Tamilogene Laherparapvec

Environmental Risk Assessment Dossier
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List of Abbreviations

Term/Abbreviation	Explanation
%CV	co-efficient of variation
°C	Degrees Celsius
α-TIF	*alpha*-transinducing factor
AML	Acute Myelogenous Leukemia
APCs	Antigen Presenting Cells
BHK	baby hamster kidney
BSL-1	Biosafety Level 1
BSL-2	Biosafety Level 2
cDNA	complementary deoxyribonucleic acid
cGMP	Current Good Manufacturing Practices
CMV	cytomegalovirus
CNS	Central Nervous System
CRO	Contract Research Organization
CT	Computed Tomography
DMEM	Dulbecco Modified Eagle Medium
DNA	Deoxyribonucleic Acid
DOT	US Department of Transport
ECACC	European Collection of Cell Cultures
ELISA	Enzyme-Linked ImmunoSorbent Assay
FAM	6-carboxyfluorescein
FBS	Fetal Bovine Serum
FDA	Food and Drug Administration of the United States
FITC	fluorescein isothiocyanate
gC, gD, gH, gG, gL	Glycoproteins (type C, D, H, G, or L)
GCP	Good Clinical Practice
GFP	Green Fluorescent Protein
GLP	Good Laboratory Practice
GM-CSF	Granulocyte Macrophage Colony Stimulating Factor
GMP	Good Manufacturing Practice
GTAC	Gene Therapy Advisory Committee
GxP	Good Practice guidelines, where “x” may be “M” for manufacturing, or “C” for clinical, or “L” for laboratory, *etc*.
hCMV IE	Human Cytomegalovirus Immediate Early

Term/Abbreviation	Explanation
hGM-CSF	Human Granulocyte Macrophage Colony Stimulating Factor
HGMP	Human Genome Mapping Project
HHV-1	Human Herpes Virus Type 1
HIV	Human Immunodeficiency Virus
HSV	Herpes Simplex Virus
HSV-1	Herpes Simplex Virus, Type 1
HSV-2	Herpes Simplex Virus, Type 2
HVEM	herpesvirus entry mediator
IRL	long internal repeated sequence
IRS	short internal repeated sequence
i.l.	Intralesional
i.t.	Intratumoural
i.v.	Intravenous
IATA	International Air Transport Association
IC50	Inhibitory Concentration At 50%
ICH	International Conference on Harmonization
IPIM	Investigational Product Instruction Manual
IEX	Ion Exchange Chromatography
IgG, IgM	Immunoglobulin G or M
IMP	Investigational Medicinal Product
LATs	Latency-Associated Transcripts
LD50	median lethal dose
mGM-CSF	Murine Granulocyte Macrophage Colony Stimulating Factor
MHC I or II	Major Histocompatability Complex Type I or Type II
MOI	Multiplicity of infection
miRNA	micro ribonucleic acids
mRNA	Messenger Ribonucleic Acid
MVL	Micro Virology Laboratories
MVSS	Master Viral Seed Stock
NF12	U.S. National Formulation 12
NSN	New Substances Notification
OncoVEX $^{GM-CSF}$	OncoVEX virus expressing hGM-CSF
OOS	Out Of Specification

Term/Abbreviation	Explanation
PAP	prostatic acid phosphatase
PCR	Polymerase Chain Reaction
PFU	Plaque Forming Unit
Ph Eur	European Pharmacopoeia
PKR	Protein Kinase R
PPE	Personal Protective Equipment
QA	Quality Assurance
QC	Quality Control
QMS	Quality Management System
QP	Qualified Person
qPCR	Quantitative Polymerase Chain Reaction
RNA	ribonucleic acid
RT	real-time
S	short
s.c.	Subcutaneous
SCCHN	Squamous Cell Cancer of the Head and Neck
SDS	sodium dodecyl sulfate
SEC	Size Exclusion Chromatography
SEM	Skin, Eyes and/or Mouth
SOP	Standard Operating Procedure
TAMRA	tetramethylrhodamine
TFF	Tangential Flow Filtration
TK	Thymidine Kinase
TRL	long terminal repeated sequence
TRS	short terminal repeated sequence
UK	United Kingdom
U_L	long unique region
USP	United States Pharmacopeia
U_S	short unique region
U.S.	United States
VHS	virion host shutoff protein

Introduction

Talimogene laherparepvec (JS1/ICP34.5-/ICP47-/hGM-CSF), formerly known as OncoVEX^{GM-CSF}, is a disabled recombinant herpes simplex type 1 virus (HSV-1). Talimogene laherparepvec was generated by modifying the wild type HSV-1 genome (new isolate JS1) to functionally delete both copies of ICP34.5 and the ICP47 gene from the viral backbone and to insert an expression cassette encoding the human granulocyte macrophage colony-stimulating factor (hGM-CSF) gene in both ICP34.5 regions.

Full Technical and Scientific Information on the GMO (talimogene laherparepvec) are provided in a separate document in accordance with Annex IIIA of Directive 2001/18/EC.

Objective

The objective of this Environmental Risk Assessment (ERA) is to identify and evaluate potential adverse effects of talimogene laherparepvec on human health and the environment which conducting a clinical trial with the GMO may exert, in accordance with Annex IIA of Directive 2001/18/EC.

Talimogene laherparepvec is intended as an investigational medicinal product in a proposed phase 1, multicenter, open-label, single-arm study to evaluate the safety of the investigational medicinal product when injected into liver tumours (Protocol 20140318). Talimogene laherparepvec is intended for intrahepatic injection into hepatocellular carcinoma (HCC) and metastatic liver tumors (non-HCC) by a trained medical professional in a medical study site facility.

Injections will be performed using the coaxial injection technique under ultrasound or CT guidance. A needle of larger diameter than the talimogene laherparepvec dosing syringe needle (introducer needle) will first be inserted into the lesion. Talimogene laherparepvec will be administered via a filled dosing syringe through the introducer needle.

Methodology

This ERA has been performed according to the precautionary principle using the methodology set down in Commission Decision 2002/623/EC. These general principles are:

- Identified characteristics of the GMO and its use which have the potential to cause adverse effects should be compared to those presented by the non-modified organism from which it is derived and its use under corresponding situations;
- The ERA should be carried out in a scientifically sound and transparent manner based on available scientific and technical data;
- The ERA should be carried out on a case-by-case basis;

- An analysis of the 'cumulative long-term effects' relevant to the release and the conduct of the clinical trial.

1. Identification of Characteristics which may Cause Adverse Effects

1.1 Characteristics of the Parental Virus, Modified Virus and the Receiving Environment

1.1.1 Characteristics of the Parental Virus

Wild type HSV-1 is a globally endemic pathogen of humans, which is usually initially transmitted in childhood via nonsexual contact, though it may be acquired in young adulthood through sexual contact. The seroprevalence in adults is estimated to be 70% in developed countries and 100% in developing countries (Gupta et al, 2007). Orolabial herpes has an infection rate of approximately 33% in developing countries and 20% in developed countries (Chayavichitsilp et al, 2009).

Its mode of transmission 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).

HSV-1 survives in the environment in the host species (humans) as a persistent infection 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.

Several wild type HSV-1 mediated conditions may occur, as summarised below.

Herpes labialis/cold sores: Primary infections with HSV-1 are acquired usually in childhood and may be asymptomatic or subclinical (Drew, 2004; Jerome & Morrow, 2007; Kimberlin, 2005). Symptomatic primary infections present mainly as gingivostomatitis, with fever, sore throat, fetor oris, anorexia, cervical adenopathy, and mucosal edema and vesicular and ulcerative painful lesions involving the buccal mucosa, tongue, gums, and pharynx (Drew, 2004; Jerome & Morrow, 2007; Kimberlin, 2005; Miller & Dummer, 2007). Ulcers heal without scarring within 2-3 weeks (Drew, 2004; Jerome & Morrow, 2007). Recurrent infections have generally milder symptoms and clinical course (Jerome & Morrow, 2007). Recurrent lesions due to HSV-1 occur mainly on a specific area of the lip (vermillion border of the lip), and are called "cold sores" or "fever blisters" (Drew, 2004; Kimberlin, 2005). The lesions heal in approximately 8-10 days (Kimberlin, 2005).

Herpetic whitlow: Characterised by formation of painful vesicular lesions on the nail or finger area (Drew, 2004), and more commonly seen in healthcare professionals (eg. dentists).

Infections of the eye: Characteristic dendritic ulceration occurs on conjunctiva, and cornea (Drew, 2004). HSV infection may cause other ocular diseases, including blepharitis/dermatitis, conjunctivitis, dendritic epithelial keratitis, and corneal ulceration (Green & Pavan-Langston, 2006).

Encephalitis: Serious infections of the CNS, affecting both children and adolescents (Whitley, 2006). Encephalitis is a rare complication, affecting approximately 1 in 500,000 people per year (Rozenberg et al, 2011). It may occur due to primary or latent infection with HSV-1 virus (Drew, 2004; Whitley, 2006). HSV encephalitis affects one temporal lobe, leading to focal neurologic signs and edema. The disease can be fatal (mortality rate of 70%), if left untreated (Drew, 2004; Whitley, 2006).

Genital herpes: Genital herpes is caused mainly by HSV-2, although HSV-1 has become as common as HSV-2 in primary genital infections in developed countries. It is transmitted sexually through genital-genital or oro-genital contact.

Antiviral medicinal products like acyclovir, valacyclovir, and famciclovir can be used to inhibit wild type HSV-1 replication (Drew, 2004; Usatine & Tinitigan, 2010). The standard antiviral drug used against HSV-1 is acyclovir. Inhibition of viral replication by acyclovir depends on the viral thymidine kinase (TK) gene, which catalyzes the first step necessary to convert acyclovir from an inactive to an active form. Valacyclovir and famciclovir can be used to inhibit wild type HSV-1 replication (Usatine & Tinitigan, 2010). In rare cases, HSV can mutate its viral kinases to gain resistance to acyclovir. In these cases, the anti-viral drug Foscarnet (phosphonoformic acid) which does not require activation by viral kinases can be used. Foscarnet directly inhibits the viral DNA polymerase.

Effects in special populations (neonates and immunocompromised individuals) are discussed below.

Neonatal HSV infection causes significant morbidity and mortality despite significant advances in treatment (reviewed in Kimberlin, 2004; Thompson & Whitley, 2011). The current estimated rate of occurrence of neonatal HSV disease in the United States is approximately 1 in 3,200 deliveries. 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). 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*). Patients with disseminated disease and SEM disease present earliest, generally at 10–12 days of life, whereas CNS disease presents during the second or third week of life. Since the advent of antiviral therapy the prognosis of neonatal HSV has improved. Prior to antiviral therapy, 85% of patients with disseminated HSV disease and 50% of patients with CNS disease died within 1 year. 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). The emergence of HSV resistance to acyclovir, a phenomenon which is mainly observed among immunocompromised patients due to the long-term treatment they receive, is also a concern. However, no case of encephalitis due to an acyclovir-resistant HSV strain has been reported to date (Rozenberg et al, 2011).

No integration of the viral genome with the cellular genome occurs during replication or latency. The linear viral genome circularises shortly after infection and this circular DNA is the template for replication and remains as an extrachromosomal episome, not integrated into the host genome (Efstathiou et al, 1986; Mellerick & Fraser, 1987; Rock & Fraser, 1985). Circularization likely results from direct ligation mediated by pre-existing cellular factors or components of the incoming virion (Mocarski & Roizman, 1982).

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). A review by Kramer et al (2006) reports that HSV-1 can survive on dry inanimate surfaces for periods ranging from a few hours to 8 weeks (the latter citing survival on a dried surface, Mahl & Sadler (1975)). However, individual publications report much lower survival times under conditions of higher humidity. Nerurkar et al (1983) reports survival times of 4 hours in tap water, and 4.5 hours on plastic surfaces at high humidity. A series of publications by Bardell (1989, 1990, 1993, 1994) report a marked (2-3 log) reduction in viral titer of HSV-1 within 1 hour on plastic surfaces (doorknobs) and chrome plated surfaces (faucet/tap handles), though infectious virus was still recoverable after 2 hours (the maximum timepoint studied). Infectious virus could also be recovered from human skin at least 2 hours after it was introduced.

Humans are the only natural host for HSV-1 infection. Amgen performed a literature search to identify reports on HSV-1 infections in non-human species. The literature search revealed that non-human infection is rare, but identified that HSV-1 infection has been reported in a variety of species including rodents, rabbits, hedgehogs, and non-human primates (Weissenbock et al, 1997; Grest et al, 2002; Huemer et al, 2002; Wohlsein et al, 2002; Allison et al, 2002; Lefaux et al, 2004; Muller et al, 2009; Longa et al, 2011).

1.1.2 Characteristics of the Genetically Modified Virus - Talimogene Laherparepvec

The genetic modifications introduced into the wild type HSV-1 clinical isolate (JS1) to produce talimogene laherparepvec are summarised below.

Talimogene laherparepvec was generated by modifying the wild type HSV-1 genome (new isolate JS1) to functionally delete both copies of ICP34.5 and the ICP47 gene from the viral backbone and to insert an expression cassette encoding the human granulocyte macrophage colony-stimulating factor (hGM-CSF) gene in both ICP34.5 regions.

The functional deletion of ICP34.5 in talimogene laherparepvec significantly decreases virulence compared to wild type HSV-1. Talimogene laherparepvec is therefore able to replicate in tumour cells but is 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

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 (Study 4647-00041). 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.

The key features of talimogene laherparepvec are:

- HSV-1 is a non-integrating virus, so treatment with talimogene laherparepvec does not result in any change to the patient's DNA.
- Talimogene laherparepvec is based on a newly isolated strain of HSV-1 (strain JS1) that has been shown to kill human tumour cells more effectively than laboratory strains of HSV-1 (Liu et al, 2003a).
- The HSV-1 protein ICP34.5 normally promotes neurovirulence by overcoming host defense pathways and allowing the virus to replicate in non-dividing cells such as neurons (see Section II.A.10(b)). Both copies of ICP34.5 are functionally deleted from talimogene laherparepvec, preventing the virus from replicating efficiently in non-dividing cells. In tumour cells, these host defence pathways are often impaired, so ICP34.5 is dispensable for replication (Aita et al, 1999; Farassati et al, 2001; Liang et al, 1999).
- The HSV-1 protein ICP47 normally inhibits antigen processing in infected cells, allowing the virus to hide from the immune system (Hill et al, 1995). ICP47 is deleted from talimogene laherparepvec in order to improve the presentation of viral and tumour antigens following tumour-selective virus replication, enhancing any anti-tumour immune response (Liu et al, 2003a).
- Removal of ICP47 from talimogene laherparepvec causes the increased expression of another viral protein, US11 that has some functional redundancy with ICP34.5 (Mohr and Gluzman, 1996). Increased US11 expression enhances the replication of ICP34.5-deleted HSV-1 in tumour cells without loss of tumour selectivity (Mohr et al, 2001).
- Talimogene laherparepvec expresses the immune stimulatory protein hGM-CSF to augment the immune response to released tumour antigens by aiding the differentiation and proliferation of dendritic cell precursors in and around the injected tumour (Dranoff et al, 1993).
- The HSV thymidine kinase (TK) gene remains intact which renders talimogene laherparepvec susceptible to anti-viral agents such as acyclovir. Therefore, acyclovir could be used to block replication of talimogene laherparepvec.

1.1.3 Characteristics of the Receiving Environment

The purpose of the release is to conduct a phase 1 multicenter, open-label trial to evaluate the safety of talimogene laherparepvec when injected into hepatocellular carcinoma (HCC) and metastatic liver tumors (non-HCC).

In this trial the product will be administered by a medical professional in a study site facility.

The product will be stored prior to administration in a secure, temperature monitored freezer at -70°C or below in the pharmacy or other appropriate secure location.

Following the injection procedure, the injection sites will be covered by an occlusive dressing before the patients return to their home environment.

1.2 Characteristics which may Cause Adverse Effects

1.2.1 Effects on Human Health

1.2.1.1 Direct Effects on Human Health

1.2.1.1.1 Transmission of Talimogene Laherparepvec to an Unintended Human Recipient

Wild type HSV-1 is a globally endemic pathogen of humans, which survives in the environment in the host species as a persistent infection 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 replication of virus and sometimes (though not always) clinical symptoms (see Section 1.1.1).

Its mode of transmission is through direct contact with infected secretions or mucous membranes/skin with lesions from an asymptomatic or symptomatic patient shedding the virus. Transmission of HSV-1 can also occur by respiratory droplets (see Section 1.1.1).

Talimogene laherparepvec is highly attenuated compared to wild type HSV-1 in terms of virulence and pathogenicity through the functional deletion of the wild type ICP34.5. Similarly, the establishment of latency is likely to be impaired by the functional deletion of ICP34.5 (Perng et al, 1996), likely due to deficient replication of the virus in the peripheral tissues innervated by the neurons. Wild type levels of reactivation can be achieved from an ICP34.5 deleted virus if 1000 times more virus than wild type is used (Perng et al, 1996). In these experiments, the ICP34.5 null virus was still avirulent, even at the 1000-fold higher infectious dose, indicating that latency/reactivation and virulence are separable.

Despite this, the transmission of talimogene laherparepvec to an unintended human recipient and establishment of latency/re-activation must be considered a potential risk.

Initial transmission would potentially have an immediate effect on human health, while any reactivation would be observed as a delayed effect.

1.2.1.1.2 Capacity for Genetic Transfer Between Humans and Talimogene Laherparepvec

Wild type HSV-1 DNA circularises as an extrachromosomal episome, and is not integrated into the host cell genome (see Section 1.1.1). The ability of the HSV-1 genome to circularise does not require the viral terminal repeat sequences, synthesis of any viral proteins, or viral replication. The efficiency and kinetics of talimogene laherparepvec genome DNA circularization would therefore be expected to be the same as wild type HSV-1, with no integration into the host cell genome.

1.2.1.2 Indirect Effects on Human Health

1.2.1.2.1 Transmission of a Genetic Variant of Talimogene Laherparepvec to an Unintended Human Recipient

Generation of wild type HSV-1 during manufacture is not possible as none of the gene deletions in talimogene laherparepvec require complementation for growth in tissue culture. This means that the cells used to manufacture talimogene laherparepvec do not contain the DNA sequences encoding the deleted genes, preventing repair of the mutations during virus production. Each batch of the medicinal product is strictly controlled throughout production and rigorously tested to confirm its identity.

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. Genetic stability testing has been achieved through repeat sequencing of small areas of the talimogene laherparepvec genome from 2001 to 2012. For example, the GM-CSF in the talimogene laherparepvec genome has been completely covered by large scale sequencing projects from both BHK-derived and Vero cell-derived talimogene laherparepvec and found to be 100% conserved.

The genetic stability of talimogene laherparepvec in vivo is expected to be the same as wild type HSV-1.

Experimentally, non-homologous recombination (recombination between different regions of two viral genomes) has been shown not to occur at detectable levels between replication incompetent and replication competent viruses (Smith et al, 2003).

However, homologous genomic recombination may occur spontaneously in nature between the viral genomes of wild type HSV-1 strains. For this to occur, it would be essential for a (human) cell to be infected simultaneously by two different strains.

Thus, there exists the theoretical potential for homologous recombination events between talimogene laherparepvec and wild type HSV-1, leading to the generation of a genetic variant of talimogene laherparepvec in an individual, and the possibility of transmission of that genetic variant. The possible products of homologous recombination between talimogene laherparepvec and WT HSV-1 are summarised in Table 1. The potential for the creation of stable genetic variants with unintended characteristics is minimised by the design of the talimogene laherparepvec genetic construct, as described below.

Table 1. Theoretical Stable Genetic Variants of Talimogene Laherparepvec Created by Homologous Recombination

Gene	ICP34.5	GM-CSF	ICP47	US11 upregulation
Function	Virulence	Immune stimulation	Immune evasion	Increases replication of ICP34.5 null viruses in tumours
Genetic variant				
Talimogene Laherparepvec	-/-	+/+	-	+
ICP47 restoration	-/-	+/+	+	-
Homozygous ICP34.5 restoration	+/+	-/-	-	+
ICP47 restoration and homozygous ICP34.5 restoration (wild type HSV-1)	+/+	-/-	+	-

In the unlikely event that homologous recombination between wild type HSV-1 and talimogene laherparepvec occurred and was in the region of ICP34.5, the insertion site of the hGM-CSF cassette (which is in place of ICP34.5) dictates that the transfer of the hGM-CSF gene would result in functional deletion of the ICP34.5 gene of the wild type HSV-1 during the recombination process. The result of this recombination event would therefore be a wild type virus (ICP47+) expressing hGM-CSF but functionally deleted for ICP34.5 and therefore only capable of tumour-selective replication. Expression of US11 would be equivalent to wild type since the natural US11 regulatory regions are located within ICP47. The variant of talimogene laherparepvec simultaneously produced in this scenario would have ICP34.5 restored but would not encode or express hGM-CSF. ICP47 (and US11 regulatory regions located within it) would remain deleted, such that US11 expression remained upregulated. Recombinant viruses that contain the US11 upregulation mutation in a wild type ICP34.5 genetic background have been generated (Mohr et al., 2001). These data demonstrated that deletion of the ICP34.5 gene is

completely responsible for the attenuated phenotype of the US11 upregulated/ICP34.5 deleted virus and there is little contribution of US11 upregulation in the presence of ICP34.5. This suggests that should ICP47 be deleted in the context of an ICP34.5 positive virus, the contribution of the US11 upregulation to replication of such a virus would be negligible. In terms of replication competency, the resulting viruses would therefore be the same as the starting materials.

In the unlikely event that homologous recombination between wild type HSV-1 and talimogene laherparepvec occurred in the region of ICP47, a version of talimogene laherparepvec containing hGM-CSF in place of both copies of ICP34.5 but with a repaired copy of ICP47 could be produced. As ICP47 deletion does not significantly affect replication in non-tumour cells, this virus would be expected to be similarly impaired to talimogene laherparepvec, however, the virus would be less able to replicate in tumour cells, as the recombination event would replace the natural US11 regulatory regions (which are located within ICP47). The variant of wild type HSV-1 simultaneously produced in this scenario would be deleted for ICP47 (and US11 regulatory regions located within it) and therefore much less able to block antigen presentation and evade the immune system. Indeed, a virus lacking ICP47 but still expressing US11 was found to be equivalent to wild type in terms of replication and virulence at the inoculation site and surrounding skin. However, the virus lacking ICP47 was less neurovirulent than wild type following corneal injection, and this was found to be due to an inability to inhibit a protective $CD8^+$ T cell response (Goldsmith et al, 1998).

Homologous recombination at both ICP34.5 and ICP47 regions would result in either wild type HSV-1 or talimogene laherparepvec.

It is theoretically possible that individual recombinant virions containing DNA with one copy of ICP34.5 and one copy of GM-CSF could be generated (since ICP34.5 is present in two copies in the wild type HSV-1 genome, one in each of the long repeat regions). While heterozygosity within the repeat regions has been observed, these heterozygote species are not stable and revert to homozygotes at a high frequency.

One example of this is with the gene encoding ICP4, located in the short repeat region of HSV-1. ICP4 is an immediate-early polypeptide essential for the expression of delayed-early and late viral genes. Temperature sensitive mutations affecting this diploid gene, which are necessarily present in both copies of the gene, are recovered at a reasonable frequency (Schaffer et al, 1978). As it is extremely improbable that both copies of ICP4 acquired the same mutation independently, this observation suggests

that a mechanism exists which generates mutant homozygotes from heterozygotes at a high frequency. This was confirmed by propagating virus stocks descended from a heterozygotic repeat mutant of HSV-1 (where the two inverted repeats flanking the short segment of viral DNA differed in length by approximately 60 base pairs). It was observed that the progeny of this heterozygote contained approximately 1/3 heterozygotes and 2/3 homozygotes after growth from 1 to 10^7 PFU, confirming that a mechanism exists which generates homozygotes from heterozygotes at a high frequency (Varmuza & Smiley, 1984). The heterozygote can therefore be viewed as an intermediate in a chain of events starting with the introduction of a change in one of the diploid sequences, followed by later acquisition of this change in the other diploid sequence. This instability is likely due to the ongoing recombination between the HSV repeat regions within that individual viral genome and those of the other virions which initiated the infection, since more than one virion is needed for productive replication to proceed. Further evidence that heterozygotic repeat viruses do not persist within a population comes from analysis of ICP4 deletion mutants isolated on a cell line providing ICP4 *in trans*. These mutants all contain the same deletion within both copies of ICP4 and are therefore homozygotes (DeLuca et al, 1985).

The instability of heterozygotic ICP34.5 regions was investigated when two different strains of HSV-1 were used to generate a heterozygous virus containing different long repeat regions (where ICP34.5 is located). The resultant heterozygotic virus segregated to both classes of homozygotes equally and at a high frequency (Umene, 1987). In order to artificially assess the virulence of virus containing a single copy of ICP34.5, the gene was cloned under the control of a different promoter into a unique region of an ICP34.5 deleted virus (Holman & MacLean, 2008). This virus, despite expressing more ICP34.5 than wild type virus, did not have its virulence restored to wild type levels.

Oncolytic viruses expressing only one copy of ICP34.5 have been artificially generated. These viruses are only able to stably exist due to extensive genomic deletions that prevent homologous recombination. For example, in the oncolytic virus NV1020, the joint region of the long (L) and short (S) regions is deleted, meaning one copy of ICP34.5 is lost (Meignier et al, 1988). The deleted region was replaced with a fragment of HSV-2 DNA from the unique short region. This fragment does not contain any sequences from the deleted repeat region and therefore does not provide a template for homologous recombination.

As with the possibility of transmission of talimogene laherparepvec itself, initial transmission of a variant of talimogene laherparepvec would be an immediate (rather

than delayed) effect. However, since HSV-1 may also enter a non-replicative latent state, any reactivation would be observed as a delayed effect on human health.

1.2.1.2.2 Capacity for Genetic Transfer Between Humans and a Genetic Variant of Talimogene Laherparepvec

No conceivable genetic variant of talimogene laherparepvec produced by homologous recombination would be expected to alter the efficiency and kinetics of viral genome DNA circularization or its existence as an extrachromosomal episome, and consequently no integration into the host cell genome is expected.

1.2.2 Effects on the Environment

1.2.2.1 Effects on Environmental Processes

Humans are the only natural host for wild type HSV-1 infection It does not infect plants, rarely animals, and does not contribute to environmental ecosystems or processes. It does not respire and does not contribute to any primary production or decomposition process. In its virion form, it does not display any metabolic activity.

None of the genetic modifications made to wild type HSV-1 during construction of talimogene laherparepvec would be expected to alter its effect on environmental processes.

1.2.2.2 Transmission to Non-human Organisms in the Environment

Humans are the only natural host for wild-type 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 virus.

The potential for talimogene laherparepvec to exhibit biological interactions or elicit a shift in the established wild type HSV-1 host range is negligible. Even a potential recombination event between talimogene laherparepvec and wild type virus could only result in a reversion to wild type like interactions in the host species (humans).

HSV-1 enters cells by interaction of specific viral glycoproteins with cell surface receptors. The inserted gene in talimogene laherparepvec is hGM-CSF, an immune-stimulatory protein that will not affect expression of viral glycoproteins and therefore would not be expected to alter the host range or cell tropism of the virus. The gene deletions in talimogene laherparepvec impair the ability of the virus to replicate in non-tumour cells, but do not affect the viral glycoproteins so would be expected to have no effect on host range or cell tropism. In line with this, it has been demonstrated that talimogene laherparepvec has identical host range and tropism to wild type HSV-1 when

particular cell types were tested. For example, CHO cells (which lack the HSV-1 entry receptors) and undifferentiated U937 cells (which allow viral entry but display a block at the level of RNA synthesis) were found to be non-permissive for both wild type HSV-1 and talimogene laherparepvec, while known permissive lines such as the human squamous cell carcinoma line, FaDu, were found to support equivalent replication of both viruses. In summary, the host range and tropism of talimogene laherparepvec (or any variant) is expected to be identical to wild type HSV-1.

1.2.2.3 Transfer of Genetic Material into the Environment

Wild type HSV-1 is not known to transfer genetic material to organisms other than humans under natural conditions. The virus is not known to be zoonotic or reverse zoonotic under natural conditions. DNA replication occurs in the cell nucleus. No integration of the viral genome with the cellular genome occurs during replication or latency (see Section 1.1.1).

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 (see Section 1.1.1).

None of the genetic modifications made to wild type HSV-1 during construction of talimogene laherparepvec would be expected to enable the transfer or maintenance of genetic material into the environment (outside its obligate host species), or have an effect on sensitivity to inactivating agents or survivability in the environment. The HSV thymidine kinase (TK) gene remains intact which renders talimogene laherparepvec sensitive to anti-viral agents such as acyclovir.

Wild type HSV-1 itself does not present specific resistance to antibacterials. The virus does not contain any gene that confers resistance to antibacterials of interest in terms of human or animal health. No genes conferring resistance to antibacterials are present in talimogene laherparepvec, nor were any antibacterial resistance genes used as markers in its construction.

1.3 Conclusions

Based on the nature of the, parental organism, the genetic modifications resulting in talimogene laherparepvec and the receiving environment, the potential adverse effects which talimogene laherparepvec may exert by conducting the clinical trial are limited to:

- Direct effects of the transmission of talimogene laherparepvec to an unintended human recipient which may be immediate or delayed.

- Indirect effects of the transmission of a genetic variant of talimogene laherparepvec to an unintended human recipient which may be immediate or delayed.

No potential adverse effects of talimogene laherparepvec on non-human organisms in the environment, ecosystems or environmental processes have been identified.

2. Evaluation of Potential Consequences / Magnitude of Effect

The potential consequences of the possible adverse effects on human health identified in Section 1 of the ERA are considered in this section.

2.1 Direct Effects of the Transmission of Talimogene Laherparepvec to an Unintended Human Recipient

2.1.1 Magnitude of Effect

Cases of the transmission of talimogene laherparepvec to an unintended human recipient are likely to be isolated. The medicinal product will be administered to (and administered by) a limited number of individuals.

The most likely individuals who may be at risk from inadvertent transmission would be:

- Healthcare workers involved in the administration of talimogene laherparepvec
- Healthcare workers or others involved in caring for the patient, which may include washing affected areas and changing dressings.
- Close contacts of the treated individual (partners and family members)

The capacity for widespread dissemination of talimogene laherparepvec is expected to be severely limited due to:

- Administration directly into the tumours of eligible patients; replication in the patient will be self-limiting, dependant on tumour burden
- Low incidence of shedding of infective virus from individuals treated with talimogene laherparepvec (see Section 3.1.3)
- Low persistence and viability outside the host organism; high sensitivity to physical and chemical agents (see Section 1.1.1)
- Natural mode of transmission (direct contact)
- Attenuation of the virus by deletion of ICP34.5, rendering it highly restricted for replication in normal cells (see Section 1.1.2)
- Existing immunity to wild type HSV-1 in a substantial proportion of the population
- Compromised ability to evade the host immune system, conferred by deletion of ICP47 (see Section 1.1.2).

2.1.2 Consequences of Transmission of Talimogene Laherparepvec to an Unintended Individual

2.1.2.1 Virulence and Pathogenicity

The functional deletion of ICP34.5 in talimogene laherparepvec significantly decreases virulence compared to wild type HSV-1. Talimogene laherparepvec is therefore able to replicate in tumour cells but is 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 in a variety of animal models and also in several human clinical trials as detailed below.

In any eventuality, toxicity would be no greater than that observed for wild type HSV-1 (see Section 1.1.1) and talimogene laherparepvec remains sensitive to anti-viral agents such as acyclovir (see Section 1.1.2).

2.1.2.1.1 Non-clinical Safety Data Obtained with HSV-1 ICP34.5 Null Mutants

Intracerebral inoculation of mice has been used to investigate the degree of attenuation conferred by the deletion of ICP34.5. Estimates range from 25 to 90 fold more attenuated than wild type (Bolovan et al, 1994; although the mutant used in this reference contained an inserted stop codon in the gene encoding ICP34.5, such that some level of read-through may have occurred) to an impairment of over 100,000 fold (Chou et al, 1990; MacLean et al, 1991).

Based on the observations from animal studies, ICP34.5 null viruses are anticipated to have enhanced safety over wild type HSV-1 strains in therapy of a number of human tumours (Chambers et al, 1995; Kesari et al, 1998; Martuza et al, 1991; Miller et al, 2001; Thomas & Fraser, 2003). It has been demonstrated that ICP34.5 deleted HSV-1 is unable to replicate or cause disease in non-dividing cells (Brown et al, 1994b) and is attenuated for causing encephalitis (Whitley et al, 1993). Additionally, ICP34.5 deleted HSV-1 may have reduced virulence in certain immune-deficient mice (Valyi-Nagy et al, 1994); this issue is discussed in further detail in “Safety in immunodeficient mice”, below. Furthermore, the safety of ICP34.5 deleted viruses has also been demonstrated in a variety of species, including mice (McKie et al, 1998), rabbits (Perng et al, 1995) and non-human primates (Hunter et al, 1999; Varghese et al, 2001).

2.1.2.1.2 Clinical Safety Data Obtained with HSV-1 ICP34.5 Null Mutants

Several strains of HSV-1 lacking ICP34.5 have been extensively utilised without incident and have been found to be non-pathogenic in several published human clinical trials.

No patients in any of these clinical studies developed HSV encephalitis or required treatment with acyclovir. Using viruses deleted only for ICP34.5, no toxicity was observed following i.t. administration of up to 10^5 PFU of virus into 21 patients with malignant glioma (Papanastassiou et al, 2002; Rampling et al, 2000) and 5 patients with metastatic melanoma (MacKie et al, 2001). None of the patients in these studies experienced any adverse symptoms attributable to virus administration. The same virus was later injected into the resection cavity rim in 12 patients with surgically treated glioma (Harrow et al, 2004), or intratumourally in 20 patients with oral squamous cell carcinoma (Mace et al, 2008). Again, there was no clinical evidence of toxicity associated with the virus administration in any of these patients.

There have also been several clinical trials with several related ICP34.5 null versions of HSV. One of these viruses, G207, also has the gene encoding ribonucleotide reductase deleted so results are not directly comparable to an ICP34.5 null virus. Nonetheless, up to 10^9 PFU of G207 has also been found to be safe in 27 patients with malignant glioma (Markert et al, 2000; Markert et al, 2009).

In addition to the functional deletion of ICP34.5, talimogene laherparepvec is deleted for ICP47 and has a hGM-CSF expression cassette inserted in place of ICP34.5. As described above, the hGM-CSF expression cassette is an immune stimulatory modification and will therefore not affect virulence or further attenuate the virus. Deletion of ICP47 indirectly affects the ability of the virus to replicate in tumour cells, but does not reverse the attenuation. This distinction is possible because in addition to allowing antigen presentation to occur in infected cells, deletion of ICP47 causes upregulation of the neighboring gene, US11. US11 is normally regulated as a late gene, but deletion of ICP47 places US11 under the control of the ICP47 regulatory sequences, causing it to be expressed as an immediate early gene (Mohr & Gluzman, 1996). This is an important aspect of the mechanism of action of talimogene laherparepvec, as US11 upregulation allows talimogene laherparepvec to replicate more efficiently in tumours. However, it has been proven that upregulation of US11 in the background of an ICP34.5 deleted virus does not affect virulence in non-tumour tissue – in immune competent or immune compromised mice an ICP34.5 null virus with US11 upregulated is equally attenuated as an ICP34.5 null virus alone. In one study examining this issue, 100% of immune compromised mice that received 10^6 PFU of wild type HSV-1 virus were dead by 8 days post intraperitoneal injection. In contrast, the ICP34.5 null virus with US11 upregulated was indistinguishable from the ICP34.5 null virus, with 100% of the mice surviving

administration of 10^6 PFU of either virus for at least 21 days post injection (Mohr et al, 2001).

2.1.2.2 Potential to Cause Encephalitis

To thoroughly assess the risk of HSV encephalitis, studies have been performed to assess the ability of ICP34.5 null viruses to replicate in ependymal cells, which are dividing cells that line the ventricles of the brain, but are non-tumour in origin. Despite still being greatly attenuated compared to wild type virus, ICP34.5 null viruses can replicate to a low level in ependymal cells (Kesari et al, 1998; Markovitz et al, 1997; Mehta et al, 2010). Exposure of these particular cell types to virus would only occur if the virus leaked or was inadvertently inoculated into the cerebral ventricle, so is highly unlikely to occur as a result of i.l. injection. However to assess the potential consequences of this, a high dose of ICP34.5 null virus (G207, in which ribonucleotide reductase is also deleted) was injected into the cerebral ventricles of mice. 100% of mice injected with the deleted virus survived for over 20 weeks with no apparent symptoms of disease, where as 80% of mice injected with 10,000 times less wild type virus died within 10 days (Sundaresan et al, 2000). Furthermore, in one human clinical using the same virus, G207, for malignant gliomas an inadvertent protocol deviation resulted in inoculation of virus into the cerebral ventricle rather than directly into the glioma for one clinical trial subject (Markert et al, 2009). This patient required treatment with dexamethasone, but an MRI found no changes indicative of encephalitis, and acyclovir was not required.

2.1.2.3 Potential Effects of the Transgene

The only gene inserted into talimogene laherparepvec is hGM-CSF, the product of which is not a known toxin and is a United States (US) Food and Drug Administration (FDA) approved pharmaceutical.

2.1.2.3.1 Potential Toxicity of hGM-CSF

hGM-CSF is also a naturally occurring, endogenous and well-characterised protein that stimulates the production and maturation of macrophages and dendritic cells. The expression of hGM-CSF mediated by talimogene laherparepvec is intended to stimulate immune cells to attack tumour cells and display tumour antigens, thereby alerting the immune system and resulting in tumour-specific immunity (Liu et al, 2003b).

Administration of hGM-CSF has been extensively tested and found to be safe in a variety of species (Baiocchi et al, 2001; Davis et al, 1990; Liu et al, 2003a; Liu et al, 2003b; Nemunaitis et al, 1991; Rowe et al, 1995; Soiffer et al, 2003; Wang et al, 2002). Human

GM-CSF has also been widely used in several clinical trials and has been found to be safe and effective against several malignancies (Bendandi et al, 1999; Daud et al, 2008; Jager et al, 1996; Sato et al, 2008; Schmittel et al, 1999; Spitler et al, 2009). Most recently, a clinical trial involving over 800 patients concluded that hGM-CSF was safe and effective, as measured by disease free survival of patients with resected high-risk melanoma (Lawson et al, 2010).

Recombinant human GM-CSF (Leukine®; sargramostim) was approved by the FDA in 1991, and again in a different formulation in 1996, for stimulation of white blood cell recovery following chemotherapy or bone marrow transplantation. The doses of hGM-CSF delivered by this therapy are typically much higher than would be expected to be generated following treatment with talimogene laherparepvec. Adverse effects resulting from intravenous infusion or subcutaneous injection of hGM-CSF in humans are usually mild, consisting primarily of fever, muscle pain, and injection site reaction. Provenge®, autologous peripheral blood mononuclear cells activated with PAP-GM-CSF (sipuleucel-T) for use in ex-vivo maturation and loading of dendritic cells with prostate-specific antigens for use in prostate cancer patients, was granted marketing authorisation in the EU on 6 September 2013. On 6 May 2015, the European Commission withdrew the marketing authorisation for Provenge® at the request of the marketing authorisation holder, Dendreon UK Ltd, for commercial reasons. Provenge® has also been approved in the USA since April 2010.

Recently, EmbryoGen®, a medium for in vitro fertilization containing GM-CSF was found to aid embryo implantation in women who are at high risk for miscarriage. EmbryoGen® was CE-marked in the European Union in 2011 and FDA 510k clearance was obtained in late 2012.

2.1.2.3.2 Potential Oncogenicity of hGM-CSF

hGM-CSF (Leukine®; sargramostim) is a US licensed pharmaceutical product that is in routine clinical use in the US. GM-CSF is not a known oncogene, but it does inhibit apoptosis and induce proliferation of specific short-lived target cells which express the hGM-CSF receptor. When certain cells that do not normally express the hGM-CSF receptor were engineered to overexpress it, and then exposed to hGM-CSF, some of the cells underwent transformation (Areces et al, 1993; Lang et al, 1985). However, in these studies the recipient cells were already immortalised, suggesting that they had already undergone at least one major step in the transformation process. hGM-CSF has been found to be well tolerated clinically (see above). However, in one trial assessing long-term treatment with hGM-CSF, two out of 98 subjects developed acute

myelogenous leukemia (AML) (Spitler et al, 2009). It is important to note that these patients were treated with much higher doses of hGM-CSF than would be expressed from talimogene laherparepvec. Furthermore, it is unclear from this study whether these cases resulted from a hGM-CSF induced uncontrolled growth of myeloblasts or pre-existing conditions (one of the two patients had a chromosomal translocation and the other was a sheet metal worker with heavy industrial exposure to carcinogens). No cases of AML have been reported in patients treated with talimogene laherparepvec in clinical trials to date.

2.1.2.4 Observed Effects of Administration of Talimogene Laherparepvec During Development

2.1.2.4.1 Non-clinical Safety Data Obtained with Talimogene Laherparepvec

A full version of this section, including confidential information, is provided in the full version submitted to the Belgian Authorities.

A comprehensive program evaluating the safety and biodistribution of talimogene laherparepvec has been conducted. This program was designed to comply with ICH Considerations for Oncolytic Viruses (September 2009), gene therapies, and biotechnology-derived therapeutics.

2.1.2.4.2 Clinical Safety Data Obtained with Talimogene Laherparepvec

At the time of the study-specific data cutoff dates for the IB Edition 13, dated 22 September 2015, 486 subjects have received talimogene laherparepvec and have provided safety data across 15 studies. Amgen has published clinical data from a number of these including safety data on 292 subjects with unresected stage IIIB to IV melanoma, in the Phase 3 study (005/05) (Andtbacka et al 2015), 30 subjects with relapsed disease from various solid tumour cancers (Hu et al, 2006), 50 subjects with metastatic melanoma (Senzer et al, 2009) and 17 subjects with squamous cell cancer of the head and neck (Harrington et al, 2010). No subjects in any of these studies or any of the on-going studies have developed HSV encephalitis. Overall, most adverse events reported in subjects administered talimogene laherparepvec are non-serious and primarily include flu-like symptoms and injection site reactions. Most fatal adverse events reported in subjects administered talimogene laherparepvec were reported in the setting of disease progression. The concurrent use of talimogene laherparepvec, cisplatin, and external beam radiation therapy, in the 17 subjects with squamous cell cancer of the head and neck (Harrington et al, 2010), did not result in more frequent or more severe adverse events beyond those typically encountered with these other anti-cancer therapies alone.

Further details of the safety profile of talimogene laherparepvec are provided in the current Investigator's Brochure.

Four accidental exposure incidents with talimogene laherparepvec (or its murine equivalent) have been reported to date. Three of these were needle-stick injuries; one of which resulted in the development of a “herpetic whitlow like” lesion at the puncture site on the finger. This lesion was confirmed as talimogene laherparepvec by PCR as would be predicted from a needle stick with a neat dose of talimogene laherparepvec. The other two needle-stick injuries (one with talimogene laherparepvec, one with its murine equivalent) produced no signs or symptoms. Of these three needle-stick injuries, two were treated with anti-HSV-1 medication. The fourth accidental exposure was a face splash of talimogene laherparepvec to the investigator, who subsequently completed a course of prophylactic antiviral ophthalmic solution and did not develop any signs or symptoms related to the exposure.

2.1.2.5 Likely Effects of Talimogene Laherparepvec in Unintended Individuals

In the case of transmission to an unintended human recipient, the consequences for the individual are expected to be minimal. However, it is appropriate to consider potential scenarios in immune-competent healthy individuals, immune-compromised individuals (the very young or elderly) and those who are immunosuppressed due to an underlying condition or therapy (eg. HIV patients, transplant recipients, cancer patients undergoing certain therapy). It is also important to consider possible effects on neonates in the case of transmission to a pregnant woman.

2.1.2.5.1 Effects in Immune-competent Individuals

Exposure of immune-competent individuals to talimogene laherparepvec is unlikely to produce any adverse effects, due to the severely attenuated nature of the virus. At worst, an adverse event profile similar to that observed in clinical trials where talimogene laherparepvec was used as a single agent would be expected. However, such reactions would be expected to be both less severe and less frequent in an unintended recipient, since:

- The ability of talimogene laherparepvec to replicate in non-tumour cells is severely attenuated
- Viral replication and expression of GM-CSF in a non-tumour environment will be considerably lower than that which occurs in patients
- Initial exposure will be considerably less than patients and likely on a single occasion as opposed to multiple injections received by patients

2.1.2.5.2 Effects in Immune-compromised Individuals

A full version of this section, including confidential information, is provided in the full version submitted to the Belgian Authorities.

Almost all cases of serious complications of wild type HSV infections in humans occur in immunocompromised individuals (see Section 1.1.1). In these cases, the immune system fails to control the infection, and it becomes disseminated.

Immunocompromised individuals unintentionally exposed to talimogene laherparepvec may therefore experience toxicity, though, the consequences of transmission to those who are severely immuncompromised are considered no greater than that posed by wild type HSV-1.

An exclusion criterion is included in the clinical trial protocol to exclude subjects if there is evidence of clinically significant immunosuppression. In addition, the latest version of the IB and information materials provided to subjects include a warning that individuals who are immunocompromised (eg. pregnant) or immunosuppressed should not come into direct contact with a talimogene laherparepvec injection site or its protective dressing.

If clinically indicated, acyclovir, the treatment for a disseminated wild type HSV-1 infection in immunocompromised people, may be used since talimogene laherparepvec remains susceptible due to the retention of the TK gene in the construct (see Section 1.1.2). However as per protocol guidance, dosing should be permanently discontinued if, in the opinion of the investigator, the subject develops clinical evidence of any serious herpes infection (such as HSV, hepatitis, encephalitis, or disseminated infection).

2.1.2.5.3 Effects in Neonates

A full version of this section, including confidential information, is provided in the full version submitted to the Belgian Authorities.

The consequences of wild type HSV-1 infection in neonates can be severe (see Section 1.1.1), due to a greater reliance on innate rather than adaptive (acquired) immunity in the first weeks of life. The infection is more likely in cases where mothers are initially exposed to HSV-1 in the last trimester of pregnancy, such that the fetus does not acquire passive immunity from the mother.

While the risk to the newborn of acquiring viral infection from a mother exposed to talimogene laherparepvec has not been established, the risk of viral transmission and infection in the neonate is likely lower than that seen with wild type HSV-1.

Pregnant women have been excluded from talimogene laherparepvec clinical studies and no reports of accidental exposures in pregnant or lactating women have been received to date. Exclusion criteria relating to pregnancy, breast-feeding, intention to become pregnant and unwillingness to use contraception are included in the clinical trial protocol.

2.2 Indirect Effects of the Transmission of a Genetic Variant of Talimogene Laherparepvec to an Unintended Human Recipient

2.2.1 Magnitude of Effect

As with talimogene laherparepvec itself, cases of the transmission of a genetic variant of talimogene laherparepvec to an unintended human recipient are likely to be isolated (see Section 2.1.1).

The investigational medicinal product will be administered to a limited number of subjects, according to the inclusion and exclusion criteria stipulated in the clinical trial protocol .

The most likely individuals who may be a recipient of inadvertent transmission of a genetic variant of talimogene laherparepvec would be:

- Study site personnel involved with the handling and administration of the product.
- Healthcare workers or others involved in caring for the subject, which may include washing affected areas and changing dressings.
- Close contacts of the treated individual (partners and family members)

The capacity for widespread dissemination of a genetic variant of talimogene laherparepvec is expected to be severely limited due to:

- Attenuation of the virus, either by the continued deletion of ICP34.5, rendering it highly restricted for replication in normal cells, or by the continued deletion of ICP47, compromising its ability to evade the host immune system. Therefore any stable variant will be compromised in its capacity for dissemination in comparison with wild type HSV-1, though potentially not as severely as talimogene laherparepvec itself (see Section 1.1.2 for a discussion of the possible genetically stable variants of talimogene laherparepvec)
- Low incidence of shedding of infective virus from individuals treated with talimogene laherparepvec to date (see Section 3.1.3).
- Low persistence and viability outside the host organism; high sensitivity to physical and chemical agents (see Section 1.1.1).
- Natural mode of transmission (direct contact)

- Existing immunity to wild type HSV-1 in a substantial proportion of the population

2.2.2 Consequences of Transmission of a Genetic Variant of Talimogene Laherparepvec to an Unintended Individual

As described in Section 1.1.2, there are only three possible stable genetic variants of talimogene laherparepvec which can theoretically be created by homologous recombination between talimogene laherparepvec and wild type HSV-1, provided they are replicating in the same cell simultaneously.

Restoration of both ICP34.5 and ICP47 would result in a wild type virus (ie. the same as the co-infecting virus) which does not present any additional concerns beyond the potential for transmission of the co-infecting wild type virus itself.

Restoration of the ICP47 function alone would restore the variant's ability to evade the immune system (similar to that of wild type virus) but would still be unable to replicate in non-tumour cells due to the functional deletion of ICP34.5. In terms of its potential effects on unintended recipients, this variant would present no additional hazards to those described for talimogene laherparepvec itself.

Restoration of the ICP34.5 function alone would result in the removal of the transgene (GM-CSF). Thus, the potential consequences described in Section 2.1.2.3 would no longer be applicable. However, this variant would not be attenuated in its capacity to replicate in non-tumour cells and its virulence would therefore be expected to be equivalent to that of wild type virus. This variant would also retain a deletion of ICP47, thus rendering it unable to evade the immune system as effectively as the wild type virus. A virus lacking ICP47 but still expressing US11 was found to be equivalent to wild type in terms of replication and virulence at the inoculation site and surrounding skin. However, the virus lacking ICP47 was less neurovirulent than wild type following corneal injection, and this was found to be due to an inability to inhibit a protective $CD8^+$ T cell response (Goldsmith et al, 1998). In terms of its potential effects on unintended recipients, this variant would therefore present at most equivalent pathogenic effects observed in wild type HSV-1 infection.

It is important to note when assessing the potential risks associated with genetic variants of talimogene laherparepvec that in all cases, variants of talimogene laherparepvec can only be created by homologous recombination with a wild type HSV-1 simultaneously replicating in the same cell. Therefore, the transmission of any of the three possible stable genetic variants of talimogene laherparepvec described above does not constitute

an additional risk to an unintended recipient above that posed by the 'initiating' wild type infection itself.

2.3 Conclusions

The potential magnitude of unintended spread within the human population is considered low, given the attenuated nature of talimogene laherparepvec and its potential variants.

For those unintended individuals that may be exposed to talimogene laherparepvec or its possible genetic variants, the adverse effects are expected to be of lower severity than those observed with wild type HSV-1 infection which itself is universally classified as 'moderate individual risk, low/limited community risk'; see Section 4.1.

In conclusion, the potential consequences in the case of transmission of talimogene laherparepvec or its possible genetic variants are expected to be low level and isolated.

3. Evaluation of Likelihood of Occurrence of Identified Adverse Effect

The likelihood of the occurrence of the possible adverse effects on human health identified in Section 1 of the ERA is considered in this section.

3.1 Likelihood of Direct effects of the transmission of Talimogene Laherparepvec to an unintended human recipient

The potential direct adverse effects and the magnitude and consequence of those effects described in the preceding sections of the ERA are dependent on the likelihood of exposure of unintended recipients to talimogene laherparepvec.

This in turn is influenced by the manner, scale and environment of release, the potential mechanisms of exposure and specific risk management measures in place to minimise exposure, and the available data relating to shedding of and exposure to talimogene laherparepvec collected during development.

3.1.1 Manner, Scale and Environment of Release

Talimogene laherparepvec is intended as an investigational medicinal product in a proposed phase 1, multicenter, open-label study to evaluate the safety of the investigational medicinal product when injected into liver tumours (Protocol 20140318).

Talimogene laherparepvec is intended for intrahepatic injection into hepatocellular carcinoma (HCC) and metastatic liver tumors (non-HCC) by a trained medical professional in a medical study site facility (see Section 5). This phase 1 investigation is intended to be conducted in Australia, France, Spain, Switzerland and United States, subject to receiving the necessary local approvals.

The use of talimogene laherparepvec will commence as soon as possible following receipt of the necessary local approvals. The estimated average per-subject study duration is approximately 32 months.

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

Consequently, subjects receiving treatment for 12 cycles could receive an approximate maximum of 96 x10^8 PFU.

Following the injection procedure, the injection sites will be covered by an occlusive dressing before the subjects leave the clinical trial facility.

3.1.2 Potential Mechanisms of Exposure and Risk Management Measures

3.1.2.1 Mechanism of Transmission and Survivability

Wild type HSV-1 survives in the environment in the host species (humans) as a persistent infection 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.

Its mode of transmission is through direct contact with infected secretions or mucous membranes/skin with lesions from an asymptomatic or symptomatic patient shedding the virus. Transmission of HSV-1 can also occur by respiratory droplets (see Section 1.1.1).

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); see Section 1.1.1.

None of the genetic modifications made to wild type HSV-1 during construction of talimogene laherparepvec would be expected to have an effect on the mode of transmission, survivability in the environment or sensitivity to inactivating agents (see Section 1.2.2).

3.1.2.2 Potential for Exposure During Administration

Talimogene laherparepvec will be administered by intralesional injection into injectable cutaneous, subcutaneous, and nodal tumours with or without image US guidance. Administration will only be performed by medical professionals in an approved study site facility, using appropriate precautions (see Section 5).

Given the minimal manipulations required in drawing the dose from a vial into a syringe (with unlikely potential aerosol from the dead space of the needle) the potential for exposure during administration is low.

The most likely mechanism of exposure during administration will be needle-stick injuries to the medical professional.

The administration procedure and precautions (including use of Personal Protective Equipment (PPE)) are described in Section 5. The availability of appropriate PPE and established disposal procedures for potentially hazardous medical waste are universal in a medical facility.

3.1.2.3 Potential for Exposure from the Environment at the Site of Administration

Talimogene laherparepvec is to be administered in an approved study site facility. Instructions for the disposal of waste and for the handling of accidental spills and breakages are provided in Section 5.

Therefore, the potential for exposure from the environment at the site of administration is considered low.

3.1.2.4 Potential for Exposure Following Administration

Following the injection procedure, the injection sites will be covered by an occlusive dressing before subjects leave the study site facility.

There has been no detection of live virus on the outside of the occlusive dressing that is placed on top of the injected lesions in subjects participating in clinical trials (see Section 3.1.3).

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.

Therefore, the potential for exposure to talimogene laherparepvec following administration is considered low.

3.1.3 Available Data Relating to Virus Shedding and Human Exposure to Talimogene Laherparepvec

A full version of this section, including confidential information, is provided in the full version submitted to the Belgian Authorities.

Four accidental exposure incidents with talimogene laherparepvec (or it's murine equivalent) have been reported in clinical trials to date. Three of these accidental exposures were with talimogene laherparepvec; two needle-stick injuries and the third was a face splash of. Note that an additional needle-stick was with a developmental variant containing the murine GM-CSF gene also occurred outside of the clinical environment. The consequences of these incidents are described in Section 2.1.2.4.2.

3.1.4 Conclusion

Consideration of the manner, scale and environment of release, the potential mechanisms of exposure and risk management measures in place, and the available clinical data relating to shedding and exposure to talimogene laherparepvec, it is considered that the likelihood of direct effects of talimogene laherparepvec on an unintended recipient is low.

3.2 Likelihood of Indirect Effects of the Transmission of a Genetic Variant of Talimogene Laherparepvec to an Unintended Human Recipient

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.

Similarly, exposure of unintended recipients to talimogene laherparepvec would not be expected to result in infection of the mucosal tissues or neuronal ganglia, since the ability of talimogene laherparepvec to replicate in non-tumour cells is severely attenuated (see Section 1.1.2).

The possibility of the creation of stable genetic variants with unintended characteristics is also minimised by the design of the talimogene laherparepvec genetic construct as described in Section 1.2.1.2.1.

Even if a genetic variant of talimogene laherparepvec emerged in a subject of unintended recipient, it would not be expected to be widely disseminated. The mode of transmission and survivability of a genetic variant of talimogene laherparepvec will be unchanged by the potential genetic modifications which may occur through homologous recombination with a simultaneously co-infecting wild type virus.

As such the likelihood of transmission of a genetic variant of talimogene laherparepvec to an unintended human recipient is considered far lower than the likelihood of transmission of talimogene laherparepvec itself.

3.3 Overall Conclusions

Consideration of the manner, scale and environment of release, the potential mechanisms of exposure and risk management measures in place, and the available clinical data relating to shedding and exposure to talimogene laherparepvec, it is considered that the likelihood of direct effects of talimogene laherparepvec on an unintended recipient is low.

The likelihood of indirect effects caused by a genetic variant of talimogene laherparepvec in unintended recipients is considered far lower, since these events would require a combination of exposure with an additional low frequency event (homologous recombination in the patient).

4. Estimation of Risk Posed by Each Identified Characteristic

The risk posed by each of the possible adverse effects identified in Section 1 of the ERA on the health of an unintended human recipient are considered in this section.

These risks are considered by combining the estimated consequences of the effect with the estimated likelihood of effect (in accordance with 2001/18/EC and 2002/623/EC). This estimation is made with reference to the risk attributed to the parental organism (wild type HSV-1) for context. The estimate is also influenced by the degree of scientific uncertainty in those estimates, in accordance with the precautionary principle.

4.1 Risk Associated with the Parental Organism (wild type HSV-1)

Wild type HSV-1 is a globally endemic pathogen of humans which is well characterised. The mode of transmission and clinical effects of wild type HSV-1 infection in humans are well understood (see Section 1.1.1). Treatment with antivirals such as acyclovir is effective.

Wild type HSV-1 is classified in Risk Group 2 in the European Economic Community (EEC) 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’.

It should be noted that this classification does not consider genetically modified micro-organisms which are attenuated or have lost known virulence genes.

Conversely, this classification is based on the effects on healthy workers, and does not consider effects on individuals with altered susceptibility which may be as a result of pre-existing disease, medication, compromised immunity, pregnancy or breast feeding.

Similar classifications of hazard have been assigned to HSV-1 by the World Health Organisation (WHO), and in the US, Canada and Australia as summarised in Table 2.

Thus, the risk for healthy individuals working with wild type HSV-1 is widely considered to be moderate, with a low or limited risk to the community.

Table 2. Biosafety Classifications for Wild Type HSV-1 Outside of EU

Territory	Category	Definition	Reference
WHO	Risk Group 2 (moderate individual risk, low community risk).	A pathogen that can cause human or animal disease but is unlikely to be a serious hazard to laboratory workers, the community, livestock or the environment. Laboratory exposures may cause serious infection, but effective treatment and preventative measures are available and the risk of spread of infection is limited.	WHO Laboratory Biosafety Manual, 3rd Ed (2004)
US	Risk Group 2	Agents associated with human disease that is rarely serious and for which preventive or therapeutic interventions are often available.	NIH Recombinant DNA Guidelines (USA, 2011) Appendix B-II-D. CDC/NIH Guidelines (2009) "Biosafety in Microbiological and Biomedical Laboratories" 5th Edition, 2009. Section VIII-E. Not listed under 42CFR73.3 – Select Agents and Toxins
Canada	Risk Group 2 (moderate individual risk, limited community risk).	Any pathogen that can cause human disease, but under normal circumstances is unlikely to be a serious hazard to laboratory workers, the community, livestock or the environment. Laboratory exposures rarely cause infection leading to serious disease, effective treatment and preventive measures are available and the risk of spread is limited.	Canadian Laboratory Safety Guidelines (2004) Human Pathogens and Toxins Act. S.C. 2009, c. 24. Schedule II.
Australia/NZ	Group 2 (moderate individual risk, limited community risk).	A pathogen that can cause human, animal or plant disease but is unlikely to be a serious hazard to laboratory workers, the community, livestock or the environment. Laboratory exposures may cause infection, but effective treatment and preventive measures are available and the risk of spread is limited.	Standard AS/NZS 2243.3:2010. Safety in laboratories Part 3: Microbiological aspects and containment facilities. Standards Association of Australia, Sydney.

4.2 Risk Associated with the Transmission of Talimogene Laherparepvec to an Unintended Recipient

The consequences of transmission of talimogene laherparepvec to an unintended recipient are considered 'low' level and isolated (See Section 2.1) (ie. lower risk than exposure to wild type HSV-1). This assessment is based on:

- Decreased virulence and pathogenicity compared to wild type HSV-1 due to the attenuation conferred by deletion of ICP34.5 (Section 1.1.2).
- ICP34.5 deleted HSV-1 strains have been extensively utilised without incident and have been found to be nonpathogenic in a variety of animal models and also in several human clinical trials, including trials of talimogene laherparepvec (Section 2.1.2.1).
- The protein encoded by the transgene, hGM-CSF is well studied and its effects are well known through both its endogenous nature and its use in medicinal products at doses likely to be considerably greater than that which would be expected to occur through expression by talimogene laherparepvec (Section 2.1.2.3).
- Levels of both virus replication and expression of hGM-CSF in a non-tumour environment will be considerably less than occur in tumour-bearing patients.
- Initial exposure will be considerably less than patients and likely on a single occasion as opposed to multiple injections received by patients

Exposure of immune-competent individuals to talimogene laherparepvec is therefore unlikely to produce any adverse effects, due to the severely attenuated nature of the virus. At worst, an adverse event profile similar to that observed in clinical trials where talimogene laherparepvec was used as a single agent would be expected. However, such reactions would be expected to be both less severe and less frequent in an unintended recipient.

The exposure of talimogene laherparepvec to immune-compromised individuals (including neonates) may have the potential to cause adverse effects. The inadvertent transmission of talimogene laherparepvec to these populations is therefore also considered conservatively as 'moderate' (as per wild type HSV-1) although the likelihood of such transmission remains low.

In summary, the consequences of inadvertent exposure are therefore considered lower for talimogene laherparepvec than wild type HSV-1 and there is a high level of scientific certainty that this is the case, resulting from the available data on wild type HSV-1, hGM-CSF, the attenuation conferred by deletion of ICP34.5 in the construct, and clinical data from the direct administration of talimogene laherparepvec itself to tumour-bearing patients.

The likelihood of the inadvertent transmission of talimogene laherparepvec to an unintended individual is also considered 'low' (see Section 3.1) based on:

- Mode of transmission (direct contact)
- Survivability in the environment and sensitivity to physical and chemical inactivation
- Administration in an approved study site facility, with access to Personal Protective Equipment waste disposal systems and environmental controls (routine cleaning procedures)
- Available clinical data supportive of a low incidence of shedding at the administration site and no detection of virus on the exterior of the occlusive dressing
- No evidence of transmission during clinical development to date
- Data gathered in clinical development to date indicate that shedding of talimogene laherparepvec in blood and urine following administration is uncommon, low level and transient
- Infrequent detection of low levels of viral DNA in ovary and salivary gland and the absence of viral DNA in lachrymal glands, nasal mucosa, testes, or faeces as observed in non-clinical distribution data,demonstrates a low likelihood of secondary exposure to viral DNA from tears, saliva, genitalia, mucous or faeces following intralesional administration.

Thus, through a combination of the low-medium level consequences of transmission and the low likelihood of this occurring, the overall risk posed by talimogene laherparepvec to the unintended recipient is considered **LOW** (with reference to Table 3: Guideline on Environmental Risk Assessments for Medicinal Products consisting of, or containing, genetically modified organisms (GMOs)).

4.3 Risk Associated with the Transmission of a Genetic Variant Talimogene Laherparepvec to an Unintended Recipient

The consequences of transmission of a stable genetic variant of talimogene laherparepvec to an unintended recipient are considered 'low' level and isolated (See Section 2.2).

There are only three possible stable genetic variants of talimogene laherparepvec which can theoretically be created by homologous recombination between talimogene laherparepvec and wild type HSV-1, provided they are replicating in the same cell simultaneously (see Section 1.1.2):

- Restoration of both ICP34.5 and ICP47 would result in a wild type virus (ie. the same as the co-infecting virus) which does not present any additional concerns beyond the potential for transmission of the co-infecting wild type virus itself.
- Restoration of the ICP47 function alone would restore the variants ability to evade the immune system (similar to that of wild type virus) but would still be unable to replicate in non-tumour cells due to the functional deletion of ICP34.5. In terms of its

potential effects on unintended recipients, this variant would present no additional hazards to those described for talimogene laherparepvec itself.

- Restoration of the ICP34.5 function alone would result in the removal of the transgene (GM-CSF). Thus, the potential consequences of exposure to GM-CSF described in Section 2.1.2.3 would no longer be applicable. This variant would not be attenuated in its capacity to replicate in non-tumour cells but would retain a deletion of ICP47, thus rendering it less able to evade the immune system as effectively as the wild type virus. In terms of its potential effects on unintended recipients, this variant would therefore present at most equivalent pathogenic effects observed in wild type HSV-1 infection.

The consequences of exposure to genetic variants of talimogene laherparepvec are therefore considered lower than exposure to wild type HSV-1, based on the wild type 'backbone' of talimogene laherparepvec, the possible variants which could theoretically stably emerge, and the known functions of ICP34.5 and ICP47 available in the scientific literature.

However, since there are no specific clinical or non-clinical data available regarding exposure to genetic variants of talimogene laherparepvec, there is some scientific uncertainty in this conclusion. Therefore, application of the precautionary principle dictates that consideration is given to a higher classification of consequences. In the absence of clinical and non-clinical data, it is appropriate to assign a moderate level of consequence, since this would imply a similar effect to wild type virus ('moderate individual risk'; see Section 4.1).

The likelihood of the inadvertent transmission of a genetic variant of talimogene laherparepvec to an unintended individual is considered much lower than the likelihood of transmission of talimogene laherparepvec itself (see Section 3.1)

The transmission of a genetic variant of talimogene laherparepvec to an unintended recipient would be an indirect effect, relying on a chain of events, namely:

- Presence of a simultaneously co-infecting wild type virus in a host's cells. In patients, 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 a pre-existing wild type HSV-1 would be in the mucosal tissues or neuronal ganglia of the patient. Similarly, exposure of unintended recipients to talimogene laherparepvec would not be expected to result in infection of the mucosal tissues or neuronal ganglia, since the ability of talimogene laherparepvec to replicate in non-tumour cells is severely attenuated.
- Spontaneous homologous recombination between talimogene laherparepvec and wild type HSV-1 at the sites of the ICP34.5 and/or ICP47 modifications

- Transfer from the patient to an unintended recipient. The mode of transmission and survivability of a genetic variant of talimogene laherparepvec will be unchanged by the potential genetic modifications which may occur through homologous recombination with a simultaneously co-infecting wild type virus.

Therefore it is concluded that the likelihood of transmission of a genetic variant of talimogene laherparepvec to an unintended recipient is very low (see Section 3.2).

However, since there are no non-clinical or clinical data on the spontaneous creation of genetic variants of talimogene laherparepvec there is some level of scientific uncertainty in this regard. Application of the precautionary principle dictates that consideration is given to a higher classification of likelihood. In this case, it may be considered that the likelihood assigned above is an underestimate. However, it is not appropriate to assign a 'moderate' likelihood, since this would imply a greater likelihood of transmission than either talimogene laherparepvec or wild type virus. (low/limited community risk'; see Section 4.1).

Thus, through a combination of the moderate level consequences (applying the precautionary principle) of transmission and the low likelihood of this occurring, the overall risk posed by a genetic variant of talimogene laherparepvec to the unintended recipient is considered **LOW** (with reference to Table 3: Guideline on Environmental Risk Assessments for Medicinal Products consisting of, or containing, genetically modified organisms (GMOs)).

5. Application of Management Strategies for Risks

5.1 Design of Viral Construct

Multiple safety features have been incorporated into talimogene laherparepvec:

- Deletion of ICP34.5 which functions as a virulence factor during HSV infection, thus preventing the virus from replicating efficientlyin non-dividing cells.
- The possibility of the creation of stable genetic variants with unintended characteristics is minimised by the design of the talimogene laherparepvec genetic construct. Insertion of the human GM-CSF coding sequence such that it replaces the gene encoding ICP34.5 ensures that a potential recombination event between talimogene laherparepvec and wild type virus could not stably result in pathogenic virus carrying the gene for human GM-CSF. Restoration of the ICP47 function alone would result in a variant unable to replicate in non-tumour cells (due to continued deletion of ICP34.5). In the event that both ICP47 and ICP34.5 functions were restored, the resulting virus would be equivalent to wild type HSV-1 (ie. equivalent to the existing, co-infecting HSV-1).
- The HSV thymidine kinase (TK) gene remains intact which renders talimogene laherparepvec susceptible to anti-viral agents such as acyclovir. Therefore, acyclovir could be used to block replication of talimogene laherparepvec.

5.2 Control of Release

Following the approval of the Clinical Trial Application for talimogene laherparepvec, product will only be supplied to approved study sites and administered to subjects enrolled in the study by a trained medical professional, in accordance with the clinical trial protocol.

The manufacture, supply and traceability of talimogene laherparepvec is therefore controlled and monitored in accordance with pharmaceutical regulation.

The product will be stored prior to administration in a secure, temperature monitored freezer at -70°C or below in the pharmacy or other appropriate secure location.

5.3 Transportation Precautions

The following transportation classification is recommended:

IATA – UN Number: 3245

Proper shipping Name – Genetically Modified Micro-Organism

Talimogene laherparepvec is not considered infectious per the IATA definition. IATA defines infectious substances as “pathogens which can cause disease in humans or animals” but specifies that substances in which “pathogens have been neutralized or inactivated such that they no longer pose a health risk” or are “unlikely to cause disease in humans or animals” are exempt from the infectious substances definition. Talimogene laherparepvec meets both of these exemption criteria. First, talimogene laherparepvec is an attenuated version of HSV-1, modified so that replication occurs selectively in tumor cells. Because the virus is attenuated in normal cells, no adverse effects would be expected for healthy individuals or animals that inadvertently come in contact with it. Second, data from animals and humans support that talimogene laherparepvec is unlikely to cause disease, therefore exempting it from the IATA definition of infectious.

Talimogene laherparepvec is a Genetically Modified Micro-Organisms (GMMO) since it is HSV-1 that has been genetically altered in a way that does not occur in nature. Talimogene laherparepvec was generated from HSV-1 strain JS1 by deletion of ICP34.5 (required for neurovirulence and efficient replication in non-tumor cells) and ICP47 (blocks antigen presentation in HSV-1 infected cells), and insertion of the gene for GM-CSF. Deletion of ICP34.5 from HSV-1 is known to render the virus avirulent. GMMOs which do not meet the definition of toxic or infectious substances must be assigned to UN 3245.

The Talimogene Laherparepvec Safety Data Sheet (Amgen, 2015), which includes instructions for handling spills, accompanies all shipments

5.4 Handling and Administration Precautions

Administration will only be performed by trained medical professionals in an approved study site facility.

Institutional guidelines for handling, personal protective equipment, accidental spills, and waste disposal should be followed during product preparation and administration.

Definitions and requirements for appropriate handling and waste disposal may vary by site and regional jurisdiction, however, universal biosafety practices are similar amongst the guidelines and are typically followed by medical facilities when handling injectable medicinal products and medical waste, such as:

- Restricted access
- Safe storage
- Training of personnel
- Availability and use of Personal Protective Equipment (PPE; laboratory coats, gowns, gloves and safety glasses)
- Established routine practices for dealing with potentially biohazardous materials such as patient samples/fluids and medical waste (autoclaves, sharps bins, incinerators, disinfectants and appropriate cleanable surfaces).

Precautions for use are provided in the latest versions of the IB and Safety Data Sheet (SDS), issued to each study site by Amgen.

Additional Information Related to Exposure to Talimogene Laherparepvec

A needle stick injury, spill, or splash back during administration may result in accidental exposure of Healthcare Professionals to talimogene laherparepvec. The ICP34.5 gene deletion is intended to allow only tumor selective replication and limited or no viral replication in normal tissues. However, talimogene laherparepvec injection can result in signs or symptoms of primary infection at the site of exposure. A few reports of accidental exposure in study personnel have been received. In one of the cases, the exposed physician developed clinical signs/symptoms of a herpetic whitlow-like lesion at the site of the accidental needle stick injury that resolved without sequelae. An initial antibody assay was positive for an HSV-type virus. A confirmatory PCR assay was conducted 10 days after the accidental exposure and was positive for a virus with the ICP47 deletion, indicating that the virus was most likely talimogene laherparepvec. None of the other exposed individuals reported signs or symptoms of infection. In some cases oral acyclovir or valacyclovir was administered.

Accidental Exposure to Talimogene Laherparepvec

Accidental exposure to talimogene laherparepvec may lead to signs or symptoms suggestive of herpetic infection. Healthcare personnel may be exposed to talimogene laherparepvec during preparation or administration. 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. Healthcare personnel with open skin wounds or who are immunosuppressed should not administer talimogene laherparepvec or should not come into direct contact with the talimogene laherparepvec injection site(s). Healthcare personnel preparing or administering intralesional injections and applying protective dressing to injected lesions must observe safety precautions (eg, wear protective gown or laboratory coat, safety glasses, and gloves) to avoid direct contact with talimogene laherparepvec. Close contacts may be exposed to talimogene laherparepvec via direct contact with injected lesions, protective dressings, or physical contact with body fluids (eg, blood and urine) of treated subjects. Individuals with open skin lesions should not come into direct contact with the talimogene laherparepvec injection site(s). Individuals who are immunosuppressed should avoid direct contact with treated subjects. Close contacts assisting subjects in applying protective dressing to injected lesions must wear protective gloves and must observe safety precautions to avoid direct contact with talimogene laherparepvec. In the event of a secondary exposure (eg, leakage through occlusive dressing to subject or contacts) to talimogene laherparepvec, advise exposed individuals to clean the site thoroughly with soap and water and/or a disinfectant. Exposed individuals should contact healthcare provider for signs of systemic (fever, aches, nausea, and malaise) or local (for example, pain, redness and swelling) infection.

Talimogene laherparepvec is sensitive to acyclovir or any anti-viral drug that is activated by the viral thymidine kinase gene and may be administered, if clinically indicated.

Accidental Spills

Talimogene laherparepvec spills should be treated with a virucidal agent and absorbent materials. All materials contaminated with Talimogene laherparepvec must be disposed of in compliance with local institutional guidelines.

Given the attenuated nature of the modified organism, the fact that the parent organism is not known to spread through aerosols, and the minimal manipulations required in drawing the dose from a vial into a syringe (with unlikely potential aerosol from the dead space of the needle) it is not considered necessary to perform the preparation procedure

in a biosafety cabinet. Similarly, no further precautions are required when administering the product by intralesional injection.

5.5 Cleaning and Waste Management

Wild type HSV-1 is sensitive to inactivation by a variety of commonly employed/ readily available physical and chemical treatments:

Physical inactivation: Wild type HSV virus is easily inactivated outside the host by exposure to PH < 4, temperatures >56°C for 30 min, pasteurization (60°C for 10 h), and microwave heating for 4 min (Jerome & Morrow, 2007; Croughan & Behbehani, 1988).

Chemical inactivation: Wild type HSV virus is easily inactivated by lipid solvents (Jerome & Morrow, 2007). It can be inactivated by 0.5% Lysol in 5 min; by Listerine (1:1 mixtures) in 5 min; by 2,000 ppm (2,000 µl/liter) of bleach in 10 min; by isopropyl alcohol (1:1 mixtures) (Croughan & Behbehani, 1988). HSV is also susceptible to quaternary ammonium compounds (Wood and Payne, 1998). Most herpes viruses are also susceptible to 30% ethanol and isopropanol, 0.12% orthophenyl phenol, and 0.04% glutaraldehyde (Prince & Prince, 2001).

The genetic modifications made during the construction of talimogene laherparepvec from wild type HSV-1 do not affect its sensitivity to physical and chemical inactivation.

Following administration of talimogene laherparepvec at a study site, used vials, syringes, needles and any disposable instruments or other materials used during the procedure should be disposed of, as instructed in the latest version of the IB, following appropriate local/regional and institutional requirements for biohazardous waste.

In the study site facility, this will involve temporary containment in sharps bins or clearly marked bags (eg. biohazard, medical waste) prior to autoclaving and/or incineration either on or off site as per local institutional guidelines for handling potentially infectious materials.

Commonly in a medical setting, non-disposable equipment and other materials used during the procedure will be cleaned using a chemical disinfectant capable of virucidal activity for the required duration of contact, or sterilised by autoclaving consistent with local institutional guidelines for handling potentially infectious materials.

Typically, standard operating procedures for disposal within medical facilities (where the potential for contamination from other agents is potentially much more hazardous than that presented by talimogene laherparepvec) will be consistent with the guidance given in the WHO Laboratory Biosafety Manual, 3rd Ed (2004) as outlined below:

Contaminated (infectious) "sharps":

Hypodermic needles, scalpels, knives and broken glass; should always be collected in puncture-proof containers fitted with covers and treated as infectious.

After use, hypodermic needles should not be recapped, clipped or removed from disposable syringes. The complete assembly should be placed in a sharps disposal container. Disposable syringes, used alone or with needles, should be placed in sharps disposal containers and incinerated, with prior autoclaving if required. Sharps disposal containers must be puncture-proof/-resistant and must not be filled to capacity. When they are three-quarters full they should be placed in "infectious waste" containers and incinerated, with prior autoclaving if laboratory practice requires it. Sharps disposal containers must not be discarded in landfills.

Contaminated (potentially infectious) materials for autoclaving and reuse:

No pre-cleaning should be attempted of any contaminated (potentially infectious) materials to be autoclaved and reused. Any necessary cleaning or repair must be done only after autoclaving or disinfection.

Contaminated (potentially infectious) materials for disposal:

Apart from sharps, which are dealt with above, all contaminated (potentially infectious) materials should be autoclaved in leak-proof containers (eg. autoclavable, color-coded plastic bags), before disposal. After autoclaving, the material may be placed in transfer containers for transport to the incinerator. If possible, materials deriving from healthcare activities should not be discarded in landfills even after decontamination. If an incinerator is available on the laboratory site, autoclaving may be omitted; the contaminated waste should be placed in designated containers (eg. color-coded bags) and transported directly to the incinerator. Reusable transfer containers should be leakproof and have tight-fitting covers. They should be disinfected and cleaned before they are returned to the laboratory for further use.

Cleaning of the facility after use will not be necessary, provided the precautions outlined above 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.

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.

5.6 Communication of Risks and Precautions

The IPIM, Investigator's Brochure, Safety Data Sheet and materials provide to the subjects contain essential information to minimise the risk of transmission to an unintended individual, including:

- Description of the method of administration and the personal protective equipment to be used during dose preparation and administration.
- Description of actions to take following accidental occupational exposure (needle-stick or splash)
- Procedures for disposal of waste and procedures for dealing with accidental spills and breakages.
- Instructions for care of the injection site (s) and disposal of dressings by the patient
- A description of the main symptoms of wild type HSV-1 infection, with instructions to inform a medical professional should the patient or a close contact of the patient display symptoms. Instructions for the management of such an infection are included.

5.7 Monitoring Activities

A full version of this section, including confidential information, is provided in the full version submitted to the Belgian Authorities.

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.

5.8 Conclusions

Appropriate risk management strategies are in place to communicate and minimise the risks and consequences of exposure to unintended individuals. Appropriate monitoring strategies are proposed to add to the scientific certainty of the environmental risk assessment.

6. Determination of Overall Risk of the GMO

Humans are the only natural host for HSV-1 It does not infect plants, rarelyanimals, and does not contribute to environmental ecosystems or processes. It does not respire and does not contribute to any primary production or decomposition process. In its virion form, it does not display any metabolic activity. Wild type HSV-1 is not known to be zoonotic or reverse zoonotic under natural conditions.

DNA replication occurs in the cell nucleus. No integration of the viral genome with the cellular genome occurs during replication or latency (see Section 1.1.1).

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 (see Section 1.1.1).

None of the genetic modifications made to wild type HSV-1 during construction of talimogene laherparepvec would be expected to enable the transfer or maintenance of genetic material into the environment (outside its obligate host species), or have an effect on sensitivity to inactivating agents or survivability in the environment. No genes conferring resistance to antibacterials are present in talimogene laherparepvec, nor were any antibacterial resistance genes used as markers in its construction.

Therefore, no potential adverse effects of talimogene laherparepvec on non-human organisms in the environment, ecosystems or environmental processes have been identified.

Based on the nature of the parental organism, the genetic modifications resulting in talimogene laherparepvec and the receiving environment, **the potential adverse effects which talimogene laherparepvec may exert by conducting this clinical trial are limited to:**

- **Direct effects of the transmission of talimogene laherparepvec to an unintended human recipient which may be immediate or delayed**
- **Indirect effects of the transmission of a genetic variant of talimogene laherparepvec to an unintended human recipient which may be immediate or delayed.**

The potential magnitude of unintended spread of talimogene laherparepvec or its theoretical genetic variants within the human population is considered low, given the attenuated nature of talimogene laherparepvec and its variants, in addition to the clinical experience obtained to date..

For those unintended individuals that may be exposed to talimogene laherparepvec or its possible genetic variants, the adverse effects are expected to be of lower severity than those observed with wild type HSV-1 infection which itself is universally classified as 'moderate individual risk, low/limited community risk'; see Section 4.1. The consequences of exposure to severely immunocompromised individuals may be considered equivalent to exposure to wild type HSV-1 (moderate risk).

In conclusion, the potential consequences in the case of transmission of talimogene laherparepvec or its possible genetic variants are expected to be low-moderate level and isolated.

Consideration of the manner, scale and environment of release, the potential mechanisms of exposure and risk management measures in place, and the available clinical data relating to shedding and exposure to talimogene laherparepvec, it is considered that the likelihood of direct effects of talimogene laherparepvec on an unintended recipient is low.

The likelihood of indirect effects caused by a genetic variant of talimogene laherparepvec in unintended recipients is considered far lower, since such an event would require a combination of exposure with an additional low frequency event (homologous recombination in the patient or a re-activation event in an unintended recipient).

Therefore, the likelihood of direct or indirect effects of the transmission of talimogene laherparepvec or a genetic variant of talimogene laherparepvec to an unintended human recipient is considered low.

The potential risks identified for human health (specifically an unintended recipient) are considered by combining the estimated consequences of the effect with the estimated likelihood of effect (in accordance with 2001/18/EC and 2002/623/EC and with reference to Table 3: Guideline on Environmental Risk Assessments for Medicinal Products consisting of, or containing, genetically modified organisms (GMOs)).

This estimation is made with reference to the risk attributed to the parental organism (wild type HSV-1) for context. The estimate is also influenced by the degree of scientific uncertainty in those estimates, in accordance with the precautionary principle.

Thus, through a combination of the low-moderate level consequences of transmission and the low likelihood of this occurring, the **overall risk posed by talimogene laherparepvec to the unintended recipient is considered LOW**.

Similarly, through a combination of the moderate level consequences (applying the precautionary principle) of transmission and the low likelihood of this occurring, the **overall risk posed by a genetic variant of talimogene laherparepvec to the unintended recipient is considered LOW.**

Appropriate risk management strategies are in place to communicate and minimise the risks and consequences of exposure to unintended individuals, including:

- Design of the viral construct
- Control of release
- Transportation precautions
- Administration precautions
- Cleaning and waste management
- Communication of risks and precautions

Appropriate activities are proposed in order to monitor the release of talimogene laherparepvec and address uncertainties in the available data.

In conclusion, overall the environmental impact of the deliberate release of talimogene laherparepvec as an investigational medicinal product under the conditions of release proposed, and with the precautions and monitoring activities proposed, is considered acceptable.

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