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	SE
(b)	Notification number	B/../../….
(c)	Date of acknowledgement of notification	../../….
(d)	Title of the project	**Clinical Study IMDZ-04-1702:** A Phase 3, Randomized, Double-blind, Placebo-controlled Study to Determine the Efficacy and Safety of CMB305 in Unresectable Locally-advanced or Metastatic NY-ESO-1+ Synovial Sarcoma Patients Following First-line Systemic Anti-cancer Therapy
(e)	Proposed period of release	From 11/12/2018 until 11/06/2023

2. Notifier

Name of institution or company: Immune Design Corp.

3. GMO characterisation

(a) Indicate whether the GMO is a:

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

specify phylum, class

Family: Retroviridae
Subfamily: Orthoretrovirinae

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

Genus: Lentivirus
Species: Human immunodeficiency virus 1

LV305 is a purified DC-tropic, replication-incompetent, integration-deficient, third-generation lentivirus vector. LV305 will be used with G305 together in a prime-boost regimen known as CMB305:

- **LV305:** A lentiviral vector derived from Immune Design's ZVEX platform that encodes the unaltered human form of the NY-ESO-1 cancer testes antigen and is designed to prime the immune system by targeting RNA antigen delivery to dendritic cells in vivo.
- **G305:** an immune system "boost" component, which is a recombinant NY-ESO-1 protein admixed with the adjuvant Glucopyranosyl lipid A stable emulsion (GLA-SE), an agonist of toll-like receptor 4. Please note that as G305 is not a GMO, it is not discussed in this document.

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

Genetic stability of LV305 is verified by sequencing of the final cloned plasmids.

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) AT, BE, DE, DK, ES, FR, GB, IT, SE
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 ES
   - Notification number B/ES/18/17

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 (x) No (.)
   If yes:
   - Member State of notification …

   United States
   - Notification number B/../../…

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

   The potential environment impact of LV305 is minimal. LV305 will be administered at licensed healthcare facilities where there are procedures in place for administration of GMO vaccines, collection and processing of blood and serum samples, and all equipment and staff

required for the clinical evaluation of study subjects under containment level 2 (Annex V of Directive 2000/54/EC). It is not anticipated that the study vaccine or any waste associated with study procedures will affect the surrounding environment.

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

other, specify …

2. Name

(i)	order and/or higher taxon (for animals)	NA
(ii)	genus	Lentivirus
(iii)	species	Human immunodeficiency virus 1
(iv)	subspecies	NA
(v)	strain	NA
(vi)	pathovar (biotype, ecotype, race, etc.)	NA
(vii)	common name	ZVex2.0

3. Geographical distribution of the organism

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

(b) Indigenous to, or otherwise established in, other EC countries:
(i) Yes No (x)

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

Atlantic ..
Meditteranean ..

Boreal ..
Alpine ..
Continental ..
Macronesian ..

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

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

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

4. Natural habitat of the organism

(a) If the organism is a microorganism

water (.)
soil, free-living (.)
soil in association with plant-root systems (.)
in association with plant leaf/stem systems (.)
other, specify Third generation lentiviruses such as ZVex2.0 do not occur in nature and are engineered in the lab setting.

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

5. (a) Detection techniques
Test methods of LV305 include an ELISA for Vpx Content. Quantitative PCR (qPCR) methods are used to detect persistence of LV305 in human PBMCs from subjects involved in the clinical trial and it would also detect ZVex2.0 vector.

(b) Identification techniques
same as (a)

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 Per 2000/54/ EC, ZVex2.0 is a Risk Group 1 biological agent as it is unlikely to cause human disease.

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. ZVex2.0 does not replicate so it's lifecycle is not relevant.

(b) Generation time in the ecosystem where the release will take place:
Not applicable. ZVex2.0 does not exist in nature as it is made in the lab.

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

(d) Factors affecting reproduction:
Third generation lentiviruses such as ZVex2.0 are engineered to undergo a single round of infection and only express the transgene of interest. They are not designed to replicate.

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. Third generation lentiviruses such as ZVex2.0 do not replicate and are not able to form survival structures.

(b) relevant factors affecting survivability:

Third generation lentiviral vectors such as ZVex2.0 have reduced survivability since viral ORFs are separated from the transfer vector resulting in virions that can only undergo a single round of infection and express the transgene of interest. They are replication-incompetent and integration-deficient. In addition, ZVex2.0 has been engineered to specifically target dendritic cells and requires dermal penetration to initiate dendritic cell transduction.

10. (a) Ways of dissemination

Not applicable.

(b) Factors affecting dissemination

Not applicable.

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	(.)
(iii)	base substitution	(.)
(iv)	cell fusion	(.)
(v)	others, specify …	

2. Intended outcome of the genetic modification

LV305 is a dendritic cell (DC)-tropic vector that has been designed to transduce patient DCs to express and process NY-ESO-1 antigen and promote T-cell activation and proliferation. Five plasmids are used to generate the lentivirus:

A) NY-ESO-1 encoding plasmid
B) Gag/Pol packaging plasmid
C) Rev accessory protein encoding plasmid
D) Vpx accessory protein encoding plasmid
E) SINVar1 envelope encoding plasmid

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 (.) No (x)

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

6. Composition of the insert

(a) Composition of the insert
Unaltered human sequence of NY-ESO-1 (Genbank NM 139250.1)

(b) Source of each constituent part of the insert

The donor sequence is the unaltered human sequence for NY-ESO-1 (Genbank NM_139250.1) and was synthesized and cloned into a plasmid vector.

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

NY-ESO-1 is a 180-amino acid protein of 18 kDa, with a glycine-rich N-terminal region and an extremely hydrophobic C-terminal region. There is, however, no evidence of membrane association at the cellular level and the function of NY-ESO-1 is unknown. There is also no obvious predicted functional domain or binding domain to give clues on partners in function. By immunohistochemistry and RT-PCR, NY-ESO-1 expression is only found in early spermatogonia and is gradually lost with sperm cell differentiation.

NY-ESO-1 expression is regulated epigenetically via demethylation of its promoter, and is seen in approximately one-quarter to one-third of melanoma, lung, esophageal, liver, gastric, prostate, ovarian, or bladder cancers and >80% in certain subtypes of sarcoma, including synovial sarcoma and myxoid round cell liposarcoma.

A retrospective review demonstrated that NY-ESO-1 expression in soft tissue sarcomas was associated with decreased progression free survival; the negative prognostic impact of NY-ESO-1 expression has been observed in other tumor types as well, including ovarian cancer (Komarov 2017). Therefore, immunotherapy approaches directed against NY-ESO-1 have the potential to be applicable to many cancer indications, and yet be tumor-specific, due to its restricted expression in normal host tissues.

(e) Location of the insert in the host organism

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

(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 (.)
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)	…Primates
(ii)	family name for plants	…
(iii)	genus	…*Homo*
(iv)	species	…*sapiens*
(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 (.) 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 …

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

(d) 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

LV305 has been designed as a non-replicating next-generation lentiviral vector wherein the split genome ensures that the vectors are only capable of a single round of infection (Dull, 1998). It is possible, however, that one particular recombination, known as a psi-gag recombinant, can occur in standard split genome vectors between the *psi* packaging sequence of the transfer genome and the gag sequence of the *gag* gene on the *gag/pol* sequences (Sastry, 2003). However, LV305 has been designed to further minimize this homology between *psi* and *gag* sequences by deletion of a key element and by codon optimization which reduces sequence similarity to the partial *gag* in the transfer genome. Overall, LV305 remains genetically stable, which is confirmed by sequencing of the final cloned plasmids.

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)
Not applicable.

4. Description of identification and detection methods

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

No environmental detection measures are planned.

(b) Techniques used to identify the GMO

The LV305 Identity Assay by RT-PCR is used to confirm the identity of the LV305 vector that encodes NY-ESO-1.

The detection of NY-ESO-1 protein is done using ELISA. This is a two-part assay consisting of a cell post transduction culture/ transduction phase followed by the detection of the NY-ESO-1 protein using a sandwich ELISA.
A persistence assay using qPCR is used to detect reverse-transcribed LV305 vector DNA in human PBMCs in subjects at baseline during the trial Screening period, and then after dosing at the 4, 6, 12 and 24-month timepoint during the clinical trial.

F. Information relating to the release

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

LV305 will be evaluated in Phase 3 study IMDZ-04-1702 as part of the CMB305 immunotherapy regimen. CMB305 consists of two different active immunotherapy agents administered sequentially in a "prime-boost" vaccination regimen. One agent is LV305, a lentiviral vector that encodes the NY-ESO-1 cancer testis (CT) antigen and is designed to "prime" the immune system. The other agent is G305, an immune system "boost" component, which is not a GMO and therefore is not described in this document.

Approximately 248 subjects who have synovial sarcoma expressing NY-ESO-1 will be enrolled and randomly assigned in a 1:1 ratio to treatment with either CMB305 or placebo.
No significant environmental benefits are expected as a result of the release.

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

Study will be carried out in the following sites:

Site:	Skånes Universitetssjukhus i Lund
Principal Investigator:	Dr. Marie Ahlstrom
Site Address:	Kirurgiska Kliniken 22185 Lund Sweden
Pharmacy Site Name:	ApoEx AB
Pharmacy Site address:	Kliniska prövningar Blidögatan 27 211 24 Malmö Sweden

Site:	Karolinska University Hospital
Principal Investigator:	Dr. Antroula Papakonstantinou
Site Address:	Oncology Department Huddinge 14186 Stockholm Sweden
Pharmacy Site Name:	ApoEx AB NKS
Pharmacy Site Address:	Kliniska prövningar Eugeniavägen 23 171 64 Solna Sweden

(a) Geographical location (administrative region and where appropriate grid reference): Not applicable

(b) Size of the site (m^2): … m^2

(i) actual release site (m^2): … m^2 Not applicable
(ii) wider release site (m^2): … m^2 Not applicable

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

LV305 will be administered at licensed healthcare facilities where there are procedures in place for administration of GMO vaccines, collection and processing of blood and serum samples, and all equipment and staff required for the clinical evaluation of study subjects under containment level 2 (Annex V of Directive 2000/54/EC). It is not anticipated that the study vaccine or any waste associated with study procedures will affect the surrounding ecosystem.

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

Not applicable. Given the nature of the product administration (subcutaneous), and the lack of shedding expected the risk of unintended exposure of flora and fauna is negligible.

4. Method and amount of release

(a) Quantities of GMOs to be released:

Four separate 1-mL SC injections of 1 x 10^{10} vg/mL will be administered to each subject on Days 1, 22, 50, and 78. Four subjects are planned for enrollment in Sweden. Assuming all enrolled subjects receive all LV305 injections specified per protocol, a total of 64 doses of LV305 will be administered, which equates to a maximum of 64 mL LV305, containing a total of 6.4 x 10^{11} vg released during the treatment period.

(b) Duration of the operation:

Immune Design is planning initiation of Study IMDZ-04-1702 in Sweden by early 2019. Enrollment in Sweden is expected to be completed by mid-2020. Patients will continue follow-up until June 2023.

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

All principal investigators and sub-investigators participating in the study will be qualified by education, training and experience to assume responsibility for the proper conduct of the trial according to the guidelines outlined in International Conference on Harmonisation (ICH) E6 - Good Clinical Practices. Clinical sites where the study is to be conducted will be thoroughly evaluated prior to the initiation of the study to ensure that the facilities are sufficient for storing and administering the vaccine, as well as having the appropriate facilities for the collection and storage of human specimens. The evaluation will be performed at site qualification and initiation visits by Clinical Research Associates employed by Medpace (CRO) and acting on behalf of IMDZ.

Additionally, all clinical site personnel involved in the handling or administration of study vaccine will be trained according to the study protocol, and all supportive documentation, including study specific laboratory and clinical trial material manuals. A thorough study-specific training will be provided by IMDZ and Medpace (CRO) prior to the initiation of the study via a formal local investigator meeting and/or on-site study initiation visit.

The risk of transmission of recombinant viruses to exposed healthcare workers is very low. There have been no cases of transmission to healthcare personnel in any of the studies with LV305.

Procedures for preparation of the vaccine are described in the clinical protocol. LV305 is classified as a group 1 biological agent since it is unlikely to cause human disease.

Loading of syringes may be performed using standard aseptic methods as outlined in the Pharmacy Manual. IMDZ recommends routine use of standard universal precautions when directly handling the vaccine, including the wearing of a lab coat, eye protection, and gloves.

In case of spills, LV305 is readily inactivated by 1% sodium hypochlorite solution (1:10 dilution of household bleach) and can easily be contained. Material Safety Data sheets will be provided with each product and study staff will be provided with specific instructions to

address spills, including information on containment, personal protective equipment, disinfection, and disposal procedures.

5. Short description of average environmental conditions (weather, temperature, etc.)
The clinical trial of LV305 is planned to take place in countries within North America, Europe, and Asia-Pacific with the majority of sites located in arid, Mediterranean or temperate climate regions. The risk of release of LV305 into the environment is unrelated to climatic characteristics.

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.

Study Reference	Location	Number of Subjects Treated	Dose
Phase 1 Study ID-LV305-3013-001	USA	39	1×10^{10} vg
Phase 1b Protocol IMDZ-C131	USA	68	1×10^{10} vg
Phase 2 Protocol IMDZ-C232	USA	88	1×10^{10} vg

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)	Primates
(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

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

LV305 is designed to prime the immune system by targeting RNA antigen delivery to dendritic cells in vivo. LV305 expresses the cancer-testis antigen NY-ESO-1 after transduction. The vector envelope, which is a modified variant of the Sindbis virus envelope protein facilitates binding to receptors present on DCs in vivo to induce strong, polyfunctional effector and long-lasting memory T-cell responses in patients with NY-ESO-1 positive cancers to cause lysis of these cells and resolution of their tumor.

G305, which is a recombinant NY-ESO-1 protein admixed with the adjuvant GLA-SE [a fully synthetic agonist of toll-like receptor 4 (TLR4) highly expressed on dendritic cells (DCs)] is the “boost” component. TLR4 is present on many different immune cells and is critical to the activation of the innate immune system. One important activity of GLA-SE is its ability to stimulate DCs, and when administered with NY-ESO-1, this activation promotes

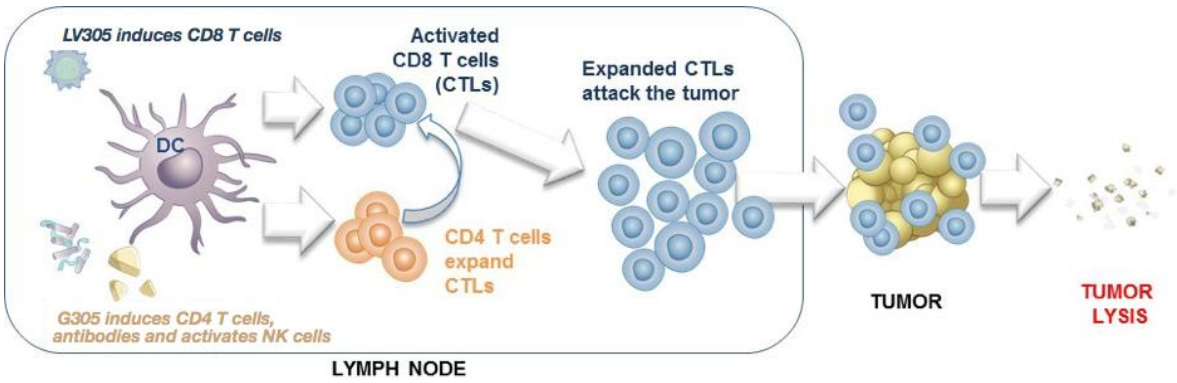
potent adaptive immune responses involving antibodies, $CD4^+$ T-cells and other effector immune cells. As noted previously, G305 is not covered in this document as it is not a GMO.

Successful antigen-directed immunotherapies for cancer are believed to require broad CD8 T cell- mediated immunity. In preclinical models, the development or "priming" of CD8 T cells with the use of a lentiviral vector, followed by the "boosting" of the immune response by a protein-adjuvant approach, can lead to potent stimulation of anti-tumor immunity. This approach capitalizes on the strengths of each agent's ability to stimulate different parts of the immune system (CD8 cytolytic T cells, CD4 helper T cells, NK cells, antibodies, and antigen presenting cells) that are required to activate and generate potent and long-lasting immunity (Ramshaw 2000, Woodland 2004).

DCs are essential for the initiation of immune responses and play a critical role in generating cellular anti-tumor immunity. A fundamental challenge for the generation of effective anti-tumor CD8 T-cell responses is the promotion of antigen presentation within the context of MHC Class I molecules by DCs, which also express co-stimulatory signals that stimulate T-cells. Lentiviral vectors offer a very attractive approach to inducing strong T-cell responses as they: (1) can transduce non-dividing cells, such as DC, (2) effectively drive the de novo transcription and translation of the antigen, resulting in MHC-I presentation, and (3) stimulate the expression of co-stimulatory molecules due to their viral elements that trigger Toll-like receptor signaling in transduced cells. IMDZ has developed a LV- based platform known as ZVex® that has been engineered to deliver tumor antigen-encoding genes to DCs in vivo. Zvex LVs are pseudotyped with Sindbis virus (SINV) envelope glycoproteins, enabling DC transduction after binding the C-type lectin receptor, DC-SIGN. As a result, the vector induces a high magnitude of functional, antigen-specific CD8 T cell immune responses after a single immunization in mice.

Research aimed at enhancing an immune response against "non-self" antigens shows that novel prime-boost protocols, i.e., triggering an immune response with one mechanism and following with a distinct mechanism that can enhance the first one, yield considerably higher responses than either mechanism alone (Nolz 2011). This is particularly true when distinct, but complementary, parts of the immune response are stimulated (Pham 2009). CMB305 is a prime-boost combination that can induce a strong CD8 T-cell response by dosing with LV305 initially given twice, followed sequentially with G305, an emulsified product composed of recombinant full-length NY ESO 1 protein mixed with a TLR4-agonist called Glucopyranosyl Lipid A formulated as a stable emulsion (GLA-SE), given two weeks later. LV305 and G305 are then dosed in an alternating sequence to augment the response. The cellular interactions are represented graphically in Figure 1.

Figure 1: Interaction of Products and Cells in a LV305, G305 Boost that Generates a Synergistic Immune Response to Lyse Tumor Cells



DCs can be efficiently stimulated by viral infection to induce strong CD8 T-cell responses to kill tumor cells. Once LV305 transduces a DC, the insert gene is expressed and its peptides are presented on the surface in the context of MHC-I to activate antigen-specific CD8 T cells. When stimulated with GLA-SE, DCs can also present peptides via MHC-II after the uptake of NY-ESO-1 protein to activate specific CD4 T cells that provide help to the CD8 T cells. Dosing with a prime-boost combination can substantially augment the CD8 T-cell response.

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

Recombination between the DNA genome of LV305 with other DNA genomes, such as the human genome or other viral genome including HIV in the infected host cell, is improbable. LV305 has been rendered integration-deficient by two redundant mechanisms (Tareen 2013).

The first involves interference with integrase enzyme activity. The second involves deletion of poly-purine tract that favors the formation of episomal dsDNA circles. These single-LTR reverse transcribed episomal dsDNA circles are a molecular form that makes them incapable of chromosomal integration. Integration deficiency was demonstrated in vitro by reduction of integration events up to 3 $\log_{10}$ compared to an integrating lentiviral vector. In addition to integration deficiency, the design of LV305 includes a *gag/pol* plasmid that has been codon optimized for the production cells and is devoid of the Rev-responsive element (RRE). The removal of the RRE minimizes the chance of psi-gag recombination and provides an added measure of safety by reducing the opportunity for the formation of a RCL during vector production.

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. LV305 will be administered in healthcare facilities under controlled conditions, and cannot establish itself outside of host cells.

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	…

The potential for LV305 interaction with other organisms in the environment would be limited to exposure via direct contact with the vaccination site or with contaminated surfaces or objects. The most likely non-target organisms would include health care workers or close contacts of subjects enrolled in the study. LV305 is intended to transduce dendritic cells and express NY-ESO-1 to stimulate an immune response. The potential effects on non-target organisms are negligible due to the specificity to dendritic cells, which are terminally differentiated and short-lived in vivo.

7. Likelihood of genetic exchange in vivo

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

The likelihood of genetic exchange from the GMO to other organisms in the release ecosystems is improbable. LV305 has been rendered integration-deficient by two redundant mechanisms (Tareen 2013). The first involves interference with integrase enzyme activity. The second involves deletion of poly-purine tract that favors the formation of episomal dsDNA circles. These single-LTR reverse transcribed episomal dsDNA circles are a molecular form that makes them incapable of chromosomal integration. Integration deficiency was demonstrated in vitro by reduction of integration events up to 3 $\log_{10}$ compared to an integrating lentiviral vector.

(b) from other organisms to the GMO:

The likelihood of genetic exchange from other organisms to the GMO is improbable. LV305 has been rendered integration-deficient by two redundant mechanisms (Tareen 2013).The first involves interference with integrase enzyme activity. The second involves deletion of poly-purine tract that favors the formation of episomal dsDNA circles. These single-LTR reverse transcribed episomal dsDNA circles are a molecular form that makes them incapable of chromosomal integration. Integration deficiency was demonstrated in vitro by reduction of integration events up to 3 $\log_{10}$ compared to an integrating lentiviral vector.

(c) likely consequences of gene transfer:

The potential consequence of LV305 integrating into the genome, transducing cells outside of dendritic cells and propagation within the target organism is minimal. LV305 has been rendered integration-deficient by two redundant mechanisms (Tareen 2013). The first involves interference with integrase enzyme activity. The second involves deletion of poly-purine tract that favors the formation of episomal dsDNA circles. These single-LTR reverse transcribed episomal dsDNA circles are a molecular form that makes them incapable of chromosomal integration. Integration deficiency was demonstrated in vitro by reduction of integration events up to 3 $\log_{10}$ compared to an integrating lentiviral vector.

However, in the unlikely event that integration occurs, LV305 is engineered to only express NY-ESO-1. NY-ESO-1, also known as CTAG1, exhibits characteristics that define a common family trait of the so-called cancer-testis (CT) antigens: these are genes with expression restricted to germ cells and no normal somatic tissue, but that are frequently expressed in cancer. By immunohistochemistry and RT-PCR, NY-ESO-1 expression is only found in early spermatogonia and placenta (villous trophoblast) before week 32 of gestation, and is gradually lost with sperm cell differentiation. Its expression in these cells is predominantly cytoplasmic. Non-gametogenic cells of the testis, including Sertoli cells, do not express NY-ESO-1, nor do other somatic cells.

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 simulated natural environments (e.g. microcosms, etc.):
Not applicable. LV305 will be administered in healthcare facilities under controlled conditions and is not expected to be released into the environment. No studies have been conducted in simulated natural environments.

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

Not applicable. LV305 is not anticipated to have any involvement in biogeochemical processes.

H. Information relating to monitoring

1. Methods for monitoring the GMOs

There are no plans for tracing LV305 or monitoring LV305 effects on patients participating in the trial, since LV305 selectively targets dendritic cells that are terminally differentiated and have a half-life of days up to a few weeks.

There are procedures in place to monitor subjects after LV305 administration to gather data to support the safety profile of LV305. Safety assessments will include solicited and unsolicited symptoms, physical examination findings, vital signs, documentation of adverse events (AEs), ECGs and/or echocardiogram as applicable, clinical laboratory evaluations, deviations or discontinuations attributed to AEs, concomitant medication use, and LV305 persistence. The testing of subject blood samples for the presence of LV305 at multiple time

points after administration may provide information regarding the risk for delayed adverse events experienced by the subject.

2. Methods for monitoring ecosystem effects

The dissemination and impact of LV305 on ecosystems is negligible because dissemination requires close contact with the vaccination site or contact with contaminated surfaces or objects. The study will be conducted at standard healthcare facilities. It is not anticipated that the study vaccine or any waste associated with study procedures will affect the surrounding ecosystem.

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

There is negligible risk of gene exchange between the GMO and other organisms, therefore, no monitoring of other organisms is planned.

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

Not applicable. No specific viral detection/monitoring of LV305 is planned.

5. Duration of the monitoring

No specific viral detection/monitoring of LV305 in the environment is planned.

The subjects' blood samples will be analyzed to detect the presence of LV305 at multiple time points after administration up to 2 years.

6. Frequency of the monitoring

No specific viral detection/monitoring of LV305 in the environment is planned.

The subjects' blood samples will be analyzed to detect the presence of LV305 at multiple time points after administration as specified in the protocol.

I. Information on post-release and waste treatment

1. Post-release treatment of the site

Procedures are in place to minimize the spread of the GMO under biosafety level 2 practises specified in Annex V of Directive 2000/54/EC. Subjects receiving LV305 are advised on measures for reducing the potential exposure of close contacts to the viral vector by minimizing their exposure to the subject’s blood or body fluids. As a general practice, subjects will be asked to wash their hands frequently, separate the use of eating utensils/dishes/clothes from others and avoid close or intimate contact for 24 hours after receiving each dose of LV305.

Clinical site staff responsible for administering LV305, collecting clinical samples, or conducting clinical evaluation of study subjects will be instructed to follow the World Health

Organization (WHO) universal precautions for the prevention of transmission of infectious agents in healthcare settings (WHO Standard Precautions, 2006).

2. Post-release treatment of the GMOs

Strict accountability of all doses of LV305 imported into Sweden will be maintained at all times.

IMDZ or delegate will review the site's policy on Investigational Product destruction, method for destruction (autoclave or incineration), and any site Standard Operating Procedures related to Investigational Product destruction. If the site is not able to destroy LV305, the site will notify the Contract Research Organization (Medpace) to return drug to an authorized depot for destruction.

3. (a) Type and amount of waste generated

Each dose of LV305 is supplied in borosilicate (2R) glass vials, which are sealed with rubber stoppers and aluminium-plastic closures. In addition to vials, other waste generated includes syringes and needles used for vaccine administration and for collection of blood samples, dressings, and other standard supplies required for physical and medical examination of subjects. Up to 16 empty vials of LV305 or placebo per subject will be generated as waste.

3. (b) Treatment of waste

All clinical study sites are licensed healthcare facilities and have standard facility controls in place for administration of vaccines, collection and processing of clinical specimens, and clinical evaluation of study subjects under containment level 2 (Annex V of Directive 2000/54/EC). Clinical site staff will be instructed to follow the World Health Organization (WHO) universal precautions for the prevention of transmission of infectious agents in healthcare settings (WHO Standard Precautions, 2006).

No risk related to LV305-related waste is anticipated. Clinical study sites will be instructed to follow the Pharmacy Manual and LV305 Administrative Guidelines for disposal of infectious biomedical waste.

J. Information on emergency response plans copy

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

In the event that the contents of the LV305 vial are accidentally released and come in contact with shipping materials, exposed skin, clothing or laboratory surfaces, sites will be instructed to follow procedures outlined in the Pharmacy Manual and LV305 Administrative Guidelines. LV305 is a virus and is killed by bleach-based disinfectants. Contaminated materials should be placed in biohazard safety bags and disposed of as biohazard waste. Surfaces in contact with LV305 should be thoroughly cleaned with freshly prepared 1% hypochlorite solution (1:10 dilution of household bleach) to kill the

virus. Cleaning materials should be disposed of as biohazard. Sites of skin contact should be cleaned with standard detergents appropriate for hand washing.

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

In case of accidental release, the source of the spill or leak should be contained. Absorbent material should be used to absorb liquid from contaminated surface. Absorbent materials must be disposed of in biohazard bags. The contaminated surface should be cleaned with 1% hypochlorite solution (1:10 dilution of household bleach) to kill the virus. Cleaning materials must be disposed of in biohazard bags.

Individuals involved in clean up should wear protective clothing including gloves, eye protection and laboratory coat.

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

The extent of exposure to non-target organisms such as plants, animals and soils is expected to be negligible. This is due to 1) The vaccine administration occurs in clinical site under controlled conditions including standard disposal procedures in biohazard bags of waste material and 1% hypochlorite solution for cleaning surfaces 2) The administration of LV305 via the subcutaneous route and subsequent bandaging of the site to contain the virus 3) Non-clinical mouse data from shedding studies indicates shedding only persists for 1-4 hours at the site of injection and 4) the design of the lentiviral vector to be replication incompetent and integration deficient by two redundant mechanisms (Tareen 2013).The first involves interference with integrase enzyme activity. The second involves deletion of poly-purine tract that favors the formation of episomal dsDNA circles. These single-LTR reverse transcribed episomal dsDNA circles are a molecular form that makes them incapable of chromosomal integration. Integration deficiency was demonstrated in vitro by reduction of integration events up to 3 $\log_{10}$ compared to an integrating lentiviral vector. Decontamination of plants, (non-human) animals and soils will not be required.

4. Plans for protecting human health and the environment in the event of an undesirable effect
Monitoring of the patients and of potential adverse events will be performed according to the study protocol.

Extensive procedural controls are in place for the transport, storage, administration, disposal, and monitoring of LV305 treatment for the duration of the clinical study.

Should any unexpected undesirable effect occur, IMDZ will follow standard procedures of assessment of the effect and decisions regarding study continuance.