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/07/010*
(c) Date of acknowledgement of notification	*March 15, 2006*
(d) Title of the project	*Study G-0034 A Phase III1 Randomized, Open-Label Study of Docetaxel in combination with CG1940/CG8711 Versus Docetaxel and Prednisone in Taxane-Naïve Patients with Metastatic Hormone-Refractory Prostate Cancer with Pain*
(e) Proposed period of release	*From 30/9/ 2006 until ~31/12/2009.*

2. Notifier

Name of institution or company:	*Christian H. Bangma, Principal Investigator* *Erasmus MC* *s-Gravendijkwal 230* *Afdeling Urologie* *Rotterdam 3015 CE* *The Netherlands* *Study Sponsor: Cell Genesys, Inc, S. San Francisco, CA USA*

3. GMO characterisation

(a) Indicate whether the GMO is a:

viroid (.)
RNA virus (.)
DNA virus (x)
bacterium (.)
fungus (.)
animal
- mammals (x)
- insect (.)
- fish (.)
- other animal (.)

specify phylum, class *GM- CSF secreting human allogeneic prostate cancer tumor cell lines*

(b) Identity of the GMO (genus and species)
NOTE: Some countries and regulatory agencies do not consider this product to be defined as a GMO according to the definition in Article 2 of Directive 2001/18/EC. (UK, BE, SE, PL)

Allogeneic, irradiated prostate cancer cell lines PC3 and LNCaP transduced with replication defective rAAV- GM- CSF. After transduction cells were cloned and selected for production of GM-CSF and negative testing for AAV and helper viruses. Subsequently, cells were banked, and clinical lots were generated for clinical use..

The human GM- CSF gene is ex vivo transferred into the prostate cancer cells using a replication deficient recombinant adeno- associated viral vector. (Family Parvovirideae, genus Dependovirus, Species adeno- associated virus 2).

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

The cell lines are genetically stable. At an early stage of culturing cell clones are tested by PCR for the presence of GM-CSF secretion, wildtype AAV and helper virus. No wildtype AAV or helper virus was detected. After expansion of the cells in culture, cells are tested again for the production of GM- CSF (Lots must meet all release criteria).

Molecular analysis showing the same DNA banding pattern from the Master Cell Bank through clinical lot production also demonstrates genetic stability of the cells.

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

Yes (x) ***NOTE: Some countries and regulatory agencies do not consider this product to be a GMO according to the definition in Article 2 of Directive 2001/18/EC***
No (.)

If yes, insert the country code(s) … *UK (study ongoing), FR (study ongoing), BE (study ongoing), SE (study ongoing), IT (study ongoing), GE (study ongoing), PL (study ongoing), NL (study ongoing)*

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

Yes (.) No (x) *Summary information has been supplied when requested.*

If yes: - Member State of notification … - Notification number B/../../…

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 …
- Notification number B/../../…

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

The potential environmental impact of the release of CG1940 and CG8711 is essentially nil. The genetic manipulation of CG1940 and CG8711 to allow expression of a therapeutic transgene resulted in a stably integrated genetic construct that is incapable of being transferred to any other organism; it is a permanent part of the genome of the cell lines, and does not change during production or after administration of the investigational product. The replication defective, recombinant AAV vector was derived by replacement of the rep and cap genes with the GM-CSF transgene and the genetic control elements needed for its expression. The only viral DNA sequences retained by the rAAV vector are the viral ITRs, flanking the heterologous gene which is integrated into the genome of the cell lines. No viral protein is expressed by these ITRs. Therefore, excretion of the vector in the environment will cannot occur after administration of CG1940 and CG8711 to patients with prostate cancer.
Also, CG1940/ CG8711 have both been irradiated and are no longer capable of cellular replication either, even in the precise temperature controlled, sterile, CO_2 saturated, cell culture environment and culture media used for growth. These culture conditions could not be recreated in the general environment; even non-irradiated cells cannot grow in the general environment.
CG1940/ CG8711 are treated as standard medical waste and disposed of according to local and national regulations for discarding medical waste, which should be considered as an additional control against environmental release.

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

other, specify *two human prostate tumor cell lines, PC- 3 (CG1940) and LNCaP (CG8711) which were obtained from the American Type Culture Collection (ATCC). PC- 3 was originally isolated from a bone metastasis and LNCaP from a prostate cancer lymph node metastasis.*

2. Name

(i) order and/ or higher taxon (for animals)	*Primates*
(ii) genus	*Homo*
(iii) species	*Sapiens*
(iv) subspecies	*NA*
(v) strain	*NA*
(vi) pathovar (biotype, ecotype, race, etc.)	*Caucasian, site prostate*
(vii) common name	*NA*

3. Geographical distribution of the organism: *Not relevant (NR)*

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

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

(i) Yes (.)

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

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

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

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

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

Yes (.) No (x)

4. Natural habitat of the organism: *Not relevant (NR)*

(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 …	

(b) If the organism is an animal: natural habitat or usual agroecosystem:
Not relevant

5. (a) Detection techniques
Not relevant

(b) Identification techniques
Not relevant

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

If yes, specify *Tumor cells Biosafety level 1 (specified on www.atcc org ; ATCC numbers CRL- 1435 (PC- 3) and CRL- 1740 (LNCaP) and the AAV vector is also Biosafety Level 1*.

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

No pathological, ecological and physiological traits are present.

8. Information concerning reproduction
Not relevant; cells are lethally irradiated before administered to patients

(a) Generation time in natural ecosystems:
NA
(b) Generation time in the ecosystem where the release will take place:
NA
(c) Way of reproduction: Sexual *NA*. Asexual *NA*

(c) Factors affecting reproduction:
NA

9. Survivability; *Not relevant for this product since no viral replication is possible in these cells.*

(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 …	

(b) relevant factors affecting survivability: NA 10. (a) Ways of dissemination
NA

(b) Factors affecting dissemination
NA

11. Previous genetic modifications of the recipient or parental organism already notified for release in the country where the notification is made (give notification numbers) …, B/NL/03/08

C. Information relating to the genetic modification

1. Type of the genetic modification

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

2. Intended outcome of the genetic modification

To insert sequences coding for the secretion of human genomic granulocyte-macrophage colony stimulating factor (hgGM- CS) leading to expression of GM-CSF from the transduced cells as a potentially therapeutic local immune adjuvant.

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

Yes (X) No (.)

If no, go straight to question 5.

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

Yes (X) No (.)

If no, go straight to question 5.

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

(a) Type of vector

plasmid	(.)
bacteriophage	(.)
virus	(x)
cosmid	(.)
transposable element	(.)
other, specify	*rAAV- MD2- hgGM- CSF vector*

(b) Identity of the vector

rAAV- MD2- hgGM- CSF vector: Recombinant replication defective adeno associated virus (rAAV): family Parvovirideae, genus Dependovirus, Species adeno-associated virus 2 (c) Host range of the vector human

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

Yes (.) No (X)

antibiotic resistance	(.)
other, specify	…

Indication of which antibiotic resistance gene is inserted

…

(e) Constituent fragments of the vector

Replication defective, recombinant AAV vectors are derived by replacement of the rep and cap genes with a heterologous transgene and the genetic control elements needed for its expression. The only viral DNA sequences retained by a rAAV vector are the viral ITRs, flanking the heterologous gene.

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

(i) transformation	(.)
(ii) electroporation	(.)
(iii) macroinjection	(.)
(iv) microinjection	(.)
(v) infection	(.)
(vi) other, specify	*ex vivo transduction*

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

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

6. Composition of the insert

(a) Composition of the insert

hg GM- CSF gene

(b) Source of each constituent part of the insert

The hgGM- CSF gene was amplified by polymerase chain reation (PCR) from a bacterial artificial chromosome (BAC). The amplified hgGM- CSF gene was non-directionally cloned into the EcoRI site of the vector, plox III- 4 CMV. Resulting clones were screened by restriction endonuclease digestion for the proper orientation of the insert, relative to the promoter. The resultant plasmid pSSV9- MD2- hgGGM-CSF contains the hgGM- CSF gene between the ß- globin intron and the ß- globin polyadenylation site. This plasmid was used to make the vector.

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

Secretion of GM- CSF will hopefully stimulate an immune response in Hormone Refractory Prostate Cancer (HRPC) patients enrolled in the study.

(d) Location of the insert in the host organism

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

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

Yes (.) No (x)

If yes, specify …

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

1. Indicate whether it is a:

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

other, specify *the human genomic hgGM- CSF gene was derived from plox III- 4 CMV/ hgGM- CSF clone # 5 by EcoRI restriction endonuclease digestion*

2. Complete name

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

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

(b) to which of the following organisms:

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

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

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

4. Is the donor organism classified under existing Community rules relating to the protection of human health and the environment, such as Directive 90/ 679/ EEC on the protection of workers from risks to exposure to biological agents at work?
Yes (.) No (x)
If yes, specify …

5. Do the donor and recipient organism exchange genetic material naturally?
Yes (.) No (x) Not known (.)

E. Information relating to the genetically modified organism

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

(a) is the GMO different from the recipient as far as survivability is concerned?
Yes (x) No (.) Not known (.)
Specify *irradiated cells will die within 1 week after injection into the skin and be eliminated by the immune system*

(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 *cells are not able to divide or reproduce due to lethal irradiation*

(c) is the GMO in any way different from the recipient as far as dissemination is concerned?
Yes (x) No (.) Not known ()
Specify *The prostate cancer cells are not able to disseminate due to irradiation.*

(d) is the GMO in any way different from the recipient as far as pathogenicity is concerned?
Yes (x) No (.) Not known (.)
Specify *CG1940 / CG8711 cells are non pathogenic.*

2. Genetic stability of the genetically modified organism
CG1940 and CG8711 cells are genetically stable, however; they will only survive maximally 1 week after injection due to lethal irradiation of the cells. The cells are allogeneic and produce GM- CSF and will be destroyed by the body's immune system.

3. Is the GMO significantly pathogenic or harmful in any way (including its extracellular products), either living or dead?
Yes (.) No (x) Unknown (.)

(a) to which of the following organisms?

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

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

4. Description of identification and detection methods

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

There is no foreseen nor expected effect on the environment. Irradiated vaccine cells are injected intradermally and locally degraded by the immune system. The cells cannot propagate nor be transmitted to persons other than the patient and thus pose no public health risk. Therefore no techniques can be described here.

(b) Techniques used to identify the GMO

Vector sequences in the vaccine cells can be detected by standard molecular techniques such as Southern hybridization or polymerase chain reaction. Once injected, the vaccine cells persist for approx. four days to a week at the immunization site and are then eliminated by the immune system. During this time presence of the cells has been detected in some patients by a temporary modest increase of serum GM- CSF levels.

F. Information relating to the release

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

The CG1940 / CG8711 cells are intended for the treatment of Hormone Refractory Prostate Cancer (HRPC) patients. It is hoped that the expression of hgGM- CSF by CG1940 / CG8711 cells will provoke an immune response.

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

Yes (.) No (x)

If yes, specify …

3. Information concerning the release and the surrounding area

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

Administrative region:

- *Rotterdam, NL*

(b) Size of the site (m2): …not applicable m2

(i) actual release site (m2): …not applicable m2

(ii) wider release site (m2): … not applicable m2

(c) Proximity to internationally recognised biotopes or protected areas (including drinking water reservoirs), which could be affected:
Not relevant

(d) Flora and fauna including crops, livestock and migratory species which may potentially interact with the GMO
Not relevant

4. Method and amount of release

(a) Quantities of GMOs to be released:
Prime dose (visit 1) of 5 x 10^8 cells consisting of equal amounts of CG1940 and CG8711 per dose and boost doses (visits 2- 10) of 3 x 10^8 cells consisting of equal amounts of CG1940 and CG8711 per dose. Maintenance doses of 3 x 10^8 cells consisting of equal amounts of CG1940 and CG8711 per dose every 28 days until death, or until new systemic therapy is required. (Experimental arm only eligible for maintenance doses; the prime and boost doses in this study are administered with chemotherapy, 2 days after each cycle of chemotherapy.)

(b) Duration of the operation:
27 weeks (10 vaccinations every 21 days and maintenance vaccination every 28 days)

(c) Methods and procedures to avoid and/ or minimise the spread of the GMOs beyond the site of the release
None. The tumor cells have been irradiated to arrest growth. It is expected that the tumor cells are not present any more 4 days to a week after the administration. Also, CG1940/ CG8711 has been irradiated and is no longer capable of cellular replication, even in the precise temperature controlled, sterile, CO_2 saturated, cell culture environment and culture media used for growth. These culture conditions could not be recreated in the general environment; even non-irradiated cells cannot grow or spread in the general environment.
CG1940/ CG8711 are treated as standard medical waste and disposed of according to local and national regulations for discarding medical waste, which should be considered as an additional control against environmental spread and release.

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

In the NL there is an average sea- climate.

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.
No potential environmental and human health impacts are present.

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	Homo
(iv)	species	H. Sapiens
(v)	subspecies	…
(vi)	strain	…
(vii)	cultivar/ breeding line	…
(viii)	pathovar	…
(ix)	common name	Man

2. Anticipated mechanism and result of interaction between the released GMOs and the target organism (if applicable)
Expression of GM- CSF from the vaccine cells at the immunization site is expected to attract cells of the immune system to that site and elicit an immune response directed towards tumor antigens expressed on the cells.

3. Any other potentially significant interactions with other organisms in the environment
None

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

5. Types of ecosystems to which the GMO could be disseminated from the site of release and in which it could become established
Not relevant. Once injected, the vaccine cells persist for approx. 4 days to a week at the immunization site t and are then eliminated by the immune system of the patient.

The possibility that the tumor cell lines would grow outside of this controlled environment is nil. After transduction and characterization, the tumor cell lines CG1940/ CG8711 are irradiated and are no longer capable of cellular replication either, even in the precise temperature controlled, sterile, CO_2 saturated, cell culture environment and culture media used for growth. These culture conditions could not

be recreated in the general environment; even non-irradiated cells cannot grow in the general environment.
CG1940/ CG8711 are treated as standard medical waste and disposed of according to local and national regulations for discarding medical waste, which should be considered as an additional control against environmental release.

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)	*Not applicable*
(ii) family name for plants	…
(iii) genus	…
(iv) species	…
(v) subspecies	…
(vi) strain	…
(vii) cultivar/ breeding line	…
(viii) pathovar	…
(ix) common name	…

7. Likelihood of genetic exchange in vivo

(a) from the GMO to other organisms in the release ecosystem:
No exchange is expected

(b) from other organisms to the GMO:
No exchange is expected

(c) likely consequences of gene transfer:
No exchange is expected

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.):
A survival advantage has been observed in some patients in phase II studies in which the CG1940/CG8711 cell lines were used alone, without chemotherapy. The results have been presented at the American Society of Clinical Oncology and other public scientific and medical meetings worldwide.

9. Possible environmentally significant interactions with biogeochemical processes (if different from the recipient or parental organism)
Not applicable

H. Information relating to monitoring

1. Methods for monitoring the GMOs
Not relevant

2. Methods for monitoring ecosystem effects
Not relevant

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

Not relevant …

4. Size of the monitoring area (m2)

Not relevant … m2

5. Duration of the monitoring

Not relevant …

6. Frequency of the monitoring

Not relevant …

I. Information on post- release and waste treatment

1. Post- release treatment of the site

No specific treatment is necessary, since risk is non existent and cells are injected into the dermis of the patient and once injected, the cells persist for approx. 4 days to a week at the immunization site and are then eliminated by the immune system.

2. Post- release treatment of the GMOs

All used vials, all unused vials and all material that comes in contact with CG1940 and CG8711 cells are treated as standard medical waste and disposed of per local and national guidelines.

3. (a) Type and amount of waste generated

Cell waste from empty 1 mL vials of CG1940/CG8711 and used syringes and needles from the injection process will become standard medical waste. For each treatment a maximum of 16 used vials of CG1940/CG8711 and approximately the same number of syringes and needles will be generated.

3. (b) Treatment of waste

Cell waste from vials and syringes used in the injection procedure will be treated by standard biosafety practices, i.e., placed into a biohazard bag or sharps container prior to medical waste disposal as required by law.

J. Information on emergency response plans

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

Not applicable …

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

Not applicable …

3. Methods for disposal or sanitation of plants, animals, soils, etc. that could be exposed during or after the spread
   Not applicable …

4. Plans for protecting human health and the environment in the event of an undesirable effect
   Not applicable …