Australian Government
Department of Health
Office of the Gene Technology Regulator

Risk Assessment and Risk Management Plan for

DIR-132

Commercial supply of a tumour-selective genetically modified virus for cancer therapy

Applicant: Amgen Australia Pty Ltd (Amgen)

August 2015

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Summary of the Risk Assessment and Risk Management Plan

for

Licence Application No. DIR-132

Decision

The Gene Technology Regulator (the Regulator) has decided to issue a licence for this application. The licence authorises import, transport, storage and disposal of the genetically modified (GM) virus, known as Talimogene laherparepvec, for the purpose of its commercial supply as a therapeutic product.

A Risk Assessment and Risk Management Plan (RARMP) for this application was prepared by the Regulator in accordance with requirements of the *Gene Technology Act 2000* (the Act) and corresponding state and territory legislation, and finalised following consultation with a wide range of experts, agencies and authorities, and the public. The RARMP concludes that this commercial release poses negligible risks to human health and safety and the environment and no specific risk treatment measures are proposed. However, general licence conditions have been imposed to ensure that there is ongoing oversight of the licenced dealings.

Before this genetically modified (GMO) can be used as a therapeutic, Amgen must also obtain regulatory approval from the Therapeutic Goods Administration (TGA). Medicines and other therapeutic goods for sale in Australia are required to be assessed for quality, safety and efficacy under the *Therapeutic Goods Act 1989* and must be included in the Australian Register of Therapeutic Goods (ARTG). The TGA are currently considering an application from Amgen to have Talimogene laherparepvec included on the ARTG. The OGTR will continue to consult with the TGA during the assessment of the application. Amgen will also need approval from the Department of Agriculture for import of the GMO.

The application

Application number	DIR-132
Applicant	Amgen Australia Pty Ltd (Amgen)
Project title	Commercial supply of a tumour-selective genetically modified virus for cancer therapy[1]
Parent organism	*Herpes simplex virus 1* (HSV-1), strain JS1
Introduced or modified genes and resulting modified traits	• deletion of *ICP34.5* gene (human therapeutic – attenuation) • deletion of *ICD47* gene (human therapeutic – enhanced immune response) • *hGM-CSF* gene encoding Granulocyte-Macrophage Colony-Stimulating Factor from humans (human therapeutic – enhanced immune response)

[1] The title of the project as supplied by Amgen is 'Commercial Release of a tumour-selective genetically modified virus for oncolytic immunotherapy.'

Proposed locations	At clinical facilities throughout Australia (subject to approval by the Therapeutic Goods Administration)
Proposed release date	Ongoing from date of approval
Proposed activities	Import, storage, transport and disposal of the GM virus for the purpose of administration by healthcare professionals as a prescription only medication for cancer therapy (administration is subject to Therapeutic Goods Administration approval)

Amgen Australia Pty Ltd (Amgen) proposes the commercial supply of a genetically modified *Herpes simplex virus 1* (HSV-1). Subject to approval by the TGA, the GMO would be used as a prescription only treatment for patients with skin cancer (metastatic melanoma) and other suitable solid tumours that are unable to be removed by surgery. The GMO will be administered to patients by injection directly into the tumour. The GMO would be manufactured overseas and imported into Australia for use in clinical facilities equipped to deal with scheduled drugs and infectious agents.

Naturally occurring HSV-1 is a human pathogen that causes local skin lesions. It is highly contagious and widespread in the environment, with around 80% of the population estimated to be seropositive for the virus. Primary infection occurs most commonly in oral mucosal tissue (e.g. cold sore) and generally prior to the age of three. The primary infection is usually mild and self-limiting, although in a minority of cases infection may be severe, including disseminated disease and encephalitis. With the exception of neonates and immune-compromised people, HSV-1 infection is not systemic and is limited to the epithelial cells and sensory ganglia of the infection site.

The GMO is an attenuated HSV-1 modified to selectively replicate in tumours (rapidly dividing cells) and enhance the immune response in treated cancer patients. To produce the GMO, HSV-1 was modified by removing specific viral genes involved in viral replication and viral antigen presentation, and by introduction of a gene encoding a human protein that stimulates certain types of immune cells.

The GMO has not previously been used commercially, however it has been used in clinical trials on skin cancer and several advanced solid tumour types in multiple countries, including the United Kingdom, Canada, South Africa and the USA. In Australia, a phase III clinical trial of the GMO, under the name OncoVEX$^{gm\text{-}csf}$, is being conducted under a GMO licence for dealings not involving intentional release (DNIR) of a GMO into the environment (licence DNIR-461). Australian patients started receiving treatment under DNIR-461 in December 2014.

Risk assessment

The risk assessment concludes that risks from the proposed dealings, either in the short or long term, to the health and safety of people, or the environment, are negligible. No specific risk treatment measures are required to manage these negligible risks.

The risk assessment process considers how the genetic modifications and proposed activities conducted with the GMOs might lead to harm to people or the environment. Risks are characterised in relation to both the seriousness and likelihood of harm, taking into account information in the application (including proposed controls), relevant previous approvals and current scientific/technical knowledge. Both the short and long term impact are considered.

To avoid duplication of regulatory oversight, the Regulator does not assess risks to people receiving or administering the GMO as a therapeutic. However, import, transport and disposal

are regulated under the *Gene Technology Act 2000* (the Act), and the Regulator has assessed risks posed to people and to the environment associated with these activities.

Credible pathways to potential harm that were considered included whether or not expression of the introduced genes and genetic modifications could: result in products that are toxic to people or other organisms; alter characteristics that may impact on the disease burden from the GM virus, or produce unintended changes in viral characteristics. The opportunity for gene transfer to other organisms, and its effects if it were to occur, was also considered.

A substantive risk is only identified for further assessment when a risk scenario is considered to have some reasonable chance of causing harm. Pathways that do not lead to harm, or could not reasonably occur, do not advance in the risk assessment process.

The risks to the health and safety of people, or the environment, from the proposed dealings with the GM virus have been assessed to be negligible. Hence, the Regulator considers that the dealings involved do not pose a significant risk to either people or the environment.

The principal reasons for the conclusion of negligible risks are that the proposed controls applicable to therapeutic goods effectively minimise unintended exposure to the GMO; the parent virus only infects humans and the genetic modifications have not altered this specificity; the genetic modifications attenuate the GM virus such that its ability to replicate, to be transmitted or persist are significantly reduced; the introduced gene is of human origin and not expected to be toxic to people or the environment.

Risk management plan

Risk management is used to protect the health and safety of people and to protect the environment by controlling or mitigating risk. The risk management plan evaluates and treats identified risks, evaluates controls and limits proposed by the applicant, and considers general risk management measures. The risk management plan is given effect through licence conditions.

As the level of risk is assessed as negligible, specific risk treatment is not required. However, the Regulator has imposed licence conditions under post-release review (PRR) to ensure that there is ongoing oversight of the release and to allow the collection of information to verify the findings of the RARMP. The licence also contains a number of general conditions relating to ongoing licence holder suitability, auditing and monitoring, and reporting requirements, which include an obligation to report any unintended effects.

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Abbreviations

Amgen	Amgen Australia Pty Ltd
APVMA	Australian Pesticides and Veterinary Medicines Authority
ARTG	Australian Register of Therapeutic Goods
bgh-PolyA	bovine growth hormone polyadenylation signal sequence
CCI	Confidential Commercial Information under section 185 of the *Gene Technology Act 2000*
CD	cluster of differentiation
CMI	Consumer Medicine Information
CMV	cytomegalovirus
DIR	Dealings involving Intentional Release
DNA	Deoxyribonucleic acid
DNIR	Dealings not involving Intentional Release
IATA	International Air Transport Association
ICP	Infected Cell Protein
FSANZ	Food Standards Australia New Zealand
GM	Genetically modified
GM-CSF	Granulocyte-Macrophage Colony-Stimulating Factor
GMO	Genetically modified organism
GTTAC	Gene Technology Technical Advisory Committee
hGM-CSF	human Granulocyte-Macrophage Colony-Stimulating Factor
HGT	Horizontal gene transfer
HSE	Herpes simplex encephalitis
HSV	*Herpes simplex virus*
HSV-1	*Herpes simplex virus 1*
HSV-2	*Herpes simplex virus 2*
IC_{50}	half maximal inhibitory concentration
LATs	Latency-Associated Transcripts
MSDS	Material Safety Data Sheet
mL	millilitre
NCCTG	National Coordinating Committee on Therapeutic Goods
µg	microgram
OGTR	Office of the Gene Technology Regulator
PFU	Plaque-forming units
PPE	Personal Protective Equipment
PRR	Post release review
RARMP	Risk Assessment and Risk Management Plan
SUSMP	Standard for the Uniform Scheduling of Medicines and Poisons
TGA	Therapeutic Goods Administration
the Act	The *Gene Technology Act 2000*
the Regulations	The Gene Technology Regulations 2001, as amended 2011

the Regulator	The Gene Technology Regulator
TK	thymidine kinase
USA	United States of America
WHO	World Health Organisation

Chapter 1 Risk assessment context

Section 1 Background

1. An application has been made under the *Gene Technology Act 2000* (the Act) for Dealings involving the Intentional Release (DIR) of genetically modified organisms (GMOs) into the Australian environment.

2. The Act in conjunction with the Gene Technology Regulations 2001 (the Regulations), an inter-governmental agreement and corresponding legislation that is being enacted in each State and Territory, comprise Australia's national regulatory system for gene technology. Its objective is to protect the health and safety of people, and to protect the environment, by identifying risks posed by or as a result of gene technology, and by managing those risks through regulating certain dealings with GMOs.

3. This chapter describes the parameters within which potential risks to the health and safety of people or the environment posed by the proposed release are assessed. The risk assessment context is established within the regulatory framework and considers application-specific parameters (Figure 1).

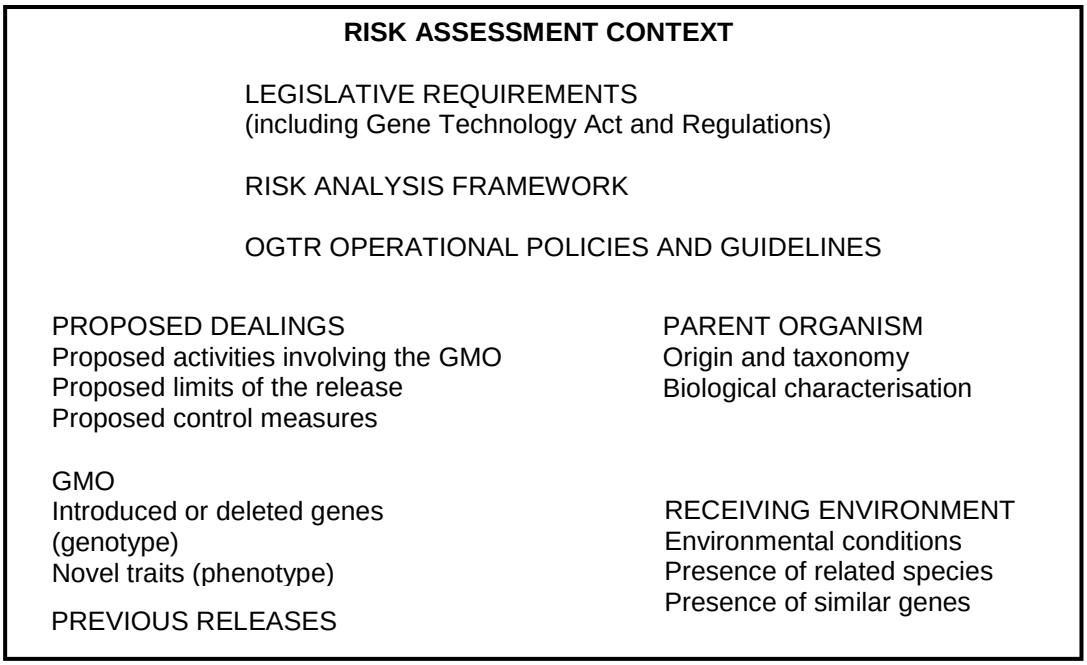

Figure 1. Summary of parameters used to establish the risk assessment context

Section 2 Regulatory framework

4. Sections 50, 50A and 51 of the Act outline the matters which the Gene Technology Regulator (the Regulator) must take into account, and consultation that is required when preparing the Risk Assessment and Risk Management Plans (RARMPs) that form the basis of decisions on licence applications. In addition, the Regulations outline matters the Regulator must consider when preparing a RARMP.

5. Since this application is for commercial purposes, it cannot be considered as a limited and controlled release application under section 50A of the Act. This means that, under section 50(3) of the Act, the Regulator was required to consult with prescribed experts, agencies and authorities to seek advice on matters relevant to the preparation of the RARMP. This first round of consultation included the Gene Technology Technical Advisory Committee (GTTAC), State and Territory Governments, Australian Government authorities or agencies

prescribed in the Regulations, local councils and the Minister for the Environment. A summary of issues contained in submissions received is given in Appendix A.

6. Section 52 of the Act requires the Regulator, in a second round of consultation, to seek comment on the RARMP from the experts, agencies and authorities outlined above, as well as the public. Advice from the prescribed experts, agencies and authorities for the second round of consultation, and how it was taken into account, is summarised in Appendix B. One public submission was received and its consideration is summarised in Appendix C.

7. The Risk Analysis Framework explains the Regulator's approach to the preparation of RARMPs in accordance with the legislative requirements (OGTR 2013). Additionally, there are a number of operational policies and guidelines developed by the Office of the Gene Technology Regulator (OGTR) that are relevant to DIR licences. These documents are available from the OGTR website.

2.1 Interface with other regulatory schemes

8. Gene technology legislation operates in conjunction with other regulatory schemes in Australia. Any dealings conducted under a licence issued by the Regulator may also be subject to regulation by other Australian government agencies that regulate GMOs or genetically modified (GM) products, including Food Standards Australia New Zealand (FSANZ), the Australian Pesticides and Veterinary Medicines Authority (APVMA), the Therapeutic Goods Administration, the National Industrial Chemicals Notification and Assessment Scheme and the Department of Agriculture. These dealings may also be subject to the operation of State legislation declaring areas to be GM, GM free, or both, for marketing purposes.

9. Medicines and other therapeutic goods for use in Australia are required to be assessed for quality, safety and efficacy under the *Therapeutic Goods Act 1989* and must be included in the Australian Register of Therapeutic Goods (ARTG). The Therapeutic Goods Administration (TGA) is responsible for administering the provisions of this legislation. The TGA also regulates the labelling, handling, sale and supply of scheduled medicines through the *Standard for the Uniform Scheduling of Medicines and Poisons* (SUSMP) (Poisons Standard 2015).

10. Where a GMO is proposed to be a registered therapeutic, the TGA has regulatory responsibility for quality, efficacy and patient safety. To avoid duplication of regulatory oversight, administration of the GMO as a therapeutic is not regulated under gene technology legislation. The Regulator notes that the TGA assesses risks to patients and manages any risks identified. Therefore, risks to people receiving or administering the GMO as a therapy are not considered as part of the Regulator's evaluation of this application; the Regulator has assessed risks posed to other people and to the environment associated with other activities. This includes risks associated with import, transport and disposal of medicines and other therapeutic goods that are GMOs, and are therefore subject to regulation under the *Gene Technology Act 2000*.

11. The Department of Agriculture administers Australian biosecurity conditions for the importation of biological products under the *Quarantine Act 1908*. Biological products include animal or microbial derived products such as foods, therapeutics, laboratory materials and vaccines (including GM vaccines). Import of the GM virus is subject to regulation by the Department of Agriculture and the Regulator.

Section 3 Proposed Dealings

12. Amgen Australia Pty Ltd (Amgen) proposes to use a live attenuated GM virus, known as Talimogene laherparepvec, as a prescription medicine for cancer treatment. The GM virus will be used as a prescription only treatment for patients with skin cancer (metastatic melanoma) and other suitable solid tumours that are unable to be removed by surgery. The GM virus will be administered to patients by intratumoural injection.

13. As therapeutic use of the GMO is subject to TGA regulation, the proposed dealings assessed by the Regulator are:

- import;
- transport;
- disposal; and
- possession (including storage) and supply of the GMO for any of the purposes above.

14. The GM virus would be imported from overseas manufacturing sites in the United States of America (USA).

15. Storage, handling and transport will be in accordance with the Regulator's *Guidelines for the Transport, Storage and Disposal of GMOs*, the *Australian code of good wholesaling practice for medicines* in schedules 2,3,4 and 8 (National Coordinating Committee on Therapeutic Goods (NCCTG 2011)) and the World Health Organisation (WHO) *Good distribution practices for pharmaceutical products* (World Health Organisation (WHO) 2010).

16. The GM virus would be packaged as a sterile frozen liquid in single use 2.0 millilitre (mL) Crystal Zenith resin vials. The single dose vials will be packaged into a secure secondary packaging carton. Amgen proposes the International Air Transport Association (IATA) shipping classification for the GM virus as Genetically Modified Micro-Organism, UN Number: 3245.

17. Transport to Australia will be carried out through the use of commercial courier companies experienced in the transportation of pharmaceutical products, which require secure handling and the maintenance of a strict temperature regime, to central storage facilities of a logistics service provider used by Amgen in Australia.

18. The logistics service provider will ensure secure storage of the GM virus is within chambers validated to maintain temperatures at or below -70^{o}C to maintain stability and efficacy of the product during storage.

19. Transport within Australia will be carried out through the use of commercial courier companies experienced in the transportation of pharmaceutical products. GM virus would be transported in insulated shipping containers with dry ice in configurations that ensure stability and efficacy of the product during transport for delivery.

20. The GM virus would be transported to treating hospitals and clinics that are registered and licenced for the purposes of handling scheduled medicines and poisons as legislated through the Poisons Standard in effect at the time and enforced through state and territory legislation.

21. The GM virus would be stored in a secure, temperature controlled freezer in the pharmacy or other appropriate secure location at treating hospital and clinics.

22. Amgen may seek to export the GM virus from the Australian logistics service provider to approved facilities in New Zealand.

23. The GM virus would be dispensed within a medical facility and in consideration of the Talimogene laherparepvec Material Safety Data Sheet (MSDS) and facility safety assessment. Typically, such facilities follow practices of the AS/NZS 2243.3:2010 Safety in laboratories - Microbiological Safety and Containment (Standards Australia/New Zealand 2010).

24. Australian Product Information and Consumer Medicine Information (CMI) documents have been submitted to the TGA as part of Amgen's application for product registration and, if approved, will be made available to healthcare professionals and consumers respectively. The Product Information document will instruct healthcare professionals in the use and storage

requirements for the GM virus. Similarly the CMI document will inform the consumer about the GM virus, its use and method of administration, and other relevant safety information.

25. Amgen has indicated that the CMI will also communicate to prescribers the nature of the product as an infectious agent, including risks of herpetic events in patients, risk of secondary transmission, safe use and handling, and how to instruct patients on these risks.

26. The GM virus will be contra-indicated in patients who are severely immunocompromised. It will also be clearly communicated to healthcare personnel who are immunocompromised not to administer the GM virus and not to come into direct contact with the injection sites or body fluids of treated patients. The CMI will provide additional communication to patients around the risk of secondary transmission, what consumers can do to mitigate this risk and measures for management of accidental exposure.

27. For handling the GM virus, the recommended personal protective equipment (PPE) include; laboratory coat, gloves and safety glasses when there is potential for direct skin contact with the virus.

28. The GM virus would be drawn into syringes from 2 mL stoppered vials in the room used for administration to the patient or appropriate room according to institutional guidelines at treating hospital and clinics.

29. Following administration at treating hospitals and clinics, all unused product and associated waste (including needles, swabs etc.) would be discarded into appropriate biohazard containers and disposed of following institutional procedures for the disposal of biohazardous material. This may include rendering all waste inert by high temperature incineration or steam sterilisation at the medical facility and/or use of registered waste contractors.

30. Patients are to be advised to avoid touching or scratching injection sites to prevent inadvertent transfer of the GM virus to other areas of the body. Caregivers are to be advised to wear protective gloves when assisting patients in applying or changing dressings.

31. When changing patient dressings outside treating hospitals and clinics, the patient dressings and associated materials used to clean the treatment area are to be placed in a sealed plastic bag and disposed of in household waste.

Section 4 The parent organism

32. The parent organism of the GM virus is human *herpes simplex virus 1* (HSV-1), strain JS1. The JS1 strain is a clinical HSV-1 isolate from a cold sore, which was found to replicate better and have improved cancer cell killing abilities in several human cancer cell lines compared to the common laboratory HSV-1 strain 17+ (Liu et al. 2003a).

33. Herpes simplex viruses are member of the *Simplexvirus* genus of the family *Herpesviridae*, subfamily *Alphaherpesvirinae*. There are two types of Herpes simplex viruses, herpes simplex virus 1 and 2 (HSV-1 and HSV-2), which share extensive genome and protein homology. HSV-1 and HSV-2 are also known as human herpesvirus 1 and 2.

34. These viruses are capable of infecting the nervous system of humans and causing neurological diseases. In addition, HSV can result in a lifelong infection by establishing a dormant state (latency) in the host sensory neurons and replicating in epithelial cells during primary infection and reactivation (Heldwein & Krummenacher 2008).

35. Herpesviruses are highly host specific and share a long synchronous evolution with their hosts. Only in rare cases does animal to human (i.e. zoonosis) or human to animal (i.e. anthroponosis) transmission occur (Epstein & Price 2009; Tischer & Osterrieder 2010). Non-human infections by HSV-1 are rare, but may occur in rodents, rabbits, hedgehogs, and non-

human primates (Allison et al. 2002; Grest et al. 2002; Huemer et al. 2002; Lefaux et al. 2004; Longa et al. 2011; Muller et al. 2009; Weissenbock et al. 1997; Wohlsein et al. 2002).

36. HSV-1 transmission and infection of marmosets and other New World monkeys usually results in fatal disease (Epstein & Price 2009; Landolfi et al. 2005). Deaths of both captive and wild marmosets have been reported (Casagrande et al. 2014; Imura et al. 2014; Longa et al. 2011). Fatal infections have also been reported for the domestic rabbit (de Matos et al. 2014; Grest et al. 2002), the chinchilla (Wohlsein et al. 2002) and both European and African pygmy hedgehogs (Allison et al. 2002; Riley & Chomel 2005).

37. In comparison Old World monkeys appear to be less susceptible to HSV-1 and infection results in clinical symptoms similar to those displayed by humans (Epstein & Price 2009; Sekulin et al. 2010).

38. Small mammals, such as rodents and rabbits have been used as a model of HSV-1 pathogenesis in humans, as they are susceptible to viral infection. Various mouse models have been established and extensively used to evaluate HSV-1 infection and pathogenesis (Anderson & Field 1983; Armien et al. 2010; Mester & Rouse 1991; Webre et al. 2012; Whitley et al. 1993).

39. Macropodid herpes viruses 1, 2 and 3 (MaHV-1, MaHV-2 and MaHV-3) infect native marsupials such as wallabies and kangaroos, are related to HSV-1 and HSV-2 and are closely associated with those herpesviruses that infect primates (Mahony et al. 1999). While there is no direct evidence that HSV-1 naturally infects marsupials, at least one study reports that marsupial cell lines are susceptible to HSV-1 (Webber & Whalley 1978).

40. A review article discussing case reports of HSV-1 causing acute fatal disease in non-human primate's notes that "recent studies of HSV-1 infection among captive animals remain scarce" (Epstein & Price 2009). A literature search conducted to establish likelihood of infectivity of HSV-1 in other species did not find evidence of naturally occurring HSV-1 infection in dogs, cats, horses, cows or other common domesticated animals, although they may be infected with other alpha herpesvirus family members (McGeoch et al. 2006).

4.1 HSV Basic biology

41. HSV-1 is an enveloped deoxyribonucleic acid (DNA) virus with a linear double stranded DNA genome. HSV-1 is a non-integrating type of virus, meaning it does not integrate into the DNA of the host. The HSV-1 and HSV-2 genomes are 152 and 154 kilobase pair in size respectively, share approximately 50% DNA nucleotide homology and each contain approximately 84 unique protein coding genes and 94 putative open reading frames (Rajcani et al. 2004). These genes encode the majority of the proteins of the mature virion (entire virus particle), including those involved in forming the capsid, viral matrix and envelope of the virus, as well as proteins controlling the replication and infectivity of the virus.

42. The viral genome is arranged in long (L) and short (S) components (Figure 2). Each component consists of a unique sequence, the long unique region (U_L) and the short unique region (U_S), bracketed by inverted repeats, R_L and R_S. The *ICP47* gene is situated in the U_S region and the two copies of the ICP34.5 gene are situated in R_L regions (Borchers et al. 1994).

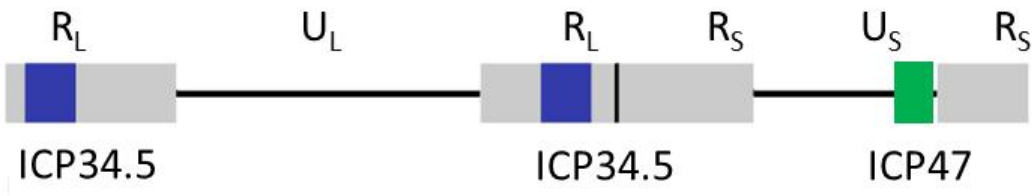

Figure 2. Basic HSV-1 genomic structure. The relative positions in the HSV-1 genome of the two *ICP34.5* genes (blue) and the *ICP47* gene (green) are highlighted.

43. Primary lytic infection of epithelial or mucosal cells results from the attachment and penetration of HSV particles to host cells. HSV enters cells by fusion of the viral envelope with the host cell membrane, involving interactions of several glycoproteins on the surface of the enveloped virus, with receptors on the surface of the host cell. The cell surface receptors include a member of the tumour necrosis factor receptor family, heparan sulphate chains on cell surface proteoglycans, and two members of the immunoglobulin superfamily related to the poliovirus receptor (Spear 2004).

44. During entry, HSV-1 releases its capsid and the tegument proteins into the cytosol of a host cell by fusing with the plasma membrane. The capsid is then transported to the nucleus, where it docks at the nuclear pore complexes (NPCs), and the viral genome is rapidly released into the nucleoplasm. Following entry of the linear dsDNA into the nucleus, the HSV genome circularizes and begins to express the lytic HSV gene functions in a highly regulated sequential cascade. The transcription of HSV genes is catalyzed by RNA polymerase II of the infected host (McGeoch et al. 2006). The herpes virus *immediate-early*, *early*, and *late* proteins are produced. Immediate early genes, which encode proteins that regulate the expression of *early* and *late* viral genes, are the first to be expressed following infection. Early gene expression follows, encoding enzymes and regulatory proteins involved in DNA replication and certain envelope glycoproteins.

45. Expression of late genes, predominantly encoding proteins that form the virion, occurs last (McGeoch et al. 2006; Pellett & Roizman 2007). New virus capsids then assemble within the nucleus, and virion maturation results in the egress of these virions from the infected cell.

46. HSV has the ability to establish a life-long latent infection in its infected host. After infection of the skin and/or mucosa at the primary site of entry, HSV is able to enter peripheral sensory neurons at their termini and is transported via retrograde axonal flow to the nucleus of the neuronal cell, where it may persist in a dormant state as a circular episome (A segment of DNA that can exist and replicate autonomously) (Pellett & Roizman 2007). This ability to persist in a quiescent form is known as *latent infection* and a complete understanding of the molecular aspects of this process have yet to be fully elucidated.

47. In the latent state, viral gene expression is restricted to a family of latency-associated transcripts (LATs) that are believed to regulate the host cell genome and interfere with natural cell death mechanisms (Jin et al. 2003). This process maintains the host cells and preserves a pool of the virus, which may allow subsequent, periodic recurrences or "outbreaks" characteristic of non-latency.

48. Periodic recurrences of HSV from latency in humans results from diverse stimuli and is thought to involve neuronal specific signaling that is not fully understood at the molecular level. Recent evidence suggests a possible role for an HSV gene called *ICP4*, which is an important trans-activator of genes associated with lytic infection in HSV-1 (Bedadala et al. 2007; Pinnoji et al. 2007).

4.2 HSV virulence

49. The virulence of HSV resides in the ability of the virus to use a peripheral port of entry (skin/mucosa) to invade the central nervous system, replicate in the neurons and cause disease. In humans, HSV causes mild disease to the skin and mucosa of infected hosts, with typical symptoms being watery blisters in the skin or mucous membranes of the mouth, lips (cold sores) or genitals. Primary infection occurs most commonly in oral mucosal tissue, most likely through contact with compromised epithelial tissue. The primary infection is usually mild and self-limiting, although in a minority of cases infection may be severe, including disseminated disease and encephalitis. With the exception of neonates and immune-compromised people, HSV infection is not systemic and is limited to the epithelial cells and sensory ganglia of the infection site.

50. The HSV virus is not considered to be transmitted by aerosol exposure. Transmission of wild type HSV-1 is through direct contact with infected secretions or mucous membranes/skin by asymptomatic or symptomatic shedding of the virus (Chayavichitsilp et al., 2009, Jerome and Morrow 2007; Whitley, 2006). Transmission of HSV-1 can also occur by respiratory droplets (Whitley, 2006).

51. The virus can be spread from the site of infection to other places (e.g. fingers and eyes) by contact with the infected area. HSV-1 infection in the fingers (herpetic whitlow) has been observed in healthcare workers who come into direct contact with the virus (i.e. in the absence of gloves). There has also been one reported case of transmission of HSV-1 by a needle-stick injury (Douglas et al. 2002). The HSV virus is not considered to be transmitted by aerosol exposure.

52. A defining characteristic of HSV is its ability to establish a latent infection and re-activation of the virus resulting in either the reappearance of the skin or mucosal lesions, or asymptomatic shedding of infectious virus. The virus can be reactivated by illnesses such as colds and influenza, eczema, emotional and physical stress, gastric upset, fatigue or injury, by menstruation and possibly exposure to bright sunlight (Ultraviolet light exposure). Genital herpes may be reactivated by friction of the affected tissue.

53. The HSV-1 diploid gene *ICP34.5* encodes the neurovirulent factor infected cell protein 34.5 (ICP34.5). The ICP34.5 protein has been identified as a major HSV virulence factor by its ability to: (i) counteract the innate immune response towards infection (Chou et al. 1990; Orvedahl et al. 2007); (ii) inhibit cellular autophagy (Jing et al. 2004; Orvedahl et al. 2007); and (iii) be involved in virus egress and release (Jing et al. 2004).

4.3 HSV Epidemiology and Pathogenesis

54. HSV is highly contagious and widespread in the environment with around 80% of the human population showing a significant level of serum antibodies, or other immunologic marker in the serum (i.e. seropositive), indicating previous exposure to the virus (Roizman et al. 2007). HSV-1 infection is common irrespective of geographic and socioeconomic location, with up to 70% to 80% of adults seropositive (Whitley & Roizman 2009). The seroprevalence of HSV-2 is estimated to be between 20% and 30% in western countries (Xu et al. 2006).

55. Recent studies indicate that in Australia, the seroprevalence of HSV-1 is approximately 76% (Cunningham et al. 2006). For HSV-1 seroprevalence, there are differences associated with age, gender and Indigenous status. The seroprevalence of HSV-2 is estimated to be approximately 12% of Australian adults. Cities in Australia have higher HSV-2 seroprevalence (13%) compared to rural populations (9%) and a higher prevalence of HSV-2 is found in Indigenous Australians (18%) in comparison to non-Indigenous Australians (12%).

56. Both HSV types can infect the mouth (producing cold sores) or the genital area (genital herpes). Primary HSV infection causes localised cell death and produces an associated inflammatory response in the area of infection, which may or may not generate disease symptoms. The resulting effects can cause lesions that may disappear within several days. The HSV infection may spread to localised skin and mucosa areas, along with migration through sensory nerves to other skin and mucosa sites distant from initial infection areas (Pellett & Roizman 2007).

57. Further replication and spread of HSV is usually inhibited by innate and antigen-stimulated immune defences, resulting in the healing of lesions present in the skin and mucosa areas. In the absence of an adapted immune response the infection may widen, with possible generation of systemic disease, due to HSV replication and release.

58. The majority of HSV infections remain asymptomatic (being primary or reactivations), however clinical manifestations may present and can be very diverse, depending on site of

infection, level of immune response, host and environmental factors. Although HSV-1 and HSV-2 infections are clinically very similar, HSV-1 reactivates less frequently in genital regions compared to oral regions. The opposite is true for HSV-2, which reactivates more frequently in genital regions compared to oral regions. This different reactivation profile is thought to be caused by type-specific differential LAT expression (Bertke et al. 2009).

59. The most common manifestation of HSV-1 infection is oropharyngeal disease, including herpetic gingivostomatitis (inflammation of the gums and lips), herpetic pharyngotonsillitis (inflammation of the pharynx and/or tonsils) and herpes labialis (cold sores, fever blisters), from infection of the skin and mucous membranes in the mouth and throat regions (Arduino & Porter 2006; Spruance et al. 1977).

60. Herpetic whitlow is a lesion on a finger or thumb caused by either HSV type 1 or 2 during primary infection and is characterised by formation of painful vesicular lesions on the nail or finger area. Herpes gladiatorum is caused by HSV-1 and is characterised by formation of skin ulcerations, usually on the face, ears, or neck.

61. Genital herpes affects the genital and surrounding areas (anus, buttocks and inside of the thighs) caused by the HSV-1 or -2. In people under 25 years of age, herpes simplex type 1 virus is more common in genital herpes, while in people 25 years of age and older, genital herpes is most often caused by herpes simplex type 2 virus.

62. Herpes simplex eye infection or ocular herpes is caused predominately by HSV-1. HSV infection may cause common manifestations including blepharitis/dermatitis, conjunctivitis, dendritic epithelial keratitis, and corneal ulceration (Green & Pavan-Langston 2006). Ocular herpes often clears without any permanent problem. In some cases the infection causes scarring to the transparent front part of the eye (the cornea) and may lead to permanent loss of vision.

63. Herpes simplex encephalitis (HSE) is a viral infection of the human central nervous system and is estimated to affect at least 1 in 500,000 individuals per year (Whitley 2006). The majority of cases of HSE are caused by HSV-1, and is thought to result from retrograde transmission of the virus from a peripheral site on the face following HSV-1 reactivation, along a nerve axon, to the brain. HSV is also the most commonly identified pathogen among hospitalized patients diagnosed with encephalitis in Australia (Huppatz et al. 2009).

64. HSE can be treated with high-dose intravenous guanosine analogue antiviral medication. Without treatment, HSE results in death in approximately 70% of cases, with survivors suffering severe neurological damage (Whitley 2006). Even when treated, HSE still causes fatalities in approximately one-third of cases, and causes serious long-term neurological damage in over half of survivors.

65. Neonatal herpes simplex is a rare but serious condition, usually caused by vertical transmission of genital HSV-1 or HSV-2 as the neonate comes in contact with the virus during passage through an infected birth canal (Corey & Wald 2009). The disease can manifest by affecting the skin, eyes, and/or mouth, a disseminated disease with visceral organ involvement or central nervous system disease without visceral organ involvement. Neonatal herpes simplex infection has high mortality and significant morbidity in disseminated disease or central nervous system disease. The likelihood of survival is greatly improved upon early diagnosis and treatment with intravenous antiviral drugs.

4.4 HSV in the environment

66. HSV-1 survival outside the host organism is limited to short periods of time (Chayavichitsilp et al. 2009). HSV-1 has been found to survive on surfaces for periods ranging from a few hours to 8 weeks [the latter being survival on a dried surface] (Kramer et al. 2006; Mahl & Sadler 1975). Various reports indicate significantly lower survival in the range of 4-5 hours in water or on plastic surfaces under conditions of higher humidity (Nerurkar et al.

1983). Several studies by Bardell (Bardell 1989; Bardell 1990; Bardell 1993; Bardell 1994) report a marked (2 to 3 log) reduction of viral titer of HSV-1 within one hour on plastic or chrome plated surfaces, though infectious virus was still recoverable after 2 hours, which was the maximum time point examined.

4.5 Susceptibility of HSV to antibiotics and other chemical agents

67. The standard treatment for HSV infection is with acyclovir, either administered topically or orally. Acyclovir is a pro-drug that is converted by the HSV thymidine kinase to cytotoxic acyclovir triphosphates which are incorporated into newly synthesised DNA resulting in a block to DNA synthesis and, therefore, viral replication (Drew 2004; Jerome & Morrow 2007).

68. In the case of occupational exposure of seropositive persons, prophylactic treatment with anti-viral drugs like foscarnet, valacyclovir, famciclovir, and penciclovir that are able to inhibit viral replication may prevent infectious herpetic lesions from developing or at least minimise the symptoms (Drew 2004; Jerome & Morrow 2007). In one report of exposure to HSV-1 in a healthcare setting, prophylactic treatment with famciclovir prevented seroconversion and the development of an herpetic whitlow infection (Manian 2000).

69. As HSV is an enveloped virus it is susceptible to chemical decontamination, such as lipid solvents, detergents and hypochlorite, and is also rapidly inactivated by desiccation (Croughan & Behbehani 1988). HSV is also susceptible to quaternary ammonium compounds (Wood & Payne 1998). Most herpes viruses are also susceptible to 30% ethanol and isopropanol, 0.12% orthophenyl phenol, and 0.04% glutaraldehyde (Prince & Prince 2001).

Section 5 The GM virus – nature and effect of the genetic modification

5.1 Introduction to the GM virus

70. The GM virus is a live attenuated HSV-1, strain JS1, modified to selectively replicate in tumours (rapidly dividing cells) and elicit an immune response for the treatment of patients with skin cancer (metastatic melanoma) and other suitable solid tumours (e.g. pancreatic cancer, squamous cell carcinoma of the head and neck) that are unable to be removed by surgery.

71. To produce the GM virus, the JS1 strain of HSV-1 has been modified by removing specific viral genes involved in neurovirulence and viral antigen presentation, and by introduction of a gene encoding a human protein that stimulates certain types of immune cells. These modifications are detailed below.

5.2 The genetic modifications and their associated effects

72. The genome modifications carried out to produce the GM virus were completed in multiple steps. The modifications involved a sequential series of *in vitro* homologous recombination events in cultured cells co-transfected with viral DNA and plasmid shuttle vectors carrying fragments of the viral genome with the desired modifications[2] (Liu BL 2003). Briefly, the genetic modifications of the GM virus include deletion of the two copies of *ICP34.5*. In place of the two deleted *ICP34.5* genes, two copies of *hGM-CSF* have been inserted. In addition, the gene *ICP47* has also been deleted from the GM virus (Figure 3).

[2] The specific details relating to the genome modifications carried out to produce the GM virus are under consideration as Confidential Commercial Information (CCI) under section 185 of the Act. Confidential information was made available to the prescribed experts and agencies.

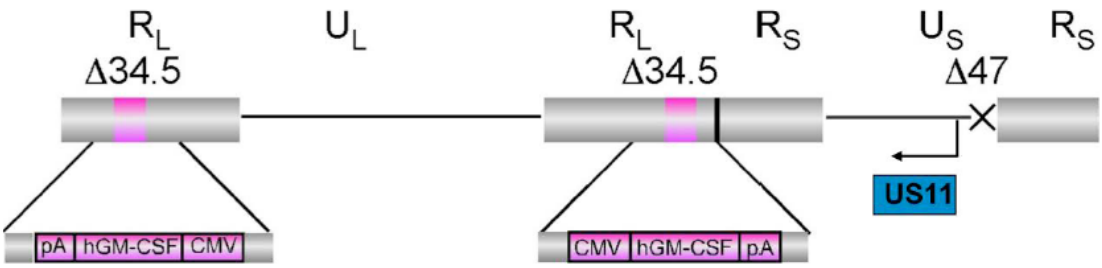

Figure 3. Genomic structure of the GM virus, Talimogene laherparepvec.

5.2.1 Deletion of ICP34.5

73. In HSV-1, neurovirulence is mediated by the ICP34.5 protein encoded by the *ICP34.5* gene. The HSV-1 ICP34.5 protein normally overcomes two important host defence pathways to promote neurovirulence, the shutdown of protein synthesis (Chou et al. 1990) and autophagy (Orvedahl et al. 2007).

74. The ICP34.5 protein is involved in the shutdown of protein synthesis by reversing the actions of Protein Kinase R (PKR), a cellular antiviral protein (Chou et al. 1990; He et al. 1997). When PKR senses that a cell is infected, it phosphorylates and inactivates the eukaryotic translation initiation factor elF2α. This is achieved by ICP34.5 recruiting a phosphatase protein that reverses the effect of PKR on elF2α and allows protein synthesis to continue during viral infection (He et al. 1997).

75. The antiviral, interferon-inducible PKR signalling pathway also promotes autophagy in response to HSV-1 infection, and the HSV-1 neurovirulence protein ICP34.5 antagonizes this response (Talloczy et al. 2002). The ICP34.5 overcomes autophagy by binding to and inhibiting the function of the Beclin protein, which functions downstream of PKR and is required for the initiation of autophagy.

76. The ability of ICP34.5 to interact with Beclin, and not the ability of ICP34.5 to regulate shutdown of protein synthesis, has been shown to be essential for HSV-1 neurovirulence (Orvedahl et al. 2007).

77. In the GM virus, both copies of ICP34.5 have been functionally deleted from the virus. This prevents the virus from replicating efficiently in non-dividing cells. In tumour cells, the host defence pathways are impaired, so *ICP34.5* is not required for replication. There have been many studies that indicate the pathogenicity of *ICP34.5*-deleted HSV-1 is substantially attenuated in animals and humans (Harrington et al. 2010; Harrow et al. 2004; Hu et al. 2006; Hunter et al. 1999; Mace et al. 2008; MacKie et al. 2001; McKie et al. 1998; Papanastassiou et al. 2002; Perng et al. 1995; Rampling et al. 2000; Senzer et al. 2009; Valyi-Nagy et al. 1994; Varghese et al. 2001; Whitley et al. 1993).

5.2.2 Introduction of hGM-CSF

78. In place of the two deleted ICP34.5 genes, two copies of the gene encoding hGM-CSF have been inserted. GM-CSF is a cytokine that is part of the immune/inflammatory response and functions to induce the proliferation and differentiation of certain types of immune cells e.g. granulocytes (neutrophils, eosinophils, and basophils) and monocytes. Monocytes are able to mature into macrophages and dendritic cells. The GM-CSF activation of immune response following intratumoural injection of the GM virus is intended to elicit an immune response by causing the influx and activation of dendritic cells. The increase in dendritic cells can also assist in the presentation of tumour-associated antigens from tumour cells, and prime tumour-specific cluster of differentiation (CD)4+ and CD8+ T-cells, to stimulate a systemic and specific anti-tumour response (Dranoff et al. 1993; Huang et al. 1994).

79. Expression of the *hGM-CSF* gene in the GM virus is driven by the non-coding viral promoter from cytomegalovirus (CMV) (Boshart et al. 1985; Foecking & Hofstetter 1986). CMV is from the herpes virus family and is a common viral infection agent. In healthy people, infection may be asymptomatic or cause a mild flu-like illness that passes harmlessly within a few days. Gastrointestinal diseases and diseases such as pneumonitis, hepatitis and retinitis may sometimes be associated with CMV infection. Human CMV can be transmitted during pregnancy from the mother to the foetus, sometimes resulting in complications including hearing loss, visual impairment, or diminished mental and motor capabilities (Revello & Gerna 2002). The non-coding viral CMV promoter (IE1 promoter/enhancer) is a well characterised constitutive promoter and is not able to cause or induce CMV viral infection.

80. The bovine growth hormone polyadenylation signal sequence (bgh-PolyA) is used in the GM virus to achieve polyadenylation of the hGM-CSF mRNA, and to help to facilitate transcriptional termination (Goodwin & Rottman 1992; Woychik et al. 1982). The bgh-PolyA is a non-coding DNA sequence and is commonly used for optimised gene expression in pharmaceutical protein production, transgenic animal research and gene therapy applications.

5.2.3 Deletion of ICP 47

81. The ICP47 protein normally blocks $CD8^+$ T cell recognition of infected cells by inhibiting the transporter associated with antigen presentation and allows the virus to hide from the immune system (Goldsmith et al. 1998). The removal of *ICP47* from the GM virus is intended to improve the presentation of viral antigens and tumour antigens in infected cells, making the infected cells better targets for the host immune response and thus improving the ability of the GM virus to induce tumour cell death.

82. The removal of *ICP47* from the GM virus also results in the increased expression of the viral protein US11 (Mohr & Gluzman 1996). The HSV-1 viral protein US11 has some functional redundancy with ICP34.5, and increased US11 expression enhances replication of *ICP34.5*-deleted HSV-1 in tumour cells, without loss of tumour selectivity (Mohr et al. 2001).

5.2.4 Susceptibility of the GMO to anti-viral therapy

83. The HSV thymidine kinase (TK) gene remains intact in the GM virus. The HSV TK enzyme is able to phosphorylate certain nucleoside analogs (e.g. non-toxic prodrugs), converting the nucleoside analogs to toxic DNA replication inhibitors that act as antiviral agents. Tumour cells that express HSV-1 TK are rendered sensitive to prodrugs due to preferential phosphorylation by this enzyme and therefore anti-viral agents can be used to block replication of the GM virus if required.

5.2.5 Toxicity or adverse response associated with the genetic modifications

84. The GM virus is a genetically modified HSV-1 virus intended for use as a prescription only therapeutic agent for patients with skin cancer (metastatic melanoma) and other suitable solid tumours. The GM virus functions to direct cytotoxic activity to the injected tumour due to the replication of the virus and cell lysis. In addition the GM virus is designed to induce an anti-tumour immune response in and around the injected tumour, directed at the infected tumour cells.

85. The deletion of both copies of the *ICP34.5* gene in the GM virus is associated with decreased virulence in normal tissues as it reduces the ability of the GM virus to replicate in non-dividing cells. In the GM virus, the two copies of one viral gene *(ICP34.5)* are functionally deleted, preventing the virus from replicating efficiently in non-dividing cells. In tumour cells, these host defence pathways are impaired, so that *ICP34.5* is dispensable for replication. Thus the GM virus is not expected to cause disease in otherwise healthy people. The fact that the GM virus is attenuated for replication in non-dividing cells significantly reduces the possibility of persistence or spread to tissues other than within the injected tumour

of a patient. Correspondingly, the ability of the GM virus to undergo transmission to and cause disease in healthy individuals or animals is substantially reduced.

86. As discussed above, the gene encoding hGM-CSF has been inserted into the GM virus in order to induce the proliferation and differentiation of certain types of immune cells. Recombinant hGM-CSF is a United States Food and Drug Administration-approved pharmaceutical. Administration of hGM-CSF has been extensively tested and found to be safe in a people and in variety of animal 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). In addition, hGM-CSF has been used in 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).

87. It is possible that GM virus-driven expression of GM-CSF could induce humoral response (an immune response involving antibodies). There are reports that patients receiving recombinant GM-CSF have anti-GM-CSF antibodies, which in some cases have neutralised the clinical efficacy of GM-CSF treatment (Ragnhammar et al. 1994; Rini et al. 2005). The mode of administration of recombinant GM-CSF compared to the GM virus-derived expression of GM-CSF is significantly different. It is therefore not possible to draw a conclusion based on recombinant GM-CSF administration relating to the risk of anti-GM-CSF antibody development in response to infection with the GM virus.

5.3 Characterisation of the GM virus

5.3.1 Genotype stability and molecular characterisation of the GM virus

88. Amgen have sequenced 99% of the GM virus genome. The modified regions of the GM virus have been sequenced at least three times between 2001 and 2011. These sequenced regions have been found to only contain the intended DNA sequence.

89. Genetic stability testing reported by Amgen has been achieved through repeat sequencing of small areas of the GM virus genome between 2001 and 2012. As an example, the region containing the GM-CSF insert of the GM virus genome has been sequenced completely by large scale sequencing from both Baby Hamster Kidney fibroblasts (BHK)-derived and Vero-cell derived GM virus and found to be 100% conserved.

5.3.2 Transmissibility of the GM Virus

90. The ability of wild type HSV-1 to enter cells is controlled by the outer membrane or envelope of the virion. Several glycoproteins in the envelope are essential for entry into cells, while others may influence this process (Spear 2004). None of the genetic modifications made to the GM virus affect surface coat proteins, and host range is not anticipated to differ from that of wild-type HSV-1.

91. Consistent with this, Amgen has performed an *in vitro* study to demonstrate that the tropism (i.e. the range of cell types that can be infected) of the GM virus is the same as wild type HSV-1[3]. In this study, cells which lack the standard HSV-1 entry receptors (for example CHO cells (Spear & Longnecker 2003)) were found to be non-permissive for both wild type HSV-1 and the GM virus, while cells which are permissive for wild type HSV-1 were equally permissive for the GM virus.

[3] The specific details relating with this study are under consideration as Confidential Commercial Information under section 185 of the Act. Confidential information was made available to the prescribed experts and agencies.

92. The deletion of both copies of the *ICP34.5* gene prevents the GM virus from replicating efficiently in non-dividing cells. Whereas in tumour cells, these host defence pathways are impaired, so that *ICP34.5* is dispensable for replication. This substantially limits the cell types in which the GM virus can efficiently replicate compared to wild-type HSV-1.

93. The GM virus has decreased pathogenicity due to deletion of both copies of *ICP34.5* gene. The *ICP34.5* gene deletions reduce the ability of the virus to replicate effectively in non-dividing cells. The GM virus will be injected intratumorally and will not be able to spread effectively to normal tissue of the patient. If a patient has a pre-existing HSV-1 infection, it would most likely be localised in skin and mucosa areas or neuronal ganglia. This reduces the possibility of the GM virus and wild-type HSV-1 infecting the same cells.

5.3.3 Non-clinical studies on HSV-1 with ICP34.5 deletions

94. Studies using intracerebral inoculation of mice have demonstrated substantial attenuation conferred by mutation or deletion of *ICP34.5*. One study found that termination of the *ICP34.5* gene (by introduction of a stop codon after the 30th codon) resulted in a mutant that was 25- to 90-fold reduced in neurovirulence, associated with restricted replication of the mutant virus in mouse brain (Bolovan et al. 1994). Similar *in vivo* mouse studies with *ICP34.5*-deleted HSV-1 indicated a reduced capacity to replicate and over 100,000-fold attenuation of neurovirulence (Chou et al. 1990; MacLean et al. 1991).

95. From animal studies, ICP34.5-deleted HSV-1 viruses are anticipated to have enhanced safety characteristics (compared to wild-type HSV-1) in therapy of a number of human tumours, including brain tumours (Chambers et al. 1995; Martuza et al. 1991), melanoma (Miller et al. 2001) and breast cancer (Thomas & Fraser 2003).

96. In rodent models of human disease, selective deletion of *ICP34.5* abolished the capacity of the HSV-1 to spread from peripheral mucosal sites to the central nervous system or to replicate in the central nervous system (Whitley et al. 1993). In this study, the *ICP34.5* mutation had diminished the capacity of the virus to replicate at mucosal sites, and subsequently, to establish latency or be reactivated *ex vivo*.

97. In addition, the decreased virulence of *ICP34.5*-deleted HSV-1 viruses has been demonstrated in a variety of species, including mice (Kesari et al. 1998; McKie et al. 1998), rabbits (Perng et al. 1995), and non-human primates (Hunter et al. 1999; Varghese et al. 2001).

5.3.4 Non-clinical studies on the GMO

98. The GM virus has been comprehensively evaluated for attenuation of infection and neurovirulence in mice. Additional toxicology and biodistribution studies of the GM virus have been conducted in rats and dogs, but not other species. Amgen reports that in immune competent mice, the GM virus had markedly reduced neurovirulence following direct intra-cerebral injection or intranasal instillation as compared to that reported for wild-type HSV-1, and has not been associated with systemic virulence following repeated exposure at doses up to 60-fold over the highest proposed clinical dose[4]. These findings, attributable to the attenuation of the virus through deletion of *ICP34.5*, are anticipated to underlie virulence in all species.

99. Non-clinical safety studies by Amgen have been conducted in dogs by intra-prostatic injection of the GM virus. Dogs are not a common model to evaluate HSV-1 infection but were used in this case because dog is a common species for evaluating therapeutics and interventions

[4] The specific details of these studies by Amgen are under consideration as Confidential Commercial Information under section 185 of the Act. Confidential information was made available to the prescribed experts and agencies.

to treat prostate cancer, due to their larger size (Lamb & Zhang 2005). A single-dose injection of GM virus into the prostate of dogs (up to $2.5x10^6$ plaque-forming units (pfu)/animal) was well tolerated (no deaths and no treatment-related effects on clinical signs, body weight, food consumption, clinical pathology, or macroscopic or microscopic changes associated with the GM virus[5]). A literature search reveals that another ICP34.5-deleted HSV-1 (a virus similar to the GM virus) was non-pathogenic following intracranial injection in dogs (Springer et al. 2001).

100. In rats, Amgen reports a single intra-arterial injection of the GM virus (up to $1x10^7$ pfu/animal) was well-tolerated. Although clinical and anatomic effects were observed in this study, they were consistent with findings expected following the surgical and dosing procedures, and were seen in all groups. There were no deaths in the study, and there were no effects on clinical signs, body weight, food consumption, clinical pathology, or macroscopic or microscopic changes attributable to the GM virus[6].

101. To examine the efficacy of the GM virus, the GMO and another version of GM HSV-1 with mouse GM-CSF replacing hGM-CSF were tested *in vitro* in human tumour cell lines and *in vivo* in mice (Liu et al. 2003a). The anti-tumour effect of the GM viruses was much greater than HSV-1 viruses not containing the full complement of genetic modifications (i.e. JS1/ICP34.5-/ICP47-/GM-CSF). *In vivo*, both injected and non-injected tumours of mice treated with the GM virus (with mouse GM-CSF) showed significant shrinkage or clearance, and mice were protected against re-challenge with tumour cells. These results indicate that the GM virus preferentially infects and kills cancer cells (oncolytic agent), which may be appropriate for the treatment of a number of solid tumour types.

102. Amgen has reported that repeated GM virus intramural injection in mice resulted in GM viral DNA being detected in tumours, blood and tissues associated with immune mediated viral clearance, as well as in tissue with high blood perfusion. There was low distribution of GM viral DNA detected in tissues capable of shedding the GM virus. In murine experiments, direct injection into implanted tumours did not cause leakage of the GM virus from the injection site.

103. The genetic modifications in the GM virus do not prevent the virus from entering latency or subsequently reactivating. Amgen's non-clinical data suggest that the GM virus can establish latency in mice. Literature shows that viruses lacking the ICP34.5 gene are able to establish latency in mice and rabbits, albeit less efficiently that wild-type HSV-1. It was initially thought that this deficiency was due to impaired reactivation rather than reduced establishment of latency (Perng et al. 1995; Robertson et al. 1992; Spivack et al. 1995). However, further work revealed reduced levels of establishment of latency, likely due to deficient replication of the *ICP34.5*-deleted virus in the peripheral tissues innervated by the neurons (Perng et al. 1996).

104. A number of non-clinical studies have been conducted by Amgen to address safety, biodistribution, and biological activity in various model systems, including *in vitro* human cell culture, mouse, mouse xenograft and mouse tumour systems[7].

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[5] The specific details of this study by Amgen are under consideration as Confidential Commercial Information under section 185 of the Act. Confidential information was made available to the prescribed experts and agencies.

[6] The specific details of this study by Amgen are under consideration as Confidential Commercial Information under section 185 of the Act. Confidential information was made available to the prescribed experts and agencies.

[7] The results of non-clinical studies were included in the licence application. These results are under consideration as Confidential Commercial Information under section 185 of the Act. Confidential information was made available to the prescribed experts and agencies.

5.3.5 Clinical trials of the GMO

105. A total of nine clinical studies have been or are currently being carried out in North America, Europe, Africa and Australia[8]. Several of these clinical studies have been completed and published. A summary of these is presented in Table 1.

Table 1 Summary of previous published clinical trials

No.	Study Design	No. Treated	Main adverse events	Reference
1	Phase I Open-label, multi-centre, single-dose study & multi-dose study investigating the safety profile, biological activity and dosing schedule. Treatment of patients with cutaneous or subcutaneous deposits of breast, head and neck and gastrointestinal cancers, and malignant melanoma	13 single-dose 17 multi-dose (30 total)	Pyrexia and associated constitutional symptoms; local reaction at the injected tumour site	(Hu et al. 2006)
2	Phase II Open-label, multi-centre, multi-dose study investigating patient survival and safety. Treatment of patients with metastatic melanoma	50	Pyrexia and associated constitutional symptoms; inflammation and erythema at injection site	(Senzer et al. 2009)
3	Phase I/ II Open-label, multi-centre, multi-dose study investigating the safety and biological activity. Treatment in combination with chemoradiotherapy in patients with squamous cell cancer of the head and neck	17	Pyrexia and fatigue: inflammation at injection site	(Harrington et al. 2010)
4	Phase III Open-label, multi-centre, multi-dose randomised study investigating durable response rate. Treatment of patients with unresected stage IIIB to IV melanoma.	436	Chills, pyrexia, nausea and fatigue; erythema at injection site	(Andtbacka et al. 2015)

106. Nine clinical trials have been or are being conducted involving a total over 400 patients treated with the GM virus. From the published studies, adverse events attributed to the GM virus were typically pyrexia (abnormal body temperature) and associated constitutional symptoms (which included indications like fever, chills, fatigue, nausea, vomiting or headache), as well as inflammation and erythema (redness of the skin) in and around the injected tumour site. These adverse events were common in trial participants and were generally mild to moderate. The adverse events were more marked in patients who were HSV-seronegative before treatment. No patients in any of these studies developed HSV encephalitis or other symptoms suggestive of infection of the central nervous system.

5.3.6 Shedding of the GM virus in the Clinical studies

107. In the Phase I study describe above, the GM virus was administered by intratumoural injection in patients with cutaneous or subcutaneous deposits of breast, head and neck and gastrointestinal cancers, and malignant melanoma (Hu et al. 2006). Thirteen patients were in a single-dose group, where doses of 10^6, 10^7, and10^8 pfu/mL were tested, and 17 patients were in a multi-dose group testing a number of dose regimens. All HSV seronegative patients strongly seroconverted 3 to 4 weeks after their first dose to a similar level to patients who were originally seropositive.

108. Presence of the GM virus was assayed on the tumours, the occlusive dressing, and new lesions by swabbing followed by plaque assay (Hu et al. 2006). In the single dosing phase of

[8] The results of clinical studies were included in the licence application. These results of currently unpublished studies are under consideration as Confidential Commercial Information under section 185 of the Act. Confidential information was made available to the prescribed experts and agencies.

the study, the GM virus was detected at low levels on the tumour surface for up to 2 weeks in 3 patients. In the multi-dosing phase, the GM virus was only detected on the surface of the tumour of one patient at a single time point at a very low level. A vesicle adjacent to the tumour and under the occlusive dressing also tested positive at a similarly low level in this same patient, which according to the authors of the study could have been due to cross-contamination from the tumour as all other vesicles from this or other patients tested negative for the presence of the GM virus.

109. The GM virus was not detected outside the occlusive dressing in any case during the study and was not routinely detectable in blood or urine in any part of the study. In the single-dose phase of the study, virus was only detected in the blood of two patients between 8 hours and 1 week after injection. In the multi-dose phase of the study, GM virus was detected in the blood of eight patients 1 to 8 hours post-dose, which was somewhat more prevalent in HSV-seronegative patients. GM virus DNA was even more rarely detected in urine with only two patients testing positive at very low levels 8 hours to 1 week after injection in the single-dose group, and no patient tested positive in the multi-dose group.

110. In the Phase II clinical trial, 50 patients with stage IIIc and stage IV metastatic melanoma were administered the GM virus (Senzer et al. 2009). Patients initially received a total intratumoural injection of up to10^6 pfu/mL, followed 3 weeks later by injections of 10^8 pfu/mL and repeated every 2 weeks. All 13 patients who were HSV seronegative at baseline strongly seroconverted by week 7. One hundred and two swabs were taken from injection sites in 19 patients at 24 to 72 hours after the first injection. Only one swab was positive at a low level and the same site tested negative after second injection, which confirmed the rare shedding seen in the Phase I study (Hu et al. 2006). Swab collection was stopped due to the low level of shedding after the initial injections. Urine samples were collected at 1 to 48 hours after the first dose from 13 patients. All 78 samples tested negative for the GM virus by qualitative polymerase chain reaction.

111. The Phase I/II Study of the GM virus in combination with radiotherapy and cisplatin was carried out on 17 patients with untreated Stage III/IVA/IVB squamous cell cancer of the head and neck (Harrington et al. 2010). Patients received chemoradiotherapy plus intra-tumoural injections, on days 1, 22, 43 and 64, with the following doses of the GM virus: 10^6, 10^6, 10^6, 10^6 pfu/mL for cohort 1; 10^6, 10^7, 10^7, 10^7 for cohort 2; 10^6, 10^8, 10^8, 10^8 for cohort 3. Seven patients were seronegative for anti-HSV antibody at the initial screening. Five of the HSV seronegative patients had seroconverted by week 3 and the remaining two were seropositive by week 6 after injection.

112. Viral shedding from the injected site occurred in three patients. The first patient (patient 1) was positive at 24 and 96 hours (one of two injection site swabs) after the first virus dose. Swabs testing for GM virus at 48, 72, 144, and 168 hours were negative. Patient 2 was positive 24 hours after the second virus injection, but swabs at 48, 72, and 96 hours were negative. Patient 3 tested positive 48 (one of two swabs) and 144 hours (one of two swabs) after the third virus injection, but swabs were negative at 168, 192, and 216 hours. Positive swab results were never obtained from the exterior of the dressing.

5.3.7 Antibiotic susceptibility of the GM virus

113. The GM virus retains the TK gene and is able to phosphorylate certain nucleoside analogs (e.g. non-toxic prodrugs), thus converting them to toxic DNA replication inhibitors that act as antiviral agents.

114. Amgen reports the sensitivity of the GM virus to the nucleoside analogue acyclovir has been tested over a range of analogue concentrations from 100 microgram (μg)/mL to 0.05 μg/mL and at two different concentrations of the GM virus in a plaque reduction assay. As an example, Amgen reports for one experiment the average half maximal inhibitory

concentration (IC_{50}) for the wild type parental strain HSV-1 JS1 and the GM virus were 0.24 μg/mL and 0.31 μg/mL respectively, indicating that both viruses have similar sensitivity to acyclovir. For comparison, acyclovir has an IC_{50} of 0.30 μg/mL against the commonly used wild type laboratory strain HSV-1 KOS and an IC_{50} of >20 μg/mL against a TK deficient strain of HSV-1 (Tebas et al. 1995).

115. In rare cases, HSV can mutate its viral kinases to gain resistance to acyclovir. Should this occur, the anti-viral drug Foscarnet, which does not require activation by viral kinases, can be used to treat HSV infection (Piret & Boivin 2011). Foscarnet directly inhibits the viral DNA polymerase. The gene deletions and insertions in the GM virus would not be expected to affect the sequence or function of the viral DNA polymerase. The GM virus whole genome sequencing projects have provided data on the gene encoding the viral DNA polymerase and it has been found to be 100% conserved at the DNA level. None of the genetic modifications in the GM virus are anticipated to increase the likelihood that it would acquire resistance over that seen with wild-type HSV-1 strains.

Section 6 The receiving environment

6.1 Relevant environmental factors

116. Subject to authorisation from other relevant regulators, the licence issued by the Regulator will permit the GM virus to be used in clinical facilities (treating hospitals and clinics) equipped to deal with scheduled drugs and infectious agents within Australia. Typically, such facilities follow AS/NZS 2243.3:2010 Safety in laboratories – Microbiological Safety and Containment.

117. The GM virus will be administered through injection into tumour tissue [skin cancer (metastatic melanoma) and other suitable solid tumours] by qualified healthcare professionals in registered clinical facilities. Unused GM virus or waste material would be disposed according to normal clinical biohazardous waste procedures within the clinical facilities and following conditions, if any, imposed by the TGA.

118. The extended receiving environment includes the dressings used by the patient and the patients' homes where the dressings will be used and disposed of. The applicant advises that the dressings and cleaning materials used on the injection sites are to be placed in a sealed plastic bag, thus providing a primary physical barrier, and disposed of in the household waste. The GM virus has been reported to be shed in the urine of patients, so excretion into the sewage system and waste water is possible. The viability of the GM virus in these environments is expected to decrease rapidly, as reported for wild-type HSV-1 (See Chapter 1, Section 5.4).

6.2 Presence of related viral species in the receiving environment

119. The family *Herpesviridae* is a large family comprising at least 100 herpesviruses which are highly disseminated among animals. Eight human herpesviruses have been described, and molecular phylogenetic analysis has established three subfamilies (McGeoch et al. 1995). These three groups correspond to the current taxonomic classification based on biological properties and include the *Alphaherpesvirinae*, *Betaherpesvirinae*, and *Gammaherpesvirinae*. HSV belongs to the *Alphaherpesvirinae* group and is classified in this subfamily on the basis of a wide host cell range, an efficient and rapid reproductive cell cycle, and the capacity to establish latency in the sensory ganglia (Roizman et al. 2007).

120. There are eight known herpesviruses that infect humans. Including HSV-1, HSV-2, varicella-zoster virus, Epstein-Barr virus, human cytomegalovirus, human herpesvirus 6, human herpesvirus 7, and Kaposi's sarcoma-associated herpesvirus.

121. Herpesviruses are highly host specific and share a long synchronous evolution with their hosts. Only in rare cases does animal to human or human to animal transmission occur (Epstein & Price 2009; Tischer & Osterrieder 2010).

6.3 Presence of the *hGM-CSF* gene and related genes in the environment

122. The *hGM-CSF* gene encodes the protein human Granulocyte-Macrophage Colony-Stimulating Factor (hGM-CSF). GM-CSFs are pleiotropic cytokines found in mammals that can stimulate the proliferation, maturation, and activation of a variety of hematopoietic cells. GM-CSFs are largely species-specific in their actions. For example, hGM-CSF is not active on mouse cells and mouse GM-CSF not active in human cells (Lee et al. 1985), however hGM-CSF is active in dog cells and weakly active in bovine cells, indicating that hGM-CSF does not exhibit absolute species specificity (Maliszewski et al. 1988; Mayer et al. 1990).

Section 7 Relevant Australian and international approvals

7.1 Australian approvals

7.1.1 Previous approvals by the Gene Technology Regulator

123. The GMO, a tumour-selective genetically modified virus for cancer therapy referred to as Talimogene laherparepvec, proposed for commercial supply was approved for clinical trials and experimental research in Australia (under the name OncoVEX$^{gm\text{-}csf}$) by the Gene Technology Regulator under a licence for dealings not involving intentional release, DNIR-461.

7.1.2 Approvals by other government agencies

124. Clinical trial of the GMO, Talimogene laherparepvec, was conducted under the TGA's Clinical Trial Notification Scheme.

125. Medicines and other therapeutic goods for use in Australia are required to be assessed for quality, safety and efficacy under the *Therapeutic Goods Act 1989* and must be included in the Australian Register of Therapeutic Goods (ARTG). The TGA is responsible for administering the provisions of this legislation, and also regulates the labelling, handling, sale and supply of scheduled medicines through the SUSMP (Poisons Standard 2015). Amgen has applied to the TGA to have Talimogene laherparepvec included on the ARTG.

126. AQIS has previously approved the importation of the GMO into Australia as a human therapeutic, for its clinical trial, under AQIS import permits IP4007399. Amgen has indicated that they intend to apply for a Department of Agriculture permit to import Talimogene laherparepvec should the TGA approve the inclusion of Talimogene laherparepvec on the ARTG.

7.2 International approvals

127. The GM virus has been or is currently being evaluated in nine clinical trials on skin cancer and several advanced solid tumour types in multiple countries, including the United Kingdom, Canada, South Africa and the USA. (Table 2). Currently, the GM virus has not been approved for commercial use in any country.

Table 2 Overseas applications and approval of trials of the GM virus for cancer therapy.

Country	Responsible agency or body
Australia	Office of The Gene Technology Regulator
United States of America	Food and Drug Administration
United Kingdom	Medicines and Healthcare Products Regulatory Agency
Canada	Health Canada
South Africa	Medicine Control Council
Germany	Paul Ehrlich Institut (application withdrawn)

Chapter 2 Risk assessment

Section 1 Introduction

128. The risk assessment identifies and characterises risks to the health and safety of people or to the environment from dealings with GMOs, posed by or as the result of gene technology (Figure 4). Risks are identified within the context established for the risk assessment (see Chapter 1), taking into account current scientific and technical knowledge. A consideration of uncertainty, in particular knowledge gaps, occurs throughout the risk assessment process.

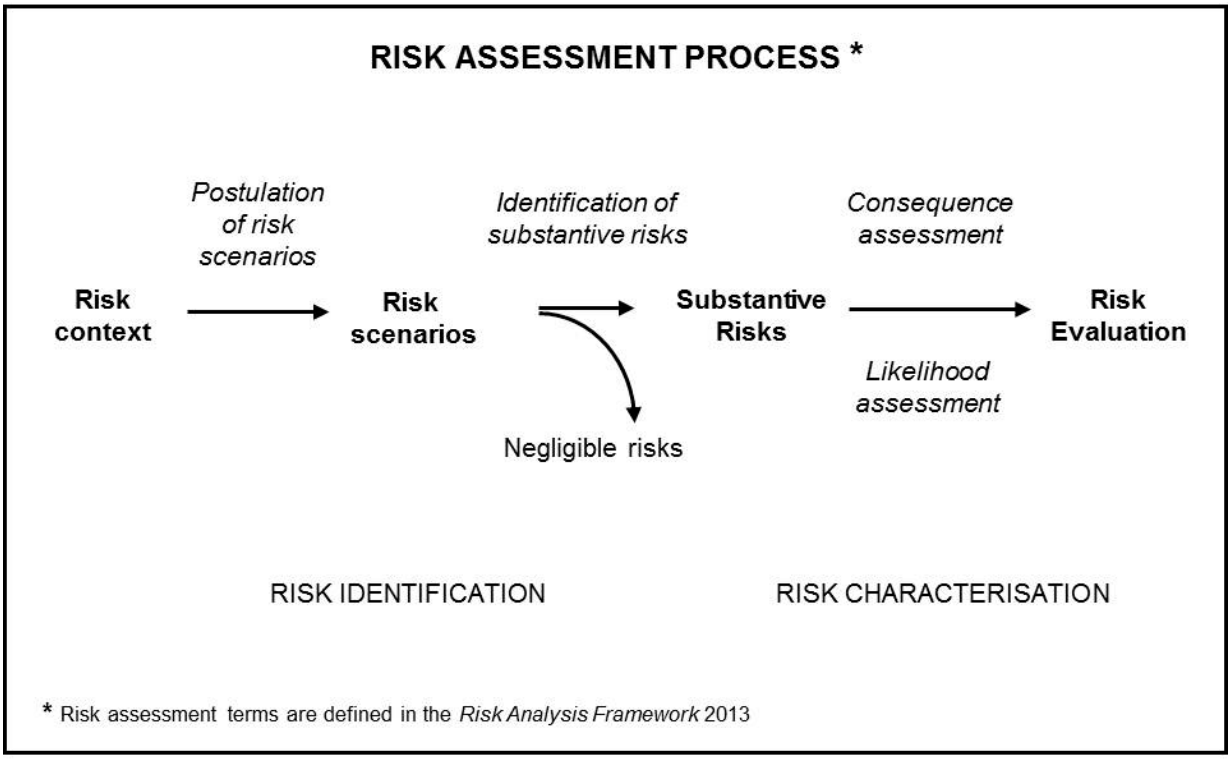

Figure 4. The risk assessment process

129. Initially, risk identification considers a wide range of circumstances whereby the GMO, or the introduced genetic material, could come into contact with people or the environment. Consideration of these circumstances leads to postulating plausible causal or exposure pathways that may give rise to harm for people or the environment from dealings with a GMO (risk scenarios) in the short and long term.

130. Postulated risk scenarios are screened to identify substantive risks that warrant detailed characterisation. A substantive risk is only identified for further assessment when a risk scenario is considered to have some reasonable chance of causing harm. Pathways that do not lead to harm, or could not plausibly occur, do not advance in the risk assessment process.

131. A number of risk identification techniques are used by the Regulator and staff of the OGTR, including checklists, brainstorming, reported international experience and consultation (OGTR 2013). In conjunction with these techniques, risk scenarios postulated in previous RARMPs prepared for licence applications of the same and similar GMOs are also considered.

132. Substantive risks (i.e. those identified for further assessment) are characterised in terms of the potential seriousness of harm (Consequence assessment) and the likelihood of harm (Likelihood assessment). The level of risk is then estimated from a combination of the Consequence and Likelihood assessments. Risk evaluation then combines the Consequence and Likelihood assessments to determine level of risk and whether risk treatment measures are required. The potential for interactions between risks is also considered.

Section 2 Risk Identification

133. Postulated risk scenarios are comprised of three components (Figure 5):

i. The source of potential harm (risk source)

ii. A plausible causal linkage to potential harm (causal pathway); and

iii. Potential harm to an object of value, people or the environment.

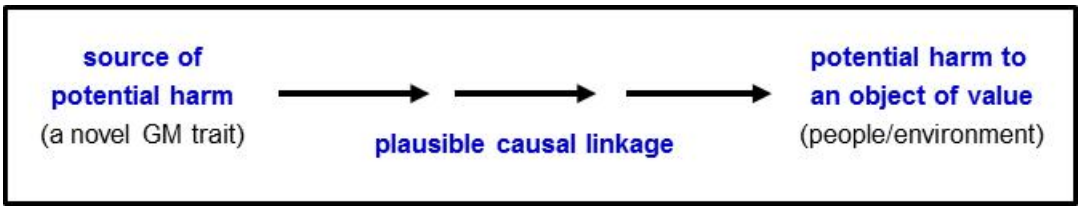

Figure 5. Risk scenario

134. In addition, the following factors are taken into account when postulating the relevant risk scenarios for this licence application:

- the proposed dealings, which are import, transport or disposal of the GMOs and the possession (including storage), supply and use of the GMOs in the course of any of these dealings;
- the proposed controls;
- characteristics of the parent organism(s);
- routes of exposure to the GMOs, the introduced gene(s) and gene product(s);
- potential effects of the introduced gene(s) and gene product(s) expressed in the GMOs;
- potential exposure to the introduced gene(s) and gene product(s) from other sources in the environment;
- the environment at the site(s) of release; and
- clinical management practices for the GMOs.

135. As discussed in Chapter 1, Section 2, the TGA has regulatory responsibility for assessing use of the GMO as a therapeutic, including patient safety. However, risks associated with exposure of people or the environment arising through transport, storage or disposal of the GMO are assessed by the Regulator.

136. Five risk scenarios were postulated and evaluated. They are summarised in Table 3, where circumstances that share a number of common features are grouped together in broader risk categories. In the context of the control measures proposed by the applicant, and considering both the short and long term, none of the risk scenarios were identified as a risk that could be greater than negligible. Therefore, they did not warrant further detailed assessment. More detail of the evaluation of these scenarios is provided later in this section.

137. As discussed in Chapter 1, Section 2.1, medicines and other therapeutic goods for use in Australia are required to be assessed for quality, safety and efficacy under the *Therapeutic Goods Act 1989*. Where a GMO is proposed to be a registered therapeutic, TGA has primary regulatory responsibility for quality, efficacy and patient safety; however authorisation is also required under gene technology legislation. The TGA also regulates the labelling, handling, sale and supply of scheduled medicines through the SUSMP (Poisons Standard 2015). Therefore, risk scenarios in the current assessment focus primarily on risks posed to people, other than people receiving the genetically modified virus, and to the environment.

Table 3 Summary of risk scenarios from dealings with GM virus

Risk Scenario				Substantive risk?	Reasons
#	Risk source	Causal Pathway	Potential harm		
Section 2.1: Increased disease burden from the GM virus					
1	GM virus	i. Exposure of clinical staff to the GM during waste disposal ⬇ ii. Establishment of infection ⬇ iii. Development of disease	Disease in clinical staff	No	• Exposure to the GM virus would be minimised by well-established clinical procedures, including the use of Personal Protective Equipment. • Limited shedding of GM. • The GM virus is attenuated and does not replicate efficiently in non-dividing cells but replication occurs in rapidly diving cells (tumours). • The inserted *hGM-CSF* gene and encoded protein are of human origin and are unlikely to be toxic or cause adverse effects to people. • Exclusion of healthcare personnel who are immunocompromised from direct or indirect contact with the GM virus. • GM virus is susceptible to anti-viral medication.
2	GM virus	i. Treated patient sheds GM virus ⬇ ii. Patient disposes of material contaminated with the GM virus ⬇ iii. Exposure of people or animals to the contaminated material ⬇ iv. Establishment of infection ⬇ v. Development of disease	Increased disease burden	No	• Patients will use clinical dressings to cover administration site and will be instructed on appropriate disposal using a primary barrier in normal waste stream. • The GM virus is attenuated and does not replicate efficiently in non-dividing cells but replication occurs in rapidly diving cells (tumours). • The GM virus is unable to replicate outside the host organism. • Viability of GM virus outside host under environmental conditions is limited. • Humans are the only known natural hosts of HSV-1. • Limited shedding of GM virus. • GM virus is susceptible to anti-viral medication.
3	GM virus	i. Exposure of people or animals to the GM virus due to unintentional release during transport or storage ⬇ ii. Establishment of infection ⬇ iii. Development of disease	Increased disease burden	No	• Transport will be according to appropriate standards for medical products. • Storage will be at secure storage or clinical facilities. • The GM virus is attenuated, does not replicate efficiently in non-dividing cells and replication only occurs in rapidly dividing cells (tumours). • The inserted *hGM-CSF* gene and encoded protein are of human origin and is unlikely to be toxic or cause adverse effects to people.

Risk Scenario				Substantive risk?	Reasons
#	Risk source	Causal Pathway	Potential harm		
Section 2.2: Unintended changes in viral characteristics					
4	GM virus	i. Accidental exposure of people to the GM virus leading to infection (see Risk Scenarios 1-3) ⬇ ii. Altered characteristics of the GM virus due to expression of the introduced genes ⬇ iii. GM virus establishes infection in new host species ⬇ iv. Development of disease	Increased disease burden	No	• Tropism of GM virus is not altered compared to naturally occurring HSV-1. • Viral surface won't be altered by the genetic modification.
Section 2.3: Horizontal transfer of genes or genetic elements to other organisms					
5	GM virus	i. Accidental exposure of people to the GM virus leading to infection (see Risk Scenarios 1-3) ⬇ ii. Person also infected with the another virus ⬇ iii. Both viruses replicate in the same host cell ⬇ iv. Recombination occurs between the viral genomes ⬇ v. Recombinant virus infects other hosts ⬇ vi. Development of disease	Increased disease burden	No	• Genetic stability of the GM virus has been confirmed by repeat sequencing of specific areas of the GM virus genome. • GM virus cannot spread effectively into normal tissue, limiting interaction with other viruses. • The GM virus is attenuated, does not replicate efficiently in non-dividing cells and replication only occurs in rapidly dividing cells (tumours). • GM virus recombination with naturally occurring HSV-1 would not result in a more pathogenic organism than the naturally occurring HSV-1. • Recombination with viruses that are not HSV-1 is highly unlikely.

2.1 Increased disease burden from the GM virus

138. The Parent organism of the GMO is HSV-1, a human pathogen. Information on HSV-1 transmissibility, virulence and pathogenicity are given in Chapter 1.

139. In summary, HSV-1 is highly contagious and widespread in the environment with an estimated 76% of the Australian population seropositive for the virus. Human HSV-1is highly host (human) specific and only in rare cases does HSV-1 anthroponosis occur.

140. Primary HSV-1 infection causes localised cell death and produces an associated inflammatory response in the area of infection, which may or may not generate disease symptoms.

141. Infection by wild type HSV-1 is generally through direct contact with infected secretions or mucous membranes/skin from asymptomatic or symptomatic shedding of the virus. The virus can be spread from the site of infection to other places (e.g. fingers and eyes) or other people by contact with the infected area or by secondary contact with items that have been contaminated by contact with the site of infection (e.g. towels, sheets, clothes, bandages). HSV-1 infection in the fingers (herpetic whitlow) has been observed in healthcare workers that come into direct contact with the virus (i.e. in the absence of gloves). There has also been one reported case of transmission of HSV-1 by a needle-stick injury (Douglas et al. 2002). The virus is not considered to be transmitted by aerosol exposure.

142. Infection by HSV-1 may lead to the establishment of a latent infection and subsequent re-activation of the virus, usually in response to illness, resulting in either the reappearance of the skin or mucosal lesions or asymptomatic shedding of infectious virus. HSV-1 DNA does not integrate into the genome of the host.

143. The GMO is a live attenuated HSV-1 that has been genetically modified to selectively replicate in tumours (rapidly dividing cells) and elicit an immune response for the treatment of patients with skin cancer (metastatic melanoma) and other suitable solid tumours. The GM virus has been modified by removing specific viral genes involved in neurovirulence and viral antigen presentation, and by introduction of a gene encoding a human protein that stimulates certain types of immune cells.

144. Patients undertaking treatment would be intentionally exposed to the GM virus. Clinical staff administering the GM virus may be accidentally exposed to the GMO. As noted in Chapter 1 Section 2.1, TGA has regulatory responsibility for use of the GMO as a therapeutic; this is not part of the Regulator's evaluation of this application. The Regulator will assess risks posed to other people and to the environment associated with other activities. Other people and animals may be exposed to the GM virus through accidental release during transport, storage or disposal, potentially including disposal of material containing GM virus shed by patients undergoing treatment.

145. Toxicity is the adverse effect(s) of exposure to a dose of a substance as a result of direct cellular or tissue injury, or through the inhibition of normal physiological processes (Arts et al. 2006).

146. An increased disease burden could be due to an increase in disease symptoms, or inappropriate immune response to the GM virus as a result of expression of the protein encoded by the introduced *hGM-CSF* gene. An inappropriate immune response would be an abnormal/unintended increase or suppression of the immune response, or an allergic response. Pathways that could lead to an increased disease burden from the GM virus include:

- exposure of staff involved in disposal of the GM virus at clinical sites, leading to viral infection and protein expression;
- exposure of contacts of trial participants (household contacts and animals) to the GM virus, leading to viral infection and protein expression;
- unintentional release of the GM virus, leading to viral infection and protein expression in other people or animals;

where expression of the introduced protein (hGM-CSF) leads to an increase in disease symptoms or an inappropriate immune response. These are discussed below.

Risk scenario 1 - Exposure of clinical staff to the GM virus resulting in infection and increased disease burden

Risk source	Causal pathway	Potential harm
GM virus	i. Exposure of clinical staff to the GM during waste disposal ⬇ ii. Establishment of infection ⬇ iii. Development of disease	Disease in clinical staff

147. Clinical staff may come into contact with the virus while disposing of unused inoculum or contaminated equipment or material, including waste from patients who have received the GMO.

148. GM virus administration would be conducted by trained medical professionals in clinical facilities equipped to deal with scheduled drugs and infectious agents. The GM virus would be dispensed within a medical facility and in consideration of the Talimogene laherparepvec MSDS and facility Safety assessment. Typically, such facilities follow AS/NZS 2243.3:2010 Safety in laboratories - Microbiological Safety and Containment.

149. For handling the GM virus, the recommended PPE in the product information documentation include laboratory coat, gloves and safety glasses when there is potential for direct skin contact with the virus. The use of gloves will minimise transmission via contact with the injection site.

150. Instructions in CMI to healthcare personnel who are immunocompromised are not to administer the GM virus and not to come into direct contact with the injection sites or body fluids of treated patients.

151. Handling of the GMO would be in accordance with *Australian code of good wholesaling practice for medicines in schedules 2,3,4, and 8* (NCCTG 2011) and the WHO *Good distribution practices for pharmaceutical products* (World Health Organisation (WHO) 2010).

152. Following administration at treating hospitals and clinics, all unused product and associated waste (including needles, swabs etc.), would be discarded into appropriate biohazard containers and disposed of following institutional procedures for the disposal of biohazardous material. This may include rendering all waste inert by high temperature incineration or steam sterilisation at the medical facility and/or use of registered waste contractors.

153. As HSV-1 is an enveloped virus it is susceptible to chemical decontamination, such as lipid solvents, detergents and hypochlorite, and is also rapidly inactivated by desiccation.

154. The level of shedding of the GM virus from patients is an important factor in determining exposure to and transmission of the GM virus. The published clinical trials found transient and limited viral shedding of the GM virus from the injection site and the urine, and GM virus shedding was not routinely detectable more than a few days after administration (Chapter 1 Section 6.3.7). The GM virus was not detected outside the occlusive dressing of treated patients in the clinical trials.

155. The GM virus has been modified such that it is not able to produce a main pathogenic determinate of HSV-1, ICP34.5 protein. The HSV-1 ICP34.5 protein normally overcomes host defence pathways to promote neurovirulence and enables the virus to replicate in non-dividing cells. The modified GM virus has both copies of *ICP34.5* removed and is attenuated for replication, such that it cannot replicate efficiently in non-dividing cells.

156. The deletion of *ICP34.5* has been shown to provide the greatest level of attenuation of any single deletion in HSV-1 that still allows the virus to replicate efficiently in culture and in animal *in vivo* tumour models (Markert et al. 2000; Sundaresan et al. 2000).

157. The GM virus is a live virus that can only replicate efficiently within tumourigenic cells and is inherently less competitive that its parental wild type HSV-1 strain. Non-clinical studies indicate that the GM virus does not have any selective advantage over naturally occurring HSV-1.

158. The removal of *ICP47* from the GM virus is intended to improve the presentation of viral antigens (and concurrently tumour antigens) in infected cells. This will make the virus more easily cleared by the host's immune system. Removal of *ICP47* also results in the increased expression of viral protein US11, which enhances replication of *ICP34.5* deleted HSV-1 in tumour cells, but does not overcome the GM viruses inability to replicate efficiently in non-dividing cells (Mohr et al. 2001).

159. The protein encoded by the inserted *hGM-CSF* gene is of human origin. The GM-CSF proteins are pleiotropic cytokines found in mammals that can stimulate the proliferation, maturation, and activation of a variety of hematopoietic cells. As discussed in Chapter 1 Section 3.2.2, administration of hGM-CSF has been extensively tested and found to be safe in people (Bendandi et al. 1999; Daud et al. 2008; Jager et al. 1996; Sato et al. 2008; Schmittel et al. 1999; Spitler et al. 2009) and a variety of animal 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). hGM-CSF has been approved by United States Food and Drug Administration as a pharmaceutical.

160. Several clinical trials of the GM virus (previously known as OncoVEX$^{gm\text{-}csf}$; now called Talimogene laherparepvec) have been carried out in Australia and elsewhere (see Chapter 1 Section 5.3.5). These trials have demonstrated that this GM virus is safe for human use, with adverse reactions such as pyrexia (abnormal body temperature) and associated constitutional symptoms (which included indications like fever, chills, fatigue nausea, vomiting or headache), as well as inflammation and erythema (redness of the skin) in and around the injected tumour site.

161. These adverse events were common in trial participants, were generally mild to moderate in severity and were more marked in patients who were HSV-seronegative before treatment. It is not possible to determine the specific element of the GM virus that is responsible for the adverse effects, however it is likely that these side effects could be, in part, mediated by the expression of GM-CSF. No patients in the clinical trials developed HSV encephalitis or other symptoms suggestive of infection of the central nervous system. The clinical studies have shown an acceptable safety profile with no medically significant virus-related adverse events for patients treated with the GM virus.

162. The applicant has tested the sensitivity of the GM virus to the nucleoside analogue acyclovir used as an anti-viral treatment and found it to be equally sensitive to acyclovir as the parental HSV-1 strain JS1.

163. In summary, the proposed controls for the commercial supply would minimise the likelihood of exposure of clinical staff to the GM virus. Human contact with GM virus prior to and during inoculation would be limited to trained and authorised staff. The staff would be wearing appropriate personal protective equipment, including a laboratory coat, gloves and safety glasses. The proposed trial sites are located within clinical facilities equipped to deal with scheduled drugs and infectious agents.

164. Even if exposure to GM virus were to occur through any of the above exposure routes, the GMO is significantly attenuated, making it less virulent. As such, exposure to the GM virus is unlikely to result in more than a transient infection. Furthermore, data from previous non-clinical studies and clinical trials suggests that even if infection does occur, expression of the hGM-CSF protein is not toxic or expected to affect the virulence and pathogenicity of the virus.

165. ***Conclusion***: The potential of the GM virus to increase disease burden following exposure and infection of clinical staff, resulting in increased disease symptoms or an inappropriate adverse immune response due to the expression of introduced gene is not identified as a substantive risk. Therefore, it does not warrant further assessment.

Risk Scenario 2 - Exposure of people or animals to the GM virus resulting in infection and increased disease burden

Risk source	Causal pathway	Potential harm
GM virus	i. Treated patient sheds GM virus ⬇ ii. Patient disposes of material contaminated with the GM virus ⬇ iii. Exposure of people or animals to the contaminated material ⬇ iv. Establishment of infection ⬇ v. Development of disease	Increased disease burden

166. Other people or animals may be exposed to the GMO through coming into contact with objects or waste which has become contaminated with the GMO shed by patients.

167. Examination of GM virus shedding in the published clinical trials indicate that transient and limited viral shedding of the GM virus can occur from the injection site, and viral DNA was detected in the urine of patients. GM virus shedding was not routinely detectable more than a few days after administration and was not detected outside the occlusive dressing of treated patients. The limited level of GM virus shedding from the patients reduces the likelihood of transmission of the GM virus to non-trial participants or animals that may come in contact with the patient.

168. HSV-1 does not form survival structures and its survival outside the host organism is limited to short periods of time, but may be up to several weeks (See Chapter 1, section 5.4). Transient and limited viral shedding of the GM virus from the injection site would be contained by clinical dressings (bandages). Patient will be instructed for care on the injection site, including how to change and dispose the dressings. Patient dressings and material used for cleaning the injection site(s) are to be placed within a sealed plastic bag before disposal in the normal household waste, mitigating the chances of accidental exposure or release into the environment.

169. The CMI will provide additional communication to patients around the risk of secondary transmission, what consumers can do to mitigate this risk and measures for management of accidental exposure.

170. Presence of DNA from the GM virus in the urine may indicate a possibility that live virus is being excreted in the urine and constitute a potential avenue for exposure. The GM virus is not able to replicate outside of a host and as an enveloped DNA virus, it is highly susceptible to chemical decontamination, such as lipid solvents, quaternary ammonium compounds, detergents and hypochlorite. The viability of the virus on common household surfaces is greatly reduced over a time frame of a few hours (see Chapter 1, Section 1.4).

171. In Australia, treatment of wastewater (including sewage) is required as per State and Territory regulations. This would significantly limit the chances of GM virus entering into the environmental waters. Septic tanks are used in some local government areas where centralised wastewater treatment is not available. Where used, septic tanks are required to be maintained in accordance with State and Territory regulations. This means that untreated sewage should not leak from the septic tank. Nevertheless, septic tank leakage can occur, and it is possible that

persons servicing the septic tank or working with the surrounding soil may come into contact with the GM virus. However, this is not considered a plausible pathway to harm for the following reasons:

- the GM virus is only shed briefly and at low levels by a small proportion of patients (Chapter 1 Section 5.3.7)
- few patients are likely to be using the septic tanks
- once in the septic tank the small amount of GM virus would be significantly diluted
- the GM virus would not persist as it does not replicate outside the human host and has limited environmental stability (Chapter 1 Section 4.4).

172. The only natural host for human HSV-1 is humans, but non-human primates and a few other mammals in captivity can be accidentally infected. Rabbits and various rodent species can be infected experimentally. The ability or likelihood of HSV-1 to infect domestic pets or animals (e.g. dogs, cats, horses, cows or other common domesticated animals) is extremely low. The insertion of two copies of the *hGM-CSF* will not change the host range of the GM virus, as this gene is not a determinant of HSV-1 host range. The attenuation of the GM virus makes the possibility for anthroponosis even less likely than for naturally occurring HSV-1.

173. As detailed in Risk Scenario 1 the GM virus has been modified such that it is significantly attenuated. It does not replicate efficiently in non-dividing cells and is inherently less competitive than naturally occurring HSV-1, and the modifications are not expected to increase disease symptoms relative to its parental HSV-1 strain. In addition, the GM virus has been found to be sensitive to standard anti-viral medication.

174. The proposed controls of the commercial supply would minimise the likelihood of transmission of the GM virus.

175. ***Conclusion*****:** The potential of the GM virus to increase disease burden due to transmission of the virus to people or animals that come into contact with patients, resulting in increased disease symptoms or an inappropriate immune response due to the expression of introduced genes is not identified as a substantive risk. Therefore, it does not warrant further assessment.

Risk Scenario 3 - Exposure of people or animals to the GM virus due to unintentional release

Risk source	Causal pathway	Potential harm
GM virus	i. Exposure of people or animals to the GM virus due to unintentional release during transport or storage ⬇ ii. Establishment of infection ⬇ iii. Development of disease	Increased disease burden

176. An unintentional release could occur as a result of a spill outside of the containment environment during import, transport or storage.

177. The GM virus proposed for commercial supply would be imported from overseas manufacturing sites in the USA. Storage, handling and transport will be in accordance with the *Australian code of good wholesaling practice for medicines in schedules 2,3,4, and 8* (NCCTG 2011) and the WHO *Good distribution practices for pharmaceutical products* (World Health Organisation (WHO) 2010).

178. The GM virus would be packaged as a sterile frozen liquid in single use 2.0 millilitre (mL) Crystal Zenith resin vials, which are highly durable vials. The single dose vials will be

packaged into a secure secondary packaging carton and imported / transported using commercial couriers to the central storage facilities of a logistics service provider used by Amgen in Australia according to IATA shipping classification UN 3245.

179. The GM virus would be transported to treating hospitals and clinics that are registered and licenced for the purposes of handling scheduled medicines and poisons as legislated through The Poisons Standard (2015) and enforced through state and territory legislation. The GM virus would be stored within the pharmacy or other appropriate secure locations at treating hospitals and clinics.

180. Any spills occurring in a clinical setting would be disinfected and cleaned according to standard clinical procedures. Spills outside of clinical facilities (i.e. during transport, storage or disposal) would be disinfected and contained according to the Talimogene laherparepvec MSDS and other appropriate regulatory standards (Chapter 1, Section 4).

181. In addition, the GM virus is supplied as purified virus particles, which have reduced capacity to survive in the environment compared to virus found in scabs and other biological specimens. The GM virus has limited environmental stability (Chapter 1 Section 4.4) and is susceptible to common chemical decontamination agents, such as lipid solvents, detergents and hypochlorite (Chapter 1 Section 4.5). Therefore there is very little potential for exposure of humans or animals to the GM viruses.

182. As noted for Scenario 1, the GMO is significantly attenuated and exposure is unlikely to result in more than a transient infection. Expression of the introduced *hGM-CSF* gene is not expected to increase the symptoms of the disease or affect the virulence and pathogenicity of the GM virus.

183. ***Conclusion:*** The potential of GM virus to increase disease burden due to infection of susceptible hosts following a spill during transport or storage, resulting in increased disease symptoms or an inappropriate immune response due to the expression of introduced genes is not identified as a substantive risk. Therefore, it does not warrant further assessment.

2.2 Unintended changes in viral characteristics

184. Both copies of *ICP34.5* have been functionally deleted from the GM virus. In place of the two deleted *ICP34.5* genes, two copies of the gene encoding hGM-CSF have been inserted. Expression of hGM-CSF from the GM virus is controlled by the non-coding CMV promoter and bgh-PolyA sequence. In addition, the viral *ICP47* gene of HSV-1 has been deleted from the GM virus resulting in the increased expression of viral protein US11. The US11 protein provides some functional redundancy with ICP34.5 in relation to viral replication. Increased US11 expression enhances replication of *ICP34.5* deleted HSV-1 in rapidly dividing cells, but not in non-dividing cells.

185. When genes are inserted into a genome, there is a possibility that the insertion may have unintended consequences on the expression of other genes. This is particularly of concern in small viruses that have a limited number of genes, as the gene products of individual genes may display pleiotropy (the genetic effect of one gene on apparently unrelated, multiple phenotypic traits (Kahl 2001)).

Risk Scenario 4 - Changes to tropism of GM virus due to the genetic modifications

Risk source	Causal pathway	Potential harm
GM virus	i. Accidental exposure of people to the GM virus leading to infection (see Risk Scenarios 1-3) ⬇ ii. Altered characteristics of the GM virus due to expression of the introduced genes ⬇ iii. GM virus establishes infection in new host species ⬇ iv. Development of disease	Increased Disease burden

186. HSV-1 is a relatively large virus, so pleiotropy is expected to be less prevalent than for viruses with small genomes. Human and animal trials involving the GM virus, and other viruses with similar genetic modifications, have not revealed unintended changes in the characteristics of the GM viruses resulting from the introduced genes or genetic modifications.

187. Amgen has provided a study indicating that the tropism (i.e. the range of cell types that can be infected) of the GM virus is the same as naturally occurring HSV-1.

188. It is also important to note that the human gene product expressed by the GM virus will not be expressed on the viral surface. Rather, following viral infection, the infected host cell will express the human gene product to be secreted as a cytokine into the local tumour microenvironment. This means that the viral surface won't be altered by the genetic modification.

189. ***Conclusion*****:** The potential for an adverse outcome as a result of altered viral structure or function is not identified as a substantive risk. Therefore, it does not warrant further assessment.

2.3 Horizontal transfer of genes or genetic elements to other organisms

190. HGT is the stable transfer of genetic material from one organism to another without reproduction (Keese 2008). All genes within an organism, including those introduced by gene technology, are capable of being transferred to another organism by HGT. HGT itself is not considered an adverse effect, but an event that may or may not lead to harm. A gene transferred through HGT could confer a novel trait to the recipient organism, through expression of the gene itself or through changes to expression of endogenous genes. The novel trait may result in negative, neutral or positive effects.

191. Baseline information on the presence of the introduced gene or similar genetic elements is provided in Chapter 1, Section 6.2. HSV is widespread in the environment, with around 80% of the human population showing a significant level of serum antibodies (indicating previous exposure), and *GM-CSF* genes are present in all mammals. Because the viral genes and the introduced *hGM-CSF* in the GMO are already available for HGT from these natural sources, their transfer to organisms other than viruses will not be assessed further. Therefore, the risk assessment will address potential HGT between the GM virus and other viruses.

192. Recombination between two viruses occurs during simultaneous infection of the same cell (DeFillipis & Villarreal 2001). Recombination can occur within and between viral types (DeFillipis & Villarreal 2001), meaning that introduced genes could be potentially transferred to other viruses, and the GMO may acquire genes from other viruses. While recombination between different classes of virus can occur, the frequency of recombination happening decreases with decreasing relationship between the viruses, i.e. the GM virus is more likely to recombine with another HSV-1 virus than with an unrelated virus. Recombination between two

live viral vaccines used for chickens, based on two strains of the same viral pathogen, has been recently reported (Lee et al. 2012).

193. Homologous recombination is a type of genetic recombination in which nucleotide sequences are exchanged between two similar or identical molecules of DNA. Homologous recombination between HSV has been previously demonstrated (Wildy 1955). Studies have shown that homologous recombination of HSV-1 occurs frequently and that HSV-1 genomes contain mosaic patterns of segments with different evolutionary origins (Norberg et al. 2011).

Risk Scenario 5 – Recombination of the GMO with other viruses

Risk source	Causal pathway	Potential harm
GM virus	i. Accidental exposure of people to the GM virus leading to infection (see risk Scenarios 1-3) ⬇ ii. Person also infected with another virus ⬇ iii. Both viruses replicate in the same host cell ⬇ iv. Recombination occurs between the viral genomes ⬇ v. Recombinant virus infects other hosts ⬇ vi. Development of disease	Increased disease burden

194. Amgen have sequenced 99% of the GM virus genome. The modified regions of the GM virus have been sequenced at least three times between 2001 and 2011. These sequenced regions have been found to only contain the intended DNA sequence. There is no evidence that the modifications are unstable or have made the GM virus prone to genetic instability.

195. The ability of replication incompetent HSV-1 and replication competent HSV-1 to undergo non-homologous recombination (recombination occurring in regions where no large-scale sequence similarity is apparent) has been examined experimentally *in vitro* and *in vivo*. This study found that non-homologous recombination between replication incompetent and replication competent HSV-1 did not occur at detectable levels (Smith et al. 2003). The replication incompetent HSV-1 used in this study was produced by deleting the *ICP27* gene. Although the gene deleted in the experiments by Smith and colleagues (Smith et al. 2003) to produce the replication incompetent HSV-1 is not *ICP34.5* (as per the GM virus in this application), the experiments do indicate that HSV-1 is not prone to non-homologous recombination between replication incompetent and replication competent strains.

196. For the GM HSV-1 to undergo recombination, a host cell would need to be concurrently infected with the GM virus and another virus. It is unlikely that a pre-existing wild-type virus would be in the same tissue as the GM virus; the GM virus will be directly injected into tumours of patients undergoing treatment and cannot spread effectively into normal tissue. If a patient has a pre-existing HSV-1 infection, it would be confined to their mucosal tissues and/or neuronal ganglia.

197. Information reported from the clinical trials indicated that the GM virus was detected in the blood of a few patients post injection. The appearance of the GM virus in the blood occurred only within a few days post-injection in a few patients, indicating the presence of the GM virus in the blood was transient and most likely due to the high level of GM virus injected into the tumours of patients at the time of treatment. The attenuation of the GM virus limits its ability produce a latent infection.

198. As detailed in risk scenarios 1 and 2, the proposed controls for the commercial supply as well as the nature of the genetic modifications would minimise the likelihood of exposure of people to the GM virus. Additionally, as noted for Scenario 1, the GM virus is significantly attenuated, making it less virulent than wild-type HSV-1. As such, exposure to the GM virus is unlikely to result in more than a transient infection, minimising the opportunity for viral recombination.

199. The possibility of recombination between the GMO and types of viruses other than HSV is even less likely. Functional differences among viruses, such as viral replication strategies, reduce the possibility for recombination between different virus types. As an example, the double stranded DNA Poxviruses and double stranded DNA herpes viruses replicate in different locations within the cell; poxviruses replicate in the cytoplasm whereas herpes viruses replicate in the nucleus (Boehmer & Lehman 1997; Mutsafi et al. 2010). This difference in replication locations minimises the potential for recombination with other viruses. As mentioned above, the GM virus will be directly injected into tumour cells and cannot spread effectively into normal tissue, reducing the possibility recombination with other viruses.

200. The ability of the GM virus to undergo homologous recombination with wild-type HSV-1 would result in a reciprocal exchange of genetic information. In theory, any combination of the introduced modifications may be generated in the genetic background of either virus involved in the exchange. The theoretically stable combinations of genetic modifications from the GMO are shown in Table 4 and discussed below. The rest of the genome of the GMO is derived from a natural isolate of HSV-1, strain JS1. Thus the rest of the genome of a recombinant between the GMO and another HSV-1 strain would be sequence from either the JS1 strain or the other naturally occurring HSV-1 strain.

Table 4 Theoretical stable genetic variants of the GM virus*.

Genetic Variant		Gene (Function)			
		ICP34.5 (Virulence)	GM-CSF (Immune stimulation)	*ICP47* (immune evasion)	US11 upregulation#
1	The GMO	-/-	+/+	-	+
2	*ICP47* restoration	-/-	+/+	+	-
3	Homozygous *ICP34.5* restoration	+/+	-/-	-	+
4	*ICP47* restoration plus homozygous *ICP34.5* restoration (Wild-type HSV-1)	+/+	-/-	+	-

***** + and - indicated presence or absence of the gene or characteristic in the unique genome regions; +/+ and -/- indicate presence or absence of two copies of the gene in the repeated regions.

ICP47 deletion leads to increased expression of the US11 gene, which increases replication of *ICP14.5*-deleted virus in rapidly dividing (tumour) cells in experimental systems.

201. In a recombinant with *ICP47* restored but retaining the *ICP34.5* deletions and hGM-CSF insertions (variant 2 in table 4), the *ICP34.5* deletion would prevent the virus from replicating efficiently in non-dividing cells and reduce neurovirulence relative to wild-type HSV-1. Many studies indicate the pathogenicity of *ICP34.5*-deleted HSV-1 is substantially attenuated in animals and humans (Harrington et al. 2010; Harrow et al. 2004; Hu et al. 2006; Hunter et al.

1999; Mace et al. 2008; MacKie et al. 2001; McKie et al. 1998; Papanastassiou et al. 2002; Perng et al. 1995; Rampling et al. 2000; Senzer et al. 2009; Valyi-Nagy et al. 1994; Varghese et al. 2001; Whitley et al. 1993) (Chapter 1 Section 5.2.1). hGM-CSF expression would have the same effect as in the GMO, inducing proliferation and differentiation of certain types of immune cells (Chapter 1 Section 5.2.2).

202. In a recombinant containing the *ICP47* deletion but with *ICP34.5* restored, the *hGM-CSF* gene insertion would be lost (variant 3 in table 4). *US11* expression would remain upregulated. Recombinant HSV-1 viruses that contain a US11 up-regulation mutation in a wild-type background have been generated and they display neurovirulence comparable to wild-type HSV-1 (Mohr et al. 2001). However, ICP47 allows the virus to hide from the immune system, so loss of *ICP47* will make infected cells better targets for the host immune response (Chapter 1 Section 5.2.3), potentially resulting if faster clearance of the virus. Additionally, as natural viral replication has a tendency to generate DNA sequence variations, including deletions, deletion of the *ICP47* gene is more likely to occur during replication of naturally occurring HSV-1 stains than through recombination between the GMO and another HSV-1 strain. Natural selection is expected to remove most of the changes generated as they are disadvantageous for the virus, while changes that are advantageous become established in the viral population.

203. Variant 1 in Table 4 would be a virus with all the genetic modification of the GMO (but with other parts of the viral genome potentially derived from a different wild-type HSV-1 strain). This viral variant would retain the attenuated characteristic of the GMO (Chapter 1 Section 5.2).

204. Variant 4 would be a virus equivalent to wild-type HSV-1, with none of the introduced genetic modifications from the GMO. Its genome could be composed of a mixture of sequences from the parent strain of the GMO, HSV-1 strain JS1, and another naturally occurring strain. Such a virus is more likely to arise from recombination events between two naturally occurring strains than from recombination involving the GMO. There is no specific data on the presence or absence of HSV-1 strain JS1 in Australia. However, HSV-1 strain JS1 is a clinical isolate and therefore present in the naturally occurring pool of HSV-1 strains. As there are no restrictions on international travel related toHSV-1 infection, HSV-1 strains present in Australia would be part of a larger pool.

205. It is possible for individual recombinant virons to contain DNA with one copy of *ICP34.5* and one copy of hGM-CSF to be generated, resulting in a virus that is heterozygous in the *ICP34.5* region (since two copies of *ICP34.5* are present in the wild-type HSV-1, one in each of the long repeat regions). While heterozygosity within the repeat regions of HSV-1 has been observed, these heterozygote genomes are not stable and revert to homozygotes at a high frequency (DeLuca & Schaffer 1985; Umene 1987; Varmuza & Smiley 1984). HSV-1 viruses expressing only one copy of *ICP34.5* have also been artificially generated, but are only able to stably exist if accompanied by extensive genomic deletions that prevent homologous recombination (Meignier et al. 1988).

206. To assess the virulence of HSV-1 containing a single copy of *ICP34.5*, the *ICP34.5* gene was cloned under the control of a different promoter into a unique region of an *ICP34.5*-deleted HSV virus (Holman & MacLean 2008). This virus did not have its virulence restored to wild-type levels.

207. In summary, none of the postulated homologous recombination events with other HSV-1 viruses are expected to give rise to a virus with pathogenicity greater than that of naturally occurring HSV-1 strains that are widespread in the environment, and the opportunity for recombination between the GMO and other viruses would be limited due to the nature of the GMO and the controls proposed.

208. ***Conclusion*:** The potential for an adverse outcome as a result of viral recombination is not identified as a substantive risk. Therefore, it does not warrant further assessment.

Section 3 Uncertainty

209. Uncertainty is an intrinsic part of risk analysis[9]. There can be uncertainty about identifying the risk source, the causal linkage to harm, the type and degree of harm, the chance of harm occurring or the level of risk. In relation to risk management, there can be uncertainty about the effectiveness, efficiency and practicality of controls.

210. Risk analysis can be considered as part of a first tier uncertainty analysis, namely a structured, transparent process to analyse and address uncertainty when identifying, characterising and evaluating risk. However, there is always some residual uncertainty that remains. If the residual uncertainty is important and critical to decision making, then this residual uncertainty may be subjected to further analysis (second tier uncertainty analysis), such as building 'worst case' scenarios, or by using meta-analysis where results from several studies are combined.

211. There are several types of uncertainty in risk analysis (Bammer & Smithson 2008; Clark & Brinkley 2001; Hayes 2004). These include:

- uncertainty about facts:
  - knowledge – data gaps, errors, small sample size, use of surrogate data
  - variability – inherent fluctuations or differences over time, space or group, associated with diversity and heterogeneity
- uncertainty about ideas:
  - description – expression of ideas with symbols, language or models can be subject to vagueness, ambiguity, context dependence, indeterminacy or under-specificity
- perception – processing and interpreting risk is shaped by our mental processes and social/cultural circumstances, which vary between individuals and over time.

212. Uncertainty can also arise from a lack of experience with the GMO itself. In regard to the GM virus, the overall level of uncertainty is low given the clinical trials carried out in Australia, the United States and several other countries. None of these trials have resulted in a serious adverse event for health and safety of people, or the environment. However, the relatively low number of clinical trial participant's is a source of uncertainty in relation to the identification of rare serious adverse events. The TGA has regulatory responsibility for quality, efficacy and patient safety of therapeutic goods and the TGA will assess risks to patients and will manage any risks identified.

213. There is lack of Australian experience with commercial use of an attenuated GM virus for oncolytic immunotherapy. However, the GM virus has been the subject of nine clinical trials, that have been or are currently being conducted and to date there have not been any confirmed adverse effects. Therefore the nature and degree of this uncertainty is not sufficient to affect the outcome of the risk assessment.

[9] A more detailed discussion is contained in the Regulator's *Risk Analysis Framework* available from the OGTR website or via Free call 1800 181 030.

Section 4 Risk Evaluation

214. Risk is evaluated against the objective of protecting the health and safety of people and the environment to determine the level of concern and, subsequently, the need for controls to mitigate or reduce risk. Risk evaluation may also aid consideration of whether the proposed dealings should be authorised, need further assessment, or require collection of additional information.

215. Factors used to determine which risks need treatment may include:

- risk criteria
- level of risk
- uncertainty associated with risk characterisation
- interactions between substantive risks

216. Five risk scenarios were identified whereby the proposed dealings might give rise to harm to people or the environment. This included consideration of whether expression of the introduced genes and genetic modifications could: result in products that are toxic to people or other organisms; alter characteristics that may impact on the disease burden of GM virus, or produce unintended changes in viral characteristics. The opportunity for gene transfer to other organisms, and its effects if this occurred were also considered.

217. A risk is only identified as substantive when a risk scenario is considered to have some chance of causing harm as a result of the gene technology. Risk scenarios that do not lead to harm, or could not reasonably occur, do not represent an identified risk and do not advance any further in the risk assessment process.

218. The characterisation of the five risk scenarios in relation to both the seriousness and likelihood of harm, in the context of the control measures proposed by the applicant, did not give rise to any substantive risks that required further assessment. The principal reasons for this include:

- Exposure to the GM virus would be minimised by well-established clinical, transport and storage procedures
- HSV-1 survival outside of a host is limited to short periods of time, and it is susceptible to common chemical decontamination agents
- Human HSV-1 does not cause disease in other organisms under natural circumstances, and is subject to anthroponosis only in rare events to a limited number of species
- The genetic changes to the GM virus do not alter its genetic stability or indicate changes in host/tissue tropism in comparison to wild-type HSV-1
- The removal specific viral genes involved in neurovirulence and evasion of host immune response make the GM virus attenuated for replication in normal tissue and significantly reduce the likelihood of unintended transmission or persistence in humans or animals
- The products of the single introduced gene are not expected to be toxic to humans or other animals
- GM virus is susceptible to anti-viral medication

219. Therefore, any risks to the health and safety of people, or the environment, from the proposed release of the GM virus into the environment are considered to be negligible. The *Risk Analysis Framework* (OGTR 2013), which guides the risk assessment and risk management process, defines negligible risks as insubstantial with no present need to invoke

actions for their mitigation. No controls are required to treat these negligible risks. Hence, the Regulator considers that the dealings involved in this proposed release do not pose a significant risk to either people or the environment.

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Chapter 3 Risk management

Section 1 Background

220. Risk management is used to protect the health and safety of people and to protect the environment by controlling or mitigating risk. The risk management addresses risks evaluated as requiring treatment, evaluates controls and limits proposed by the applicant, and considers general risk management measures. The risk management plan informs the Regulator's decision-making process and is given effect through licence conditions.

221. Under section 56 of the Act, the Regulator must not issue a licence unless satisfied that any risks posed by the dealings proposed to be authorised by the licence are able to be managed in a way that protects the health and safety of people and the environment.

222. All licences are subject to three conditions prescribed in the Act. Section 63 of the Act requires that each licence holder inform relevant people of their obligations under the licence. The other statutory conditions allow the Regulator to maintain oversight of licensed dealings: section 64 requires the licence holder to provide access to premises to OGTR inspectors and section 65 requires the licence holder to report any information about risks or unintended effects of the dealing to the Regulator on becoming aware of them. Matters related to the ongoing suitability of the licence holder are also required to be reported to the Regulator.

223. The licence is also subject to any conditions imposed by the Regulator. Examples of the matters to which conditions may relate are listed in section 62 of the Act. Licence conditions can be imposed to limit and control the scope of the dealings and to manage risk to people or the environment. In addition, the Regulator has extensive powers to monitor compliance with licence conditions under section 152 of the Act.

Section 2 Risk treatment measures of identified risks

224. The risk assessment of the risk scenarios listed in Chapter 2 concluded that there are negligible risks to people and the environment from the proposed dealings with GM virus. These risk scenarios were considered in the context of the scale of the proposed dealings, the receiving environment, and considering both the short and the long term. The risk evaluation concluded that no controls are required to treat these negligible risks.

Section 3 General risk management

225. All DIR licences issued by the Regulator contain a number of conditions that relate to general risk management. These include conditions relating to:

- applicant suitability;
- identification of the persons or classes of persons covered by the licence reporting structures
- a requirement that the applicant allows access to specified sites for purpose of monitoring or auditing.

3.1 Applicant suitability

226. In making a decision whether or not to issue a licence, the Regulator must have regard to the suitability of the applicant to hold a licence. Under section 58 of the Act, matters that the Regulator must take into account include:

- any relevant convictions of the applicant (both individuals and the body corporate)
- any revocation or suspension of a relevant licence or permit held by the applicant under a law of the Commonwealth, a State or a foreign country

- the capacity of the applicant to meet the conditions of the licence

227. On the basis of information submitted by the applicant and records held by the OGTR, the Regulator considers Amgen suitable to hold a licence.

228. The licence includes a requirement for the licence holder to inform the Regulator of any circumstances that would affect their suitability.

229. In addition, any applicant organisation must have access to a properly constituted Institutional Biosafety Committee and be an accredited organisation under the Act.

3.2 Testing methodology

230. Amgen is required to provide a method to the Regulator for the reliable detection of the presence of the GMOs and the introduced genetic materials in a recipient organism. This instrument is required prior to conducting any dealings authorised by the licence.

3.3 Identification of the persons or classes of persons covered by the licence

231. Any person, including the licence holder, may conduct any permitted dealing with the GMOs.

3.4 Reporting requirements

232. The licence obliges the licence holder to immediately report any of the following to the Regulator:

- any additional information regarding risks to the health and safety of people or the environment associated with the dealings
- any contraventions of the licence by persons covered by the licence
- any unintended effects of the release

233. The licence holder is also obliged to submit an Annual Report containing any information required by the licence.

234. There are also provisions that enable the Regulator to obtain information from the licence holder relating to the progress of the commercial supply (see Section 4, below).

3.5 Monitoring for Compliance

235. The Act stipulates, as a condition of every licence, that a person who is authorised by the licence to deal with a GMO, and who is required to comply with a condition of the licence, must allow inspectors and other persons authorised by the Regulator to enter premises where a dealing is being undertaken for the purpose of monitoring or auditing the dealing.

236. In cases of non-compliance with licence conditions, the Regulator may instigate an investigation to determine the nature and extent of non-compliance. The Act provides for criminal sanctions of large fines and/or imprisonment for failing to abide by the legislation, conditions of the licence or directions from the Regulator, especially where significant damage to health and safety of people or the environment could result.

Section 4 Post release review

237. Regulation 10 requires the Regulator to consider the short and the long term when assessing risks. The Regulator does not fix durations, but takes account of the likelihood and impact of an adverse outcome over the foreseeable future, and does not disregard a risk on the basis that an adverse outcome might only occur in the longer term. However, as with any predictive process, accuracy is often greater in the shorter rather than longer term.

238. For the current application for a DIR licence, the Regulator has incorporated a requirement in the licence for ongoing oversight to provide feedback on the findings of the

RARMP and ensure the outcomes remain valid for future findings or changes in circumstances. This ongoing oversight will be achieved through post release review (PRR) activities. The three components of PRR are:

- adverse effects reporting system (Section 4.1)
- requirement to monitor specific indicators of harm (Section 4.2)
- review of the RARMP (Section 4.3)

239. The outcomes of these PRR activities may result in no change to the licence or could result in the variation, cancellation or suspension of the licence.

4.1 Adverse effects reporting system

240. Any member of the public can report adverse experiences/effects resulting from an intentional release of a GMO to the OGTR through the Free-call number (1800 181 030), fax (02 6271 4202), mail (MDP 54 – GPO Box 9848, Canberra ACT 2601) or via email to the OGTR inbox (ogtr@health.gov.au). Reports can be made at any time on any DIR licence. Credible information would form the basis of further investigation and may be used to inform a review of a RARMP (see Section 4.3 below) as well as the risk assessment of future applications involving similar GMO(s).

4.2 Requirement to monitor specific indicators of harm

241. Additional specific information on an intentional release provides a mechanism for 'closing the loop' in the risk analysis process and for verifying findings of the RARMP, by monitoring the specific indicators of harm that have been identified in the risk assessment.

242. The term 'specific indicators of harm' does not mean that it is expected that harm would necessarily occur if a licence was issued. Instead, it refers to measurement endpoints which are expected to change should the authorised dealings result in harm. Should a licence be issued, the licence holder would be required to monitor these specific indicators of harm as mandated by the licence.

243. The triggers for this component of PRR may include risk estimates greater than negligible or significant uncertainty in the risk assessment.

244. The characterisation of the risk scenarios discussed in Chapter 2 did not identify any risks that could be greater than negligible. Therefore, they did not warrant further detailed assessment. No specific indicators of harm have been identified in this RARMP for application DIR-132. However, specific indicators of harm may also be identified once a licence is issued, through either of the other components of PRR.

245. Conditions have been included in the licence to allow the Regulator to request further information from the licence holder about any matter to do with the progress of the release, including research to verify predictions of the risk assessment.

4.3 Review of the RARMP

246. The third component of PRR is the review of RARMPs after a commercial/general release licence is issued. Such a review would take into account any relevant new information, including any changes in the context of the release, to determine if the findings of the RARMP remained current. The timing of the review would be determined on a case-by-case basis and may be triggered by findings from either of the other components of PRR or be undertaken after the authorised dealings have been conducted for some time. If the review findings justified either an increase or decrease in the initial risk estimate(s), or identified new risks to people or to the environment that needed managing, this could lead changes to the risk management plan and licence conditions.

Section 5 Conclusions of the RARMP

247. The risk assessment concludes that this proposed commercial supply of GM virus poses negligible risks to the health and safety of people or the environment as a result of gene technology.

248. The risk management plan concludes that these negligible risks do not require specific risk treatment measures. However general conditions have been imposed to ensure that there is ongoing oversight of the release.

References

Allison, N., Chang, T.C., Steele, K.E., Hilliard, J.K. (2002) Fatal herpes simplex infection in a pygmy African hedgehog (Atelerix albiventris). *J Comp Pathol* **126**: 76-78.

Anderson, J.R., Field, H.J. (1983) The distribution of herpes simplex type 1 antigen in mouse central nervous system after different routes of inoculation. *Journal of the Neurological Sciences* **60**: 181-195.

Andtbacka, R.H., Kaufman, H.L., Collichio, F., Amatruda, T., Senzer, N., Chesney, J. et al. (2015) Talimogene Laherparepvec Improves Durable Response Rate in Patients With Advanced Melanoma. *Journal of Clinical Oncology*

Arduino, P.G., Porter, S.R. (2006) Oral and perioral herpes simplex virus type 1 (HSV-1) infection: review of its management. *Oral Diseases* **12**: 254-270.

Armien, A.G., Hu, S., Little, M.R., Robinson, N., Lokensgard, J.R., Low, W.C. et al. (2010) Chronic cortical and subcortical pathology with associated neurological deficits ensuing experimental herpes encephalitis. *Brain Pathology* **20**: 738-750.

Arts, J., Mommers, C., de Heer, C. (2006) Dose-response relationships and threshold levels in skin and respiratory allergy. *Critical review in Toxicology* **36**: 219-251.

Baiocchi, R.A., Ward, J.S., Carrodeguas, L., Eisenbeis, C.F., Peng, R., Roychowdhury, S. et al. (2001) GM-CSF and IL-2 induce specific cellular immunity and provide protection against Epstein-Barr virus lymphoproliferative disorder. *J Clin Invest* **108**: 887-894.

Bardell, D. (1989) Hand-to-hand transmission of herpes simplex virus type 1. *Microbios* **59**: 93-100.

Bardell, D. (1990) Survival of herpes simplex virus type 1 on some frequently touched objects in the home and public buildings. *Microbios* **63**: 145-150.

Bardell, D. (1993) Survival of herpes simplex virus type 1 in saliva and tap water contaminating some common objects. *Microbios* **74**: 81-87.

Bardell, D. (1994) Studies on the survival and inactivation of herpes simplex virus type 1 on coins. *Microbios* **77**: 161-166.

Bedadala, G.R., Pinnoji, R.C., Hsia, S.C. (2007) Early growth response gene 1 (Egr-1) regulates HSV-1 ICP4 and ICP22 gene expression. *Cell Research* **17**: 546-555.

Bendandi, M., Gocke, C.D., Kobrin, C.B., Benko, F.A., Sternas, L.A., Pennington, R. et al. (1999) Complete molecular remissions induced by patient-specific vaccination plus granulocyte-monocyte colony-stimulating factor against lymphoma. *Nature Medicine* **5**: 1171-1177.

Bertke, A.S., Patel, A., Imai, Y., Apakupakul, K., Margolis, T.P., Krause, P.R. (2009) Latency-associated transcript (LAT) exon 1 controls herpes simplex virus species-specific phenotypes: reactivation in the guinea pig genital model and neuron subtype-specific latent expression of LAT. *Journal of Virology* **83**: 10007-10015.

Boehmer, P.E., Lehman, I.R. (1997) Herpes simplex virus DNA replication. *Annual Review of Biochemistry* **66**: 347-384.

Bolovan, C.A., Sawtell, N.M., Thompson, R.L. (1994) ICP34.5 mutants of herpes simplex virus type 1 strain 17syn+ are attenuated for neurovirulence in mice and for replication in confluent primary mouse embryo cell cultures. *Journal of Virology* **68**: 48-55.

Borchers, K., Goltz, M., Ludwig, H. (1994) Genome organization of the herpesviruses: minireview. *Acta Veterinaria Hungarica* **42**: 217-225.

Boshart, M., Weber, F., Jahn, G., Dorsch-Hasler, K., Fleckenstein, B., Schaffner, W. (1985) A very strong enhancer is located upstream of an immediate early gene of human cytomegalovirus. *Cell* **41**: 521-530.

Casagrande, R.A., Pannuti, C.S., Kanamura, C., Freire, W.S., Grespan, A., Matushima, E.R. (2014) Fatal *Human herpesvirus 1* (HHV-1) infection in captive marmosets (*Callithrix jacchus* and *Callithrix penicillata*) in Brazil: clinical and pathological characterization. *Pesquisa Veterinária Brasileira* **34**: 1109-1114.

Chambers, R., Gillespie, G.Y., Soroceanu, L., Andreansky, S., Chatterjee, S., Chou, J. et al. (1995) Comparison of genetically engineered herpes simplex viruses for the treatment of brain tumors in a scid mouse model of human malignant glioma. *Proc Natl Acad Sci U S A* **92**: 1411-1415.

Chayavichitsilp, P., Buckwalter, J.V., Krakowski, A.C., Friedlander, S.F. (2009) Herpes simplex. *Pediatrics in Review* **30**: 119-129.

Chou, J., Kern, E.R., Whitley, R.J., Roizman, B. (1990) Mapping of herpes simplex virus-1 neurovirulence to gamma 134.5, a gene nonessential for growth in culture. *Science* **250**: 1262-1266.

Corey, L., Wald, A. (2009) Maternal and neonatal herpes simplex virus infections. *New England Journal of Medicine* **361**: 1376-1385.

Croughan, W.S., Behbehani, A.M. (1988) Comparative study of inactivation of herpes simplex virus types 1 and 2 by commonly used antiseptic agents. *Journal of Clinical Microbiology* **26**: 213-215.

Cunningham, A.L., Taylor, R., Taylor, J., Marks, C., Shaw, J., Mindel, A. (2006) Prevalence of infection with herpes simplex virus types 1 and 2 in Australia: a nationwide population based survey. *Sex Transm Infect* **82**: 164-168.

Daud, A.I., Mirza, N., Lenox, B., Andrews, S., Urbas, P., Gao, G.X. et al. (2008) Phenotypic and functional analysis of dendritic cells and clinical outcome in patients with high-risk melanoma treated with adjuvant granulocyte macrophage colony-stimulating factor. *Journal of Clinical Oncology* **26**: 3235-3241.

Davis, T.A., Monroy, R.L., Skelly, R.R., Donahue, R.E., MacVittie, T.J. (1990) Differential augmentation of in vivo natural killer cytotoxicity in normal primates with recombinant human interleukin-1 and granulocyte-macrophage colony-stimulating factor. *Clinical and Experimental Immunology* **79**: 436-442.

de Matos, R., Russell, D., Van, A.W., Miller, A. (2014) Spontaneous fatal Human herpesvirus 1 encephalitis in two domestic rabbits (Oryctolagus cuniculus). *J Vet Diagn Invest* **26**: 689-694.

DeFillipis, V.R., Villarreal, L.P. (2001) Chapter 13: *Virus Evolution*. In: Knipe, D.M., Howley P.M., eds. *Fields Virology*, Edition 4th. Lippincott Williams and Wilkins Philadelphia. 353-370.

DeLuca, N.A., Schaffer, P.A. (1985) Activation of immediate-early, early, and late promoters by temperature-sensitive and wild-type forms of herpes simplex virus type 1 protein ICP4. *Mol Cell Biol* **5**: 1997-2008.

Douglas, M.W., Walters, J.L., Currie, B.J. (2002) Occupational infection with herpes simplex virus type 1 after a needlestick injury. *Medical Journal of Australia* **176**: 240.

Dranoff, G., Jaffee, E., Lazenby, A., Golumbek, P., Levitsky, H., Brose, K. et al. (1993) Vaccination with irradiated tumor cells engineered to secrete murine granulocyte-macrophage colony-stimulating factor stimulates potent, specific, and long-lasting anti-tumor immunity. *Proc Natl Acad Sci U S A* **90**: 3539-3543.

Drew, W.L. (2004) Herpesviruses. In: Ryan, K.J., Ray C.G., eds. *Sherris medical microbiology: An introduction to infectious diseases*, Edition 4. McGraw Hill. 555-576.

Epstein, J.H., Price, J.T. (2009) The significant but understudied impact of pathogen transmission from humans to animals. *Mount Sinai Journal of Medicine* **76**: 448-455.

Foecking, M.K., Hofstetter, H. (1986) Powerful and versatile enhancer-promoter unit for mammalian expression vectors. *Gene* **45**: 101-105.

Goldsmith, K., Chen, W., Johnson, D.C., Hendricks, R.L. (1998) Infected cell protein (ICP)47 enhances herpes simplex virus neurovirulence by blocking the CD8+ T cell response. *Journal of Experimental Medicine* **187**: 341-348.

Goodwin, E.C., Rottman, F.M. (1992) The 3'-flanking sequence of the bovine growth hormone gene contains novel elements required for efficient and accurate polyadenylation. *Journal of Biological Chemistry* **267**: 16330-16334.

Green, L.K., Pavan-Langston, D. (2006) Herpes simplex ocular inflammatory disease. *Int Ophthalmol Clin* **46**: 27-37.

Grest, P., Albicker, P., Hoelzle, L., Wild, P., Pospischil, A. (2002) Herpes simplex encephalitis in a domestic rabbit (Oryctolagus cuniculus). *J Comp Pathol* **126**: 308-311.

Harrington, K.J., Hingorani, M., Tanay, M.A., Hickey, J., Bhide, S.A., Clarke, P.M. et al. (2010) Phase I/II study of oncolytic HSV GM-CSF in combination with radiotherapy and cisplatin in untreated stage III/IV squamous cell cancer of the head and neck. *Clinical Cancer Research* **16**: 4005-4015.

Harrow, S., Papanastassiou, V., Harland, J., Mabbs, R., Petty, R., Fraser, M. et al. (2004) HSV1716 injection into the brain adjacent to tumour following surgical resection of high-grade glioma: safety data and long-term survival. *Gene Therapy* **11**: 1648-1658.

He, B., Chou, J., Brandimarti, R., Mohr, I., Gluzman, Y., Roizman, B. (1997) Suppression of the phenotype of gamma(1)34.5- herpes simplex virus 1: failure of activated RNA-dependent protein kinase to shut off protein synthesis is associated with a deletion in the domain of the alpha47 gene. *Journal of Virology* **71**: 6049-6054.

Heldwein, E.E., Krummenacher, C. (2008) Entry of herpesviruses into mammalian cells. *Cell Mol Life Sci* **65**: 1653-1668.

Holman, H.A., MacLean, A.R. (2008) Neurovirulent factor ICP34.5 uniquely expressed in the herpes simplex virus type 1 Delta gamma 1 34.5 mutant 1716. *Journal of Neurovirology* **14**: 28-40.

Hu, J.C., Coffin, R.S., Davis, C.J., Graham, N.J., Groves, N., Guest, P.J. et al. (2006) A phase I study of OncoVEXGM-CSF, a second-generation oncolytic herpes simplex virus expressing granulocyte macrophage colony-stimulating factor. *Clinical Cancer Research* **12**: 6737-6747.

Huang, A.Y., Golumbek, P., Ahmadzadeh, M., Jaffee, E., Pardoll, D., Levitsky, H. (1994) Role of bone marrow-derived cells in presenting MHC class I-restricted tumor antigens. *Science* **264**: 961-965.

Huemer, H.P., Larcher, C., Czedik-Eysenberg, T., Nowotny, N., Reifinger, M. (2002) Fatal infection of a pet monkey with Human herpesvirus. *Emerging Infectious Diseases* **8**: 639-642.

Hunter, W.D., Martuza, R.L., Feigenbaum, F., Todo, T., Mineta, T., Yazaki, T. et al. (1999) Attenuated, replication-competent herpes simplex virus type 1 mutant G207: safety evaluation of intracerebral injection in nonhuman primates. *Journal of Virology* **73**: 6319-6326.

Huppatz, C., Durrheim, D.N., Levi, C., Dalton, C., Williams, D., Clements, M.S. et al. (2009) Etiology of encephalitis in Australia, 1990-2007. *Emerging Infectious Diseases* **15**: 1359-1365.

Imura, K., Chambers, J.K., Uchida, K., Nomura, S., Suzuki, S., Nakayama, H. et al. (2014) Herpes simplex virus type 1 infection in two pet marmosets in Japan. *Journal of Veterinary Medical Science* **76**: 1667-1670.

Jager, E., Ringhoffer, M., Dienes, H.P., Arand, M., Karbach, J., Jager, D. et al. (1996) Granulocyte-macrophage-colony-stimulating factor enhances immune responses to melanoma-associated peptides in vivo. *International Journal of Cancer* **67**: 54-62.

Jerome, K.R., Morrow, R.A. (2007) Herpes simplex viruses and Herpes B virus. In: Murray, P.R., ed. *Manual of clinical microbiology* , Edition 9. ASM Press Washington, D.C. 1523-1536.

Jin, L., Peng, W., Perng, G.C., Brick, D.J., Nesburn, A.B., Jones, C. et al. (2003) Identification of herpes simplex virus type 1 latency-associated transcript sequences that both inhibit apoptosis and enhance the spontaneous reactivation phenotype. *Journal of Virology* **77**: 6556-6561.

Jing, X., Cerveny, M., Yang, K., He, B. (2004) Replication of herpes simplex virus 1 depends on the gamma 134.5 functions that facilitate virus response to interferon and egress in the different stages of productive infection. *Journal of Virology* **78**: 7653-7666.

Kahl, G. (2001) *The dictionary of gene technology: genomics, transcriptomics, proteomics.* Wiley-VCH, Weinheim, Germany.

Keese, P. (2008) Risks from GMOs due to horizontal gene transfer. *Environmental Biosafety Research* **7**: 123-149.

Kesari, S., Lasner, T.M., Balsara, K.R., Randazzo, B.P., Lee, V.M., Trojanowski, J.Q. et al. (1998) A neuroattenuated ICP34.5-deficient herpes simplex virus type 1 replicates in

ependymal cells of the murine central nervous system. *Journal of General Virology* **79 (Pt 3)**: 525-536.

Kramer, A., Schwebke, I., Kampf, G. (2006) How long do nosocomial pathogens persist on inanimate surfaces? A systematic review. *BMC Infect Dis* **6**: 130.

Lamb, D.J., Zhang, L. (2005) Challenges in prostate cancer research: animal models for nutritional studies of chemoprevention and disease progression. *Journal of Nutrition* **135**: 3009S-3015S.

Landolfi, J.A., Wellehan, J.F., Johnson, A.J., Kinsel, M.J. (2005) Fatal human herpesvirus type 1 infection in a white-handed gibbon (Hylobates lar). *J Vet Diagn Invest* **17**: 369-371.

Lee, F., Yokota, T., Otsuka, T., Gemmell, L., Larson, N., Luh, J. et al. (1985) Isolation of cDNA for a human granulocyte-macrophage colony-stimulating factor by functional expression in mammalian cells. *Proc Natl Acad Sci U S A* **82**: 4360-4364.

Lee, S.-W., Markham, P.F., Coppo, M.J.C., Legione, A.R., Markham, J.F., Noormohammadi, A.H. et al. (2012) Attenuated Vaccines Can Recombine to Form Virulent Field Viruses. *Science* **337**: 188.

Lefaux, B., Duprez, R., Tanguy, M., Longeart, L., Gessain, A., Boulanger, E. (2004) Nonhuman primates might be highly susceptible to cross-species infectivity by human alpha-herpesviruses. *Veterinary Pathology* **41**: 302-304.

Liu, B.L., Robinson, M., Han, Z.Q., Branston, R.H., English, C., Reay, P. et al. (2003a) ICP34.5 deleted herpes simplex virus with enhanced oncolytic, immune stimulating, and anti-tumour properties. *Gene Therapy* **10**: 292-303.

Liu, X., Tian, P.K., Ju, D.W., Zhang, M.H., Yao, M., Cao, X.T. et al. (2003b) Systemic genetic transfer of p21WAF-1 and GM-CSF utilizing of a novel oligopeptide-based EGF receptor targeting polyplex. *Cancer Gene Therapy* **10**: 529-539.

Longa, C.S., Bruno, S.F., Pires, A.R., Romijn, P.C., Kimura, L.S., Costa, C.H. (2011) Human herpesvirus 1 in wild marmosets, Brazil, 2008. *Emerging Infectious Diseases* **17**: 1308-1310.

Mace, A.T., Ganly, I., Soutar, D.S., Brown, S.M. (2008) Potential for efficacy of the oncolytic Herpes simplex virus 1716 in patients with oral squamous cell carcinoma. *Head and Neck* **30**: 1045-1051.

MacKie, R.M., Stewart, B., Brown, S.M. (2001) Intralesional injection of herpes simplex virus 1716 in metastatic melanoma. *Lancet* **357**: 525-526.

MacLean, A.R., ul-Fareed, M., Robertson, L., Harland, J., Brown, S.M. (1991) Herpes simplex virus type 1 deletion variants 1714 and 1716 pinpoint neurovirulence-related sequences in Glasgow strain 17+ between immediate early gene 1 and the 'a' sequence. *Journal of General Virology* **72 (Pt 3)**: 631-639.

Mahl, M.C., Sadler, C. (1975) Virus survival on inanimate surfaces. *Canadian Journal of Microbiology* **21**: 819-823.

Mahony, T.J., Smith, G.A., Thomson, D.M. (1999) Macropodid herpesviruses 1 and 2 occupy unexpected molecular phylogenic positions within the Alphaherpesvirinae. *Journal of General Virology* **80 (Pt 2)**: 433-436.

Maliszewski, C.R., Schoenborn, M.A., Cerretti, D.P., Wignall, J.M., Picha, K.S., Cosman, D. et al. (1988) Bovine GM-CSF: molecular cloning and biological activity of the recombinant protein. *Molecular Immunology* **25**: 843-850.

Manian, F.A. (2000) Potential role of famciclovir for prevention of herpetic whitlow in the health care setting. *Clinical Infectious Diseases* **31**: E18-E19.

Markert, J.M., Medlock, M.D., Rabkin, S.D., Gillespie, G.Y., Todo, T., Hunter, W.D. et al. (2000) Conditionally replicating herpes simplex virus mutant, G207 for the treatment of malignant glioma: results of a phase I trial. *Gene Therapy* **7**: 867-874.

Martuza, R.L., Malick, A., Markert, J.M., Ruffner, K.L., Coen, D.M. (1991) Experimental therapy of human glioma by means of a genetically engineered virus mutant. *Science* **252**: 854-856.

Mayer, P., Werner, F.J., Lam, C., Besemer, J. (1990) In vitro and in vivo activity of human recombinant granulocyte-macrophage colony-stimulating factor in dogs. *Experimental Hematology* **18**: 1026-1033.

McGeoch, D.J., Cook, S., Dolan, A., Jamieson, F.E., Telford, E.A. (1995) Molecular phylogeny and evolutionary timescale for the family of mammalian herpesviruses. *Journal of Molecular Biology* **247**: 443-458.

McGeoch, D.J., Rixon, F.J., Davison, A.J. (2006) Topics in herpesvirus genomics and evolution. *Virus Research* **117**: 90-104.

McKie, E.A., Brown, S.M., MacLean, A.R., Graham, D.I. (1998) Histopathological responses in the CNS following inoculation with a non-neurovirulent mutant (1716) of herpes simplex virus type 1 (HSV 1): relevance for gene and cancer therapy. *Neuropathology and Applied Neurobiology* **24**: 367-372.

Meignier, B., Longnecker, R., Roizman, B. (1988) In vivo behavior of genetically engineered herpes simplex viruses R7017 and R7020: construction and evaluation in rodents. *Journal of Infectious Diseases* **158**: 602-614.

Mester, J.C., Rouse, B.T. (1991) The mouse model and understanding immunity to herpes simplex virus. *Reviews of Infectious Diseases* **13 Suppl 11**: S935-S945.

Miller, C.G., Krummenacher, C., Eisenberg, R.J., Cohen, G.H., Fraser, N.W. (2001) Development of a syngenic murine B16 cell line-derived melanoma susceptible to destruction by neuroattenuated HSV-1. *Mol Ther* **3**: 160-168.

Mohr, I., Gluzman, Y. (1996) A herpesvirus genetic element which affects translation in the absence of the viral GADD34 function. *EMBO Journal* **15**: 4759-4766.

Mohr, I., Sternberg, D., Ward, S., Leib, D., Mulvey, M., Gluzman, Y. (2001) A herpes simplex virus type 1 gamma34.5 second-site suppressor mutant that exhibits enhanced growth in cultured glioblastoma cells is severely attenuated in animals. *Journal of Virology* **75**: 5189-5196.

Muller, K., Fuchs, W., Heblinski, N., Teifke, J.P., Brunnberg, L., Gruber, A.D. et al. (2009) Encephalitis in a rabbit caused by human herpesvirus-1. *Journal of the American Veterinary Medical Association* **235**: 66-69.

Mutsafi, Y., Zauberman, N., Sabanay, I., Minsky, A. (2010) Vaccinia-like cytoplasmic replication of the giant Mimivirus. *Proc Natl Acad Sci U S A* **107**: 5978-5982.

NCCTG (2011). Canberra ACT,National Coordinating Committee on Therapeutic Goods. Australian code of good wholesaling practice for medicines in schedules 2, 3, 4 & 8. Commonwealth of Australia Goverment Printer

Nemunaitis, J., Singer, J.W., Buckner, C.D., Mori, T., Laponi, J., Hill, R. et al. (1991) Long-term follow-up of patients who received recombinant human granulocyte-macrophage colony stimulating factor after autologous bone marrow transplantation for lymphoid malignancy. *Bone Marrow Transplantation* **7**: 49-52.

Nerurkar, L.S., West, F., May, M., Madden, D.L., Sever, J.L. (1983) Survival of herpes simplex virus in water specimens collected from hot tubs in spa facilities and on plastic surfaces. *JAMA* **250**: 3081-3083.

Norberg, P., Tyler, S., Severini, A., Whitley, R., Liljeqvist, J.A., Bergstrom, T. (2011) A genome-wide comparative evolutionary analysis of herpes simplex virus type 1 and varicella zoster virus. *PLoS One* **6**: e22527.

OGTR (2013) Risk Analysis Framework. No. Version 4, Document produced by the Australian Government Office of the Gene Technology Regulator.

Orvedahl, A., Alexander, D., Talloczy, Z., Sun, Q., Wei, Y., Zhang, W. et al. (2007) HSV-1 ICP34.5 confers neurovirulence by targeting the Beclin 1 autophagy protein. *Cell Host Microbe* **1**: 23-35.

Papanastassiou, V., Rampling, R., Fraser, M., Petty, R., Hadley, D., Nicoll, J. et al. (2002) The potential for efficacy of the modified (ICP 34.5(-)) herpes simplex virus HSV1716 following intratumoural injection into human malignant glioma: a proof of principle study. *Gene Therapy* **9**: 398-406.

Pellett, P.E., Roizman, B. (2007) The Family: Herpesviridae a Brief Introduction. In: Knipe, D.M., Howley P.M., Griffin D.E., Lamb R.A., Martin M.A., Roizman B., Straus S.E., eds. *Fields Virology*, Edition 5. Lippincott, Williams, and Wilkins Philadelphia, PA. 2479-2500.

Perng, G.C., Ghiasi, H., Slanina, S.M., Nesburn, A.B., Wechsler, S.L. (1996) High-dose ocular infection with a herpes simplex virus type 1 ICP34.5 deletion mutant produces no corneal disease or neurovirulence yet results in wild-type levels of spontaneous reactivation. *Journal of Virology* **70**: 2883-2893.

Perng, G.C., Thompson, R.L., Sawtell, N.M., Taylor, W.E., Slanina, S.M., Ghiasi, H. et al. (1995) An avirulent ICP34.5 deletion mutant of herpes simplex virus type 1 is capable of in vivo spontaneous reactivation. *Journal of Virology* **69**: 3033-3041.

Pinnoji, R.C., Bedadala, G.R., George, B., Holland, T.C., Hill, J.M., Hsia, S.C. (2007) Repressor element-1 silencing transcription factor/neuronal restrictive silencer factor (REST/NRSF) can regulate HSV-1 immediate-early transcription via histone modification. *Virol J* **4**: 56.

Piret, J., Boivin, G. (2011) Resistance of herpes simplex viruses to nucleoside analogues: mechanisms, prevalence, and management. *Antimicrobial Agents and Chemotherapy* **55**: 459-472.

Poisons Standard (2015). *Standard for the Uniform Scheduling of Medicines and Poisons* (*SUSMP*). known as the Poisons Standard 2015, made under paragraph 52D(2)(b) of the *Therapeutic Goods Act 1989*.

Prince, H.N., Prince, D.L. (2001) Principles of viral control and transmission. In: Block, S.S., ed. *Disinfection, sterilization and preservation* , Edition 5. Lippincott Williams & Wilkins Philadelphia, PA. 543-571.

Ragnhammar, P., Friesen, H.J., Frodin, J.E., Lefvert, A.K., Hassan, M., Osterborg, A. et al. (1994) Induction of anti-recombinant human granulocyte-macrophage colony-stimulating factor (Escherichia coli-derived) antibodies and clinical effects in nonimmunocompromised patients. *Blood* **84**: 4078-4087.

Rajcani, J., Andrea, V., Ingeborg, R. (2004) Peculiarities of herpes simplex virus (HSV) transcription: an overview. *Virus Genes* **28**: 293-310.

Rampling, R., Cruickshank, G., Papanastassiou, V., Nicoll, J., Hadley, D., Brennan, D. et al. (2000) Toxicity evaluation of replication-competent herpes simplex virus (ICP 34.5 null mutant 1716) in patients with recurrent malignant glioma. *Gene Therapy* **7**: 859-866.

Revello, M.G., Gerna, G. (2002) Diagnosis and management of human cytomegalovirus infection in the mother, fetus, and newborn infant. *Clinical Microbiology Reviews* **15**: 680-715.

Riley, P.Y., Chomel, B.B. (2005) Hedgehog zoonoses. *Emerging Infectious Diseases* **11**: 1-5.

Rini, B., Wadhwa, M., Bird, C., Small, E., Gaines-Das, R., Thorpe, R. (2005) Kinetics of development and characteristics of antibodies induced in cancer patients against yeast expressed rDNA derived granulocyte macrophage colony stimulating factor (GM-CSF). *Cytokine* **29**: 56-66.

Robertson, L.M., MacLean, A.R., Brown, S.M. (1992) Peripheral replication and latency reactivation kinetics of the non-neurovirulent herpes simplex virus type 1 variant 1716. *Journal of General Virology* **73 (Pt 4)**: 967-970.

Roizman, B., Knipe, D.M., Whitley, R.J. (2007) Herpes simplex viruses. In: Knipe, D.M., Howley P.M., eds. *Fields Virology*, Edition 5. Wolters Kluwer / Lippincott Williams & Wilkins Philidelphia. 2501-2601.

Rowe, J.M., Andersen, J.W., Mazza, J.J., Bennett, J.M., Paietta, E., Hayes, F.A. et al. (1995) A randomized placebo-controlled phase III study of granulocyte-macrophage colony-stimulating factor in adult patients (> 55 to 70 years of age) with acute myelogenous leukemia: a study of the Eastern Cooperative Oncology Group (E1490). *Blood* **86**: 457-462.

Sato, T., Eschelman, D.J., Gonsalves, C.F., Terai, M., Chervoneva, I., McCue, P.A. et al. (2008) Immunoembolization of malignant liver tumors, including uveal melanoma, using granulocyte-macrophage colony-stimulating factor. *Journal of Clinical Oncology* **26**: 5436-5442.

Schmittel, A., Keilholz, U., Max, R., Thiel, E., Scheibenbogen, C. (1999) Induction of tyrosinase-reactive T cells by treatment with dacarbazine, cisplatin, interferon-alpha +/- interleukin-2 in patients with metastatic melanoma. *International Journal of Cancer* **80**: 39-43.

Sekulin, K., Jankova, J., Kolodziejek, J., Huemer, H.P., Gruber, A., Meyer, J. et al. (2010) Natural zoonotic infections of two marmosets and one domestic rabbit with herpes simplex

virus type 1 did not reveal a correlation with a certain gG-, gI- or gE genotype. *Clin Microbiol Infect* **16**: 1669-1672.

Senzer, N.N., Kaufman, H.L., Amatruda, T., Nemunaitis, M., Reid, T., Daniels, G. et al. (2009) Phase II clinical trial of a granulocyte-macrophage colony-stimulating factor-encoding, second-generation oncolytic herpesvirus in patients with unresectable metastatic melanoma. *Journal of Clinical Oncology* **27**: 5763-5771.

Smith, J., Thomas, S.K., Coffin, R.S., Latchman, D.S. (2003) Examination of the potential interactions between herpes simplex virus vectors and replication-competent virus *in vitro* and *in vivo*. *Gene Therapy and Regulation* **2**: 29-47.

Soiffer, R., Hodi, F.S., Haluska, F., Jung, K., Gillessen, S., Singer, S. et al. (2003) Vaccination with irradiated, autologous melanoma cells engineered to secrete granulocyte-macrophage colony-stimulating factor by adenoviral-mediated gene transfer augments antitumor immunity in patients with metastatic melanoma. *Journal of Clinical Oncology* **21**: 3343-3350.

Spear, P.G. (2004) Herpes simplex virus: receptors and ligands for cell entry. *Cell Microbiol* **6**: 401-410.

Spear, P.G., Longnecker, R. (2003) Herpesvirus entry: an update. *Journal of Virology* **77**: 10179-10185.

Spitler, L.E., Weber, R.W., Allen, R.E., Meyer, J., Cruickshank, S., Garbe, E. et al. (2009) Recombinant human granulocyte-macrophage colony-stimulating factor (GM-CSF, sargramostim) administered for 3 years as adjuvant therapy of stages II(T4), III, and IV melanoma. *Journal of Immunotherapy* **32**: 632-637.

Spivack, J.G., Fareed, M.U., Valyi-Nagy, T., Nash, T.C., O'Keefe, J.S., Gesser, R.M. et al. (1995) Replication, establishment of latent infection, expression of the latency-associated transcripts and explant reactivation of herpes simplex virus type 1 gamma 34.5 mutants in a mouse eye model. *Journal of General Virology* **76 (Pt 2)**: 321-332.

Springer, S.L., Vite, C.H., Polesky, A.C., Kesari, S., Fraser, N.W., Wolfe, J.H. (2001) Infection and establishment of latency in the dog brain after direct inoculation of a nonpathogenic strain of herpes simplex virus-1. *Journal of Neurovirology* **7**: 149-154.

Spruance, S.L., Overall, J.C., Jr., Kern, E.R., Krueger, G.G., Pliam, V., Miller, W. (1977) The natural history of recurrent herpes simplex labialis: implications for antiviral therapy. *New England Journal of Medicine* **297**: 69-75.

Standards Australia/New Zealand (2010) *Safety in laboratories - Microbiological safety and containment AS/NZS 2243.3:2010.*, Edition AS/NZS 2243.3:2010 Standards Australia/New Zealand.

Sundaresan, P., Hunter, W.D., Martuza, R.L., Rabkin, S.D. (2000) Attenuated, replication-competent herpes simplex virus type 1 mutant G207: safety evaluation in mice. *Journal of Virology* **74**: 3832-3841.

Talloczy, Z., Jiang, W., Virgin, H.W., Leib, D.A., Scheuner, D., Kaufman, R.J. et al. (2002) Regulation of starvation- and virus-induced autophagy by the eIF2alpha kinase signaling pathway. *Proc Natl Acad Sci U S A* **99**: 190-195.

Tebas, P., Stabell, E.C., Olivo, P.D. (1995) Antiviral susceptibility testing with a cell line which expresses beta-galactosidase after infection with herpes simplex virus. *Antimicrobial Agents and Chemotherapy* **39**: 1287-1291.

Thomas, D.L., Fraser, N.W. (2003) HSV-1 therapy of primary tumors reduces the number of metastases in an immune-competent model of metastatic breast cancer. *Mol Ther* **8**: 543-551.

Tischer, B.K., Osterrieder, N. (2010) Herpesviruses--a zoonotic threat? *Veterinary Microbiology* **140**: 266-270.

Umene, K. (1987) Transition from a heterozygous to a homozygous state of a pair of loci in the inverted repeat sequences of the L component of the herpes simplex virus type 1 genome. *Journal of Virology* **61**: 1187-1192.

Valyi-Nagy, T., Fareed, M.U., O'Keefe, J.S., Gesser, R.M., MacLean, A.R., Brown, S.M. et al. (1994) The herpes simplex virus type 1 strain 17+ gamma 34.5 deletion mutant 1716 is avirulent in SCID mice. *Journal of General Virology* **75 (Pt 8)**: 2059-2063.

Varghese, S., Newsome, J.T., Rabkin, S.D., McGeagh, K., Mahoney, D., Nielsen, P. et al. (2001) Preclinical safety evaluation of G207, a replication-competent herpes simplex virus type 1, inoculated intraprostatically in mice and nonhuman primates. *Human Gene Therapy* **12**: 999-1010.

Varmuza, S.L., Smiley, J.R. (1984) Unstable heterozygosity in a diploid region of herpes simplex virus DNA. *Journal of Virology* **49**: 356-362.

Wang, L., Qi, X., Sun, Y., Liang, L., Ju, D. (2002) Adenovirus-mediated combined P16 gene and GM-CSF gene therapy for the treatment of established tumor and induction of antitumor immunity. *Cancer Gene Therapy* **9**: 819-824.

Webber, C.E., Whalley, J.M. (1978) Widespread occurrence in Australian marsupials of neutralizing antibodies to a herpesvirus from a parma wallaby. *Australian Journal of Experimental Biology and Medical Science* **56**: 351-357.

Webre, J.M., Hill, J.M., Nolan, N.M., Clement, C., McFerrin, H.E., Bhattacharjee, P.S. et al. (2012) Rabbit and mouse models of HSV-1 latency, reactivation, and recurrent eye diseases. *J Biomed Biotechnol* **2012**: 612316.

Weissenbock, H., Hainfellner, J.A., Berger, J., Kasper, I., Budka, H. (1997) Naturally occurring herpes simplex encephalitis in a domestic rabbit (Oryctolagus cuniculus). *Veterinary Pathology* **34**: 44-47.

Whitley, R.J. (2006) Herpes simplex encephalitis: adolescents and adults. *Antiviral Research* **71**: 141-148.

Whitley, R.J., Kern, E.R., Chatterjee, S., Chou, J., Roizman, B. (1993) Replication, establishment of latency, and induced reactivation of herpes simplex virus gamma 1 34.5 deletion mutants in rodent models. *J Clin Invest* **91**: 2837-2843.

Whitley, R.J., Roizman, B. (2009) Herpes simplex viruses. In: Richmann, D.D., Whitley R.J., Hayden F.G., eds. *Clinical Virology*, Edition 3. ASM Press Washington. 409-436.

Wildy, P. (1955) Recombination with herpes simplex virus. *Journal of General Microbiology* **13**: 346-360.

Wohlsein, P., Thiele, A., Fehr, M., Haas, L., Henneicke, K., Petzold, D.R. et al. (2002) Spontaneous human herpes virus type 1 infection in a chinchilla (Chinchilla lanigera f. dom.). *Acta Neuropathol* **104**: 674-678.

Wood, A., Payne, D. (1998) The action of three antiseptics/disinfectants against enveloped and non-enveloped viruses. *Journal of Hospital Infection* **38**: 283-295.

World Health Organisation (WHO) (2010) World Health Organisation (WHO) Good distribution practices for pharmaceutical products, WHO Technical Report Series. No. 957, **Switzerland**.

Woychik, R.P., Camper, S.A., Lyons, R.H., Horowitz, S., Goodwin, E.C., Rottman, F.M. (1982) Cloning and nucleotide sequencing of the bovine growth hormone gene. *Nucleic Acids Research* **10**: 7197-7210.

Xu, F., Sternberg, M.R., Kottiri, B.J., McQuillan, G.M., Lee, F.K., Nahmias, A.J. et al. (2006) Trends in herpes simplex virus type 1 and type 2 seroprevalence in the United States. *JAMA* **296**: 964-973.

Appendix A Summary of advice from prescribed experts, agencies and authorities on matters relevant to the preparation of the consultation RARMP[10]

The Regulator received a number of submissions from prescribed experts, agencies and authorities on matters considered relevant to the preparation of the consultation RARMP. All issues raised in submissions relating to risks to the health and safety of people and the environment were considered. The issues raised, and how they are addressed in the consultation RARMP, are summarised below.

Summary of issues raised	Comment
Acknowledges receipt of notification and makes no further comments.	Noted.
Council has no objection to the proposal.	Noted.
Notes that Council has declared its district a GMO free area and makes no further comments.	The object of the Act is to protect human health and safety and the environment by identifying and managing risks posed by or as a result of gene technology. The Regulator must decide to issue or refuse a licence based on consideration relevant to the object.
Assumes that control mechanisms, risk tolerability and legal compliance have been addressed by those holding the necessary competencies.	Regulator prepares RARMP according to the requirements of the Act and following the Risk Analysis Framework. As required by section 50(3) of the Act, the Regulator has consulted with prescribed experts, agencies and authorities to seek advice on matters relevant to the preparation of the RARMP, and will consult the same groups and the public on the RARMP that has been prepared. Submission relating to human health and the environment will be taken into consideration in making a decision on the application. TGA approval will also be required for the proposed use of the GMO as a treatment for certain cancers. TGA will address quality, patient safety and efficacy of the GMO as a therapeutic good.
Expect risk evaluation associated with GMO on environmental risk, human risk and risk to neighbouring flora and fauna arising from growth, harvest, transport, processing and consumption/use to be validated by subject matter experts.	Risk to people and the environment have been assessed. Prescribed experts, agencies and authorities and the public will be consulted on the RARMP before making a decision on the application.
If the GMO was to be grown, harvested and transported within the local government area, Council would expect the community to be consulted with prior to commencing the project. Consultation should clearly articulate purpose, what the risks are if any, how these have been evaluated and controlled, the duration of the project and an opportunity for feedback.	The GMO is proposed to be manufactured overseas and imported into Australia in single use vials. Transport will be according to appropriate standards for medical products. Prescribed authorities, including local councils, and the public will be consulted on the RARMP before making a decision on the application.
Full support for any further science, research or gene technology advances that will ultimately reduce cancer rates.	Noted.

[10] Prescribed agencies include GTTAC, State and Territory Governments, relevant local governments, Australian government agencies and the Minister for the Environment

Summary of issues raised	Comment
Has sought input from the Toxicology unit of the Department of Health and have also copied in Saint John of God Hospital Geraldton, and will refer any response given with permission. [No further response received.]	Noted.
Expressed concern about GMO being able to spread like a normal virus or to become a pathogenic organism.	The potential of the GM virus to be spread and contribute to disease has been evaluated in detail. The RARMP concludes that the risk is negligible. Reasons for this assessment include that exposure would be minimal and the GM virus is significantly attenuated compared to the parent virus (which is widespread in the Australian environment), having reduced ability to replicate, transmit or persist.
Request forwarded to the respective departments within council for investigation, and a response will be issued accordingly. [No further response received.]	Noted.
Is the modification of this virus likely to change the virulence of the general group of Herpes virus such that treatment of cold sores and genital herpes becomes more difficult?	The GM virus retains the capability to be treated with nucleoside analogue acyclovir. The RARMP concludes that the risk from the proposed dealings is negligible. Reasons for this assessment include that exposure would be minimal and the GM virus is significantly attenuated compared to the parent virus (which is widespread in the Australian environment), having reduced ability to replicate, transmit or persist.
The modified virus is unable to replicate in non-dividing cells. Skin and mucosa do contain dividing cells, therefore is it likely that a cross over into these areas of the body could occur from the treatment site?	
Human herpes simplex virus belongs to the same family of viruses as the feline and canine herpes viruses and the RARMP should consider the potential of the modified virus to be transmitted to a wider host range.	The potential of the GM virus to spread to unintended hosts (e.g. domestic pets) was evaluated in the RARMP, which concludes that risk from the proposed dealings is negligible.
Product is well characterised and evaluated. It appears to be low risk and can be controlled by drug therapy - immunocompromised individuals are the only human risk group.	Noted.
How host specific is the GMO and can domestic pets become infected with the modified virus?	The potential of the GM virus to spread to unintended hosts (e.g. domestic pets) was evaluated in the RARMP, which concludes that risk from the proposed dealings is negligible.
Sees no technical problems with the release of this GMO from an IBC view. Notes that TGA approval will be the key issue in terms of actual use in Australia.	Noted.
Herpesviruses have the capacity to form latent infections - the capacity or lack of capacity for the modified HSV-1 (Talimogene laherparepvec) to form latent infections or capacity to reactivate from latency if it occurs.	Infection with the GM virus could potentially lead to the establishment of latency, however deletion of the ICP34.5 gene prevents efficient replication in non-dividing cells and reduces neurovirulence therefore the likelihood of developing a latent infection is significantly reduced compared to the parent virus.
In the quoted study 4648-00024, the parent virus and the GMO had IC50 to acyclovir of 0.22ug/ml and 0.39ug/ml respectively. Why the difference?	The sensitivity of GM virus to the nucleoside analogue acyclovir has been tested over a range of analogue concentrations from 100 µg/mL to 0.05 µg/mL and at two different concentrations of the GM virus in a plaque reduction assay. The difference on concentrations highlighted is not significant considering that the sensitivity of HSV-1 to acyclovir without the viral TK gene is ~50-100 fold higher.

Summary of issues raised	Comment
Have the applicants reisolated Talimogene laherparepvec from treated patients? If so have these viruses been tested for nucleotide analogue sensitivity?	The GM virus has been isolated from a limited number of swab samples from treated patients. Based on the small sample size, and unlikeliness of loss of nucleoside analogue sensitivity, Amgen has not tested these isolates for nucleoside analogue sensitivity.
Pathways to harm that relate to environmental release, persistence, gene transfer and host range should be considered in the RARMP [as detailed below].	Noted
Environmental release during transport. Application states it is not an infectious agent and is therefore not an IATA-regulated material, increasing likelihood of environmental exposure.	Transport will be according to appropriate standards for medical products. Amgen has specified that the GM virus would be packaged in single-dose vials within a secure secondary packaging carton. Amgen proposes the International Air Transport Association (IATA) shipping classification for the GM virus as Genetically Modified Micro-Organism, UN Number: 3245.
Environmental release after treatment through excretion from patients.	The published clinical trials found transient and limited viral shedding of the GM virus from the injection site and the urine, and GM virus shedding was not routinely detectable after more than a few days post administration. The GM virus was not detected outside the occlusive dressing of treated patients in the clinical trials. This issue will be considered by the TGA in their assessment of GMO as a therapeutic good.
Environmental release from inappropriate or accidental disposal of waste material contaminated with GM virus following administration to patients.	The RARMP concludes that the risk associated with waste disposal is negligible, including because proposed disposal methods will minimise exposure, and the GM virus is significantly attenuated compared to the parent virus (which is widespread in the Australian environment), having reduced ability to replicate, transmit or persist.
Broad host range of the GM virus. Minor genetic changes can result in dramatic changes in host spectrum compared to the unmodified virus.	The potential of the GM virus to spread to unintended hosts was evaluated in detail. Human and animal trials involving the GM virus, and other viruses with similar genetic modifications, have not demonstrated unexpected changes in the characteristics of the GM viruses resulting from the introduced genes or genetic modifications. The RARMP concludes that this risk is negligible.
HSV-1 can survive for up to eight weeks outside of the host.	Proposed disposal methods will minimise exposure, and the GM virus is significantly attenuated compared to the parent virus (which is widespread in the Australian environment), having reduced ability to replicate, transmit or persist.
When the GM virus is broken down in the environment, the naked DNA can be released, making horizontal gene transfer of the GM virus possible.	The parent virus and the introduced gene are widespread in the environment are already available for horizontal gene transfer from these natural sources.
The Regulator should consider the evidence for potential routes of accidental exposure and potential risks, including in relation to clinical waste.	The GM virus will be packaged into a secure secondary packaging carton. Transport will be according to appropriate standards for medical products. Transmission of the GM virus via viral shedding will be minimised through the route of inoculation (intratumoural), bandaging of the injection site and appropriate training of healthcare workers, patients and caregivers. At clinical sites, waste would be disposed of according to standard practice for infectious material. The GM virus is significantly attenuated compared to the parent virus (which is widespread in the Australian environment), having reduced ability to replicate and spread.

Summary of issues raised	Comment
The Regulator should consider the potential for recombination with other viruses	The introduced gene in the GM virus is a human gene and therefore widespread in the environment. The remainder of the GM virus is derived from a natural isolate of HSV-1, which is widespread in the environment. The reduced capacity of the GM virus to replicate and spread will limit potential for co-infection and recombination. Even if recombination were to occur, the resulting virus would not be more pathogenic than naturally occurring HSV-1. Therefore the risk from recombination with other viruses is considered to be negligible.
Notes role of TGA in assessment and potential commercial approval of the GMO as a therapeutic, including interactions with other immune treatments, and the provisions for reciprocal advice in the respective assessment processes	Noted

Appendix B Summary of advice from prescribed experts, agencies and authorities on the consultation RARMP[11]

The Regulator received several submissions from prescribed experts, agencies and authorities on the consultation RARMP. All issues raised in submissions that related to risks to the health and safety of people and the environment were considered in the context of the currently available scientific evidence and were used in finalising the RARMP that formed the basis of the Regulator's decision to issue the licence. Advice received is summarised below.

Abbreviations: **Act**: *Gene Technology Act 2000*; **Ch**: chapter; **GM**: genetically modified; **HSV**: *Herpes simplex virus*; **RARMP**: Risk Assessment and Risk Management Plan; **TGA**: Therapeutic Goods Administration.

Summary of issues raised	**Comment**
Noted the regulatory information sufficiently covers the needs of inquiry; not able to provide any further specific feedback.	Noted.
Indicated that a condition should be applied to ensure that waste generated from the use of the GMO should be disposed of appropriately.	The RARMP concludes that the risk associated with waste disposal is negligible, see Risk Scenario 2. The GM virus is significantly attenuated compared to the parent virus (which is widespread in the Australian environment), having reduced ability to replicate and decreased potential for transmission or persistence. The proposed disposal methods, to be communicated to clinical staff and patients via the product documentation, will minimise exposure and are considered appropriate.
No objection to the issuance of a licence or to the proposed licence conditions for DIR-132.	Noted.
Indicated that the Council recommends that local health professionals should be consulted.	In addition to consultation with State and Territory Governments, prescribed Australian Government agencies, relevant local councils and the Gene Technology Technical Advisory Committee, a call for submissions on the RARMP was advertised in *The Australian*, the *Australian Government Gazzette* and the OGTR website, as well as sent directly to people and organisations registered with the OGTR to receive such notifications.
No concerns for the health and safety of people or the environment with the proposed management and control measures. Commented that the statement "like HSV-1, the GMO is not able to infect other animals" in the "Questions & Answers" document is not correct. Some detail should be provided.	To more accurately reflect the RARMP, the Q&A in relation to the decision on this application that humans are the only known natural hosts of HSV 1, so the GMO is extremely unlikely to infect animals.

[11] Prescribed agencies include GTTAC, State and Territory Governments, relevant local governments, Australian Government agencies and the Minister for the Environment.

Summary of issues raised	Comment
Noting that the GMO is shed by some patients, and information on the stability of HSV-1 in the environment, disposal of patient dressings into household waste needs further consideration.	Clinical trials for the GM virus found transient and limited viral shedding from the injection site in some patients. The GM virus was not detected outside the occlusive dressing. Patients would be provided with information on how to mitigate the risk of transmission, including disposal of dressings. HSV-1 is short-lived under moist conditions, and the GM virus is significantly attenuated compared to the parent virus, having reduced ability to replicate and decreased potential for transmission or persistence. For these reasons, the RARMP concludes that the risk associated with waste disposal is negligible.
Considered that the ability of virus to survive outside host and the potential of shedding of the virus through disposal of dressing containing GM virus into household waste poses a risk to the environment which needs further consideration.	
Noted that the GM virus was detected in the blood of multiple patients, suggesting that infection is not always localised to the injection area. Thus it is possible that the GM virus and naturally occurring HSV-1 will infect the same cell, providing a feasible route for viral recombination, potentially forming strains with increased virulence and ultimately increasing the disease burden of HSV-1.	The GM virus could only be detected transiently (within few days post-injection) in blood of a few patients, most likely due to the high level of GM virus injected into the tumours at the time of treatment. The ability of the GM virus to undergo recombination, potentially forming strains with increased virulence was examined in Risk Scenario 5. Additional discussion has been added to reflect this consideration. The potential for an adverse outcome as a result of viral recombination was not identified as a substantive risk. The safety of the GM virus as a human therapeutic will be assessed by the TGA.
While herpes viruses are generally very host-specific, infection of other species can occur with serious consequences. While the RARMP states that there are no reports of naturally occurring HSV-1 in domestic animals, the same point should be addressed for native Australian animals.	The potential of the GM virus to spread to unintended hosts was evaluated in detail. The RARMP has been updated to recognise potential exposure of the GM virus to native Australian animals. The GM virus is significantly attenuated compared to the parent virus (which is widespread in the Australian environment), having reduced ability to replicate and decreased potential for transmission or persistence. The RARMP concludes that the risk of transmission to unintended hosts is negligible.
Council is grateful for opportunity to comment. Understands TGA will be examining safety of the GMO relating to patients. Trusts the RARMP will clarify the potential risk the GMO poses to public health and the environment as being negligible based on proper and rigorous scientific assessment.	A Risk Assessment and Risk Management Plan (RARMP) for this application was prepared by the Regulator in accordance with requirements of the *Gene Technology Act 2000* (the Act) and corresponding state and territory legislation. The risk assessment concludes that the commercial supply of GM virus poses negligible risks to the health and safety of people or the environment as a result of gene technology.
Not supplying a response as GMO is a vaccine and is not food related.	Noted.
Supportive of the application as the RARMP indicates that the proposed release poses negligible risks to people or the environment. Notes that approval is required from the Therapeutic Goods Administration for use as a therapeutic product and the Australian Department of Agriculture for importation.	Noted.
Agreed with the overall conclusions of the RARMP that the proposed GMO dealings pose negligible risk to the health and safety of people and the environment.	Noted.
Suggested clarification in the RARMP of background information and control measures regarding latency and containment of the GMO be considered.	The RARMP has been updated to address specific aspects of the background information, the GM virus' potential for latency and containment.

Appendix C Summary of submissions from the public on the consultation RARMP

The Regulator received one submission from the public on the application, and none on the consultation RARMP. The issues raised in this submission are summarised in the table below. All issues raised in the submission that related to risks to the health and safety of people and the environment were considered in the context of currently available scientific evidence in finalising the RARMP that formed the basis of the Regulator's decision to issue the licence.

Abbreviations: **Act**: *Gene Technology Act 2000*; **Ch**: chapter; **GM**: genetically modified; **HSV**: *Herpes simplex virus*; **RARMP**: Risk Assessment and Risk Management Plan; **TGA**: Therapeutic Goods Administration.

Issues raised: **B**: Benefits; **C**: consultation; **D**: data availability/quality; **E**: environment; **Hhs**: human health and safety; **Rf**: regulatory framework; **T**: Transparency.

Issue	Summary of issues raised	Comment
T	Concern over redacted details in public version of application.	Under Section 54 of the *Gene Technology Act 2000* (the Act), and the *Privacy Act 1988,* confidential commercial information, relevant convictions and personal details in application DIR-132 have been redacted. However, the redacted information has been considered by the Regulator during the evaluation of the application and has been made available to the prescribed authorities consulted on this application.
D	Why is Part 17 ['Additional information – live GM vaccine for use in animals'] of the application not completed? Suggest that this GMO should be classified as a "live gm vaccine" for injection into humans.	For Part 17 of the DIR application states, "You must only respond to this part if you are proposing to deal with a GMO that is a live vaccine for use in animals." Application DIR-132 relates to a GMO which is not a vaccine, and is intended for use in humans and not animals (the term "animals" as defined in the Gene Technology Regulations, 2001 does not include humans). Note that TGA has regulatory responsibility for quality, efficacy and safety of therapeutic products. A separate application has been submitted by Amgen to TGA, addressing their specific data requirements.
E; Hhs	Concern related to human health and safety and the environment, as detailed below.	A Risk Assessment and Risk Management Plan (RARMP) for this application was prepared by the Regulator in accordance with requirements of the Act and corresponding state and territory legislation. The RARMP was finalised following consultation with State and Territory Governments, prescribed Australian Government agencies (including the TGA), relevant local councils and the public. The Gene Technology Technical Advisory Committee (GTTAC) were also consulted in the preparation and finalisation of the RARMP. The risk assessment concludes that the commercial supply of GM virus poses negligible risks to the health and safety of people or the environment as a result of gene technology.
Hhs	Risk of GM virus infection for healthcare workers, family members of patients and the general public.	The potential of the GM virus to be spread and contribute to disease has been evaluated in detail. The RARMP concludes that the risk is negligible. Exposure would be minimal as the GM virus is significantly attenuated compared to naturally occurring HSV-1 (which is widespread in the Australian environment), having reduced ability to replicate and decreased potential for transmission or persistence. Consequences of exposure would also be minimised for the same reasons. The GMO cannot be used as a therapeutic until it is approved by the TGA, which has regulatory responsibility for quality, efficacy and safety of therapeutic products.

Issue	Summary of issues raised	Comment
Hhs	Concern regarding ability of the GMO to exhibit latency and persist in an infected individual.	Infection with the GM virus could potentially lead to the establishment of latency, however deletion of the ICP34.5 gene prevents efficient replication in non-dividing cells and reduces neurovirulence. As neurons are the site of latent infection, the likelihood of developing a latent infection is therefore significantly reduced compared to the parent virus. In the clinical studies conducted, no patient developed symptoms suggestive of infection of the central nervous system. See also above response.
T	Questions why specific data related to shedding of the GMO provided in the application has not been provided in the public version of the application.	Some details relating to the clinical studies are under consideration as Confidential Commercial Information under section 185 of the Act, as the reports provide commercially sensitive information. All confidential information has been made available to the prescribed experts and agencies that were consulted on this application. Clinical trials found transient and limited viral shedding of the GM virus from the injection site and the urine, and GM virus shedding was not routinely detectable after more than a few days post administration. The GM virus was not detected outside the occlusive dressing of treated patients. This has been considered in the RARMP and will be further considered by the TGA in their assessment of GMO as a human therapeutic.
D	Concern regarding the ability of the GMO to be more virulent than wild type HSV-1 in rapidly diving cells, including normal cells (e.g. reproductive cells, skin cells, blood cells progenitors, gastrointestinal cells) due to the introduced genes. The most obvious concern is expectant mothers, due to dividing cells in the foetus, and children. Suggests the gene technology regulator should require data from animal model studies showing the rates of spontaneous abortions and birth defects in infected animals.	The GMO has been modified by removing specific viral genes involved in neurovirulence and viral antigen presentation. The GMO is attenuated for replication and is not as virulent as wild type HSV-1. There is no evidence, nor reason to expect, that the GMO will be more virulent in rapidly dividing cells than naturally occurring HSV-1. The Regulator has imposed licence conditions to ensure that there is ongoing oversight of the release, including an obligation to report any unintended effects. The GMO cannot be used as a therapeutic until it is approved by the TGA, which has regulatory responsibility for quality, efficacy and safety of therapeutic products. The TGA will consider relevant data in their assessment. A number of clinical studies, as well as non-clinical studies in various model systems (including *in vitro* human cell culture, mouse, mouse xenograft and mouse tumour systems), have been conducted by the Amgen to address safety, biodistribution, and biological activity.
Hhs, Rf	Concern regarding the applicant's intention to monitor pregnant and lactating mothers receiving the GM virus post-market, and not prior to approval.	The GMO cannot be used as a therapeutic until it is approved by the TGA, which has regulatory responsibility for quality, efficacy and safety of therapeutic products.
Hhs	Concern regarding potential for autoimmune-like conditions to develop over time in infected individuals. Normal antigens linked with viral antigens may be targeted by the immune response.	The GM virus functions to improve the presentation of both viral and tumour antigens in the infected tumour cells, and to direct cytotoxic activity to the tumour. This will make the virus more easily cleared by the host's immune system and stimulate a systemic and specific anti-tumour response in treated individuals. The GM virus is attenuated and the GM virus cannot effectively spread from injected tumours into normal tissues. The Regulator has imposed licence conditions to ensure that there is ongoing oversight of the release, including an obligation to report any unintended effects. The GMO cannot be used as a therapeutic until it is approved by the TGA, which has regulatory responsibility for quality, efficacy and safety of therapeutic products.

Issue	Summary of issues raised	Comment
Hhs	Concerned regarding specificity of the immune response elicited by the GM virus in relation to reported adverse effects (nausea, vomiting, diarrheal and similar general symptoms).	Several clinical trials of the GMO have been carried out in Australia and elsewhere. These trials have demonstrated that no serious adverse events attributable to the GM virus were observed. Minor adverse reactions associated with treatment included pyrexia (abnormal body temperature) and associated constitutional symptoms (which included indications like fever, chills, fatigue nausea, vomiting or headache), as well as inflammation and erythema (redness of the skin) in and around the injected tumour site. These adverse events were common in trial participants, were generally mild to moderate in severity and were more marked in patients who were HSV-seronegative before treatment. It is not possible to determine the specific element of the GMO that is responsible for the adverse effects, however it is likely that these effects are, in part, mediated by the expression of the introduced GM-CSF. This information is considered in the RARMP. The Regulator has imposed licence conditions to ensure that there is ongoing oversight of the release, including an obligation to report any unintended effects. The GMO cannot be used as a therapeutic until it is approved by the TGA, which has regulatory responsibility for quality, efficacy and safety of therapeutic products. The TGA will consider relevant clinical data in their assessment.
Hhs; B	Expresses an opinion that a balance needs to be drawn regarding the GMOs efficacy as a therapeutic and with the risks of the gm-virus establishing in the population generally.	The Regulator is required to assess and manage risks but does not consider potential benefits from GMOs. The risk assessment concludes that the commercial supply of GM virus poses negligible risks to the health and safety of people or the environment. The efficacy of the GM virus will be considered by the TGA in their assessment of GMO as a human therapeutic.
Hhs	Queries if the gm-viral DNA is capable of integrating into the host DNA because of homology with the DNA inserted into the GMO.	HSV-1 is a non-integrating type of virus and does not integrate into the DNA of the host. The modified regions of the GMO have been sequenced at least three times between 2001 and 2011. These sequenced regions have been found to only contain the intended DNA sequence, demonstrating the stability of the GMO. There is no evidence from non-clinical or clinical studies to suggest that the genetic modification has altered the non-integrating phenotype of HSV-1.