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	**Slovenia**
(b)	Notification number	**B/SI/18/01**
(c)	Date of acknowledgement of notification	**17. 12. 2018**
(d)	Title of the project	**Evaluation of the GluSense Glyde CGM safety and performance in subjects with Type 1 Diabetes Mellitus**
(e)	Proposed period of release	**From May 2019 until 31/12/2020**

2. Notifier

Name of institution or company: **Adax International d.o.o., Bravničarjeva 13, 1000 Ljubljana, Slovenia**

3. GMO characterisation

(a) Indicate whether the GMO is a:

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

specify phylum, class **Chordate, Mammals**

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

Genetically engineered cell line (C710/16) derived from the human retina (*Homo sapiens*)

(c) Genetic stability – according to Annex IIIa, II, A(10)
The genetically modified cell line C710/16 has been assessed in accordance with the applicable guidance for evaluation of genetic stability (ICH Q5D Guideline). The number of copies and the insertion sites of the biosensor gene are stable over all important cultivation periods.

4. Is the same GMO release planned elsewhere in the Community (in conformity with Article 6(1)), by the same notifier?
Yes (.) No **(x)**
If yes, insert the country code(s) …

5. Has the same GMO been notified for release elsewhere in the Community by the same notifier?
Yes (.) No **(x)**
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 environmental impact of genetically modified cell line C710/16 is not expected as the release of the cells is limited to patient administration in hospital settings. The cell line is embedded and contained in the Glyde implant which is designed for long term use. The modified cell line is not capable to survive in environment in general. The accidental release of the cell line from the implant into the human body fluid will be a subject of allogenic cellular immune response against non-cell tissue. The potential risk to human health and the environment of cell line C710/16 is therefore considered to be minimal or negligible.

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

1. Recipient or parental organism characterisation:

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

(select one only)

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

other, specify

Chordate, Mammals. The ARPE-19 cell line (NTC-200) is a spontaneously immortalized cell line derived from the human retina pigmented epithelium of the eye. The cell line is obtained from the ATCC and is widely used in research laboratories.

2. Name

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

3. Geographical distribution of the organism

Not applicable. Laboratory derivation.

(a) Indigenous to, or otherwise established in, the country where the notification is made:

Yes (.) No (.) 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 (.)

(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 applicable. It is artificially developed and does not have natural habitat.

(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 parental cell line can only live in laboratory cell culture**

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

5. (a) Detection techniques

Antibody assisted flow cytometry of ARPE-19 specific markers: CRALBP (retin-aldehyde binding protein 1); RPE-65 (retinal pigmented epithelium specific 65kDa protein).

(b) Identification techniques

Antibody assisted flow cytometry of ARPE-19 specific markers: CRALBP (retin-aldehyde binding protein 1); RPE-65 (retinal pigmented epithelium specific 65kDa protein).

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:
Not applicable.

(b) Generation time in the ecosystem where the release will take place:
Not applicable.

(c) Way of reproduction: Sexual .. Asexual **(x)**
Not applicable.

(d) Factors affecting reproduction:
Not applicable.

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	

Not applicable.

(b) relevant factors affecting survivability:
The survival of the ARPE-19 cells requires a combination of complex culture media and incubation at controlled temperature and CO_2 levels. The environmental conditions outside the host (body) are substantially different and highly variable, and will not support the cells' survival (temperature, pH, UV and different biophysical and bio-chemical conditions).

10. (a) Ways of dissemination

The host cells can only be transmitted between individuals through active transfer by injection.

(b) Factors affecting dissemination

Dissemination of the cell in the environment is negligible due to containment and fast inactivation in the body. Therefore, lack of a natural exit route from, or entry route into the body which would allow dissemination into environment (e.g. respiratory, digestive, urinary, reproductive tract), is negligible.

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)

Not applicable.

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

The cell expression and secretion of a glucose binding fluorescent biosensor molecule, contained within a subcutaneously implanted medical device.

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

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

If no, go straight to question 5.

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

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

If no, go straight to question 5.

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

(a) Type of vector

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

(b) Identity of the vector

In the process of preparing of GMO, two vectors were used:

(i) the DNA plasmid D320 incorporates a transposon carrying the biosensor expression and selection cassette flanked by inverted terminal repeat (ITR) sequences at each end; and

(ii) the DNA plasmid D319 encodes and expresses the Sleeping Beauty transposase enzyme gene that facilitates the integration of the D320 transposon into the host cell genome. This mechanism inserts only the transposon sequence bearing the biosensor expression and selection cassette flanked by the ITRs into the host cell genome. None of sequences of the D319 plasmid and none of the sequences of D320 plasmid other than the transposon with its ITRs are transferred to the host genome; these non-transferred sequences are not propagated at cell division and are therefore eliminated from the population.

(c) Host range of the vector

E. coli **(for the production and reproduction of both DNA plasmids)**
Mammalian cells (for the transposition mechanism)

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

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

antibiotic resistance (**x**)
other, specify …

Indication of which antibiotic resistance gene is inserted
Puromycin (puromycin N-acetil-transferase)

(e) Constituent fragments of the vector

Plasmid D319:
• Bacterial origin of replication
• Chloramphenicol resistance cassette
• Sleeping Beauty transposase expression cassette
Plasmid D320:
• Bacterial origin of replication
• Kanamycin resistance cassette
• Biosensor/puromycin resistance expression cassette (with LTRs)

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

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

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

(i) transformation (.)

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

6. Composition of the insert

(a) Composition of the insert

The genetic insert transposed into the recipient cell genome comprises the two flanking ITRs, the expression cassette for puromycin resistance and the expression cassette for the glucose sensor molecule. The full sequence of the 7,959 base pair insert is known. As well as the required expressed gene coding sequences, the expression cassettes each contain transcription, translation and post-translation processing control elements which are in common use for the expression of proteins in mammalian cells in laboratory and clinical research. These elements are derived from a variety of mammalian, viral and fungal sources, none of which are pathogenic.

(b) Source of each constituent part of the insert

Table 1. Source and function of the constituent parts of the genetic modification

Constituent	***Source***	***Function***
ITR-R, ITR-L	*XX*	*Inverted terminal repeat recognition sequences enabling insertion of the cassette by the Sleeping Beauty artificial DNA transposon system*
Puromycin resistance expression cassette		
P_PGK	*Mouse*	*Constitutive phosphoglycerate kinase I transcription promoter*
Puromycin-r	*Streptomyces alboniger*	*M_Puromycin-r- resistance gene encoding a puromycin N-acetyl-transferase*
Poly A	*Human*	*Beta-globin gene polyA signal for transcription termination.*
Biosensor expression cassette		
CAG promoter	*Cytomegalovirus, chicken, rabbit*	*Hybrid source expression (transcription and translation) control element comprising: CMV early enhancer; chicken beta-actin gene first exon and the first intron; and rabbit beta-globin gene splice acceptor site*
SP-signal peptide	*Human*	*Consensus model protein secretion signal peptide*
Biosensor		***Glucose biosensor coding sequence***
Human scaffold-attachment region (SAR)	*Human*	*Anchors the chromosomal DNA to the nuclear matrix. SARs enhance stable expression of transcribed genes.*
HRPE	*Hepatitis B virus*	*A cis-acting post-transcriptional regulatory element which facilitates the cytoplasmic localization of intronless*

		transcripts and contributes to high gene expression
Poly A	*Rabbit*	*Beta-globin gene polyA signal for transcription termination.*

(c) Intended function of each constituent part of the insert in the GMO
See Table 1 in (b).

(c) Location of the insert in the host organism

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

(d) Does the insert contain parts whose product or functions 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 (**x**)
bacterium (.)
fungus (**x**)
animal
- mammals **(x)**
- insect (.)
- fish (.)
- other animal (.)
(specify phylum, class) **See Table 1 in C 6(b)**

other, specify

2. Complete name
See Table 1 in C 6(b)

(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:

(a) to which of the following organisms:

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

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

(b) is the GMO in any way different from the recipient as far as mode and/or rate of reproduction is concerned?
Yes (.) No (**x**) Unknown (.)
Specify …

(b) is the GMO in any way different from the recipient as far as dissemination is concerned?
Yes (.) No (**x**) Not known (.)
Specify …

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

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

Specify …

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 **N/A**

(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.…

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

Not applicable

4. Description of identification and detection methods

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

Polymerase Chain Reaction (PCR) assays of characteristic genome sequence; visualization under a fluorescence microscope using GFP and RFP filters; resistance to puromycin.

(b) Techniques used to identify the GMO

Polymerase Chain Reaction (PCR) assays of characteristic genome sequence and visualization under a fluorescence microscope using GFP and RFP filters. The GMO can be distinguished from the parent cell line by resistance to puromycin and the expression of the biosensor molecule.

F. Information relating to the release

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

The clinical trial will evaluate the safety of the Glyde implantable medical device containing the GMO cells for continuous monitoring of tissue glucose levels in patients with type 1 diabetes. The GMO cells are contained within a sealed compartment of Glyde implant. They are separated from the host tissue by a selective permeable barrier which allows for bi-directional diffusion of nutrients, waste products and glucose, while preventing the egress of cells and biosensor protein molecules into the surrounding tissue of the host. The GMO cells within the implant constitutively produce the biosensor molecule which enables accurate and long term continuous monitoring of tissue glucose levels. A single device will be subcutaneously implanted into one arm of each study participant where it will remain for no longer than 6 months before removal and return to the sponsor's laboratories for examination. No environmental benefit is expected.

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

Not relevant. There is no natural habitat or ecosystem for the GM cell lines C710/16.

3. Information concerning the release and the surrounding area

(a) Geographical location (administrative region and where appropriate grid reference):
University medical Center Ljubljana, University Children's Hospital, Bohoričeva 20, Ljubljana, Slovenia.

(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 applicable.

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

(f) Flora and fauna including crops, livestock and migratory species which may potentially interact with the GMO
Not applicable.

4. Method and amount of release

(a) Quantities of GMOs to be released:
Each implanted device will contain up to 20,000 GMO cells, 1 device will be implanted.

(b) Duration of the operation:
Each device will remain implanted for up to 6 months before removal and transfer to the sponsor's laboratory.

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

The GMO cells are contained in and confined within the device by encapsulation inside in a semi-permeable membrane which is mechanically protected by a surrounding PTFE mesh. At the site the receipt, handling, storage, dispensing, implantation, explantation, packaging and return of the devices to the sponsor's laboratories and disposal of the devices and potentially contaminated clinical waste will be undertaken only by trained individuals according to written protocols and procedures to ensure physical and biological containment. Institutional standard cleaning and disinfection procedures will ensure sanitization of the clinic treatment room environment. A continuous chain of custody monitoring system ensures tracking of each device before, during and after the proposed release.

5. Short description of average environmental conditions (weather, temperature, etc.)
Hospital treatment room environment.

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.
None. This is the first evaluation of this GMO in a clinical trial.

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)	**Mammalia,**
(ii)	family name for plants	…
(iii)	genus	***Homo***
(iv)	species	***sapiens***
(v)	subspecies	**…**
(vi)	strain	…
(vii)	cultivar/breeding line	…
(viii)	pathovar	…
(ix)	common name	**Human (Type 1 diabetes patient)**

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

The device is designed and manufactured to ensure that the GMO cells and the biosensor protein molecules they produce remain inside the cell compartment. The selectively permeable membrane sealing the compartment retains the cells and the intact biosensor protein molecule (~100KDa). It is possible that the small components and degradation products of lysed senescent cells and fragments of degraded biosensor molecules may diffuse out of the membrane and come into contact with the host tissues outside the device. This could cause a local inflammatory reaction. In two animal models only a minor degree of foreign body response was observed around the implant, attributed to the device materials and not to the GMO cell components. Any GMO specific response in the patient is likely to be minimal in humans since the GMO is derived from a human cell.

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 A7 in B 8-10.

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 applicable.

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	…

(j)	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	…

Not applicable.

7. Likelihood of genetic exchange in vivo

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

(b) from other organisms to the GMO:

(c) likely consequences of gene transfer:

The likelihood of genetic exchange in vivo is considered negligible.

8. Give references to relevant results (if available) from studies of the behaviour and characteristics of the GMO and its ecological impact carried out in stimulated natural environments (e.g. microcosms, etc.):
No such studies have been conducted.

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
GMO cells are contained and sealed in capsule of the implant and likelihood of its release into the environment is negligible. Therefore, no direct monitoring of the GMO is planned during the trial, other than for the intended function of tissue glucose level measurement which dependent on the viability of the GMO within the device. Patients receiving the implanted devise will be closely monitored for general safety parameters adverse events.

2. Methods for monitoring ecosystem effects
No monitoring of ecosystems is planned.

3. Methods for detecting transfer of the donated genetic material from the GMO to other organisms
No monitoring of other organisms is planned.

4. Size of the monitoring area (m^2)
… m^2
Not applicable.

5. Duration of the monitoring
Trial participants will be monitored for up to 6 months while they have the device implanted and for a further up to 1 month after the device is removed. In case of any mechanical failure in the implant that may expose the GMO to the host tissue, the follow up duration will be extended to 3 month on a monthly visiting basis.

6. Frequency of the monitoring
Patients will be monitored for safety effects and device function at least once every one month after implantation and up to 1 month after the device is removed.

I. Information on post-release and waste treatment

1. Post-release treatment of the site
No specific procedures are required for post-release in addition to the standard of institutional cleaning, hygiene and clinical waste management procedures. All working surfaces that came into contact with the device (before implantation or after removal from the patient) will be disinfected according to institutional practice.

2. Post-release treatment of the GMOs
No post-release treatment of the GMO is necessary.

3. (a) Type and amount of waste generated

Clinical waste includes items such as the primary packaging of the device, storage and cleaning agents and materials used to collect biological samples (e.g. blood, biopsies etc.). The total amount of waste per patient is estimated to be less than 1 kilogram. Surgical blades for single use; sewing material, surgical swabs and wound care materials.

(b) Surgical and Biological waste:
Disposable surgical blades; suture material, surgical swabs and wound dressings.

(c) Treatment of waste
Sharps such as needles will be disposed of in adequate sharp containers and incinerated. Disposables such as syringes, tubing, catheters and surgery waste (gloves, compresses) will be treated as and disposed of as GMO waste. All the surgical materials (surgery tools, linens) will be collected and autoclaved before washing or will be treated as and disposed of as GMO waste. All non-disposable surgical equipment will be cleaned using a chemical disinfectant with proven virucidal activity (e.g. hypochlorite solution. 70% ethanol) and subsequently treated according to standard practice of the institution.

J. Information on emergency response plans

1. Methods and procedures for controlling the dissemination of the GMO(s) in case of unexpected spread
In case of spillage, the affected area will be swabbed with absorbent material, and decontaminated using appropriate disinfectants. A spill kit will be available at all times during the device preparation, implantation and removal procedures. Detailed descriptions of the handling of the device, storage, administration and removal procedures are given in the Study Manual provided to the site during the site initiation and training visit (prior to starting the study).

2. Methods for removal of the GMO(s) of the areas potentially affected
Standard operating procedures for performing biologic waste decontamination apply.

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 other than emergency response in case of accidental injection of medical personnel, this is disinfection of injection site and follow up for symptoms related to inflammatory or immune reaction.