulence of the wide-type virus in its natural hosts.[26]

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

   Yes (.) No **(x)**

   If yes, specify …

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

   Yes (.) No (.) Not known **(x)**

E. Information relating to the genetically modified organism

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

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

Yes (.) No (.) Not known (**x**)

Specify …

Survival of pBR322 or the VCL-1005 pDNA in the natural environment has not been studied. Based on the data and considerations provided under A(3)(c) and B(8)(d) above, the potential for survival of the VCL-1005 pDNA in the natural environment as a consequence of the intended use is considered remote.

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

Yes (**x**) No (.) Unknown (.)

Specify

The bacterial origin of DNA replication of VCL-1005 is derived from pBR322 and confers the ability to replicate in E. coli. The fragment of pBR322 used in VCL-1005 represents the region from pBR322 base number 2244 to base number 3193.[27] This fragment provides a bacterial origin of DNA replication originating from the naturally occurring plasmid Col E1 and does not contain any open reading frames known to be expressed in bacterial or animal cells. To increase pDNA yield during manufacture in E. coli, the pBR322 region of VCL-1005 was modified to change a single base from G to A at position 4236. This single-base change is located in the region controlling plasmid replication and effects a modest increase in the plasmid copy numbers obtained in E. coli.[28] Replication of the VCL-1005 pDNA in E. coli is otherwise unaltered relative to pBR322.

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

Yes (.) No (.) Not known (**x**)

Specify

Dissemination of pBR322 or the VCL-1005 pDNA in the natural environment has not been studied. Based on the data and considerations provided under A(3)(c) and B(8)(d) above, the potential for dissemination of the VCL-1005 pDNA in the natural environment as a consequence of the intended use is considered remote, and no greater than that of pBR322.

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

Yes (.) No (**x**) Not known (.)

Specify

The pathogenicity of pBR322 in humans is not known. The modifications to pBR322 embodied in the VCL-1005 pDNA component are considered non-pathogenic based on the clinical and nonclinical safety experience with Allovectin-7® described below.

Clinical safety experience. **Allovectin-7® has been evaluated in multiple clinical trials and has shown minimal toxicity at doses ranging from 5 µg to 2 mg. Based on data obtained from 711 subjects dosed with Allovectin-7®, there is no evidence that Allovectin-7® poses any significant health risks to humans.**

Allovectin-7® appears to be a relatively safe therapy with no incidence of drug-related, Grade 3 or 4 adverse events (AEs) at the current 2-mg dose. The most common AEs occurring in >10% of the subjects who received Allovectin-7® at the 2-mg dose include: injection site reactions, fatigue, chills, arthralgia, myalgia, nausea, headache, injection site pain, fever, and dizziness.

Of the reported SAEs from 711 subjects dosed with Allovectin-7® in previous trials, 18 have been deemed to be associated with the use of Allovectin-7®, and 16 of these 18 were determined to be likely related to the injection procedure. However, most of these 16 SAEs were due to injection into highly vascularized organs such as the liver, injection into the lungs, or biopsy of a tumor. Consequently, for the 133 subjects treated with the 2-mg dose in the completed VCL-1005-208 trial and for all subjects enrolled in the ongoing LX01-315 trial, only injections into cutaneous, subcutaneous, or nodal lesions have been allowed (no lung or visceral lesion injections) and no biopsies have been taken to eliminate any potential risks associated with these procedures. The two SAEs deemed by an investigator to be potentially associated with the use of Allovectin-7® and not related to the injection procedure were 1 case of non-specific arthritis and 1 case of increased abdominal ascites. Other reported serious adverse events have been attributed to the underlying disease, toxicity of other drug products, such as DTIC, or other causes clearly not associated with the use of Allovectin-7®.

Nonclinical safety experience. **The pathogenicity of Allovectin-7® in animals has been evaluated at doses substantially higher (on a mg/kg basis) than the 2-mg human dose. The nonclinical efficacy, pharmacology, and safety studies of Allovectin-7® completed in animals to date are summarized below. Intravenous injection was utilized in most of these studies as a "worst-case" scenario whereby all injected material was available to disseminate systemically. The single-dose and repeat-dose toxicity studies evaluated the toxicity of Allovectin-7® following intravenous injection. The results of these studies demonstrated that Allovectin-7® is well tolerated in both large and small animal models. The results of the biodistribution study indicated clearance of VCL-1005 pDNA from blood and major organs, including gonads, and progressive clearance over time from skeletal muscle following intravenous administration.[5] The results of the**

mass balance study indicated that 24 hours after intravenous injection of VCL-1005 complexed with ^{3}H-DMRIE-DOPE, the tissues containing the highest total amount of radioactivity were the liver, spleen, and lungs. At 96 hours post-injection, the majority of the radioactivity remained in the carcass (82.66% of the administered dose) and skin (3.14% of the administered dose).[29] Collectively, these findings support a favorable risk/benefit ratio for the intended clinical use of Allovectin-7®.

Summary of Pharmacology and Toxicology Studies of Allovectin-7®

Type of Study	Species and Strain	Method of Administration	Dosing Regimen	Doses (mg/kg)	Gender and Number
Non-GLP mass balance	**CD-1 mice**	**Intravenous injection**	**Day 1[a]**	**2**	**8M/group**
Results: Twenty-four hours after intravenous injection, the tissues containing the highest total amount of radioactivity were the liver (75.54% of the dose), spleen (5.28% of the dose), and lungs (1.67% of the dose). The tissue-to-plasma ratios for these tissues ranged from approximately 35.7 to 5.69, indicating retention of radioactivity. The radioactivity content of the liver and spleen were not affected by lyophilization. The heart, brain, and muscle contained < 0.13% of the administered dose with tissue-to-plasma ratios < 0.63, indicating a low uptake of radioactivity. The radioactivity content in the heart and lungs decreased by 8-12% following lyophilization of these tissues to remove water, suggesting that the extent of ^{3}H-exchange to $^{3}H_2O$ was low. However, a significant fraction of the radioactivity content of the brain and muscle (77% and 48%, respectively) was eliminated by lyophilization, indicating that most of the radioactivity in these tissues was due to $^{3}H_2O$. The low radioactivity uptake and high proportion of $^{3}H_2O$ in the brain and muscle indicated that the transfer of ^{3}H-DMRIE in these tissues was low. During the 96-hour period post-injection, most of the eliminated radioactivity was excreted via the feces (12.7%) and very little radioactivity was excreted via urine (≤ 0.9%). At 96 hours post-injection, the majority of the radioactivity remained in the carcass (82.66% of the administered dose) and skin (3.14% of the administered dose). Following lyophilization, approximately 5% and 11% of the radioactivity was eliminated from the carcass and skin samples, respectively, indicating that only a small fraction of the retained radioactivity was due to $^{3}H_2O$. The total recovery of radioactivity at 96 hours post-dose was 102% of the administered dose, indicating that a complete mass balance had been achieved following the intravenous administration of VCL-1005/ ^{3}H-DMRIE-DOPE. The overall low proportion of radioactivity eliminated by lyophilization (0-12%) in plasma, liver, spleen, heart, lung, skin, and carcass suggested that the extent of in vivo ^{3}H-exchange was low.					
Non-GLP Biodistribution	**ICR mice**	**Intravenous injection**	**Day 1**	**2**	**2M, 2F 4/group; 3F (blood)**
Results: The half-life of intact pDNA in blood was less than 5 minutes. The degradation products of the pDNA (< 330 bp) were undetectable in the blood within 1 hour post-administration. By one hour post-administration, pDNA was detected in the bone marrow, heart, kidney, liver, lung, spleen and muscle but not in the intestines, ovaries/testes or brain. Plasmid DNA could no longer be detected by Southern blot in any tissues 7 days post-administration. Plasmid DNA presence was measured with the increased sensitivity of PCR analysis (sensitivity ~50 plasmid copies per µg genomic DNA) in tissue samples from Days 7, 28 and 6 months. At Days 7 and 28, pDNA was detected to the greater extent in the bone marrow, heart, kidney, liver, lung, spleen, and muscle. Between these two time points, there was up to a 128-fold decrease in the amount of pDNA in all tissues. The largest decrease from Day 7 was found in the liver. By Day 28, the range of residual pDNA had diminished to < 0.5 fg/µg in the brain, liver, intestines, and gonads to about 16 fg/µg in the bone marrow, heart, lung and spleen, equivalent to about 125 to 4,000 pDNA copies/µg genomic DNA. Approximately 125 plasmid copies per µg genomic DNA were present in gonadal tissues at 28 days after intravenous injection, with clearance to levels at or below the sensitivity of the PCR assay by 6 months after injection. At 6 months, pDNA was only detected in the muscle at approximately 2,000 pDNA copies/µg genomic DNA. Despite the fact that at Days 1 and 7 the heart, kidney, liver, lung, and spleen contained some of the highest levels of intact VCL-1005 pDNA as detected by Southern blot and PCR analysis, none of these tissues were positive for HLA-B7 expression by immunohistochemistry.					
Non-GLP Toxicology	**BALB/c**	**Direct intrahepatic injection**	**1× per week for up to 4 weeks**	**0. 04 0.4**	**10/group**
Results: Direct intrahepatic injection did not appear to result in significant histopathology of the liver or significant changes in liver enzymes even after repeated administration. Direct intrahepatic administration of Allovectin-7® may have prolonged tissue healing.					

Summary of Pharmacology and Toxicology Studies of Allovectin-7®

Type of Study	Species and Strain	Method of Administration	Dosing Regimen	Doses (mg/kg)	Gender and Number
GLP Single-Dose Toxicity	**Mice CD-1®**	**Intravenous injection**	**Day 1**	**0.004 0.04 0.4 2**	**5M 5F 10/group**
Results: The administration of test article did not produce any signs of acute toxicity or signs of residual toxicity during the 14 day post-administration observation period.					
GLP Repeat-Dose Toxicity	**Mice CD-1®**	**Intravenous injection**	**Daily for 14 days**	**0.003 0.03 0.3**	**10M 10F 20/group**
Results: The effect of the test article on clinical chemistry, hematology, and organ weight parameters was mild. There were no test-article-related changes observed in any of the tissues evaluated based on histopathological examination. The in-life data and histopathological analysis did not reveal the presence of overt systemic toxicity at any of the administered dose levels.					
GLP Repeat-Dose Toxicity	**Cynomolgus macaques (Macaca fascicularis)**	**Intravenous injection**	**Day 0, 7 and 14**	**0.1**	**3M, 3F 6/group**
Results: In-life results revealed no overt toxicity and no test-article-related effects on behavior, appetite, body weights, morbidity or mortality, serum chemistry, anti-nuclear antibody, antibodies to double-stranded DNA, or complete blood count (CBC). There were no macroscopic changes, differences in organ weights, or histopathological lesions observed that could be attributed to the test article. The repeated intravenous administration of Allovectin-7® did not produce any signs of acute or residual toxicity in cynomolgus macaques during the 28 days of observation.					

[a] VCL-1005 pDNA used in this study was formulated at a DNA:DMRIE mass ratio of 5:1 using ^{3}H-DMRIE.

The pathogenicity of Allovectin-7® in plants has not been evaluated. The VCL-1005 pDNA lacks the structural genes and sequence elements of plasmid vectors typically used for stable transformation of plants via Agrobacterium tumefaciens,[30] and is not predicted to replicate autonomously in plants or fungi based on theoretical and experimental data.[31,32] Moreover, the likelihood of pDNA uptake by plant cells is low.[33]

2. Genetic stability of the genetically modified organism

See A(3)(c) above.

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

Yes (.) No (**x**) Unknown (.)

(a) to which of the following organisms?

humans (.)
animals (.)
plants (.)
other …

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

Annex III A, point II(A)(11)(d): Pathogenicity: infectivity, toxigenicity, virulence, allergenicity, carrier (vector) of pathogen, possible vectors, host range including non-target organism. Possible activation of latent viruses (proviruses). Ability to colonise other organisms.

Based on the clinical and nonclinical safety experience with Allovectin-7® described under E(1)(d) above, the VCL-1005 pDNA is not pathogenic in humans or animals. Activation of latent proviruses in humans or animals has not been observed. Based on the data and considerations provided under A(3)(c), B(8)(d), and E(1)(d) above, the potential for colonisation of bacteria or other organisms by VCL-1005 is considered remote.

Annex III A, point II(C)(II)(i):

Toxic or allergenic effects of the GMOs and/or their metabolic products. **Based on clinical trial data published to date[13,34,35] and the clinical and nonclinical safety experience described under E(1)(d) above, no toxic or allergenic effects are expected as a consequence of treatment with Allovectin-7®.**

Comparison of the modified organism to the donor, recipient or (where appropriate) parental organism regarding pathogenicity. **Expression of the human HLA-B7 and chimpanzee β-2 microglobulin genes of Allovectin-7® is intended to provide a therapeutic benefit to recipients in the treatment of cancer. These genes are not considered pathogenic to humans or animals based on the data provided under E(1)(d) above. Relevant pathogenic donor organisms comprise the parental RSV and encephalomyocarditis viruses from which the RSV promoter and CITE sequences of the VCL-1005 pDNA originate.**

The RSV promoter sequence of VCL-1005 was subcloned from the plasmid pRSV-β-globin, which was cloned directly from the plasmid pRSVcat.[36] The RSV LTR sequences present in pRSVcat have been tested and shown to have no intrinsic oncogenic activity in transgenic mice.[37,38,39] With the exception of 3 base pairs modified to inactivate the polyadenylation signal sequence of the RSV 3′ LTR, the VCL-1005 RSV promoter contains the entire RSV promoter sequence present in pRSVcat. The remainder of the RSV LTR-derived sequences are unmodified from their parental form. The RSV 3′ LTR-derived sequences of VCL-1005 do not contain transforming oncogenes or other retroviral coding sequences that could lead to the pathogenic effects of retroviruses observed in mammalian or avian hosts.

The CITE sequence of VCL-1005 is derived from murine encephalo-myocarditis virus and functions to enhance internal ribosomal entry and translation of the β-2 microglobulin coding sequences in the bicistronic mRNA expressed from the pDNA. The encephalomyocarditis-derived CITE sequence of VCL-1005 encodes the translational start and first 4 amino acids of the β-2 microglobulin signal peptide fused to downstream signal peptide

sequences derived from the chimpanzee β-2 microglobulin cDNA, but is otherwise non-codogenic. Based on the clinical and nonclinical safety experience with Allovectin-7® described under E(1)(d) above, the CITE sequence of VCL-1005 does not lead to the pathogenic effects of encephalomyocarditis virus observed in swine or rodents.

Capacity for colonisation. **The VCL-1005 pDNA of Allovectin-7® is not expected to colonise bacterial hosts for the reasons stated under A(3)(c) and B(8)(d) above.**

If the organism is pathogenic to humans who are immunocompetent: diseases caused and mechanism of pathogenicity including invasiveness and virulence, communicability, infective dose, host range, possibility of alteration, possibility of survival outside of human host, presence of vectors or means of dissemination, biological stability, antibiotic-resistance patterns, allergenicity, availability of appropriate therapies. **The absence of pathogenic effects in cancer patients treated with Allovectin-7® is reviewed above under E(1)(d) above. While cancer patients may in some cases be immunodeficient, the results of the nonclinical safety studies of Allovectin-7® performed in healthy animals [also reviewed under E(1)(d) above] provide additional evidence suggesting that Allovectin-7® is not pathogenic in immunocompetent humans as well.**

Other product hazards. **None.**

4. Description of identification and detection methods

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

PCR assays utilizing primers specific for VCL-1005 sequences have previously been developed for detection of the pDNA in mammalian tissue samples[5] and in principle could be employed for detection of the VCL-1005 pDNA in samples obtained from other organisms.

(b) Techniques used to identify the GMO

In addition to the PCR assays described above, the presence of the VCL-1005 pDNA may be identified by Southern blot analysis with specific probes.

F. Information relating to the release

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

   The purpose of the release is a phase 3 clinical trial in human subjects with metastatic melanoma.

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

   Yes (.) No (.)

   If yes, specify

   Not relevant. There is no natural habitat or ecosystem for the VCL-1005 pDNA.

3. Information concerning the release and the surrounding area

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

   University Medical Center Groningen
   Hanzeplein 1
   9713 GZ Groningen
   PO Box 30.001
   9700 RB Groningen
   The Netherlands

   (b) Size of the site (m^2): … m^2
   (i) actual release site (m^2): … m^2
   (ii) wider release site (m^2): … m^2

   Not relevant (see below).

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

   Not relevant (see below).

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

   Sections (b)-(d) above are not considered relevant. Environmental release of the Allovectin-7® product is not intended beyond the treatment of trial subjects. Subjects will be administered the product by intralesional injection into melanoma tumors. Treatment will be on an outpatient basis. Proximity to internationally recognised biotopes or protected areas (including drinking water reservoirs) cannot be specified under these circumstances as the movement of treated subjects will not be restricted. Flora and fauna including crops, livestock and migratory species which may potentially interact with Allovectin-7® or the

VCL-1005 pDNA under these circumstances include bacteria present on or in human subjects as detailed under B(8)(d) above.

4. Method and amount of release

(a) Quantities of GMOs to be released:

Subjects treated at clinical sites in the Netherlands will receive multiple injections of the Allovectin-7® product at weekly intervals in accordance with instructions provided in the LX01-315 clinical protocol.

The treatment course for Allovectin-7® is a minimum of two cycles. Each cycle will consist of a 1 mL direct intralesional injection of a 2-mg dose of Allovectin-7® to a single lesion once every week for six weeks followed by two weeks of observation and assessments. Subjects may receive additional cycles of Allovectin-7® treatment as long as their disease remains stable or continues to respond to treatment.

(b) Duration of the operation:

The duration of the clinical trial will not exceed ten years.

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

Safety containment measures for handling of the Allovectin-7® product will follow institutional guidelines. Subjects will not be isolated or subjected to containment following treatment.

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

The product will be administered to trial subjects at clinical sites in an enclosed facility at ambient temperature.

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

Potential environmental impacts of Allovectin-7® have not been assessed at clinical sites in the U.S. Based on the clinical and nonclinical experience reviewed under E(1)(d) above, Allovectin-7® poses no significant risk to human or animal health.

G. Interactions of the GMO with the environment and potential impact on the environment, if significantly different from the recipient or parent organism

1. Name of target organism (if applicable)

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

Human:

- **Kingdom: Animalia**
- **Phylum: Chordata**
- **Class: Mammalia**
- **Order: Primates**
- **Family: Hominidae**
- **Genus: Homo**
- **Species: H. sapiens**

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

Allovectin-7® is intended to augment the innate and adaptive immune responses toward melanoma. Direct intratumoral injection of Allovectin-7® has been shown to reduce both injected and distal untreated tumors by mobilizing the immune system against the melanoma cells, including those that have metastasized to other organs. As a pDNA/lipid complex encoding HLA-B7 and β-2 microglobulin, Allovectin-7® may accomplish this by the following mechanisms:

- **In HLA-B7 positive or negative recipients, enhanced HLA-B7 and β-2 microglobulin surface expression by transfected tumor cells could increase antigen presentation to tumor antigen-specific T cells resulting in both local and systemic antitumor effects. Loss or mutations in β-2 microglobulin have been reported as a possible mechanism for deficient MHC class I expression in tumor cells. An enhancement of an autologous T-cell response to tumor antigens may occur in both HLA-B7 negative and HLA-B7 positive recipients if the introduction of exogenous β-2 microglobulin from Allovectin-7® enhances or reconstitutes surface expression of endogenous non-HLA-B7 alleles. This could be an important mechanism given that melanoma often exhibits HLA down-regulation or elimination.[40, 41]**

- **In HLA-B7 negative recipients, an allogeneic T-cell response may be initiated by the introduction of a foreign HLA, similar to that observed in tissue transplant rejections. A subsequent T-cell/inflammatory-cell infiltration and eradication of HLA-B7 positive tumor cells possessing the foreign HLA-B7 could then catalyze**

an "antigenic spreading" from the alloantigen to tumor antigen specificities. In human biopsies in which HLA-B7 protein expression is observed following Allovectin-7® treatment, CD3+ tumor-infiltrating lymphocytes (primarily CD8+ T cells) as well as mature (CD80+ CD86+) dendritic cells have been identified.[13]

- **In any recipient, a pro-inflammatory response may ensue following intratumoral injection of the pDNA/lipid complex as demonstrated in animal tumor models,[42,43] and may be additive to both of the mechanisms described above. Leukocytes of the innate and/or adaptive arms of the immune response may be recruited to the tumor and participate in a localized direct antitumor response. Pro-inflammatory cytokines (e.g., interferons) can augment HLA expression and facilitate cross priming of antigens which increase the immunogenicity of tumor cells.**

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

Other potentially significant interactions with other organisms in the environment are not expected for the reasons given under A(3)(c), B(8)(d), and E(1)(d) above.

4. Is post-release selection such as increased competitiveness, increased invasiveness for the GMO likely to occur?
Yes (.) No (**x**) Not known (.)
Give details

See A(3)(c), B(8)(d), and E(1)(d) above.

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

The VCL-1005 pDNA component of Allovectin-7® could potentially disseminate into soils and wastewater through accidents in transport or improper handling and disposal at clinical sites. The possibility of dissemination and establishment in soil or wastewater ecosystems (e.g., uptake and replication in permissive bacteria) in these cases is considered remote based on the data and considerations provided above under A(3)(c), B(8)(d), and E(1)(d) above.

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

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

No significant harm to non-target organisms is anticipated.

7. Likelihood of genetic exchange in vivo

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

See G(7)(b) below.

(b) from other organisms to the GMO:

The likelihood of genetic exchange between VCL-1005 and bacteria present on or in treated human subjects is considered remote for the reasons stated under A(3)(c) and B(8)(d) above.

Because the RSV and encephalomyocarditis viruses are not normally present in humans,[44,45,46] the likelihood of genetic exchange between VCL-1005 and these viruses in treated human subjects is considered remote. The likelihood of homologous recombination between human retroviruses and picornaviruses and the RSV- or encephalomyocarditis virus-derived elements of the VCL-1005 pDNA is limited by the generally low sequence homology between human viruses and these elements the low likelihood that such viruses and VCL-1005 would be present in the same cell, and the fact that genetic recombination among these viruses usually occurs within RNA intermediates.[26]

(c) likely consequences of gene transfer:

In the case of homologous recombination between the virally derived elements of VCL-1005 and other viruses, the likely consequences of gene transfer are considered minimal because the RSV- and encephalomyocarditis virus-derived elements of the VCL-1005 pDNA are essentially unmodified from their natural forms. In theory, gene transfer of the various genetic elements of VCL-1005 poses no greater risk to the environment than that posed by the natural existence of the parental forms of these elements, which have been released into the environment for millenia as a consequence of the death and decomposition of their natural hosts.

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

   No studies of Allovectin-7® or the VCL-1005 pDNA have been performed in simulated natural environments.

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

   No significant interactions with biogeochemical processes are anticipated.

H. Information relating to monitoring

1. Methods for monitoring the GMOs
…

2. Methods for monitoring ecosystem effects
…

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

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

5. Duration of the monitoring
…

6. Frequency of the monitoring
…

Methods for monitoring include PCR assays utilizing primers specific for VCL-1005 pDNA sequences that have previously been developed for detection of the pDNA in mammalian tissue samples.[5] However, based on the nonclinical and clinical safety experience with Allovectin-7® described under E(1)(d) above, no monitoring is planned.

I. Information on post-release and waste treatment

1. Post-release treatment of the site

No post-release treatment of the site is planned. A post-injection clinical evaluation of trial subjects (vital signs and symptom-directed exam) will be performed after each administration. The subject may leave the clinic once the investigator deems the subject to be clinically stable. Periodic evaluations will be performed at scheduled visits throughout the duration of treatment. All subjects are required to have a follow-up safety assessment four weeks after their termination visit.

2. Post-release treatment of the GMOs

Disposal of used syringes, gloves or other items that may potentially contain residual amounts of medicinal product will be performed in accordance with institutional procedures of the clinical site.

3. (a) Type and amount of waste generated

The amount of medical waste generated on a daily basis at clinical sites will generally not exceed the contents of a few vials (< 20 mg pDNA).

3. (b) Treatment of waste

Treatment of medical waste will be performed in accordance with institutional procedures of the clinical site.

J. Information on emergency response plans

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

   Instructions on spill response procedures will be provided to clinical sites. Site personnel responsible for spill cleanup will be instructed to wear proper personal protective equipment (protective gloves, safety glasses, and clothing).

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

   Spilled liquids will be absorbed with common absorbent materials and placed in appropriate containers for disposal.

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

   Disposal of spilled liquids will be performed in accordance with institutional procedures. Disposal or sanitation of plants, animals, soils, etc. exposed to the product is not mandated due to the low risk of resultant harm.

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

   Because the medicinal product does not appear to pose a significant risk to human health or the environment, specific plans for protection have not been prepared.

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