



Pexastimogene Devacirepvec

(Pexa-Vec, formerly JX-594)

**SUMMARY NOTIFICATION INFORMATION FORMAT
(SNIF) FOR RELEASES OF GENETICALLY MODIFIED
ORGANISMS OTHER THAN HIGHER PLANTS IN
ACCORDANCE WITH ARTICLE 11 OF DIRECTIVE
2001/18/EC**

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LIST OF ABBREVIATIONS

<i>AmpR</i>	Ampicillin resistance gene
BSC	Best supportive care
CDC	US Centers for Disease Control and Prevention
DNA	Deoxyribonucleic acid
<i>E. coli</i>	<i>Escherichia coli</i>
EU	European Union
GMO	Genetically modified organism
hGM-CSF	Human granulocyte macrophage-colony stimulating factor
HCC	Hepatocellular Carcinoma
HEPA	high-efficiency particulate air
IT	Intratumoral
IV	Intravenous
<i>lacZ</i>	β -galactosidase coding gene
PCR	Polymerase chain reaction
Pexa-Vec (JX-594)	Recombinant Vaccinia Virus
pfu	Plaque forming unit
PPE	Personal Protective Equipment
SAE	Serious Adverse Event
TK	Thymidine kinase
TKL	ATP-binding domain sequence of TK
TKR	Nucleoside-binding domain sequence of TK
VV	Vaccinia virus
X-gal	5-Bromo-4-chloro-3-indolyl galactopyranoside

A. General Information

1. Details of Notification

Member state of notification: Germany

Notification number: B/DE/16/PEI2680

Date of acknowledgement of notification: 01.03.2016

Title of the project: Clinical Study JX594-HEP024, “A Phase 3 Randomized, Open-Label Study Comparing Pexa-Vec (Vaccinia GM CSF / Thymidine Kinase-Deactivated Virus) Followed by Sorafenib Versus Sorafenib in Patients with Advanced Hepatocellular Carcinoma (HCC) Without Prior Systemic Therapy”.

Proposed period of release: From 01-Oct-2016 until 30-Jun-2020 (date of study completion)

2. Notifier

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USA

Contact: Ursula Fritsch
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3. GMO characterization

a. Indicate whether the GMO is a:

- | | | |
|------------------------|-------------------------------------|----------------|
| Viroid | <input type="checkbox"/> | |
| RNA virus | <input type="checkbox"/> | |
| DNA virus | <input checked="" type="checkbox"/> | Vaccinia virus |
| Bacterium | <input type="checkbox"/> | |
| Fungus | <input type="checkbox"/> | |
| Animal | <input type="checkbox"/> | |
| Other (please specify) | <input type="checkbox"/> | |

b. Identity of the GMO (genus and species)

Genus: Orthopoxvirus

Species: Vaccinia virus (VV)

The GMO is a viral suspension of the recombinant virus Pexa-Vec (formerly called JX-594 in previous submissions performed in European Union (EU) countries by Transgene S.A.). Pexa-Vec is an oncolytic therapeutic vaccinia virus (Wyeth strain) designed to selectively replicate in and destroy cancer cells, while at the same time stimulating a systemic antitumoral immune response through the expression of its transgene, human granulocyte-macrophage colony stimulating factor (hGM-CSF) in the context of tumor lysis. Three genetic modifications are present in Pexa-Vec:

- 1 thymidine kinase (TK) gene deactivation,
- 2 GM-CSF insertion under control of the synthetic early-late promoter and,
3. *lac-Z* gene insertion under control of the p7.5 promoter.

Pexa-Vec is a therapeutic anticancer candidate to be administered in patients with advanced stage of cancers.

c. Genetic stability

Double-stranded DNA viruses, such as VV, typically have very low rates of mutation from one passage to the next (*Nalca A. and Zumbun E., 2010*). Dryvax[®], from which the Pexa-Vec virus was prepared, is a mixed population of vaccinia clones. During the manufacturing process of Pexa-Vec virus, one clone was selected (LVB Clone 1). The genetic elements of the Pexa-Vec expression cassette have been sequenced and were shown to be identical to GenBank sequences for the hGM-CSF and *lacZ* genes and the associated promoters and for the VV TK gene with the following exceptions: one nucleotide difference in the TKR region (which is not expressed in Pexa-Vec) and one additional nucleotide (G, position 48) in a non-critical part of the p7.5E/L promoter.

4. Is the same GMO release planned elsewhere in the Community (in conformity with Article 5 (1))?

Yes No

If yes, insert the country code(s): AT, FI, FR, IT, PL, PT and UK

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

Yes No

If yes – Member State of notification:

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

Yes No

If yes – specify countries: AUSTRALIA, CANADA, CHINA, ISRAEL, KOREA (Republic of), NEW ZEALAND, SINGAPORE, TAIWAN, THAILAND and the USA

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

The likelihood of Pexa-Vec becoming persistent and invasive in natural habitats is low for the following reasons:

- Due to the inactivation of its TK gene, Pexa-Vec replicates preferentially in actively dividing cells. Pexa-Vec is therefore expected to propagate mostly in cancer cells. Pexa-Vec could bring back its genome up to the structure of its parent by eliminating the expression cassette inserted in the TK gene. Current genetic stability studies on Pexa-Vec have not detected spontaneous revertants of Pexa-Vec.
- Pexa-Vec remains exclusively in the cytoplasm of infected cells thus eliminating any risk of integration of the viral DNA into the host genome.
- Shedding of infectious particles into the environment and potentially to the public can occur during the proposed release. However, dispositions will be taken in this clinical trial to minimize dissemination and inadvertent transmission.
- No environmental concern was raised during the smallpox vaccination campaign during which hundreds of millions of people were administered with the nonattenuated wild type virus (i.e. parental virus of Pexa-Vec).

B. Information Relating To The Recipient Or Parental Organisms From Which The GMO Is Derived

Recipient or parental organism characterization:

1. Indicate whether the recipient or parental organism is a

- | | |
|------------------------|--|
| Viroid | <input type="checkbox"/> |
| RNA virus | <input type="checkbox"/> |
| DNA virus | <input checked="" type="checkbox"/> Vaccinia Virus |
| Bacterium | <input type="checkbox"/> |
| Fungus | <input type="checkbox"/> |
| Animal | <input type="checkbox"/> |
| Other (please specify) | <input type="checkbox"/> |

2. Complete name

Order and/or higher taxon (for animals):	Poxviridae
Genus:	Orthopoxvirus
Species:	Vaccinia virus
Subspecies:	Not applicable
Strain:	Wyeth
Pathovar:	Not applicable
Common name:	Not applicable

3. Geographical distribution of the organism

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:

Yes No Not known

c. If Yes, indicate the type of ecosystem in which it is found

- | | |
|----------------|-------------------------------------|
| Atlantic | <input type="checkbox"/> |
| Mediterranean | <input type="checkbox"/> |
| Boreal | <input type="checkbox"/> |
| Alpine | <input type="checkbox"/> |
| Continental | <input type="checkbox"/> |
| Macaronesian | <input type="checkbox"/> |
| ii) No | <input checked="" type="checkbox"/> |
| iii) Not Known | <input type="checkbox"/> |

The ecology of VV is not known. It is commonly thought that VV is not naturally found in the environment.

d. Is it frequently used in the country where the notification is made?

Yes

No

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

Yes

No

4. *Natural habitat of the organism*

a. If the organism is a micro-organism

Water

Soil, free-living

Soil in association with plant-root systems

In association with plant-leaf/stem systems

In association with animals

Other (specify)

The parental organism is not naturally found in the environment.

b. If the organism is an animal: natural habitat or usual agro-ecosystem –
Not applicable.

5a. *Detection techniques*

Not applicable. There is no detection or identification technique able to discriminate the wild type VV from the GMO except by:

- Restriction mapping of the *Hind III* region
- Sequencing of the TK gene.

5b. *Identification techniques*

See 5a.

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

Yes

No

If yes, specify

In terms of classification of hazard, VV is considered as a Group 2 biological agent as per the European Union classification for the protection of workers with biological agents (Directive 2000/54/EC). The Group 2 designation applies to agents that can cause human disease and might be a hazard to workers, that are unlikely to spread to the community and for which there is usually effective prophylaxis or treatment available.

Examples of other Group 2 biological agents include the measles virus, salmonellae, and the influenza viruses (types A, B and C).

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

Yes

No

Not known

If yes,

a. To which of the following organisms:

Humans

Animals

Plants

Others

VV has the longest and most extensive history of use in humans acquired during the smallpox vaccination campaign in the 1960s up to the early 1980s with hundreds of millions of people vaccinated. The fact that VV was massively used in the smallpox eradication campaign means that there is unprecedented information on its behaviour in humans, including the identification of populations which are at risk for rare adverse events (*Cono J. et al., 2003; Kretzschmar M. et al., 2006*). Rare complications included eczema vaccinatum, disseminated vaccinia rash, progressive vaccinia and encephalitis (*Fields B.N., 1996*). The individuals who have been identified with an increased risk of serious adverse effects are:

- Children less than 12 months old
- Pregnant or breastfeeding women
- People with exfoliative skin conditions (e.g. severe eczema, ectopic dermatitis or similar skin disorder) that requires systemic therapy
- People with significant immunodeficiency due to an underlying illness (e.g. HIV/AIDS) and/or medication (e.g. systemic corticosteroids or other immune-suppressive medications including cortisone, dexamethasone, hydrocortisone, prednisone, prednisolone, interferon, cisplatin, doxorubicin, fluorouracil, etc.).

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

There is no known natural reservoir host of VV. Humans, cows, buffaloes, camels, foxes, raccoons, pigs, etc. have already been infected by VV. It is however believed that VV does not produce latent infection and once the infection arises, the virus is rapidly cleared from the host.

VV can infect a wide range of human tissues but does not cause any known human disease except for vaccination complications (see section 7. a). A number of approved or experimental antiviral agents are available to treat poxvirus infections in case of an adverse response. Vaccinia immune globulin and cidofovir are efficient therapies recommended by the US Centers for Disease Control and Prevention (CDC) for certain serious smallpox vaccine reactions.

VV replication exclusively occurs in the cytoplasm thus eliminating any risk of integration of the viral DNA into the host genome (*Moss B., 2007*).

8. *Information concerning reproduction*

a. Generation time in natural ecosystems

Not relevant as VV is not naturally found in the environment.

b. Generation time in the ecosystems where the release will take place

Not relevant.

c. Way of reproduction

Sexual Asexual Other - Viral

d. Factors affecting reproduction

Vaccinia viruses are rapidly inactivated by a number of disinfectants. In addition to chemical agents, these viruses are inactivated by exposure to ultraviolet light and by exposure to increasing temperatures. For example, when stored at 25°C, viruses lose viability over a period of weeks when stored in water, and over a period of days when stored dried.

9. *Survivability*

a. Ability to form structures enhancing survival or dormancy

- i. endospores
- ii. cysts
- iii. Sclerotia
- iv. asexual spores (fungi)
- v. sexual spores (fungi)
- vi. eggs
- vii. pupae
- viii. larvae
- ix. Other, please specify

Not relevant.

b. Relevant factors affecting survivability

VV viruses are sensitive to inactivation by either physical or chemical methods of disinfection. Heat is the most effective antimicrobial agent (viable counts of a VV are reduced 10^7 fold by exposure to 60°C at ambient pressure within an hour or less). Vaccinia is rendered noninfectious following treatment in an autoclave. Hospital-grade chemical disinfectants are also effective against lipophilic viruses such as VV.

10a. Ways of dissemination

Wild type VV has the ability to widely disseminate in human body. VV is known not to produce latent infection. So, after an initial period with some viral shedding in biological fluids, the virus becomes completely cleared from the host.

TK gene inactivation in the recombinant Pexa-Vec virus tends to limit its dissemination to tumors (*Puhmann M. et al., 2000*). In humans, Pexa-Vec could disseminate from biological fluids, the injection site and vaccine pustules.

The presence of Pexa-Vec has already been monitored in human blood, urine and throat swab. The recombinant virus was detected in throat swabs following intravenous administration and blood following intravenous or intratumoral administration up to 2 and 3 weeks respectively after patient administration. Pexa-Vec was also identified in pustules exudates of patients who developed skin pustules after Pexa-Vec intravenous or intratumoral administration. No data are available yet regarding the presence of Pexa-Vec in faeces.

10b. Factors affecting dissemination

Instructions on how to prevent dissemination and contamination will be given in the dedicated Infection Control Guidelines which will be provided to investigators, pharmacists, and all personnel involved in handling of the product. The patients will receive these instructions as part of their informed consent process. The injection site (the skin through which Pexa-Vec is to be administered), as well as any skin ulcers, acneiform pustules or rashes, will be covered with a bandage and clothing for a period of 7 days after treatment, or until resolution of any Pexa-Vec related skin ulceration, pox or rash, whichever is longer. If superficial oral mucous membrane lesions develop, patients should adhere to conservative management of their pustules and wear a mask when around other people until resolution of the oral pustules. Patients will be instructed to avoid direct contact with pustules followed by contact with other parts of the body (e.g., the eyes, nose, or other areas). Spills of potentially contaminated biological fluids will be handled according to standard institutional procedures for handling spills of potentially infectious material. Patients while they are at home will be instructed to wipe the surfaces that come in contact with the pustule with a bleach solution or with any other active disinfectant. Clothing, towels and bed linens will be washed using the hot water cycle with detergent. Frequent hand washing, avoidance of direct contact with saliva (e.g. kissing), avoidance of sharing of household

items (e.g. eating utensils), and the avoidance of direct physical contact with people in at risk groups [i.e. children <12 months of age, pregnant or breastfeeding women, immunocompromised populations (e.g. organ transplant recipients, HIV-positive individuals, or those receiving chronic immunosuppressive medication), and those with inflammatory skin conditions (e.g. eczema requiring previous treatment, atopic dermatitis)] is recommended during the study participation.

Sexual secondary transmission of VV from a vaccination recipient has already been described (*MMWR, 2004; MMWR, 2010*). This report together with Pexa-Vec pre-clinical data showing viral DNA distribution and abscesses in the rabbits' testes emphasizes the importance of sexual contact precautions which have been implemented for patients who will be participating in the proposed Phase III trial. Patients will be requested to use a barrier method for at least 6 weeks after each Pexa-Vec injection.

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 genetic modification

- a) insertion of genetic material
- b) deletion of genetic material
- c) base substitution
- d) cell fusion
- e) other, specify

2. Intended result of the genetic modification

Pexa-Vec is a GMO developed as a therapeutic candidate to treat patients with cancer. The vaccinia TK gene was inactivated in Pexa-Vec to enhance the *in vivo* tropism of the VV for cancer cells versus normal cells. The therapeutic transgene encoding hGM-CSF was inserted in order to increase the anti-cancer efficacy of Pexa-Vec locally and against distant tumor metastases by stimulating a systemic anti-tumor immunity. The gene for *Escherichia coli* β -galactosidase was inserted to provide a marker for viral replication in histopathological examination of tissue biopsies from treated patients.

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

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

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

pSC65/hGM-CSF

c. Host range of the vector – *Escherichia coli*.

d. Presence in the vector of sequences giving a selectable or identifiable phenotype,

Yes No Not known

Antibiotic resistance

Yes No Not known

Other specify

Indication of which antibiotic resistance gene is inserted:

e. Constituent fragments of the vector

The vector pSC65/hGM-CSF contains DNA sequences coding for the hGM-CSF and β -galactosidase proteins and for their respective promoters. In addition, the transgene sequences are flanked by two VV genomic regions (TKL and TKR) that allow homologous recombination between the transfer plasmid and VV.

f. Method for introducing the vector into the recipient organism:

electroporation, macroinjection, microinjection, infection, or other?

- | | | |
|-------------|------------------------|-------------------------------------|
| i. | Transformation | <input type="checkbox"/> |
| ii. | Electroporation | <input type="checkbox"/> |
| iii. | Macroinjection | <input type="checkbox"/> |
| iv. | Microinjection | <input type="checkbox"/> |
| v. | Infection | <input type="checkbox"/> |
| vi. | Other, specify | <input checked="" type="checkbox"/> |

Homologous recombination between pSC65/hGM-CSF and the parent VV virus (Wyeth strain, Dryvax[®]) in CV-1 monkey kidney cells.

5. *If the answer to questions 3(a) and (b) is no, what was the method used to introduce the insert into the recipient/parental cell?*

Not applicable.

6. *Information on the insert*

a. Composition of the insert

The insert contains the genes which encode for hGM-CSF and β -galactosidase. The insert also contains VV promoters for transgenes expression (i.e., synthetic PsE/L, p7.5K). Of note, following recombination between the VV and pSC65, the AmpR gene is not part of the insert.

b. Source of each constituent part of the insert

Constituent of the Insert	Source	Intended Function
hGM-CSF	Human	Stimulates anti-cancer immune response
PsE/L	Synthetic	Early/late promoter
<i>lacZ</i>	<i>Escherichia coli</i>	Safety marker gene
p7.5K	VV	Early/late promoter

c. Intended function of each constituent part of the insert in the GMO

The therapeutic transgene encoding hGM-CSF was inserted in order to increase the anticancer efficacy of Pexa-Vec locally and against distant tumor metastases by stimulating a systemic anti-tumor immunity. The gene for *Escherichia coli* β -galactosidase was inserted to facilitate selection of recombinant plaques and to allow monitoring of viral replication in tumor tissue.

d. Location of the insert in the host organism

- on a free plasmid
- Integrated in the chromosome
- other, specify

The insert is fully integrated in the VV genome by homologous recombination in the TK gene.

e. Does the insert contain parts whose products or functions are not known?

Yes No

If yes, specify

D. Information On The Organism From Which The Insert Is Derived

1. Indicate whether it is a:

- Viroid
- RNA virus
- DNA virus Vaccinia virus for p7.5K
- bacterium *E. coli* for *lacZ*
- fungus
- animal
- mammals *Homo sapiens* for hGM-CSF (not described in the sections below)
- insect
- fish
- other animal specify phylum, class

other, specify

2. Complete name

	<u>lacZ</u>	<u>p7.5K</u>
Order and/or higher taxon (for animals):	Proteobacteria	Poxviridae
Family name (for plants):	Enterobacteriaceae	
Genus:	<i>Escherichia</i>	Orthopoxvirus
Species:	<i>Escherichia coli</i>	Vaccinia virus
Subspecies:		
Strain:		Wyeth
Cultivar/Breeding line:		
Pathovar:		
Common name:	<i>E.coli</i>	

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

Yes No Not known

If yes, specify the following

a) To which of the following organisms?

- Humans
- Animals
- Plants
- Other

E. coli is a gram negative bacterium that is commonly present in the intestines of humans and animals. Most strains of *E. coli* are harmless, but there are exceptions with strains causing severe diarrhea. The most common symptoms of infection with *E. coli* are abdominal cramping and diarrhea. In an uncomplicated case, the illness should recover in about 5 to 10 days without any antibiotic treatment. In routine practice, antibiotic treatment must be started empirically based on the site and severity of infection and then modified based on antibiotic susceptibility testing.

VV: see sections B.7.a) & B.7.b).

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 related to exposure to biological agents at work?*

Yes

No

If yes, specify

E. coli is classified as a group 2 biological agent in the European Union classification for the protection of workers with biological agents (Directive 2000/54/EC).

VV: see section B.6.

5. *Do the donor and recipient organism exchange genetic material naturally?* – No

Yes

No

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? If yes, please specify.

Yes

No

Not known

Pexa-Vec is engineered to replicate preferentially in tumor cells by virtue of thymidine kinase gene interruption so its potential survival range is narrower than the parental organism.

b. Is the GMO in any way different from the recipient as far as mode and/or rate of reproduction is concerned? If yes, please specify.

Yes

No

Not known

Since Pexa-Vec selectively reproduces only in tumor cells; it is different to the parental organism.

c. Is the GMO in any way different from the recipient as far as dissemination is concerned? If yes, please specify.

Yes

No

Not known

The genes coding for hGM-CSF and β -galactosidase are inserted into the viral TK gene, thus inactivating the TK gene. TK inactivation decreases VV virulence (*Buller R. et al., 1985*) by restricting viral replication to proliferating cells. This also targets dissemination of the virus to tumors (*Puhlmann M. et al., 2000*).

d. Is the GMO in any way different from the recipient as far as pathogenicity is concerned?

Yes

No

Not known

Pexa-Vec has been attenuated by deletion of the TK gene.

2. *Genetic stability of the genetically modified organism*

β -galactosidase expression can be used for the evaluation of the genetic stability and purity of the product and for histopathological examination of biopsies. β -galactosidase expression is confirmed by infecting U2OS cells with Pexa-Vec in a modified plaque assay format and incubating the cultures with X-gal substrate to visualize β -galactosidase activity. β -galactosidase expressing plaques appear blue and non-expressing plaques appear as white colorless holes in the monolayer.

More than 500 plaques have been evaluated for β -galactosidase expression from two clinical lots of Pexa-Vec. The results show that the genetic stability is comparable from a clinical lot to another.

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

Yes

No

Not known

a. To which of the following organisms:

Humans

Animals

Plants

Others

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

The hGM-CSF is a pro-inflammatory cytokine which is involved in immune inflammatory reactions. It could theoretically participate in exacerbating the immune response of a patient to an allergen. To date, over 306 patients (including 7 compassionate use patients) have been treated with Pexa-Vec administered via direct intratumoral (IT) injection and/or intravenous (IV) infusion in 13 clinical studies with no report of treatment-related allergic effect. There is no known pathological trait attributed to β -galactosidase.

Non-clinical (in mice and rabbits) and clinical studies (in more than 306 patients with advanced stage of cancer) performed with the GMO until now have shown an acceptable safety profile of Pexa-Vec. The most notable effect from non-clinical experience was the GMO persistence in rabbits' testes for 3 weeks. To date, 15 to 20% subjects enrolled in Pexa-Vec clinical trials have developed small (< 1 cm) skin pustules after intravenous or intratumoral treatment, which were later confirmed to be Pexa-Vec related. However, secondary transmission and shedding to the environment has never been reported with the GMO.

4. Description of identification and detection methods

a. Techniques used to detect the GMO in the environment

The presence of the VV backbone can be detected by Polymerase Chain Reaction (PCR) specific for the E9L gene that codes for the vaccinia DNA polymerase.

b. Techniques used to identify the GMO – See 4a, above

A *Hind III* restriction map is used to identify the recombinant virus and demonstrate genetic integrity.

F. Information Relating To The Release

1. Purpose of the release.

The proposed release will be the administration of the investigational product, in a hospital or clinic setting, by IT injections to patients as part of an international, multicenter clinical trial. This clinical trial is a Phase III trial in patients with Advanced Hepatocellular Carcinoma (HCC) without prior systemic therapy.

Approximately 40 clinical sites in the EU will enroll patients in the JX594-HEP024 (PHOCUS) study. Additional clinical sites in Australia, Canada, China, Israel, Korea (Republic of), New Zealand, Singapore, Taiwan, Thailand and the USA will also participate in the study. A total of 600 patients will be recruited in this clinical trial with an expectation to enroll 200 patients in EU countries. Among them, 300 patients (i.e. approximately 100 patients in EU) will receive Pexa-Vec by Intratumoral (IT) injections.

The 300 patients randomized to the investigational arm will be treated by IT injections of Pexa-Vec at the dose of 1×10^9 pfu (9.0 log pfu) on Days 1, 15 and 29. In the control arm, the 300 patients will not receive Pexa-Vec.

After study completion, all patients will be followed up for survival.

The release will be performed by dedicated and trained medical and pharmacy personnel. The potential for viral shedding from patients biological fluids will be closely monitored. Detailed instructions on how to prevent contamination by the virus have been written on the basis of the medical knowledge acquired during the smallpox eradication campaign. These instructions will be provided to all personnel involved in handling of the product and the patients.

2. Is the site of the release different from the natural habitat or from the ecosystem in which the recipient organism is regularly used, kept or found? Yes No

If yes, specify

Not applicable. The GMO and VV are not naturally found in the environment. The current release can be compared to the use of VV during smallpox eradication campaign.

3. Information concerning the release and the surrounding area

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

The following are clinical study sites within Germany where study vaccine will be administered:

Site 1

The GMO will be administered at Medizinische Klinik und Poliklinik IV, II. Medizinische Klinik und Poliklinik Klinikum rechts der Isar TU München, 81675 München.

Site 2

The GMO will be administered at Universitätsklinikum Würzburg, Oberdürrbacherstrasse 6, 97080 Würzburg.

Site 3

The GMO will be administered at Universitätsklinikum Tübingen, Otfried-Müller-Strasse. 10, 72072 Tübingen.

Site 4

The GMO will be administered at Universitätsklinikum Freiburg, Hugstetter Strasse 55, 79106 Freiburg.

Site 5

The GMO will be administered at Medizinische Hochschule Hannover (MHH) (Hannover Medical School) - Klinik für Frauenheilkunde und Geburtshilfe, Medical School Hannover Carl-Neuberg-Strasse 1, 30625 Hannover.

Site 6

The GMO will be administered at Universitätsklinikum Hamburg-Eppendorf, Martinistrasse 52, 20246 Hamburg.

Site 7

The GMO will be administered at Klinikum der Johann-Wolfgang Goethe-Universität, JWG University Hospital, Department of Medicine 1 Theodor-Stern-Kai 7, Bldg. 11, 60596 Frankfurt.

Site 8

The GMO will be administered at Universitätsmedizin der Johannes Gutenberg-Universität Mainz, I. Med. Klinik und Poliklinik Gastrointestinale Onkologie, Gastroenterologie, Endosonographie, Langenbeckstrasse 1, 55101 Mainz.

Site 9

The GMO will be administered at Medizinischen Klinik III, Universitätsklinikum RWTH Aachen, Pauwelsstrasse 30, 52074 Aachen.

b. Size of the site (m²)

- i. **Actual release site (m²)** – Not applicable; Pexa-Vec will be administered at licensed healthcare facilities listed above.
- ii. **Wider release area (m²)** – Not applicable; Pexa-Vec will be administered at licensed healthcare facilities.

Universal (Standard) Precautions [[CDC 2007 Guideline](#), [Health Canada 1999 Guideline](#)] should be implemented for all patients treated with Pexa-Vec. **These are the same precautions routinely followed with any patient seen in the hospital/clinic** to reduce the risk of transmission of infectious organisms from both recognized and unrecognized sources in the clinical setting. These precautions for handling of patients, body fluids, and contaminated materials include (but are not limited to):

- Use of personal protective equipment (PPE) (e.g., gloves, gown, etc., as appropriate based on risk assessment).
- Safe handling of potentially contaminated equipment or surfaces in the patient environment.
- Implementation of respiratory hygiene and cough etiquette for two weeks after the first treatment.
- Good Hand Hygiene: washing hands with soap and warm water or with hand rubs containing at least 60% alcohol are recommended methods of decontamination following contact with material containing vaccinia ([Casey 2006](#); [Grabenstein 2003](#)).
- Proper Environmental Cleaning: Vaccinia viruses, like most enveloped viruses, are sensitive to inactivation by standard physical and chemical methods of disinfection. A standard hospital-grade disinfectant should be used to clean non-critical patient care equipment or medical devices between each patient use (e.g., bedpans, commodes, blood pressure cuffs, oximeters, glucose meters [[Infection Control Guidelines 1998](#)]). Hospital-grade disinfectants include 3% hydrogen peroxide, ≥60% alcohol, hypochlorite (1000 ppm), 0.5% accelerated

hydrogen peroxide, quaternary ammonium compounds, iodophors (75 ppm), phenolics, and aldehydes. The manufacturer's instructions should be followed to ensure adequate contact time and confirm the ability of the equipment to withstand the disinfectant used. Critical and semi-critical devices should be cleaned and reprocessed according to institutional guidelines.

- Proper Sharps Handling and Waste Management: All contaminated material (e.g., syringes, catheters, needles, tubing, gloves, containers, bandages, etc.) should be disposed of in a clearly-marked biomedical waste container and discarded according to regular institution procedure for infectious waste i.e.: autoclaving, incineration, or treatment with sodium hypochlorite solution. Textiles and fabrics can be decontaminated by laundering using routine protocols for healthcare facilities (e.g., hot water washing with detergent and hot air drying).
- c. **Proximity to internationally recognized biotypes or protected areas (including drinking water reservoirs), which could be affected.**

Not applicable.

- d. **Flora and fauna, including crops, livestock and migratory species, which may potentially interact with the GMO.**

Not applicable; the study vaccine will be administered at licensed healthcare facilities. It is not anticipated that the study vaccine or any waste associated with study procedures will affect the surrounding ecosystem.

4. Method and amount of release

- a. **Quantities of GMOs to be released.**

The maximal dose administered will be 1×10^9 pfu per infusion. Each patient will receive three (3) injections during the first month of treatment on days 1, 15 and 29.

Considering:

- the maximal dose administered per patient, i.e. 1×10^9 pfu (9.0 log pfu) per injection,
- the maximum total number of patients planned to be treated with Pexa-Vec in the study, i.e. 300 patients
- the maximal number of Pexa-Vec injections per patients, i.e. 3 injections

the maximum quantity of GMO expected to be released during the whole study is 9×10^{11} pfu.

Note: All these Pexa-Vec doses will not be released in EU clinical sites (approximately 1/3 of the GMO study release in EU).

b. Duration of the operation.

See 4. a)

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

Pharmacies, hospitals, and clinics routinely administer infectious organisms (e.g., live viral vaccines, and live BCG for bladder cancer) and Pexa-Vec preparation and administration handling recommendations are also based on CDC guidelines for preparation of the standard vaccinia vaccine ([CDC 2009](#)). Pexa-Vec is classified as a biohazard safety level 1 or 2 (BSL-1 or 2) agent based on the very low pathogenicity and the potential to spread only by direct physical contact (**NOTE**: BSL-1 vs BSL-2 classification is country dependent). Other examples of BSL 2 agents commonly encountered in the routine clinical practice of medicine include: *Escherichia coli*, *Helicobacter pylori*, *Staphylococcus aureus*, and Herpes simplex virus. Regardless of the BSL classification, the following are recommended as conservative measures for Pexa-Vec preparation, decontamination and storage:

1. Individuals excluded from handling (preparation and administration):

- pregnant or breastfeeding women
- immunocompromised individuals (e.g., organ transplant recipient, HIV-positive individual, or receiving chronic immunosuppressive medication)
- individuals with ongoing severe inflammatory skin condition requiring medical treatment or history of severe eczema requiring medical treatment.

2. Limit access:

a. Place a biohazard sign in the preparation area

b. Limit access to the designated area (e.g., the hood used to prepare Pexa-Vec) during preparation

3. Equipment

The following are recommended as conservative measures for Pexa-Vec preparation, decontamination and storage [To be adapted to local requirements]:

a. Prepare in a Class IIA Biological Safety Cabinet (e.g., a standard chemotherapy hood equipped with a properly maintained high-efficiency particulate air [HEPA] filter)

b. Wear standard PPE - gloves, goggles, mask, and gown while preparing the agent (e.g., as with standard chemotherapy precautions)

4. Recommended Hood Cleaning and Decontamination:

- a. Prepare the hood for Pexa-Vec preparation utilizing standard institutional requirements for cleaning and decontamination after chemotherapy mixing.
- b. Pexa-Vec is inactivated by standard physical and chemical methods of disinfection, including those noted below [*Infection Control Guidelines 1998*].

After Pexa-Vec preparation, completely wipe down the inside of hood with:

- i. At least 60% alcohol, OR
- ii. Bleach solution (with at least 0.6% of active chlorine) followed by at least 60% alcohol, OR
- iii. Other institution recommended agents (other hospital-grade disinfectants include: 3% hydrogen peroxide, \geq 60% alcohol, hypochlorite (1000 ppm), 0.5% accelerated hydrogen peroxide, quaternary ammonium compounds, iodophors (75 ppm), phenolics, and aldehydes).

The manufacturer's instructions should be followed to ensure adequate contact time and confirm the ability of the equipment to withstand the disinfectant used.

All contaminated material should be disposed of in a clearly-marked biomedical waste container and discarded according to regular institution procedure for infectious waste.

5. Storage:

- a. Store Pexa-Vec at -60°C or below in an alarmed, temperature-monitored, secured freezer with restricted access.
- b. Pexa-Vec can be stored with other therapeutics, but should be separate from lab samples.

All study patients will receive detailed instructions (similar to those outlined above) as part of the informed consent process. As previously noted these recommendations are conservatively based on the US CDC guidelines for the standard (non-attenuated) vaccinia vaccine. Recall from information provided elsewhere in this document that Pexa-Vec is a weaker, attenuated vaccinia vaccine.

Person-to-person transmission of Pexa-Vec has not been reported despite development of superficial pustules in approximately 20% – 25% of patients following Pexa-Vec treatment. Nevertheless, SillaJen has applied many of the same conservative guidelines to minimize the theoretical potential of Pexa-Vec transmission to other people who might be in direct physical contact specifically with a patient who has developed a pustule.

All patients treated with Pexa-Vec (with or without a pustule) should:

1. Practice good hand hygiene with soap and warm water or with hand rubs containing at least 60% alcohol.
2. Use barrier contraception method for at least 6 weeks after each treatment of Pexa-Vec

Only for patients who develop a Pexa-Vec related superficial skin (or oral mucosa) pustule:

In addition to the guidelines listed for all patients treated with Pexa-Vec above, the following should be in place until pustule resolution (e.g., the lesion has scabbed over and fallen off):

1. Cover skin pustule with a non-occlusive bandage (e.g., gauze)
2. Do not allow others or patient to have direct physical contact with the pustule(s) itself and any potentially contaminated material (e.g., pustule dressing, clothing, sheets). If patient care is necessary (e.g., changing pustule dressing), gloves should be worn and hands should be washed afterward with soap and warm water or hand rub containing at least 60% alcohol.
3. Avoid direct physical contact (especially with the pustule[s] and any potentially contaminated material) with children <12 months of age and the Excluded Individuals population (pregnant or breastfeeding women, individuals with ongoing severe inflammatory skin condition requiring medical treatment or history of severe eczema requiring medical treatment, immunocompromised individuals [e.g., organ transplant recipients, HIV-positive individuals, or those receiving chronic immunosuppressive medication]).
4. Avoid touching other parts of your body (e.g., eyes, nose, or other areas) after touching a pustule or any other potentially contaminated material (e.g., after changing pustule dressing).
5. If a mouth pustule is present:
 - a. Wear a mask when around other people
 - b. Do not share items such as tooth brushes, eating utensils, etc.
6. Dispose of contaminated materials (e.g., gauze, bandages) in a sealed container or ziplock bag in regular trash. Fabrics (e.g., clothing, sheets, towels) that have touched an uncovered pustule can be laundered in hot water with detergent. Alternatively, you may use bleach in warm or cold water to inactivate the virus (one cup per wash load).

Study personnel should instruct patients to tell the study doctor if they notice they have developed a pustule or if they notice anything unusual or different from what they've been told to expect.

Patients who cannot comply with the above guidelines if they were to develop a Pexa- Vec related pustule should not enroll in a Pexa-Vec trial.

ACCIDENTAL EXPOSURE TO PEXA-VEC

If an accidental human exposure to Pexa-Vec occurs, no specific interventions other than local wound care, as needed, and close observation are indicated. Specifically, the following is recommended:

1. Implementation of local, institutional needle stick, or other exposure guidelines.
2. Wash area thoroughly with soap and water.
3. Cover area with non-occlusive dressing until complete resolution.
4. Report the event to the Principal Investigator, the institution's Biosafety Specialist, and/or physician knowledgeable in the care of individuals experienced with vaccinia infection.

To date, four inadvertent exposures have been documented to date with Pexa-Vec without clinically significant symptoms or need for specific treatment other than minor local exposure site observation. In the event of a percutaneous exposure in an otherwise healthy individual no clinically significant sequelae are expected aside from potential local skin redness and gradual uncomplicated healing. If a pustule develops, the usual course following intentional vaccination is anticipated, which includes pustule development within 7–14 days followed by scab formation and resolution within 21–28 days total. The pustule(s) should be covered with a non-occlusive dressing.

5. *Short description of average environmental conditions (weather, temperature, etc.)*
Not applicable.

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*

Since its entry in clinical development Pexa-Vec more than 306 patients (including 7 compassionate use patients) have been injected with Pexa-Vec so far. Pexa-Vec was shown to be generally safe and well tolerated during these clinical trials with the most frequent adverse events reported being vaccine-related reactions like fever, chills, anorexia, aches/pain, fatigue, headache and/or nausea. Acute, mild to moderate hypotension is expected within 1 hour following intravenous treatment and is then observed intermittently (with maximum severity between 8 to 12 hours) during the first 24 hours following treatment with Pexa-Vec. Acute, moderate to severe fever is expected within 4 to 6 hours posttreatment and has a typical duration of 18 to 24 hours. At the tumor or injection sites, the following toxicities are possible: pain, necrosis, ulceration and inflammation. As reported in section E.3.b), 15 to 20% of subjects have developed small (< 1 cm) skin pustules which were later confirmed to be Pexa-Vec related. However, secondary transmission and shedding to the environment has never been reported with the GMO.

G. Interactions Of The GMO With The Environment And Potential Impact On The Environment

1. Complete name of target organisms

Order and Higher Taxon:	Primates
Family:	Hominidae
Genus:	Homo
Species:	Sapiens
Subspecies:	Not applicable
Strain:	Not applicable
Pathovar:	Not applicable
Common name:	Human

2. Anticipated mechanism and result of interaction between the released GMOs and the target organism.

Pexa-Vec is an oncolytic therapeutic vaccinia virus designed to selectively replicate in and destroy cancer cells, while at the same time stimulating a systemic anti-tumoral immune response through the expression of its transgene, hGM-CSF. The expression of β -galactosidase also provides a marker for viral replication in the histological examination of tumour biopsies, using immuno-histochemistry, and shedding or transfer of the virus to caregivers, through the detection of antibodies specific to β -galactosidase.

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

There is extremely low potential for gene transfer to other species under the proposed release. As mentioned in Section F, the GMO will be released in a hospital operating room and is unlikely to come in contact with other animal species.

Recombination events with other organisms are unlikely since this would require the presence of other poxviruses which are not naturally found in the environment.

Pexa-Vec remains exclusively in the cytoplasm of infected cells thus eliminating any risk of integration of the viral DNA into the host genome.

4. Is post-release selection such as increased competitiveness, increased invasiveness for the GMO likely to occur?

Yes

No

Not known

Give details

Not applicable. The replicative and propagative characteristics of vaccinia virus have been attenuated in Pexa-Vec with the disruption of the thymidine kinase gene which renders the

modified organism dependent of highly dividing cells such as cancer cells. Therefore Pexa-Vec should have reduced competitiveness and invasiveness compared to vaccinia virus.

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

Pexa-Vec is anticipated not to interact with non-target organisms due to the conditions of the proposed release. Indeed, the GMO will be confined to the hospital site, including the operating room, pharmacy, clinical laboratory, and autoclaving/incineration area. In the unlikely event of inadvertent administration to non-target organisms, further spread would be unlikely as there were only rare cases of secondary transmissions during the smallpox vaccination campaign with wild type vaccinia virus and the pathogenicity of Pexa-Vec is reduced compared to the wild type vaccinia virus.

6. *Complete name of non-target organisms which may be affected unwittingly*

The most likely non-target organisms which may be accidentally exposed to the GMO are human clinic staff members or close patient contacts.

Order and Higher Taxon:	Primates
Family:	Hominidae
Genus:	Homo
Species:	Sapiens
Subspecies:	Not applicable
Strain:	Not applicable
Pathovar:	Not applicable
Common name:	Human

It is possible that hospital staff may be injected by accident and that secondary transmission occurs in patients' family members. Infection would be harmful in at risk populations (see section B.7.b) but patients who cannot avoid direct physical contact with people in those at risk groups as well as healthcare personnel in those at risk groups will be excluded from study participation. To date, studied healthcare personnel who have come in contact with treated patients, have not seroconverted due to the exposure to Pexa-Vec.

7. *Likelihood of genetic exchange in vivo*

a. From the GMO to other organisms in the release ecosystem

There is minimal potential for gene transfer to other species under the proposed release of the GMO. The GMO will be released to be administered to patients in hospital operating rooms and is unlikely to come in contact with other animal species. Furthermore Pexa-Vec remains localized in the cell cytoplasm up to the lysis of the infected cell. There is no possible genetic exchange with other human poxviruses as they are not endemic in humans. In animals

susceptible to infection by the vaccinia virus, the opportunity for genetic recombination with animal poxviruses is probably low since, to our knowledge, this has never been reported during the smallpox eradication campaign.

b. From other organisms to the GMO

See response to 7a, above.

c. Likely consequences of gene transfer

See response to 7a, above.

8. *Give references to relevant results from studies of the behaviour and characteristics of the GMO and its ecological impact carried out in simulated natural environments (eg microcosms, etc).*

No studies have been conducted on the ecological impact of Pexa-Vec in simulated natural environments.

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.

Monitoring of the direct and indirect effects of the GMO in patients will be achieved using the following clinical assessments: physical examinations, adverse event reporting, clinical laboratory assessments throughout the clinical study for all patients.

2. Methods for monitoring ecosystem effects.

Not planned as the GMO and the parental vaccinia virus are not naturally found in the environment.

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

Method not available - The probability for a transfer of the donated genetic material to other organisms is unlikely since Pexa-Vec has no nuclear localization and there is no known human endemic virus able to complement, to recombine or to exchange genetic material with the vaccinia genome.

4. Spatial extent of the monitoring area (m²).

Not applicable: the GMO will be administered to patients by intravenous injections in hospital operating rooms.

5. Duration of the monitoring.

Safety assessments will be performed all along the patient's participation in the clinical trial and up to 4 weeks after treatment discontinuation.

6. Frequency of the monitoring.

The patient's safety will be monitored in an ongoing manner for the complete course of the study: From start of treatment to 28 days after the last dose of any study medication. There is also an IDMC in place for safety oversight.

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I. Information On Post-Release And Waste Treatment

1. Post-release treatment of the site

The biological safety cabinet where the product will be prepared for injection will be decontaminated before and after the manipulation with 70% isopropyl alcohol or with any other active disinfectant.

All material dedicated to the clinical trial will be disposed of after use and will then be autoclaved, incinerated, or treated with sodium hypochlorite solution by personnel who are trained to dispose of biohazard waste.

The material not dedicated to the clinical trial will be sterilized or cleaned with an active disinfectant (e.g. bleach solution at 0.6% of active chlorine or any other active disinfectant) followed by 70% isopropyl alcohol before using it for other purposes.

Following the patient's discharge home, the hospital room (surfaces and floor) and the bathroom will be cleaned in a standard way using a hospital grade disinfectant.

2. Post-release treatment of the GMOs

For clinical waste treatment, see I.3.(b).

3a. Type and amount of waste generated

The virus titer of the clinical batch which will be used for the JX594-HEP-024 (PHOCUS) trial is 1.0×10^9 pfu/mL. The virus is suspended in a total volume of 2.3 mL from which 2.0 mL are extractable. The total dose which will be injected to patients in the Pexa-Vec arm (Arm A) of the proposed clinical trial will be 3.0×10^9 pfu. As a consequence, the quantity of waste per injection will not be more than 3×10^8 pfu, which is considered limited.

3b. Treatment of waste

The waste is to be deactivated by:

- Autoclaving/incineration

or

- Use of a disinfectant [e.g. bleach solution at 0.6% of active chlorine or any other active disinfectant].

J. Information On Emergency Response Plans

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

It will be recommended to personnel involved in Pexa-Vec handling to act as recommended below in case of incident with the use of Pexa-Vec.

- Accidental spill:

The spill area will be contained with barriers to avoid traffic within the area. Personnel that are involved in the clean-up of the spill should wear personal protective equipment. Spill should be absorbed with paper towels and an active disinfectant (e.g. bleach solution at 0.6% of active chlorine or any other active disinfectant) should be applied. The contact with the disinfectant will be allowed for 20 to 30 minutes. Contaminated paper towels will then be replaced by fresh paper towels soaked in disinfectant.

- Eye splash:

The eyes should be rinsed with clean water or physiological saline solution (NaCl 0.9%) and one drop of trifluridine 1% should be instilled every 2 hours.

- Intact skin splash:

An absorbent tissue should be placed immediately on the affected area. After removing the tissue, the skin should be washed with mild soap thoroughly and rinsed abundantly with water. Then the skin should be covered for 5 minutes with a pad soaked with either a solution of bleach at 0.45% of active chlorine or a solution of 4% iodine. Then the skin should be rinsed again abundantly with water. The contaminated tissue and pad should be treated as infectious material.

- Cut or puncture:

Allow bleeding from the wound before flushing it under a running stream of clean, and preferably sterile, water.

2. Methods for decontamination of the areas affected.

See J.1.

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

Not applicable.

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

Patients will be monitored for the occurrence of adverse events and serious adverse events (SAE) according to the clinical protocol. Each SAE will be recorded and assessed by the hospital staff and the study sponsor, and Health Authorities will be notified when applicable.

Replicative and propagative characteristics of vaccinia virus have been attenuated in Pexa-Vec with the disruption of the TK gene which makes the virus replication dependent on actively dividing cells such as cancer cells. Therefore the probability of propagation of Pexa-Vec outside patients' tumors is very low.

The clinical information available to date suggests that Pexa-Vec is safe at the clinical dose of 1×10^9 pfu (10,000-fold higher than smallpox vaccine dose) and has not spread to caregivers in contact with the treated patients. Should shedding occur, the level of exposure would be predicted to be low compared to the doses received by patients in the proposed trial, and extremely low compared to doses of non-attenuated vaccines administered to the public (e.g. vaccines against smallpox). In addition, exposed individuals over the age of 35 will likely have been previously immunized with vaccinia. In the highly unlikely event that an exposed individual were to demonstrate virus-associated toxicity, therapy could be initiated with vaccinia immune globulin and/or cidofovir. Therefore, public health risks with this virus are extremely low and in fact should be lower than with standard vaccination procedures. To date, no reports of transmission to health care personnel from vaccinia recipients have been published. Routine barriers nursing approaches should be used per institutional guidelines for infectious organisms (e.g. such as for *M. tuberculosis*, *Pseudomonas*); these include gloves, gown, face mask and safety goggles.

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