PART 1 (COUNCIL DECISION 2002/813/EC)

SUMMARY NOTIFICATION INFORMATION FORMAT FOR THE RELEASE OF GENETICALLY MODIFIED ORGANISMS OTHER THAN HIGHER PLANTS IN ACCORDANCE WITH ARTICLE 11 OF DIRECTIVE 2001/18/EC

In order to tick one or several possibilities, please use crosses (meaning x or X) into the space provided as (.)

A. General information

1. Details of notification

(a) Member State of notification: Belgium
(b) Notification number: B/BE/17/BVW2
(c) Date of acknowledgement of notification: ../../….
(d) Title of the project
A Phase 1b Study of Talimogene Laherparepvec in Combination With Atezolizumab in Subjects With Triple Negative Breast Cancer and Colorectal Cancer With Liver Metastases (20140299)
(e) Proposed period of release: January 2018 to December 2021

2. Notifier

Name of institution or company: Amgen Limited, UK, on behalf of Amgen Inc. (study sponsor)

3. GMO characterisation

(a) Indicate whether the GMO is a:

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

specify phylum, class …

(b) Identity of the GMO (genus and species)
Genus: Simplexvirus
Species: Talimogene laherparepvec is a recombinant of a wild type Herpes simplex virus 1 (HSV-1) strain JS1, with genes ICP34.5 and ICP47 deleted and hGM-CSF inserted

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

In general, DNA viruses have greater genetic stability than RNA viruses. Firstly, DNA is more thermodynamically stable than RNA; secondly, replication of DNA is a much less error-prone process than the replication of RNA; and thirdly, more mechanisms exist in the host cell for repairing errors in DNA than in RNA.
The overall mutation rate for the wild type HSV-1 is low and has been estimated to be 1.8×10^{-8} mutations per nucleotide, per genomic replication (Duffy et al., 2008).The genetic stability of talimogene laherparepvec in vivo is expected to be the same as wild type HSV-1. The genetic stability of talimogene laherparepvec in isolation (ie in the absence of a co-infecting different strain of HSV-1) has been demonstrated and continues to be monitored. The possibility of the creation of stable genetic variants with unintended characteristics is also minimised by the design of the talimogene laherparepvec genetic construct.
However, homologous genomic recombination may occur spontaneously in nature between the viral genomes of HSV-1 strains. For this to occur, it would be essential for a (human) cell to be infected simultaneously by two different strains. There is evidence that this may be a common occurrence in natural infections with wild type viruses (Bowden et al, 2004, Norberg et al, 2006). However, all strains of HSV-1 which were investigated and considered to have potentially evolved by this mechanism remained recognisably HSV-1 in their familial characteristics and pathogenicity.

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

Yes (X) No (.)

If yes, insert the country code(s) BE, FR, DE, ES, GR, NL, PT, SE (Contained use: AT, IT, PL, GB),

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

Yes (X) No (.)

If yes:

Member State of notification Notification number	BE B/BE/15/BVW1 (study 265) B/BE/15/BVW2 (study 232)
Member State of notification Notification number	FR B/FR/15/GT06 (study 325) B/FR/15/GT07 (study 266) B/FR/15/GT01 (study 264)
Member State of notification Notification number	DE B/DE/14/PEI2133 (study 264) B/DE/14/PEI2194 (study 325)
Member State of notification Notification number	ES B/ES/15/04 (study 328) B/ES/14/08 (study 266) B/ES/14/05 (study 325) B/ES/14/06 (study 265) B/ES/16/01 (study 261) B/ES/15/14 (study 232) B/ES/15/07 (study 318)

Member State of notification Notification number	GR Pending confirmation
Member State of notification Notification number	HU B/HU/14/01 (325)
Member State of notification Notification number	NL Pending confirmation
Member State of notification Notification number	GR Pending confirmation
Member State of notification Notification number	SE B/SE/14/EU-2014-000185-22 (study 265)
Member State of notification Notification number	AT (CU), Pending confirmation
Member State of notification Notification number	IT (CU), Pending confirmation
Member State of notification Notification number	PL (CU) Pending confirmation
Member State of notification Notification number	GB (CU) Pending confirmation

Please use the following country codes:
Austria AT; Belgium BE; Germany DE; Denmark DK; Spain ES; Finland FI; France FR; United Kingdom GB; Greece GR; Ireland IE; Iceland IS; Italy IT; Luxembourg LU; Netherlands NL; Norway NO; Portugal PT; Sweden SE

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

Yes (X) No (.)

If yes:

-

Member State of notification Notification number	USA IND 12412
Member State of notification Notification number	Canada EAU-741
Member State of notification Notification number	South Africa 39.4 (2/11/314-5)
Member State of notification Notification number	Switzerland Pending confirmation

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

Wild type HSV-1 is a human pathogen which is not known to be involved in environmental processes. It does not respire and does not contribute to primary production or decomposition processes. In its virion form, it does not display any metabolic activity. There are no known indigenous vectors of HSV-1, other than human beings. The presence of natural mobile genetic elements such as proviruses, transposons or plasmids related to HSV-1 has not been reported. The genetic modifications made to produce talimogene lahreparepvec do not affect its impact on the environment.

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

1. Recipient or parental organism characterisation:

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

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

other, specify …

2. Name

(i)	order and/or higher taxon (for animals)	Herpesvirales
(ii)	genus	Simplexvirus
(iii)	species	Herpes simplex virus 1 (HSV-1)
(iv)	subspecies	…
(v)	strain	JS1 (ECACC Accession Number 01010209)
(vi)	pathovar (biotype, ecotype, race, etc.)	…
(vii)	common name	HSV-1

3. Geographical distribution of the organism

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

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

(i) Yes (X)

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

Atlantic	X
Mediterranean	X
Boreal	X
Alpine	X
Continental	X
Macaronesian	X

(ii) No (.)

(iii) Not known (.)

(c) Is it frequently used in the country where the notification is made?

Yes (.) No (X)

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

Yes (.) No (X)

4. Natural habitat of the organism

(a) If the organism is a microorganism

water	(.)
soil, free-living	(.)
soil in association with plant-root systems	(.)
in association with plant leaf/stem systems	(.)
other, specify	Humans are the only natural host for HSV-1 infection; non-human infection is rare, but HSV-1 infection has been reported in a variety of species including rodents, rabbits, hedgehogs, and non-human primates. In the available case reports, the infection occurred subsequent to close contact with humans actively shedding.

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

Not applicable.

5. (a) Detection techniques

The diagnosis of HSV-1 infection is usually made by the appearance of the lesions and the patient's history. However, if the clinical pattern of the lesions is not specific to HSV, its diagnosis can be made by viral culture, Polymerase Chain Reaction (PCR), viral antigen detection, Tzanck test or serology.

(b) Identification techniques

See section 5(a). PCR is the most sensitive method in the identification of HSV-1 (99% specific for HSV-1; Whitley et al, 1998).

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

Yes (X) No (.)

If yes, specify

Wild type Herpes Simplex virus Type 1 is classified in Risk Group 2 in the European Union (EU) according to Directive 2000/54/EC on the protection of workers from risks related to exposure to biological agents at work. A Risk Group 2 biological agent is defined in the EU as 'one that can cause human disease and might be a hazard to workers; it is unlikely to spread to the community; there is usually effective prophylaxis or treatment available'.

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

Yes (X) No (.) Not known (.)

If yes:

(a) to which of the following organisms:

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

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

Humans are the only natural host for HSV-1 infection; non-human infection is rare, but HSV-1 infection has been reported in a variety of species including rodents, rabbits, hedgehogs, and non-human primates. In the available case reports, the infection occurred subsequent to close contact with humans actively shedding.

The incubation period for orolabial HSV-1 infection is 2 to 12 days, with an average of 4 days (Miller & Dummer 2007).

The following wild type HSV-1 mediated conditions may occur: herpes liablis/cold sores; herpetic whitlow; infections of the eye; encephalitis; genital herpes.

HSV-1 also travels to the sensory ganglia, where latency is established. Oral HSV-1 infections reactivate from the trigeminal sensory ganglia, affecting the facial, oral, labial, oropharyngeal, and ocular mucosa (Usatine & Tinitigan, 2010).

Recurrent infections may be precipitated by various stimuli, such as stress, fever, exposure to sunlight, extremes in temperature, ultra-violet radiation, immunosuppression, or trauma. The virus remains dormant for a variable amount of time. On reactivation, generally the duration of symptoms is shorter and the symptoms are less severe (Usatine & Tinitigan, 2010).

Neonatal HSV infection causes significant morbidity and mortality (reviewed in Kimberlin, 2004; Thompson & Whitley, 2011). HSV infections in newborns can be classified into three patterns, which occur with roughly equal frequency. These comprise disseminated disease involving multiple visceral organs, including lungs, liver, adrenal glands, skin, eyes, and the brain; central nervous system (CNS) disease, with or without skin lesions; and disease

limited to the skin, eyes, and/or mouth (SEM disease). With the use of high-dose acyclovir, 12-month mortality has reduced to 29% for disseminated neonatal HSV disease and to 4% for CNS HSV disease (reviewed in Kimberlin, 2004; Thompson & Whitley, 2011). The majority of neonatal HSV infections are caused by HSV-2, but approximately 15 to 30 percent are thought to be caused by HSV-1 Neonatal Herpes Simplex Virus Infections (Rudnick & Hoekzema 2002).

In the immunocompromised host, including those with HIV infection, HSV disease can be particularly severe, resulting in chronic, persistent, active infection and in some cases, life-threatening disease (Stewart et al, 1995). Immunosuppressed patients, especially those with impaired T-cell immunity, develop severe lesions that persist longer than those in the normal host; these lesions can progress to visceral disease. As a result of this, almost all examples of serious complications of wild-type HSV infections in humans occur in immunocompromised individuals. In these cases, the immune system fails to control the infection, and it becomes disseminated. Susceptible immunocompromised individuals include patients receiving cytotoxic therapy, transplant recipients, and patients with human immunodeficiency virus (HIV) (reviewed in Brady & Bernstein, 2004).

8. Information concerning reproduction

(a) Generation time in natural ecosystems:
HSV-1 does not persist in natural ecosystems, relying on its host organism for asexual replication with a short reproductive cycle (~18 hr.).

(b) Generation time in the ecosystem where the release will take place:
Replication outside the host organism (human) does not occur and it is not known to infect species other than human under natural circumstances.

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

(c) Factors affecting reproduction:
See section 8(a).

9. Survivability

(a) ability to form structures enhancing survival or dormancy:

(i)	endospores	(.)
(ii)	cysts	(.)
(iii)	sclerotia	(.)
(iv)	asexual spores (fungi)	(.)
(v)	sexual spores (funghi)	(.)
(vi)	eggs	(.)
(vii)	pupae	(.)
(viii)	larvae	(.)
(ix)	other, specify	Not applicable

(b) relevant factors affecting survivability:
Wild type HSV-1 survives in the environment as a persistent infection in the host species (humans) or as a latent infection in the nucleus of some infected cells (principally neurons of the trigeminal ganglion), where it may remain inactive indefinitely, or be reactivated giving rise to secretion of virus and sometimes (though not always) clinical symptoms.

Outside of the host, HSV-1 is an enveloped virus which is sensitive to and rapidly inactivated by both physical inactivation (dehydration, heat, low pH) and disinfectants (lipid solvents and mild detergents). It does not form survival structures and its survival outside the host organism is limited to short periods of time (Chayavichitsilp et al, 2009).

10. (a) Ways of dissemination
The mode of transmission of wild type HSV-1 is through direct contact with infected secretions or mucous membranes/skin with lesions from an asymptomatic or symptomatic patient shedding the virus (Jerome & Morrow 2007, Chayavichitsilp 2009, Whitley 2006). Transmission of HSV-1 can also occur by respiratory droplets (Whitley 2006).

(b) Factors affecting dissemination
See section 10(a).

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)

The parental strain of HSV-1 used in the construction of talimogene laherparepvec was named JS1. This strain was a new isolate taken from a healthy individual and subsequently banked (ECACC Accession Number 01010209). There are no known previous genetic modifications of this strain of HSV-1.

C. **Information relating to the genetic modification**

1. Type of the genetic modification

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

2. Intended outcome of the genetic modification

The intended outcome of the modification is to functionally delete both copies of ICP34.5 and the ICP47 gene from the viral backbone of wild type HSV-1 (strain JS1) and to insert an expression cassette encoding the human granulocyte macrophage colony-stimulating factor (hGM-CSF) gene in both ICP34.5 regions. The intended therapeutic strategy is to produce a direct oncolytic effect by replication of the virus within the tumour, and induction of an anti-tumour immune response, enhanced by the local expression of hGM-CSF.

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

Yes (.) No (X)

There is no mobile genetic vector in talimogene laherparepvec. Shuttle vectors (plasmids) were used to construct the recombinant virus which was subsequently plaque purified.

If no, go straight to question 5.

(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

…

(c) Host range of the vector

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

Yes (.) No

antibiotic resistance (.)
other, specify …

Indication of which antibiotic resistance gene is inserted
…

(e) Constituent fragments of the vector
…

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

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

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

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

6. Composition of the insert

(a) Composition of the insert
The hGM-CSF expression cassette contains a human cytomegalovirus immediate-early (hCMV IE) promoter, hGM-CSF gene and bovine growth hormone polyadenylation (bGH polyA) signal.

(b) Source of each constituent part of the insert
The hCMV IE promoter sequence was derived from pcDNA3, a commercial plasmid from Invitrogen.

An IMAGE clone of the hGM-CSF gene was obtained from the Human Genome Mapping Project (HGMP) Resource Centre, UK.

The bGH polyA signal was derived from pcDNA3, a commercial plasmid from Invitrogen.

(c) Intended function of each constituent part of the insert in the GMO
The hCMV promoter is used to drive expression of hGM-CSF gene.

hGM-CSF augments the immune response to released tumour antigens by aiding the differentiation and proliferation of dendritic cell precursors in and around the injected tumour.

The bGH polyA signal facilitates hGM-CSF mRNA transport and stability.

(d) Location of the insert in the host organism
- on a free plasmid (.)
- integrated in the chromosome (.)
- other, specify Integrated in the HSV-1 genome

(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:
The following information relates to the organism from which the inserted gene (hGM-CSF) is derived.

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

other, specify …

2. Complete name

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

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

(a) to which of the following organisms: Not Applicable

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

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

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

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

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

E. Information relating to the genetically modified organism

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

(a) is the GMO different from the recipient as far as survivability is concerned?
Yes (.) No (X) Not known (.)
Specify …

(b) is the GMO in any way different from the recipient as far as mode and/or rate of reproduction is concerned?
Yes (X) No (.) Unknown (.)
Specify The HSV-1 protein ICP34.5 normally promotes neurovirulence by overcoming host defence pathways and allowing the virus to replicate in non-dividing cells such as neurons. Both copies of ICP34.5 are functionally deleted from talimogene laherparepvec, preventing the virus from replicating efficiently in non-dividing cells.

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

Yes (X) No (.) Not known (.)

Specify The functional deletion of ICP34.5 in talimogene laherparepvec significantly decreases the ability of the virus to replicate in non-dividing cells.

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

Yes (X) No (.) Not known (.)

Specify The functional deletion of ICP34.5 in talimogene laherparepvec significantly decreases virulence compared to wild type HSV-1. Talimogene laherparepvec is therefore significantly attenuated in normal cells. Virus mediated toxicity is therefore likely to be minimal.

Strains of HSV-1 lacking ICP34.5 have been extensively utilised without incident and have been found to be non-pathogenic in a variety of animal models and also in several human clinical trials.

2. Genetic stability of the genetically modified organism

The genetic stability of talimogene laherparepvec is expected to be the same as wild type HSV-1.i.e. stable in isolation but with the potential for homologous recombination with other HSV-1 viruses if they simultaneously infect the same (human) cell.

The genetic stability of talimogene laherparepvec in isolation (ie. in the absence of a co infecting different strain of HSV-1) has been demonstrated and continues to be monitored. A spontaneously occurring genetic variant of talimogene laherparepvec would require an initial recombination event(s) leading to the creation of the genetic variant itself. It is unlikely that a wild type virus would be in the same tissue as talimogene laherparepvec since the latter is directly injected into tumour cells and cannot spread effectively into normal tissue, while the pre-existing HSV-1 would be in the mucosal tissues or neuronal ganglia of the patient. The possibility of the creation of stable genetic variants with unintended characteristics is also minimised by the design of the talimogene laherparepvec genetic construct.

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

Yes (.) No (X) Unknown (.)

(a) to which of the following organisms?

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

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

…

4. Description of identification and detection methods

(a) Techniques used to detect the GMO in the environment
The same techniques used to detect the parental organism can be used to detect talimogene laherparepvec.

Viral culture (plaque assay; Dulbecco, 1952) is routinely used to detect talimogene laherparepvec in swabs or other samples, although this assay only detects live virus and does not discriminate between talimogene laherparepvec and wild type HSV-1.

A qPCR assay has been developed which can be used to detect talimogene laherparepvec specifically.

(b) Techniques used to identify the GMO
In the proposed clinical trial (20140299), qPCR (described above) will be used for detection and identification of talimogene laherparepvec in specified human samples as defined in the clinical trial protocol.

F. Information relating to the release

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

The purpose of the release is to evaluate the safety, as assessed by incidence of dose limiting toxicities (DLTs), of intrahepatic injection of talimogene laherparepvec into liver metastases in combination with intravenously administered atezolizumab separately in subjects with triple-negative breast cancer and colorectal cancer (protocol 20140299).

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 (X) No (.)
If yes, specify: The site of release will be a medical facility approved to conduct this clinical trial.

3. Information concerning the release and the surrounding area

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

Site Name	Address
UCL Cliniques Universitaires Saint Luc	Avenue Hippocrate 10, 1200 Bruxelles
Universitair Ziekenhuis Gent	De Pintelaan 185, 9000 Gent

(b) Size of the site (m^2): … m^2
(i) actual release site (m^2): … m^2
(ii) wider release site (m^2): … m^2

The size of each site will vary but it is important to note that contamination of the site at which the administration is performed is expected to be minimal, when suitable precautions are adhered to.

(c) Proximity to internationally recognised biotopes or protected areas (including drinking water reservoirs), which could be affected:
Given the nature of the product administration directly into the subject's tumours, and procedures for waste treatment, the exposure to significant biotopes, protected areas and drinking water supplies is expected to be minimal. Since the parental organism is an obligate pathogen of humans with no known vector, proximity to other biotopes is not a concern. The genetic modifications made to the parental virus in the construction of talimogene laherparepvec do not affect its selectivity to the host species.

The stability of talimogene laherparepvec in the environment is also unchanged from that of wild type HSV-1, and will rapidly lose viability outside the target species.

(d) Flora and fauna including crops, livestock and migratory species which may potentially interact with the GMO
Since the parental organism is an obligate pathogen of humans with no known vector, proximity to other flora and fauna is not a concern.

4. Method and amount of release

(a) Quantities of GMOs to be released:

Vials containing 1.15 mL of talimogene laherparepvec will be supplied to study site pharmacies in two strengths; 10^6 PFU/mL or 10^8 PFU/mL.

The maximum volume of talimogene laherparepvec administered at any dose is 4.0 mL for any individual lesion. The maximum dose in any one treatment is 4.0 mL.

(b) Duration of the operation:

The complete administration procedure from preparation of the dosing syringe to completing the injection procedure is expected to take 2 hours.

(c) Methods and procedures to avoid and/or minimise the spread of the GMOs beyond the site of the release
Talimogene laherparepvec is an investigational medicinal product for use only in approved clinical trials by trained medical professionals at an authorised study site. All involved personnel on site will be trained in best biosafety practices to be applied for the storage and during the preparation in the pharmacy, transportation to the administration room, precautions during administration and disposal of any biological waste. Such training involves, among others, wearing adapted protective clothing, gloves and goggles, the constant presence of a spill kit and the decontamination of waste prior to disposal.

Talimogene laherparepvec will be supplied directly to the study site and appropriate records/ traceability of shipments will be maintained in line with the requirements of Good Clinical Practice (GCP).

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

Environmental conditions which may affect survival of talimogene laherparepvec outside the host are temperature, pH and environmental humidity, as for wild type HSV-1.

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.

The biodistribution and shedding of intralesionally administered talimogene laherparepvec are being investigated in a melanoma study. Interim results from 30 patients show that talimogene laherparepvec DNA was detected at transient and low concentrations in blood in 90% of patients and in urine in 20% of patients in the study. The proportion of patients with detectable talimogene laherparepvec DNA in blood and urine was highest during the second cycle. Talimogene laherparepvec DNA was detected in samples from injected lesions in approximately 90% of patients. However, only 14% of patients tested positive for infective virus by 50% Tissue Culture Infectious Dose (TCID50) assay, all within 8 days of treatment administration. Seventeen percent of samples from the exterior of occlusive dressing tested positive for talimogene laherparepvec DNA but none tested positive for presence of infective virus. Among samples of oral mucosa, only 1 sample had detectable talimogene laherparepvec DNA during the study, but the sample did not test positive for presence of infective virus.

Based on the low level of virus shedding observed and the attenuated nature of talimogene laherparepvec, the likelihood for persistent or systemic infection of personal contacts of the patients and health care personnel is considered low. If this were to occur it is not expected that any significant clinical manifestations would be evident as effects in non-tumour tissue in talimogene laherparepvec treated patients have not been noted.

G. Interactions of the GMO with the environment and potential impact on the environment, if significantly different from the recipient or parent organism

1. Name of target organism (if applicable)

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

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

Talimogene laherparepvec has been engineered to replicate selectively in tumours, killing tumour cells by viral lysis, followed by spread of talimogene laherparepvec within the tumour and further tumour cell lysis. Additionally, the oncolysis of tumour cells by talimogene laherparepvec also releases and exposes an array of antigens to initiate a systemic immune response, and this is augmented through the expression of an immune stimulatory protein, hGM-CSF from the virus. Released tumour antigens are expected to be taken up by antigen presenting cells (APCs) which then traffic to lymph nodes and present to T cells, inducing an immune response. hGM-CSF increases the activity of APCs, enhancing the immune responses. This immune response is intended to provide a systemic anti-tumour effect, including the shrinkage of tumours which do not come into direct contact with talimogene laherparepvec, reduction of micrometastatic disease, and protection against future relapse.

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

Talimogene laherparepvec is an attenuated, non-pathogenic version of HSV-1, modified so that replication occurs selectively in tumour cells in the target human population and is therefore self-limiting. As with wild type HSV-1 it is not known to colonise other species, nor are other species known to be carriers or vectors under natural conditions. Considerable literature shows that HSV-1 deleted for ICP34.5 is non-pathogenic in animals and humans.

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

Yes (.) No (X) Not known (.)

Give details

Talimogene laherparepvec is modified so that replication occurs selectively in tumour cells in the target human population and is therefore self-limiting.

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

No ecosystems and habitats are targeted in the use of talimogene laherparepvec. Ecosystems and habitats are not expected to be affected, due to the obligate human nature of the virus and its rapid inactivation outside the host species.

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

None

(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	…

7. Likelihood of genetic exchange in vivo

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

Humans are the only natural host for wild-type HSV-1 infection. No transfer into other organisms is expected.

The transfer of genetic material is limited to the virally mediated (non integrative) transfer of the viral DNA between humans and theoretically, genetic exchange between two wild type HSV-1 strains by homologous recombination which could only occur if human cells were simultaneously infected with both strains.

A spontaneously occurring genetic variant of talimogene laherparepvec would require an initial recombination event(s) leading to the creation of the genetic variant itself. It is unlikely that a wild type HSV-1 virus would be in the same tissue as talimogene laherparepvec since the latter is directly injected into tumour cells and cannot spread effectively into normal tissue, while the pre-existing HSV-1 would be in the mucosal tissues or neuronal ganglia of the patient. The possibility of the creation of stable genetic variants with unintended characteristics is also minimised by the design of the talimogene laherparepvec genetic construct.

(b) from other organisms to the GMO:

Highly unlikely. See section G.7(a) above.

(c) likely consequences of gene transfer:

The design of the talimogene laherparepvec genetic construct is such that the inserted gene is located in the region of the ICP 34.5 deletions. Thus, restoration of ICP34.5 will cause a simultaneous deletion of the hGM-CSF insert (and vice versa). It is considered that any stable (homozygous) produced in this way will not pose a greater hazard than the wild type HSV-1 co-infection itself.

8. Give references to relevant results (if available) from studies of the behaviour and characteristics of the GMO and its ecological impact carried out in stimulated natural environments (e.g. microcosms, etc.):

Talimogene laherparepvec is an attenuated version of wild type HSV-1. The genetic modifications do not affect its natural host range, which is restricted to humans.

No specific studies have been conducted regarding transmission of talimogene laherparepvec between humans as such studies would be unethical.

It is not possible to model human transmission between treated and untreated animals since transmission of wild type HSV-1 is not known to occur in nature, and the genetic modifications made to wild type HSV-1 resulting in talimogene laherparepvec attenuate the virus, further reducing the likelihood of transmission.

Biodistribution and virus shedding has however been monitored in both humans and animals following administration of talimogene laherparepvec (see Section F.6)

9. Possible environmentally significant interactions with biogeochemical processes (if different from the recipient or parental organism)
None known or predicted.

H. Information relating to monitoring

1. Methods for monitoring the GMOs
Monitoring of the direct and indirect effects of talimogene laherparepvec in subjects will be achieved by the clinical assessments defined in the clinical trial protocol. Study investigators will monitor subjects throughout treatment and will report adverse effects to Amgen Global Safety according to the requirements stipulated in the protocol.

Amgen will conduct a surveillance program to aid the assessment of any potential risks to third parties following treatment of subjects with talimogene laherparepvec.

2. Methods for monitoring ecosystem effects
Since humans are the only natural host for wild-type HSV-1 infection, no further monitoring of ecosystem effects is proposed.

3. Methods for detecting transfer of the donated genetic material from the GMO to other organisms
See Section E.4.

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

5. Duration of the monitoring
Monitoring will occur throughout the subject's participation in the study, including a period of safety follow-up, as defined in the study protocol, upon permanent discontinuation from the study.

6. Frequency of the monitoring
Clinical assessments will be made according to the predefined schedule detailed in the study protocol. At each visit, the subject must be interviewed regarding their knowledge of any possible exposures or events that may have occurred in their close contacts.

The investigator is responsible for ensuring that all SAEs and AEs observed by the investigator or reported by the subject that occur after signing of the informed consent through to a predefined period after the last dose of study medication are recorded in the

subject's medical record and are submitted to Amgen. Any SAE must be submitted to Amgen within 24 hours following the investigator's knowledge of the event.

I. Information on post-release and waste treatment

1. Post-release treatment of the site

Treatment of the study site facility after use will not be necessary, provided the advised handling precautions are adhered to when administering the product or when dealing with accidental spillages and breakages. However, work surfaces shall be decontaminated using a chemical disinfectant capable of virucidal activity following preparation and dosing of talimogene laherparepvec.

2. Post-release treatment of the GMOs

Please refer to section I.3.(b).

3. (a) Type and amount of waste generated

Waste generated from i.l. administration of talimogene laherparepvec will be limited to:

- Used vials and needles
- Used swabs and items used to clean injected area
- Used dressings applied to the injection sites
- Personal Protective Equipment used at the point of administration and when replacing or removing the used dressing.

Vials containing 1.15 mL of talimogene laherparepvec supplied to pharmacies will be provided in two strengths; 10^6 PFU/mL or 10^8 PFU/mL.

The maximum total dose for an individual patient is less than 5 mL (or 4 vials) per treatment. A treatment duration of 34 weeks (12 cycles), would require a total of 48 vials per subject.

Each administration will result in the waste identified above.

3. (b) Treatment of waste

Talimogene laherparepvec is sensitive to inactivation by a variety of commonly available physical and chemical methods (see Section F.4.(c)).

Since talimogene laherparepvec will be administered in a medical facility, all associated waste will be disposed of in line with standard practice for medical waste.

The information leaflet provided to each subject instructs that disposal of any soiled dressings should occur via the study site at their next scheduled visit. The subject is provided with additional dressings, disposable gloves and resealable bags, and specific instructions to be followed to minimise the risk of unintended exposure to the environment.

J. Information on emergency response plans

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

The only organism which may act as a mechanism for the spread of talimogene laherparepvec are humans, since the parental organism is an obligate species-specific pathogen of human beings and is non-zoonotic under natural conditions.

In the unlikely event of the transmission of talimogene laherparepvec to an unintended human recipient, the affected patient can be treated with approved antiviral treatments such as acyclovir, if clinically indicated to alleviate any symptoms of primary infection and potential recurrence (if deemed necessary). Further spread from the individual can be mitigated by educational materials to increase awareness of the infection and preventative measures which can be taken to prevent transmission to close contacts.

Any spread of talimogene laherparepvec to unintended human recipients is likely to be isolated to single cases in discrete geographical locations. The risk of widespread infection is considered negligible.

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

Talimogene laherparepvec cannot persist outside its host organism for long periods and maintain viability. Since it is sensitive to even moderately harsh conditions, it is considered highly unlikely that spread would occur in the environment from fomites and talimogene laherparepvec would quickly be rendered non-viable by the prevailing conditions. In the event of an accidental occupational exposure through a splash to the eyes or mucous membranes, flush with clean water for at least 15 minutes. In the event of exposure to broken skin or needle stick, clean the site thoroughly with soap and water and/or disinfectant such as 1% sodium hypochlorite or Virkon®. Spills should be treated with a virucidal agent and absorbent

Decontamination of areas in which a recently treated patient had frequented (their home and or examination room at a medical facility) could be implemented by applying chemical disinfectants capable of virucidal activity to areas of likely contact.

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

Decontamination of plants, (non-human) animals and soils is not expected

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

No undesirable effects are expected

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