



JX-594

**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**

14 September 2011

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LIST OF ABBREVIATIONS

<i>Amp^R</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>
GMO	Genetically modified organism
hGM-CSF	Human granulocyte macrophage-colony stimulating factor
JX-594	Recombinant VV
<i>lacZ</i>	β -galactosidase coding gene
PCR	Polymerase chain reaction
TK	Thymidine kinase
TK _L	ATP-binding domain sequence of TK
TK _R	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

- a) Member State of notification *Germany*
- b) Notification number B/DE/11/PEI 1491
- c) Date of acknowledgement of notification 08.11.2011
- d) Title of the project

The project, JX594-HEP018 clinical trial, is entitled “A Phase 2b Randomized, Open-Label Trial of JX-594 (Vaccinia GM-CSF / TK-deactivated Virus) Plus Best Supportive Care Versus Best Supportive Care in Patients with Advanced Hepatocellular Carcinoma Who Have Failed Sorafenib Treatment (JX594-HEP018)”.

e) Proposed period of release *From 01 April 2012 until 31 August 2013 (date of study completion)*

2. Notifier

Name of institution or company

*Sponsor's legal representative: Transgene SA
Boulevard Gonthier d'Andernach
Parc d'Innovation
CS80166
67405 Illkirch Graffenstaden cedex - France*

3. GMOs characterization

a) Indicate whether the GMO is a:

- | | | |
|----------------|-------------------------------------|-----------------------|
| viroid | <input type="checkbox"/> | |
| RNA virus | <input type="checkbox"/> | |
| DNA virus | <input checked="" type="checkbox"/> | <i>Vaccinia virus</i> |
| bacterium | <input type="checkbox"/> | |
| fungus | <input type="checkbox"/> | |
| animal | <input type="checkbox"/> | |
| - mammals | <input type="checkbox"/> | |
| - insect | <input type="checkbox"/> | |
| - fish | <input type="checkbox"/> | |
| - other animal | <input type="checkbox"/> | specify phylum, class |

other, specify (kingdom, phylum and class)

b) Identity of the GMO (genus and species)

Genus: *Orthopoxvirus*

Species: *Vaccinia virus (VV)*

The GMO is a viral suspension of the recombinant virus JX-594. JX-594 is a non-integrative, replicative, propagative, recombinant VV derived from the commonly used commercial vaccine Wyeth strain. JX-594 differs by three genetic modifications from the wild type Wyeth strain: 1) disruption of the viral thymidine kinase (TK) gene by, 2) insertion of the human granulocyte macrophage-colony stimulating factor (hGM-CSF) gene and 3) insertion of the lacZ gene.

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

Double-stranded DNA viruses, such as VV, typically have very low rates of mutation from one passage to the next (Nalca A. and Zumbrun E., 2010). Dryvax[®], from which JX-594 virus was prepared, is a mixed population of vaccinia clones. During the manufacturing process of JX-594 virus, one clone was selected (LVB Clone 1). The genetic elements of JX-594 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 TK_R region (which is not expressed in JX-594) 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 6 (1)), by the same notifier?*

Yes

No

If yes, insert the country code(s): *DE, IT and ES*

Please use the following country codes:

Austria AT; Belgium BE; Bulgaria BG; Cyprus CY; Czech Republic CZ; Denmark DK; Estonia EE; Finland FI; France FR; Germany DE; Greece GR; Hungary HU; Ireland IE; Italy IT; Latvia LV; Lithuania LT; Luxembourg LU; Malta MT; Netherlands NL; Poland PL; Portugal PT; Romania RO; Slovak Republic SK; Slovenia SI; Spain ES; Sweden SE; United Kingdom GB.

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
- Notification number

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

Yes

No

If yes:

- Member State of notification *United States of America, Canada, Australia, Russia, South Korea, Taiwan, Hong Kong*
- Notification number

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

The likelihood of JX-594 becoming persistent and invasive in natural habitats is low for the following reasons:

- *Due to the inactivation of its TK gene, JX-594 replicates preferentially in actively dividing cells. JX-594 is therefore expected to propagate mostly in cancer cells. JX-594 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 JX-594 have not detected spontaneous revertants of JX-594.*
- *JX-594 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 non-attenuated wild type virus (i.e. parental virus of JX-594).*

B. INFORMATION RELATING TO THE RECIPIENT OR PARENTAL ORGANISMS FROM WHICH THE GMO IS DERIVED

1. *Recipient or parental organism characterization:*

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

- | | | |
|----------------|-------------------------------------|-----------------------|
| viroid | <input type="checkbox"/> | |
| RNA virus | <input type="checkbox"/> | |
| DNA virus | <input checked="" type="checkbox"/> | <i>Vaccinia virus</i> |
| bacterium | <input type="checkbox"/> | |
| fungus | <input type="checkbox"/> | |
| animal | <input type="checkbox"/> | |
| - mammals | <input type="checkbox"/> | |
| - insect | <input type="checkbox"/> | |
| - fish | <input type="checkbox"/> | |
| - other animal | <input type="checkbox"/> | specify phylum, class |

other, specify

2. Name

- (i) Order and/or higher taxon (for animals) *Poxviridae*
- (ii) Genus *Orthopoxvirus*
- (iii) Species *Vaccinia virus*
- (iv) Subspecies
- (v) Strain *Wyeth*
- (vi) Pathovar (biotype, ecotype, race, etc.)
- (vii) Common name

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:

(i) Yes

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

(iii) Not known

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

c) 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 microorganism

Water
Soil, free-living
Soil in association with plant-root systems
In association with plant leaf/stem systems
In association with animal

other, specify

The parental organism is not naturally found in the environment.

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

Not applicable.

5. (a) *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 HindIII region*
- Sequencing of the TK gene.*

5. (b) *Identification techniques*

See 5. (a).

6. *Is the recipient organism classified under existing Community rules 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 Economic Community 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 significantly 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

Other

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:

- Pregnant or breastfeeding women*
- Children less than 12 months old*
- 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 ecosystem where the release will take place:

Not relevant.

c) Way of reproduction: Sexual Asexual

Not relevant.

d) Factors affecting reproduction:

Not relevant.

9. Survivability

a) ability to form structures enhancing survival or dormancy:

- | | |
|-----------------------------|--------------------------|
| (i) endospores | <input type="checkbox"/> |
| (ii) cysts | <input type="checkbox"/> |
| (iii) sclerotia | <input type="checkbox"/> |
| (iv) asexual spores (fungi) | <input type="checkbox"/> |
| (v) sexual spores (fungi) | <input type="checkbox"/> |
| (vi) eggs | <input type="checkbox"/> |
| (vii) pupae | <input type="checkbox"/> |
| (viii) larvae | <input type="checkbox"/> |
| (ix) other, specify... | <input type="checkbox"/> |

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 10⁷ fold by exposure to 60°C at ambient pressure within an hour or less). Vaccinia is rendered non-infectious following treatment in an autoclave. Hospital-grade chemical disinfectants are also effective against lipophilic viruses such as VV.

10. (a) 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 JX-594 virus tends to limit its dissemination to tumors (Puhmann M. et al., 2000). In humans, JX-594 could disseminate from biological fluids, the injection site and vaccine pustules.

The presence of JX-594 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. JX-594 was also identified in pustules exudates of patients who

developed skin pustules after JX-594 intravenous or intratumoral administration. The presence of JX-594 in feces was not monitored until now but has been planned in the proposed clinical trial.

10. (b) 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, all personnel involved in handling of the product, the patients and their family. The injection site (the skin through which JX-594 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 JX-594 related skin ulceration, pox or rash, whichever is longer. 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 their body fluids with a 1-in-10-part dilution of chlorine bleach. Clothing, towels and bed linens will be washed using the hot water cycle with detergent followed by hot air drying. Contaminated clothing should be stored separately from clean linen. 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 contact with immunosuppressed individuals is recommended for 14 days after treatment with JX-594 as a precaution. Similar to precautions exercised by individuals with upper respiratory infections, wearing face shields or surgical masks is recommended if individuals are coughing or sneezing during this time period.

Sexual secondary transmission of VV from a vaccination recipient has already been described (MMWR, 2004; MMWR, 2010). This report together with JX-594 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 to the proposed phase IIb trial. Patients will be requested to abstain from sexual activity for a minimum of 7 days after each injection and to use barrier method during the treatment period and for at least 6 weeks after the last JX-594 treatment.

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

Not applicable.

C. INFORMATION RELATING TO THE GENETIC MODIFICATION

1. Type of the genetic modification

- i. Insertion of genetic material
- ii. Deletion of genetic material
- iii. Base substitution
- iv. Cell fusion
- v. Other, specify

2. *Intended outcome of the genetic modification*

JX-594 is a GMO developed as a therapeutic candidate to treat patients with hepatocellular carcinoma. The vaccinia TK gene was inactivated in JX-594 to enhance the in vivo tropism of the VV for cancer cells vs. normal cells. The therapeutic transgene encoding hGM-CSF was inserted in order to increase the anti-cancer efficacy of JX-594 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 No

If no, go straight to question 5.

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

Yes No

If no, go straight to question 5.

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

a) Type of vector

Plasmid
Bacteriophage
Virus
Cosmid
Transposable element

Other, specify

b) Identity of the vector

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

Antibiotic resistance

Other, specify

Indication of which antibiotic resistance gene is inserted : *Ampicillin resistance (AmpR) gene. However, the AmpR sequence is finally not contained in the DNA fragment which is inserted in the recipient.*

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 2 VV genomic regions (TK_L and TK_R) that allow homologous recombination between the transfer plasmid and VV.

f) Method for introducing the vector into the recipient organism

- i. transformation
- ii. electroporation
- iii. macroinjection
- iv. microinjection
- v. infection
- vi. other, specify

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 B.3 (a) and (b) is no, what was the method used in the process of modification?*

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

6. *Composition of the insert*

a) Composition of the insert

The 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

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

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 anti-cancer efficacy of JX-594 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 product or function are not known?

Yes No

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 *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

	<i>lacZ</i>	<i>p7.5K</i>
i. Order and/or higher taxon (for animals)	<i>Proteobacteria</i>	<i>Poxviridae</i>
ii. Family name (for plants)	<i>Enterobacteriaceae</i>	
iii. Genus	<i>Escherichia</i>	<i>Orthopoxvirus</i>
iv. Species	<i>Escherichia coli</i>	<i>Vaccinia virus</i>
v. Subspecies		
vi. Strain		<i>Wyeth</i>
vii. Cultivar/breeding line		
viii. Pathovar		
ix. Common name	<i>E. coli</i>	

3. *Is the organism significantly 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	<input checked="" type="checkbox"/>
Animals	<input checked="" type="checkbox"/>
Plants	<input type="checkbox"/>
Other	<input type="checkbox"/>

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 5-10 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 into the European Economic Community 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?*

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?

Yes No 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 Unknown

Specify

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

Yes No Not known

Specify

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

Specify

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 JX-594 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 JX-594. The results show that the genetic stability is comparable from a clinical lot to another.

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

Yes No 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)

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. JX-594 had been administered in more than 90 patients to date 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 90 patients with advanced stage of cancer) performed with the GMO until now have shown an acceptable safety profile of JX-594. The most notable effect from non-clinical experience was the GMO persistence in rabbits' testes for 3 weeks. To date five subjects enrolled in JX-594 clinical trials have developed small (< 1 cm) skin pustules after intravenous or intratumoral treatment at the 1×10^9 pfu dose, which were later confirmed to be JX-594 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

A HindIII restriction map is used to identify the recombinant virus and demonstrate genetic integrity.

F. INFORMATION RELATING TO THE RELEASE

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

The release will be the administration of the product, in hospital operating rooms, by intravenous and intratumoral injections to patients as part of an international, multicenter clinical trial.

This clinical trial is a phase IIb trial in patients with advanced hepatocellular carcinoma who have failed sorafenib treatment, the only approved chemotherapy in the European Union in

this patient population. The study has been designed to assess whether JX-594, added to the best supportive care (BSC), prolongs the survival of patients. 120 patients will be recruited into the study. Among them, 80 patients will receive JX-594 plus BSC and 40 patients will receive BSC only. 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, the patients and their family.

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

Yes

No

If yes, specify

Not 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):
JX-594 will be administered at the following clinical sites:*

- *Hôpital de la Croix-Rousse 103 grande rue de la Croix Rouse
69317 Lyon cedex 04

Service d'Hépatologie
Professeur Philippe MERLE*
- *Nouvel Hôpital Civil 1 place de l'Hôpital
67091 Strasbourg

Centre d'Investigation Clinique
Docteur François HABERSETZER*
- *Hôpital Saint André 1 rue Jean Burguet
33000 Bordeaux

Docteur Jean-Frédéric BLANC*
- *Centre Eugène Marquis Avenue de la bataille Flandres Dunkerque
CS44229
35042 Rennes cedex

Service d'Oncologie Médicale
Docteur Evelyne BOUCHER*
- *Hôpital Purpan Place du Docteur Baylac
31000 Toulouse*

*Service d'Hépatogastro-Entérologie
Docteur Jean-Marie PERON*

- *Institut Paoli-Calmettes 232 boulevard Sainte Marguerite
13009 Marseille*

*Service d'Oncologie
Professeur Jean-Luc RAOUL*

- *Hôpital la Pitié-Salpêtrière 47-83 boulevard de l'Hôpital
75651 Paris cedex 13
Docteur Vlad RATZIU*

b) Size of the site (m²):

i. Actual release site (m²):

See below.

ii. Wider release area (m²):

No specific size is required for the clinical site. However all zones in which JX-594 will be handled and administered to the patients and in which the patients will be hospitalized after dosing with JX-594 must have restricted access (i.e. access to these zones will be controlled and limited to authorized hospital staff who has received training on measures to control infection). The international biohazard symbol will be affixed at each entrance of the restricted zones. The site pharmacy and laboratory must be equipped with a vertical biological safety cabinet in accordance with biosafety level 2 handling guidelines. The patient will stay into a private room for 8 and 24 hours after each intratumoral and intravenous administration, respectively. Dedicated, private bathroom facilities must be allocated to patients having received JX-594 treatment.

Environmental surfaces, hospital rooms, patients' care areas, patients-care equipment and medical devices should be routinely cleaned with a hospital-grade disinfectant. Following the patient's discharge home, all surfaces of the room and bathroom should be wiped down with a hospital grade disinfectant. Items such as dishes, utensils, textiles and fabrics will be decontaminated with hot water (>70°C) and detergent. Any and all waste should be autoclaved, incinerated, or treated with sodium hypochlorite solution by personnel who are trained to dispose of biohazard waste.

c) Proximity to internationally recognized biotopes 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

4. Method and amount of release

a) Quantities of GMOs to be released

The dose administered is 10⁹ pfu per injection. Each patient will receive 6 injections over an 18-week period.

b) Duration of the operation

See 4.a)

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

The GMO is released for clinical use only, supplied in closed vials and labeled appropriately. The administration is under the responsibility of the medical staff, according to the clinical protocol and in respect of the Good Clinical Practice.

The product must be prepared in aseptic conditions compliant with injectable solutions. JX-594 will be prepared under a vertical biological safety cabinet of type II in a laboratory or pharmacy under the direction of an accredited pharmacist. The safety cabinet will be decontaminated before and after manipulation with 70% isopropyl alcohol or with any other active disinfectant.

All staff involved in handling of JX-594 or any material or linen potentially contaminated with JX-594 must wear personal protective equipment (i.e. waterproof gloves, gown, surgical/procedure mask and safety goggles with side shields). All transfers of JX-594 must be done using a sealed plastic transport bag or other sealed, leak-proof secondary container displaying a clearly marked biohazard symbol. Furthermore, the site staff will follow the standard hospital policy recommended for the manipulation of live virus vaccines.

In case of 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. 10% bleach solution or any other active disinfectant) should be applied. The contact with the disinfectant will be allowed for 20-30 minutes. Contaminated paper towels will then be replaced by fresh paper towels soaked in disinfectant. All personnel involved in handling the product is informed that in case of:

- Eye Splash: the eyes should be rinsed with clean water at an eyewash station and one drop of trifluridine 1% should be instilled every 2 hours.*
- Intact skin splash: the area should be decontaminated with alcohol and then flushed under a running stream of water.*
- Cuts or Punctures: bleeding from the wound should be allowed before flushing it under a running stream of clean, and preferably sterile, water.*

The individual should be referred to and medically monitored by a physician knowledgeable in the care and treatment of patients with vaccinia infections.

A subset of up to 50 staff involved with JX-594 preparation and/or administration (e.g. pharmacists, doctors, interventional radiology staff, nurses involved in administration of drug or monitoring of patients on trial) will have blood collected within one month prior to JX-594 exposure or to patients treated with JX-594. Samples will then be collected approximately 6 months and 12 months later in order to assess anti-vaccinia and anti- β -galactosidase antibody titers. Development of anti- β -galactosidase antibodies would be an indication of unintentional exposure and/or infection of care-givers by JX-594 as wild-type vaccinia virus does not encode β -galactosidase.

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, JX-594 has been released in a clinical setting closed to the proposed one in 8 prior occasions. More than 90 patients have been injected with JX-594 so far. JX-594 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 8-12 hours) during the first 24 hours following treatment with JX-594. Acute, moderate to severe fever is expected within 4-6 hours post-treatment and has a typical duration of 18-24 hours. At the tumor or injection sites, the following toxicities are possible: pain, necrosis, ulceration and inflammation. As reported in section E.3.b), five subjects to date have developed small (< 1 cm) skin pustules which were later confirmed to be JX-594 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, IF SIGNIFICANTLY DIFFERENT FROM THE RECIPIENT OR PARENT ORGANISM

1. *Name of target organisms (if applicable)*

- i. Order and/or higher taxon (for animals)
- ii. Family name (for plants)
- iii. Genus
- iv. Species
- v. Subspecies
- vi. Strain
- vii. Cultivar/breeding line
- viii. Pathovar
- ix. Common name

The target organisms are the human beings.

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

JX-594 is an armed 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 tumor 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.

JX-594 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 JX-594 with the disruption of the thymidine kinase gene which renders the modified organism dependent of highly dividing cells such as cancer cells. Therefore JX-594 should have reduced competitiveness and invasiveness compared to vaccinia virus.

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

JX-594 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 JX-594 is reduced compared to the wild type vaccinia virus.

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 nature

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 with family members in those at risk groups as well as healthcare personnel in those at risk groups will be excluded from study participation.

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 JX-594 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 7 (a).

(c) Likely consequences of gene transfer:

No data are available.

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.):

No data are available regarding the behaviour and characteristics of JX-594 in the mentioned environments.

9. Possible environmentally significant interactions with biogeochemical process (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.

Furthermore, JX-594 shedding in throat swabs, urine and feces will be analyzed in up to 25 patients treated with JX-594 at different time points. In throat swabs and urine JX-594 will be detected by plaque formation while it will be detected by quantitative PCR in feces samples.

Secondary transmission will also be monitored in up to 50 hospital staff (e.g. pharmacists, doctors, interventional radiology staff, nurses involved in administration of drug or monitoring of patients on trial) as described in section F.4.c.

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 JX-594 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. *Site of the monitoring area (m2)*

Not applicable: the GMO will be administered to patients by intravenous and intratumoral 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 disease progression.

6. *Frequency of the monitoring*

Monitoring visits, during which safety will be assessed, are planned every week during 4 weeks then every 3 weeks during 18 weeks and then every 6 weeks up to the end of the follow up. Additional monitoring visits will be performed at each GMO injection.

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. 10% bleach solution 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).

3. (a) *Type and amount of waste generated*

JX-594 titer in the clinical batch will be within the required specifications (i.e., 1.0×10^7 to 5.0×10^9 pfu/mL). JX-594 is suspended in a total extractable volume of 0.8 mL. The total dose which will be injected to patients in the proposed clinical trial is 1.0×10^9 pfu. As a consequence, the quantity of waste per injection will not be more than 3.0×10^9 pfu.

There will be a total of 120 patients in this clinical trial. It has been estimated that a given clinical site would recruit approximately 2 patients over the recruitment period. Each patient will receive 6 injections of 10^9 pfu of JX-594. The overall quantity of waste per site will be 36×10^9 pfu, which is considered limited.

3. (b) Treatment of waste

The waste is to be deactivated by:

- *Autoclaving/incineration*

or

- *Utilisation of a disinfectant [e.g. sodium hypochlorite (1:10 dilution of sodium hypochlorite (e.g. Clorox) for a working concentration of 0.615% sodium hypochlorite) or any other active disinfectant].*

J. INFORMATION ON EMERGENCY RESPONSE PLAN

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

It will be recommended to personnel involved in JX-594 handling to act as recommended below in case of incident with the use of JX-594.

- 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. 10% bleach solution or any other active disinfectant) should be applied. The contact with the disinfectant will be allowed for 20-30 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 at an eyewash station and one drop of trifluridine 1% should be instilled every 2 hours.

- Intact skin splash:

The area should be decontaminated with alcohol and then flushed under a running stream of water.

- Cut or puncture:

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

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

See J.1.

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

Not applicable.

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

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 JX-594 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 JX-594 outside patients' tumors is very low.

*The clinical information available to date suggests that JX-594 is safe at the clinical dose tested 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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