Australian Government

Department of Health

Office of the Gene Technology Regulator

Risk Assessment and Risk Management Plan for

DIR 140

Clinical trial of a genetically modified virus for treatment of liver cancer

Applicant: Clinical Network Services (CNS) Pty Ltd

March 2016

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Summary of the Risk Assessment and Risk Management Plan

for

Licence Application No. DIR 140

Decision

The Gene Technology Regulator (the Regulator) has decided to issue a licence for this application to conduct a Phase 3 clinical trial of a genetically modified virus in patients with advanced liver cancer. A Risk Assessment and Risk Management Plan (RARMP) for this application was prepared by the Regulator in accordance with the requirements of the *Gene Technology Act 2000* (the Act) and corresponding state and territory legislation, and finalised following consultation with a range of experts, agencies and authorities, and the public. The RARMP concludes that this clinical trial poses negligible to low risks to human health and safety and the environment, and that any risks posed by the dealings can be managed by imposing conditions on the conduct of the trial.

As part of Australia's integrated approach to the regulation of gene technology, regulation under the Act is not intended to override or duplicate the regulatory oversight of agencies that have responsibility for genetically modified organisms (GMOs) or GM products based on their intended use.

Clinical trials in Australia must be conducted in accordance with requirements of the *Therapeutic Goods Act 1989*, which is administered by the Therapeutic Goods Administration (TGA). Clinical trials must have approval from the Human Research Ethics Committee at each trial site before the trial commences, and must be conducted in accordance with the National Statement on the Ethical Conduct in Research Involving Humans.

The application

Application number	DIR 140
Applicant	Clinical Network Services (CNS) Pty Ltd
Project title	Clinical trial of a genetically modified virus for treatment of liver cancer[1]
Parent organism	*Vaccinia virus,* New York City Board of Health (NYCBH) vaccine strain
Introduced genes and modified traits	- inactivation of viral *thymidine kinase* (*TK*) gene (human therapeutic – attenuation) - insertion of human *Granulocyte-Macrophage Colony-Stimulating Factor* (*hGM-CSF*) gene (human therapeutic - enhanced immune response) - insertion of bacterial *lacZ* gene from *Escherichia coli* (*E. coli*) (reporter gene expression)

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[1] The title of the licence application submitted by Clinical Network Services (CNS) Pty Ltd is 'A Phase 3 Randomized, Open-Label Study Comparing Pexa Vec (Vaccinia GM CSF / Thymidine Kinase-Deactivated Virus) Followed by Sorafenib Versus Sorafenib in Patients with Advanced Hepatocellular Carcinoma (HCC) Without Prior Systemic Therapy (JX594-HEP024)'.

Proposed release dates	The clinical trial is anticipated to occur over a 12 month period, commencing in 2016. A total period of 5 years has been requested to accommodate follow-up studies, if required.
Proposed location	For administration by medical professionals in hospitals throughout Australia (specific locations are not yet finalised, but would be chosen so as to provide access to patients with advanced liver cancer).
Primary purpose	To assess the effectiveness and safety of the GMO as a treatment for advanced liver cancer when provided in conjunction with a standard treatment, compared with the standard treatment alone.

CNS has proposed a clinical trial of a live GM *vaccinia virus* (VACV) intended to preferentially kill cancer cells and trigger an immune response against the tumour. Up to 50 adult volunteers with advanced hepatocellular carcinoma (HCC), a common type of liver cancer, would be treated with the genetically modified organism (GMO). The purpose of the trial is to assess the effectiveness and safety of the GMO as a treatment for advanced HCC when provided in conjunction with a standard treatment, compared with the standard treatment alone.

The parent organism, VACV, is a member of the Poxvirus family, infects a range of mammalian species and is mildly pathogenic in humans. It is not known to be circulating in any human population but was used world-wide as a vaccine to protect against smallpox infection. VACV has attracted recent interest as a potential cancer therapeutic as it is thought to show a natural selectivity for cancer cells without causing significant adverse effects in the patient.

The GMO is based on the Dryvax^{TM} smallpox vaccine (Wyeth Laboratories), which was used extensively in the USA during the smallpox eradication campaign in the 1960s and 70s. Dryvax^{TM} was prepared from the New York City Board of Health (NYCBH) *vaccinia* strain and contains a mixture of closely related viruses of relatively low pathogenicity. The GMO is derived from a single clone isolated from this mixture.

The GMO has been modified so as to enhance its specificity for cancer cells. The vaccinia *thymidine kinase* (*TK*) gene, encoding a thymidine kinase enzyme required for DNA synthesis and viral replication, has been disrupted by insertion of the *GM-CSF* and *lacZ* genes. As a result, the GMO can only reproduce in host cells which produce their own TK. TK is expressed at low levels during normal cell division and at higher levels in most cancer cells, but not in non-dividing cells. Thus, the GMO is expected to replicate well in tumours while displaying attenuation in normal tissues, leading to preferential destruction of cancer cells.

A single copy of the human *GM-CSF* gene has been inserted within the viral *TK* gene, so as to promote an anti-cancer immune response. hGM-CSF is a naturally occurring cytokine that encourages the proliferation of certain types of immune cell. When tumour cells are killed by the GMO, hGM-CSF is expected to enhance the immune response to tumour antigens by stimulating specific immune cells in the immediate vicinity. This may also lead to a therapeutic effect on tumours located elsewhere in the body.

A single copy of the *E. coli lacZ* reporter gene has also been inserted within the viral *TK* gene. This gene encodes a β-galactosidase enzyme which can be detected using a biochemical assay, allowing quick and easy detection of the GMO in blood and tissue samples collected from trial participants.

The GMO has not previously been trialled in Australia. However, it has been evaluated overseas in 13 completed and ongoing clinical trials, in countries that include the USA, Canada and South Korea. The proposed study would form part of an international multi-centre Phase 3 clinical trial.

Risk assessment

The risk assessment concludes that risks from the proposed dealings to the health and safety of people, or to the environment, are negligible to low. It is proposed that risk treatment measures be applied to manage these risks.

The risk assessment process considers how the genetic modification and proposed activities conducted with the GMOs could lead to harm to people or the environment. Plausible causal or exposure pathways are postulated that may give rise to harm for people or the environment from dealings with a GMO (risk scenarios) in the short and long term. This included consideration of the absence of the parent organism from the Australian environment and the potential for: expression of the introduced genes and genetic modifications to impact on the disease burden caused by the GM virus; infection of at-risk individuals; infection of animals; and gene transfer to other organisms.

A risk is only identified for further assessment when a risk scenario is considered to have some chance of causing harm. Pathways that do not lead to an adverse outcome, or could not reasonably occur, do not advance in the risk assessment process. Identified risks are characterised in relation to both the likelihood and seriousness of harm, taking into account information in the application (including proposed limits and controls), relevant previous approvals and current scientific/technical knowledge.

The TGA, the trial sponsor, the investigators and the Human Research Ethics Committee (HREC) at each trial site all have roles to play in ensuring participants' safety under the *Therapeutic Goods Act 1989*. Therefore, the Regulator focuses primarily on risks posed to people other than those participating in the clinical trial, and to the environment.

Risks to the health and safety of at-risk individuals from exposure to the GMO through contact with trial participants who are shedding the virus were estimated as negligible to low. Risk evaluation proposed that risk treatment should be applied to mitigate the low risk. No other substantive risks were identified.

Important factors in reaching the conclusions of the risk assessment included: that the GM virus is expected to be attenuated relative to unmodified VACV; the introduced genes have not been associated with toxicity; unintended exposure would be minimised by precautions proposed by the applicant; and previous experience with the GMO in earlier clinical trials.

As risks to the health and safety of people, or the environment, from the proposed clinical trial of the GM virus have been assessed as negligible to low, the Regulator considers that the dealings involved do not pose a significant risk to either 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 options for treatment of identified risks, evaluates limits and controls proposed by the applicant, and considers general risk management measures. The risk management plan is given effect through licence conditions.

Treatment measures to mitigate the identified negligible to low risks to human health and safety were considered and licence conditions imposed. These conditions require patients participating in the trial to avoid contact with certain at-risk people following their initial treatment with the GMO, until they have been assessed for the presence of GMO-related lesions by their clinician. These measures are considered sufficient to manage the identified negligible to low risks.

As this is a limited and controlled release, the licence also includes conditions that limit the scope and duration of the trial as well as controls in line with those proposed by the applicant,

including administration of the GMO via intratumoural inoculation and by trained medical staff, exclusion of participants and staff at higher risk of adverse reactions and more likely to shed the GMO, educating trial participants about methods to minimise transmission of the GMO, appropriate containment and disposal of waste, destroying excess GMO that is not required for further studies, and transporting and storing the GMO in accordance with the Regulator's *Guidelines for the Transport, Storage and Disposal of GMOs*.

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.

These licence conditions will manage risks to so as to protect human health and safety and the environment.

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Abbreviations

ACIP	Advisory Committee on Immunization Practices
APVMA	Australian Pesticides and Veterinary Medicines Authority
ARTG	Australian Register of Therapeutic Goods
BSC	biological safety cabinet
CCI	Confidential Commercial Information under section 185 of the *Gene Technology Act 2000*
CNS	Clinical Network Services (CNS) Pty Ltd
CRO	Clinical research organisation (in this case PPD Australia Pty Ltd)
DIR	Dealings involving Intentional Release
DNA	Deoxyribonucleic acid
DNIR	Dealings not involving Intentional Release
ECOG	Eastern Cooperative Oncology Group
IATA	International Air Transport Association
ICH-GCP	*Guidelines for Good Clinical Practice* of the International Council for Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use
IV	intravenous
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
IC_{50}	half maximal inhibitory concentration
mL	millilitre
µg	microgram
OGTR	Office of the Gene Technology Regulator
pfu	plaque-forming units
PPD	PPD Australia Pty Ltd
PPE	Personal Protective Equipment
RARMP	Risk Assessment and Risk Management Plan
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
US CDC	United States Center for Disease Control
USA	United States of America
VACV	*Vaccinia virus*
VIG	*Vaccinia Immunoglobulin*
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).

RISK ASSESSMENT CONTEXT

LEGISLATIVE REQUIREMENTS
(including Gene Technology Act and Regulations)

RISK ANALYSIS FRAMEWORK

OGTR OPERATIONAL POLICIES AND GUIDELINES

PROPOSED DEALINGS	PARENT ORGANISM
Proposed activities involving the GMO	Origin and taxonomy
Proposed limits of the release	Biological characterisation
Proposed control measures	
GMO	RECEIVING ENVIRONMENT
Introduced or deleted genes (genotype)	Environmental conditions
Novel traits (phenotype)	Presence of related species
	Presence of similar genes
PREVIOUS RELEASES	

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.   In accordance with section 50A of the Act, this application is considered to be a limited and controlled release application, as its principal purpose is to enable the applicant to conduct experiments and the applicant has proposed limits on the size, locations and duration of the release, as well as controls to restrict the spread and persistence of the GMOs and their genetic material in the environment. Therefore, the Gene Technology Regulator (the Regulator) was not required to consult with prescribed experts, agencies and authorities before preparation of the RARMP (see section 50 of the Act).

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6.   Section 51 of the Act and regulation 9A of the Regulations outline the matters the Regulator must take into account in preparing a RARMP.

7.   Section 52 of the Act requires the Regulator to seek comment on the RARMP from the States and Territories, the Gene Technology Technical Advisory Committee, Commonwealth authorities or agencies prescribed in the Regulations, the Minister for the Environment, relevant local council(s), and the public. Advice from the prescribed experts, agencies and authorities, and how it was taken into account, is summarised in Appendix A. No submissions were received from members of the public.

8.   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

9.   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.

10.   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. Clinical trials of therapeutic products that are experimental and under development, prior to a full evaluation and assessment, are also regulated by the TGA through the Clinical Trial Exemption (CTX) scheme or the Clinical Trial Notification (CTN) scheme.

11.   For clinical trials, TGA has regulatory responsibility for the supply of unapproved therapeutic products. In terms of risk to individuals participating in a clinical trial, TGA (as the primary regulatory agency), the trial sponsor, the investigators and the Human Research Ethics Committee (HREC) at each trial site all have roles in ensuring participants' safety under the *Therapeutic Goods* Act *1989*. However, where the trial involves a GMO, authorisation is also required under gene technology legislation. To avoid duplication of regulatory oversight, and as risks to trial participants are addressed through the above mechanisms, the Regulator's focus is on assessing risks posed to people other than those participating in the clinical trial, and to the environment. This includes risks to people preparing and administering the GM virus, and risks associated with import, transport and disposal of the GMO.

12.   The International Council for Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use - *Guidelines for Good Clinical Practice* (ICH-GCP) is an international ethical and scientific quality standard for designing, conducting, recording and reporting trials that involve the participation of human subjects. The guideline was developed with consideration of the current good clinical practices of the European Union, Japan, and the United States, as well as those of Australia, Canada, the Nordic countries and the World Health Organization (WHO). TGA has adopted the ICH-GCP in principle as *Note for Guidance on Good Clinical Practice* (designated CPMP/ICH/135/95), which provides overarching guidance for conducting clinical trials in Australia which fall under TGA regulation.

13.   The National Health and Medical Research Council (NHMRC) has issued the *National Statement on the Ethical Conduct in Research Involving Humans* (National Health and Medical Research Council 2013). This document sets the Australian standard against which all research involving humans is reviewed. The *Therapeutic Goods Act 1989* requires that the use of a therapeutic good in a clinical trial must be in accordance with the ethical standards set out in this document.

14.   Approval by a HREC is also a fundamental requirement of a clinical trial. HRECs conduct both ethical and scientific assessment of the proposal and in addition often consider issues of research governance. Other elements of governance of clinical trials that are considered by HRECs include appropriate informed consent, specific inclusion and exclusion criteria, data monitoring and vaccine accounting and reconciliation.

15.   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

16.   CNS has proposed a clinical trial of a genetically modified (GM) live virus (JX-594; also known as Pexa-Vec). The GM virus is being developed as a treatment for solid tumours of various origin and has been tested in patients with metastatic melanoma, and liver, renal and colorectal cancers. In the proposed trial, the GMO will be tested for efficacy against advanced hepatocellular carcinoma (HCC), a common type of liver cancer. It will be administered to cancer patients by direct injection into solid tumours located in the liver.

3.1   The proposed clinical trial

3.1.1   Details of the clinical trial

17.   The purpose of the trial is to assess the efficacy and safety of the GM virus when provided in conjunction with a standard treatment for HCC (the chemotherapy drug sorafenib), compared with the standard treatment alone.

18.   The proposed study is part of an international multi-centre Phase 3 clinical trial. A total of 600 patients globally are to take part in the study, which will be conducted at approximately 120 sites worldwide, including in North America, Asia and Europe.

19.   The international trial sponsor is SillaJen, Inc., based in The Republic of Korea. SillaJen Inc. has contracted Clinical Network Services (CNS) Pty Ltd to manage regulatory compliance for the Australian component of the trial, and PPD Australia Pty Ltd (PPD) to act as the Clinical Research Organisation (CRO) and liaise with individual study sites. CNS will audit trial sites periodically and ensure compliance with regulatory requirements.

20.   The Australian component of the trial will involve 40-50 adult volunteers at approximately 10-15 study sites. The applicant plans to conduct the trial in hospitals that provide specialised cancer-treatment services and access to patients with liver cancer.

21.   The trial will be divided into three stages:

1) <u>Screening</u>: medical history and tumour status of prospective volunteers will be examined and their suitability to participate in the study assessed.

2) <u>Treatment</u>: participants will undergo a series of three treatments over a four week period. At each treatment, a total dose of 1×10^9 plaque-forming units (pfu) of the GM virus will be injected into one or more liver tumours. Patients will be observed for at least eight hours after each treatment before being released from the hospital.

At subsequent visits, participants will be asked to report any adverse reactions experienced since the onset of treatment. Before the second and third treatments, specimens (e.g. blood samples) will be collected for laboratory analysis. These will be exported for analysis overseas, but may if required be analysed in Australia.

3) <u>Follow-up and monitoring</u>: conventional therapy will commence two weeks after the final treatment with the GMO and participants will thereafter be assessed at 3-week intervals. At each visit, blood samples will be collected for laboratory analysis and any adverse reactions reported.

22. Under the *Gene Technology Act 2000*, the proposed clinical trial involves the following dealings:

a) importing the GMO;

b) conducting experiments with the GMO;

c) transporting the GMO;

d) disposing of the GMO; and

e) possession (including storage) and use of the GMO for the purpose of any of the above activities.

3.1.2 Selection of trial participants

23. Relevant inclusion criteria to be used by study site investigators include:

a) adults of any gender;

b) a performance status on the Eastern Cooperative Oncology Group (ECOG) scale of either 0 (fully active, able to carry on all pre-disease activities without restriction) or 1 (restricted in physically strenuous activity but ambulatory and able to carry out work of a light or sedentary nature).

24. Relevant exclusion criteria include:

a) pregnant or breastfeeding women;

b) persons with known significant immunodeficiency due to underlying illness (e.g. HIV/AIDS) and/or high dose immunosuppressive medication;

c) persons with an ongoing and severe inflammatory skin condition requiring medical treatment;

d) persons with a history of severe eczema requiring prior medical treatment;

e) persons who have previously experienced a severe systemic reaction or side effect due to previous vaccination with VACV; and

f) persons unable or unwilling to comply with the requirements of the trial protocol.

25. For the purpose of this document, individuals meeting the criteria listed in paragraph 24 (a) – (d) will be referred to as 'Excluded Individuals'.

26. Assessment of a prospective participant's willingness to comply with trial requirements will be based on their acceptance of the information contained in the Participant Information Sheet/Consent Form and explained by a medical member of the study team, and their provision of signed consent. A standard Participant Information Sheet, and a Discussion Checklist to aid in briefing of patients, will be used across all Study Sites. The Investigator at each site may exclude a person who, in their professional judgement, appears unable or unwilling to comply with mandated instructions.

3.1.3   Instructions to trial participants

27.   Trial participants treated with the GMO will receive instructions intended to minimise interpersonal spread of the GM virus – in particular, to individuals at risk of developing severe disease (i.e. 'Excluded Individuals' (paragraph 25)). The applicant has confirmed that these behaviours are a required part of the clinical trial protocol, and unwillingness or inability to comply is grounds for exclusion from the trial (see paragraph 24 (f)).

28.   For all trial participants, these include:

   a)   frequent hand washing with soap or hand disinfectant containing at least 60% alcohol;

   b)   respiratory hygiene and cough etiquette followed for two weeks after initial treatment; and

   c)   refraining from blood, tissue or organ donation while taking study medications.

29.   Patients who develop GMO-related lesions will also be expected to:

   a)   cover skin lesions with a non-occlusive dressing (e.g. gauze);

   b)   where mouth lesions are present, wear a mask and avoid sharing items such as toothbrushes and eating utensils;

   c)   avoid, and ensure others avoid, direct physical contact with lesions and any potentially contaminated material except where necessary for patient care. When caring for the lesion, disposable gloves should be worn and hands washed or disinfected afterwards;

   d)   prevent self-inoculation by avoiding touching other body parts (e.g. eyes, nose) after direct contact with the lesion or with potentially contaminated material; and

   e)   avoid any direct physical contact with children under 12 months of age and 'Excluded Individuals' (see paragraph 25) until the lesions have healed.

30.   Patients will be informed that anything coming into contact with the outer side of the dressing could potentially be contaminated, and that clothing, towels, bedding and other items that may have come in direct contact with a skin lesion or with drainage from a lesion are to be treated as described in Section 3.1.6.

31.   The above information will be explained to prospective participants during screening (see Section 3.1.2). It will also be provided in writing by means of the Participant Information Sheet, and a Patient Card that includes details of the GMO and contact precautions. Home carers and family members will not receive formally-documented education on lesion care and contact precautions, however, the written information will be available to them via the patient.

32.   The applicant has advised that trial participants and clinical staff will be provided with an instructional leaflet which details procedures to follow should transmission (e.g. development of a lesion) to a human or animal contact be suspected.

3.1.4   Transport and storage of the GMO

33.   The GMO will be manufactured according to Good Manufacturing Practice (GMP) guidelines in France and imported into Australia. Concentrated virus will be supplied in small volumes in sealed pharmaceutical-quality vials.

34.   Vials will be labelled to indicate the contents, quantity and clinical trial details. Shipping cartons will be labelled to indicate that they contain GMOs and with the applicant's contact information, in accordance with the Regulator's *Guidelines for the Transport, Storage and Disposal of GMOs*.

35.   For transport during import and distribution within Australia, primary containers will be sealed within leak-proof secondary packaging and further packed to meet the requirements of International Air Transport Association (IATA) shipping classification <u>Biological Substance, Category B, UN 3373</u>. These satisfy the containment requirements outlined in the Regulator's *Guidelines for the Transport, Storage and Disposal of GMOs*.

36.   Packages will be transported by commercial courier companies experienced in the transport of pharmaceutical products, and in accordance with IATA Dangerous Goods Regulations and, within Australia, the *Australian Dangerous Goods Code* (edition 7.3).

37.   The GMO will be transported from the point of import either directly to clinical sites or to a central storage facility (e.g. Flinders Clinical Trial Services, Adelaide SA) for later distribution. Individual sites will typically hold small quantities of the GMO at any one time, stored in a secure location with access restricted to staff in the dispensing pharmacy or laboratory, and otherwise in accordance with the Regulator's *Guidelines for the Transport, Storage and Disposal of GMOs*.

38.   For all transport within the clinical site, the GM virus will be sealed within a primary container (e.g. vial, capped syringe or intravenous (IV) infusion bag) and placed within a leak-proof secondary container or bag clearly marked with a biohazard symbol.

39.   Blood and tissue samples collected from trial participants during the study will be exported for analysis overseas. These samples may contain the GMO, and will be transported in accordance with the Regulator's *Guidelines for the Transport, Storage and Disposal of GMOs*.

3.1.5   Handling of the GMO

40.   Hospital staff conducting the dealings will be trained in product handling by a representative of SillaJen, Inc. and in specific GMO-related requirements by a CNS representative.

Procedures

41.   The GM virus will be dispensed within a hospital pharmacy or laboratory by staff designated and trained for this study, and only with written authorisation from the lead investigator at the site. The international trial sponsor will provide guidance on product handling, safety precautions and decontamination procedures.

42.   Concentrated GM virus will be drawn into a syringe, diluted with saline solution in a mixing container then drawn into one or more dosing syringes according to the number of tumours to be injected. The procedure will require the use of sharps and, depending on local procedures, could involve removal of contaminated needles from filled syringes. Syringes will be capped and transported to the clinic for administration to the patient.

43.   Appropriately qualified medical staff will administer the GMO by image-guided intratumoural injection. The needle will be fully inserted into each tumour before the syringe containing the GM virus is attached to the injection apparatus via flexible tubing. Before withdrawing the needle, the apparatus will be flushed with saline solution to reduce GMO leakage from the injection site.

Safety considerations

44.   Clinical trial documents[2] prepared by the international trial sponsor outline specific precautions to be taken by clinical staff involved in the trial.

45.   Hospital staff who meet the criteria listed in paragraph 24 (a) – (d) will be excluded from directly handling the GMO, administering it to patients, and caring for patients who present with GMO-related lesions.

46.   When handling the GMO, personal protective equipment (PPE) including gown, gloves, eye protection and a surgical mask must be worn. In addition, relevant institutional policies and procedures should be followed. Vaccination of staff with VACV is not required.

47.   Additional requirements while dispensing concentrated GMO include the use of a Class II biological safety cabinet (BSC) and PC2 work practices.

48.   Administration of the GMO and subsequent care of inoculated patients will be, at a minimum, in accordance with Universal Standard Precautions (World Health Organisation 2007) and the Australian Guidelines for the Prevention and Control of Infection in Healthcare (National Health and Medical Research Council 2010). These aim to reduce transmission of infectious organisms from both recognized and unrecognized sources in the clinical setting. Appropriate practices include (but are not limited to) hand hygiene, use of PPE as appropriate and based on risk assessment, safe sharps handling and disposal practices, safe handling of potentially contaminated equipment or surfaces in the patient environment, respiratory hygiene/cough etiquette and correct cleaning and waste management.

49.   Where returning patients present with GMO-related lesions, from which the GM virus may be shed, study sites are advised to implement additional 'Contact Precautions' based on local risk assessment. Examples include additional PPE, dedicated bedside diagnostic devices and use of single patient rooms. Where single rooms are unavailable, patients could be placed in rooms with patients who are not Excluded Individuals (paragraph 25).

3.1.6   Disposal of the GMO (including waste contaminated with the GMO)

50.   Disposable materials contaminated with the GM virus (e.g. syringes, catheters, needles, tubing, gloves, vials, bandages etc) will be discarded into appropriate biomedical waste containers and disposed of following standard institutional procedures for infectious clinical waste and in accordance with applicable State and Territory legislation. This may include rendering the waste inert by steam sterilisation, high temperature incineration or chemical treatment, and may take place at the medical facility or involve the use of registered waste contractors. Waste generated at clinical sites will not be left unattended in a public area.

51.   After handling the GMO, work surfaces will be decontaminated with an appropriate chemical disinfectant, following standard institutional procedures. Contaminated textiles (e.g. linens, towels and clothing) will be laundered using routine protocols for healthcare facilities (e.g. hot (71°C) water with detergent and hot air drying).

52.   When changing dressings at home, patients will be instructed to seal soiled dressings and other contaminated items in a container or zip-loc plastic bag and place them in a biohazard container provided by the clinical site. Patients will be required to return the container to the

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[2] Clinical trial documents provided as part of the application include the *Study Protocol, Investigator's Brochure, Pexa-Vec Guidelines, Pexa-Vec Guidelines for Pharmacy Staff* and *Pexa-Vec Guidelines for Clinical Staff* With the exception of *Pexa-Vec Guidelines for Clinical Staff,* these documents are under consideration as Confidential Commercial Information (CCI) under section 185 of the Act. Any confidential information will be made available to the prescribed experts and agencies.

hospital at each visit, at which time it will be disposed of by the site as clinical waste. Patients will be issued replacement containers until no longer required.[3] Contaminated textiles generated in patients' homes are to be laundered in hot water with detergent or treated with dilute bleach.

53.   Any unused GM virus will be destroyed, according to written instructions provided by the international trial sponsor.

3.1.7   Contingency plans

54.   In the event of accidental human exposure to the GMO, the applicant has proposed the following:

a)   implement institutional guidelines, such as documented in the *Australian Guidelines for the Prevention and Control of Infection in Healthcare*;

b)   wash the area thoroughly with soap and water;

c)   cover affected area with a non-occlusive dressing until complete resolution; and

d)   report the incident to the study investigator and trial sponsor.

55.   Should trial participants who have developed GMO-related lesions suspect that transmission to a human or animal contact has occurred, they are to inform staff at the clinical site. Any reports of potential transmission will be investigated by a medical or veterinary professional. The applicant has advised that a Transmission Handling Protocol has been developed, which describes the investigation of reported adverse effects suspected to be due to transmission of the GMO.

56.   In the event of a clinically-significant reaction that may be related to the GMO, the study investigator should immediately contact the trial sponsor and seek appropriate infectious disease expertise.

57.   Medications recommended for treating some adverse reactions to VACV infection (see Section 4.9.1) are available in Australia and from the international trial sponsor. Country-specific treatment protocols will be provided to clinical sites. The applicant has stated that at least one course of Vaccinia Immunoglobulin (VIG) treatment is available in Australia. A stockpile sufficient for three patients will be maintained in Singapore and can be transported to study sites in Australia within 24 hours. The anti-viral drug Cidofovir is available within Australia and is suggested as an off-label second line of treatment (see paragraph 114).

3.1.8   Record keeping

58.   Each site will maintain records of all GMO received, dispensed and destroyed. These records will be verified by the CRO and licence holder during regular site visits.

59.   Each site will also track and maintain a record of the dispensation, return and destruction of biohazard containers provided to trial participants.

60.   On completion of the study at each trial site, the sponsor and licence holder will verify that all used vials have been decontaminated and disposed of, all unused GMO has been destroyed, and no GMO remains at the site.

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[3] The applicant originally proposed that patients would place soiled dressings and other contaminated items in a sealed container or zip-loc plastic bag and dispose of them with general household waste. Following consultation on the draft RARMP, this proposal was amended as outlined in paragraphs 52 and 59.

61.   A pharmacovigilance reporting system will capture information regarding any GMO-related lesions or other adverse events experienced by trial participants. The international trial sponsor will maintain a record of all suspected transmission events to both human and animal contacts. Any confirmed transmission events will be reported to the Regulator.

3.2   The proposed limits on the dealings (scope, scale, locations, duration and people)

62.   The trial would take place in hospitals throughout Australia. While clinical sites have not been finalised, participating hospitals are likely to be located in Brisbane, Sydney, Melbourne and Adelaide. The study will run from the date of issue of the licence until after the required number of participants have been enrolled, treated and any follow-up studies undertaken. The applicant intends to enrol a maximum of 50 patients in the Australian component of the trial.

3.3   The proposed controls to restrict the spread and persistence of the GM vaccine and its genetic material in the environment

63.   The applicant has proposed a number of controls to limit exposure to the GM virus, and to restrict its spread and persistence in the environment. These include:

- excluding prospective participants at higher risk of severe reaction to VACV, which is characterised by extensive lesion formation and increased opportunity for viral shedding into the environment;

- excluding staff who are at higher risk of severe reaction to VACV from handling the GMO and caring for patients presenting with GMO-related lesions;

- administering the GM virus via intratumoural injection, which is associated with reduced viral shedding compared with intravenous infusion;

- requiring that the GMO be administered by appropriately trained medical staff in a hospital setting and in accordance with WHO *Standard Universal Precautions* and ICH-GCP;

- requiring that staff handling the GMO and caring for patients presenting with lesions wear and use appropriate protective clothing and equipment;

- requiring trial participants to take precautions intended to minimise transmission of the GMO;

- requiring that trial participants who develop GMO-related lesions avoid direct physical contact with individuals at risk of severe reaction to VACV infection;

- ensuring contingency plans are in place to detect, investigate and report any inadvertent transmission of the GMO to human or animal contacts of the trial participants;

- ensuring contingency plans are in place to manage any exposure to the GM virus and treat any severe vaccinia-related illness that may eventuate;

- transporting and storing the GM virus in accordance with relevant guidelines and regulations[4];

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[4] The Regulator's *Guidelines for the Transport, Storage and Disposal of Genetically Modified Organisms*, or IATA Transportation Regulations

- requiring that trial participants who develop GMO-related lesions store potentially-contaminated waste under two levels of containment and return it to the hospital for disposal;

- track and record the dispensation, return and destruction of waste containers provided to trial participants for this purpose;

- disposing of waste generated at the clinical trial site and at participants' homes in accordance with standard clinical waste disposal practices as required by the relevant local and state legislation; and

- destroying unused GMO on completion of the study, and maintaining records at each clinical site of all GMO received, dispensed and destroyed.

64.	The suitability of these controls and the limits outlined above is assessed in Chapter 3.

## Section 4	The parent organism

65.	*Vaccinia virus* (VACV) is a member of the *Orthopoxvirus* genus of the family *Poxviridae*, subfamily *Chordopoxvirinae*. This genus also includes the human pathogen *Variola virus* (causative agent of smallpox), as well as *Monkeypox virus* (monkeypox)*, Cowpox virus* (cowpox)*, Horsepox virus* (horsepox)*, Ectromelia virus* (mousepox) and others. It should be noted that the human disease known as chickenpox is caused by the *Varicella zoster virus,* which is not a member of family *Poxviridae*.

66.	There is extensive clinical experience with VACV as it was used globally as a vaccine against smallpox prior to the latter's declared eradication in 1980 (Jacobs, 2009). VACV is not known to exist naturally in the environment. Its origin is unknown, and it may have evolved from other related poxviruses at some point during 150 years of propagation and use as a vaccine. It is genetically distinct from both cowpox virus and variola virus, and most closely related to horse pox virus (Jacobs et al. 2009; Shchelkunov 2013).

67.	Due to their evolution in different parts of the world over 150 years of smallpox vaccination, many strains of VACV exist (e.g. Paris, Copenhagen, Bern, Ankara, Lister and New York City Board of Health (NYCBH) strains). These differ in viral characteristics, host range, pathogenicity and prevalence of adverse reactions to vaccination. Overall, vaccination with the NYCBH strain caused the lowest rate of adverse reactions (Jacobs et al. 2009; Kretzschmar et al. 2006; Osborne et al. 2007; Shen & Nemunaitis 2005).

68.	JX-594, the GMO that is the subject of the proposed clinical trial, is derived from the Dryvax[TM] smallpox vaccine (Wyeth Laboratories), also known as 'Wyeth' strain. Dryvax[TM] was prepared from the NYCBH VACV strain and contains a highly heterogeneous population of closely related viruses with varying pathogenicity. Its phenotype is therefore the aggregate consequence of infection with this diverse collection (Nalca & Zumbrun 2010; Osborne et al. 2007). JX-594 was isolated by plaque purification after the recombination event that created the GMO (see Section 5). Therefore, it is clonally pure but not entirely characterised.

### 4.1	Basic biology of *Vaccinia Virus*

69.	The lifecycle of a virus involves transmission of infective virus particles to a new host organism, attachment and entry into susceptible host cells, replication of the viral genome, production of viral proteins, assembly of new virus particles and, finally, release of progeny virus particles – often accompanied by cell lysis. Viruses have co-evolved with their host species and are often specific for a narrow range of host organisms and infect only certain cell types within those hosts.

70.	Like other poxviruses, VACV is a large enveloped virus with a linear double-stranded DNA genome, approximately 192 kilo bases (kb) in length. The genome encodes around 200

proteins with roles in viral entry, transcription of viral genes, DNA synthesis, assembly of virus particles, and suppression of the host anti-viral response (Liu et al. 2014).

71.   VACV is a non-integrating virus, meaning it does not integrate into the genome of cells it infects, and its entire life cycle takes place within the cytoplasm of host cells. This means VACV cannot utilise host enzymes for DNA replication and gene transcription, as these reside in the cell nucleus. It must therefore encode its own.

72.   VACV genes are expressed in three highly coordinated temporal waves: early, intermediate and late, with each wave controlled by a specific set of transcription factors. The viral core contains the entire machinery needed to commence transcription of early genes, while expression of intermediate and late genes relies on transcription factors synthesised during the early and intermediate phases, respectively. A fourth group of genes (termed early-late) are expressed constitutively. The promoters for each group of genes have distinctive sequence elements recognized by specific viral proteins, providing the basis for a programmed cascade of gene regulation (Baur et al. 2010; Lefkowitz et al. 2006; Shen & Nemunaitis 2005).

73.   Replication of viral DNA yields 10 000 copies per cell within hours of infection. In the final stages of the VACV lifecycle, new virions assemble and acquire one or more outer membranes to form one of three distinct types of infectious particle. The entire lifecycle of VACV is usually complete with 24 hours and ends with lysis of the infected cell and the release of as many as 10 000 virus particles (Lefkowitz et al. 2006; Shen & Nemunaitis 2005; Thorne et al. 2005).

4.2   Host range

74.   VACV is considered a laboratory virus with no known natural host or reservoir, but can infect a wide range of mammals. These include humans, several monkey species, a variety of rodents, rabbits, cattle, buffalo, sheep, horses, and domestic cats and dogs (Abrahao et al. 2010; Adams et al. 2007; Artois et al. 1990; Bennett et al. 1989; Brochier et al. 1989; de Sant'Ana et al. 2013a; Dumbell & Richardson 1993; Felipetto et al. 2012; Oliveira et al. 2015; Robinson & Mercer 1988; Riyesh et al. 2014).

75.   VACV is capable of causing disease symptoms in human, mice, rabbits, cattle, horses and buffalo. The related *cowpox virus* infects and causes disease symptoms and viraemia in domestic cats – in fact, cats are the most frequently recognised host of *cowpox virus* in Great Britain. Dermal pock formation due to VACV (Lister strain) has been demonstrated in cats, however the infectious dose was higher than that of *cowpox virus* (Bennett et al. 1989).

76.   Vaccinia can infect and replicate to some degree in canines, including domestic dogs, as genetically modified VACVs are effective therapeutics in these species (Autio et al. 2014; Rupprecht et al. 2005). A VACV strain found in Brazil (see Section 4.3), which causes clinical symptoms in humans and cattle but not in mice, is also associated with subclinical or asymptomatic infection in dogs (Peres et al. 2016; Peres et al. 2013). It is unclear whether any unmodified VACV strains can cause disease in dogs.

77.   Birds are not known as a target for VACV, but a study of a GM VACV-based rabies vaccine (Section 5.2.1) demonstrated sufficient viral replication in several Canadian bird species to permit seroconversion (Artois et al. 1990).

4.3   Global distribution

78.   Although used extensively in the past as a smallpox vaccine, persistence of VACV in the environment has been limited. Naturally-occurring infections with VACV or close relatives have been documented only in India and Brazil.

79.   *Buffalopox virus*, which appears to have become established in India, is considered a variant of VACV (Baxby 1996; Condit 2010; Moussatche et al. 2008).

80.   VACV appears to have become endemic in Brazil, and causes an occupational disease in humans, primarily associated with the handling of infected dairy cattle (Abrahao et al. 2015; Costa et al. 2015; World Health Organisation 2007). Outbreaks of zoonotic disease caused by VACV-like viruses and affecting cattle and rural workers have occurred since 1999 (de Sant'Ana et al. 2013a), and significant levels of VACV infection have been found in remote Amazonian wildlife (Abrahao et al. 2009a; Abrahao et al. 2010; Condit 2010; Oliveira et al. 2015). The origin of these viruses is unclear, but they appear genetically distinct from the VACV vaccine strains used during the post-1966 smallpox eradication campaign. Their origins may lie with earlier VACV imports beginning in the early 1800s, however strains indigenous to South America have not been ruled out (Drumond et al. 2008; Shchelkunov 2013; Trindade et al. 2007).

4.4   VACV pathology

4.4.1   Pathogenesis of poxvirus infection

81.   Natural infection with most of the pathogenic poxviruses occurs via the respiratory tract, although VACV appears to be an exception (see paragraph 90). Infection is also caused by inoculation into the skin or contact with broken skin, and possibly through mucosal membranes. The virus replicates at the site of inoculation and causes dermal hyperplasia and leukocyte infiltration (Baxby 1996). Orthopoxviruses display tropism for epithelial cells and tend to produce cutaneous lesions as described below (Section 4.4.2) (Moussatche et al. 2008).

4.4.2   Normal reactions to VACV infection in humans

82.   In humans, pathology related to VACV is best understood in the context of smallpox vaccination. VACV is generally administered by scarification, i.e. by scratching the skin with a two-pronged needle dipped in virus solution. The vaccinee normally develops a single lesion at the site of exposure around 3-4 days post-vaccination. Over 2-3 weeks, the lesion progresses through the following stages, typical of orthopoxviral lesions: macule, papule, vesicle, pustule and crusted scab (Figure 2). This is often accompanied by flu-like symptoms (fever, malaise, headache, nausea and muscle aches), swelling and redness around the vaccination site, swelling and tenderness of the draining lymph node, and less commonly, an urticarial-like rash. These reactions resolve spontaneously and require only observation and symptomatic treatment. Historically, about 21% of first time vaccinees were sufficiently concerned about their symptoms to seek medical attention (Cono et al. 2003; Fulginiti et al. 2003; Maurer et al. 2003).

83.   Zoonoses associated with outbreaks of bovine vaccinia in Brazil demonstrate natural human infection with VACV. In this context, farmers, milkers and close contacts have developed painful skin lesions on the hands, forearms, legs and face (Figure 3). These are accompanied by systemic symptoms such as fever, headache, myalgia, and swelling of the lymph nodes (Assis et al. 2013; de Assis et al. 2013; Kroon et al. 2011; Silva-Fernandes et al. 2009). Disease can be severe, with 12 out of 26 infected rural workers hospitalised in an outbreak in 2010 (Abrahao et al. 2015).
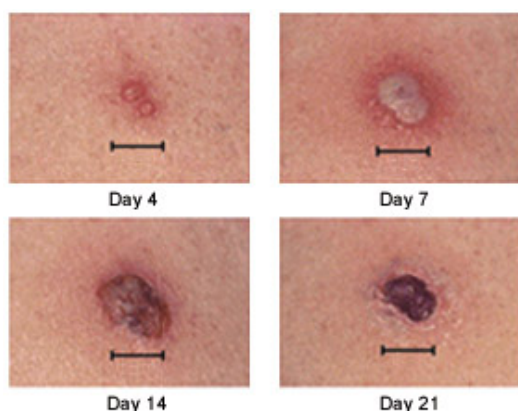
Figure 2. Natural progression of response to smallpox vaccine: papule (day 4), vesicle (day 7), pustule (day 14) and scab (day 21) (from (Maurer et al. 2003)).

Figure 3. Lesion on the arm of a Brazilian dairy worker caused by a naturally-occurring VACV infection (from (Peres et al. 2016)).

4.4.3   VACV infection in animals

84.   VACV infection in animals is best documented in the context of naturally-occurring disease in dairy cows. The infection is characterised by development of papules and vesicules on the teats and/or udder that progress to coalescent ulcers and scabby lesions. These are often associated with swelling and severe local pain. Ulcers have also occurred on the muzzle, tongue, hard palate and gums of suckling calves. Symptoms may be accompanied by fever and lymph node swelling, and usually resolve after 3-4 weeks (de Sant'Ana et al. 2013a; de Sant'Ana et al. 2013b; Quixabeira-Santos et al. 2011).

4.5 VACV shedding from infected hosts

85.   In the context of smallpox vaccination, VACV can be shed from the primary lesion from at least the third to twenty first days post-vaccination – from the time the papule develops until the scab drops off, and possibly longer. Maximal shedding occurs between days 4 and 14, and peak titres of 10^7 pfu/ml have been detected (Cummings et al. 2008; Cooney et al. 1991; Wharton et al. 2003).

86.   Limited historical data suggests that viremia (viral presence in the blood), viruria (viral presence in urine) and pharyngeal shedding in association with the NYCBH strain were uncommon, although such reactions were documented with more virulent vaccine strains used in Europe and Asia (e.g. (Gurvich et al. 1974)). Viremia and viruria do occur in patients with progressive vaccinia and eczema vaccinatum (see Section 4.4.3) (Lane & Fulginiti 2003).

87.   In the context of non-human hosts, VACV is shed into the milk and faeces of experimentally infected dairy cattle, suggesting a systemic infection (de Oliveira et al. 2015; Guedes et al. 2013; Rivetti, Jr. et al. 2013). Virus continued to be shed even after lesions on the teats and udders had healed. VACV of both high and low pathogenic strains is shed into the faeces and urine of experimentally infected mice (Ferreira et al. 2008).

4.6 Host to host transmission

4.6.1   Transmission between humans

88.   VACV is generally transmitted between humans through direct physical contact with a lesion or vaccine inoculation site, or contact with a contaminated object (e.g. bandages, clothing, sheets and towels). An infected person may spread the virus from the site of initial infection by touching other body parts or people with contaminated hands, or through such every day activities as shaving (Armed Forces Health Surveillance Center (AFHSC) 2014;

CDC 2013; Egan et al. 2004; Oliveira et al. 2014). Transmission usually required close interaction and occurred most often in the home (Wharton et al. 2003). Zoonotic transmission through direct physical contact has also been documented during outbreaks of bovine vaccinia in South America (Lum et al. 1967; Silva-Fernandes et al. 2009).

89.   Oral transmission via drinking contaminated milk has been observed in humans (Damaso, 2000) and demonstrated experimentally in mice (Rehfeld et al. 2015). A VACV-based rabies vaccine (discussed in Section 5.2.1) also relies on oral transmission for efficacy.

90.   Natural infections with other orthopox viruses such as *variola virus* (smallpox) commonly occur via the respiratory tract (Fenner 1989). However, in the context of vaccination with the NYCBH strain, aerosol transmission of VACV is considered unlikely and has not been documented. The millions of vaccinations performed in the past provided extensive opportunity for viral spread within families. However, relatively few transmissions were reported and all involved physical contact. Airborne transmission has never been documented (Lane & Goldstein 2003a; Lane & Fulginiti 2003).

91.   Average transmission rates from historical smallpox vaccination campaigns in the USA and UK are reported as 20-60 per million primary vaccinations (Neff et al. 2002). However, vaccinees were generally young children and the majority of contacts would have been immune or had previous exposure to vaccinia due to ongoing vaccination campaigns. Therefore, not every exposure would have resulted in observable infection. While it would be reasonable to expect a higher transmission rate from adults to the predominantly unvaccinated population of today, a recent report estimates that, among health care workers and military personnel vaccinated between 2003 and 2011, the rate of transmission to non-vaccinees was still 54 per million vaccinees (Wertheimer et al. 2012).

92.   Reported cases predominantly involved transmission between family members or other close contacts (e.g. school friends), and transmission in a hospital setting. The latter involved transmission to clusters of patients from recently vaccinated health care workers or patients hospitalised with a vaccine-related complication (Sepkowitz 2003). In more recent (post-2000) vaccination programmes involving health care workers in Israel and the USA, there were no reports of transmission to patients (Lane & Fulginiti 2003).

4.6.2   *Transmission to and between animals*

93.   Outbreaks of VACV infection amongst dairy cows are the best documented examples of transmission between humans and animals. Viral transmission occurs mainly via direct contact between milkers and cattle. Daily and intensive hand-milking leads to infection of dairy workers by infected cows, and further transmission to cows by milkers with lesions on their hands and fingers (de Sant'Ana et al. 2013a; de Sant'Ana et al. 2013b; Quixabeira-Santos et al. 2011).

94.   Calves of infected dairy cows have developed oral infections, likely due to direct contact with infected teats or with infected milk ((Quixabeira-Santos et al. 2011; de Sant'Ana et al. 2013a) and paragraph 89).

95.   Faecal shedding from experimentally infected cows has been demonstrated (Rivetti, Jr. et al. 2013), and mice exposed to bovine faeces displayed signs of viral replication (D'Anunciacao et al. 2012). VACV strains of both high and low pathogenicity can also be shed by and transmitted amongst laboratory mice via their excrement, even where the mice appeared asymptomatic (Ferreira et al. 2008). Murine faeces exposed to environmental conditions retained infectious VACV particles for at least 20 days (Abrahao et al. 2009b). These data suggest that horizontal transmission via contaminated faeces is possible, and that faeces could provide a means for viral dissemination into the environment (Abrahao et al. 2009b; D'Anunciacao et al. 2012).

96.   Asymptomatic VACV infection has been documented in domestic dogs and wild opossums co-located with outbreaks of bovine vaccinia. A recent serological study conducted in Brazil also reported dogs as the domestic species with the highest rate of seropositivity (22.8%), leading to suggestions that they (and potentially other mammalian species) could act as a reservoir for the virus, acquiring and transmitting it without showing clinical signs. Alternatively, they could be incidental hosts' that nonetheless could spread VACV to the environment (Peres et al. 2013; Peres et al. 2016).

97.   Peri-domestic rodents have also been hypothesised to act as VACV hosts, acting as a conduit between domestic animals and wildlife, and initiating VACV outbreaks temporally and spatially distant from previously infected areas (Abrahao et al. 2009a; Franco-Luiz et al. 2014).

98.   In the context of smallpox vaccination, the US Centres for Disease Control and Prevention (US CDC) has advised that there is potential for transfer of VACV to animals from a human with an unhealed vaccination site. Should an animal develop an active vaccinia lesion, further transmission is possible. Avoiding exposure of domestic animals to unhealed vaccination sites or to fomites contaminated with fluid from a vaccination site is recommended[5] (Centres for Disease Control and Prevention 2009).

4.7   VACV persistence in infected hosts

99.   VACV is thought unable to persist in a latent state within an infected host. The large poxviral genome is apparently unstable in host cells, and the large size of virus particles encourages their clearance by phagocytic cells of the immune system (Buller & Palumbo 1991).

4.8   VACV-host interactions

100.   VACV infection in immune-competent hosts is generally self-limiting and involves an interplay between the host immune response and viral evasion strategies in which the host ultimately prevails.

101.   The host response to VACV infection is multifactorial. Immediately after viral invasion, nonspecific mechanisms such as apoptosis induction, complement, interferons, cytokines and natural killer cells serve as a first line of defence. Subsequently, adaptive immune responses mediated by cytotoxic and helper T cells develop. Although neutralizing antibodies contribute to host protection (especially in preventing subsequent infection with VACV), the T cell response is particularly potent and may be critical for viral clearance. The response is mediated

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[5] Recommendations from the US CDC and other health authorities (Centres for Disease Control and Prevention 2009; Wisconsin Department of Health Services 2003) for avoidance of human to animal transmission after vaccination include:
- Do not let animals sniff or have direct contact with a vaccination site or the bandages, clothing, towels, sheets etc that have been in direct contact with the vaccination site or scab;
- Keep pets out of the room when changing bandages or clothes;
- Before allowing a pet back into the room after changing a bandage, place it in a sealable plastic bag and store it in a place that pets cannot access until it is disposed of –make sure pets and other animals do not have access to trash containers that have bandages in them;
- Launder (using hot water and detergent or bleach) any clothing, towels or other materials that have touched the vaccination site;
- Wash hands after touching the vaccination site or other items that have touched the vaccination site (e.g. bandages or clothing);
- People who come into contact with livestock should ensure their vaccination site is covered with a bandage and with clothing, and should wash their hands thoroughly before caring for animals or handling equipment such as bridles or buckets.

by T helper 1 (T_h1) cells, which enhance the cytolytic activity of macrophages and cytotoxic T cells which can then kill virally infected cells. In contrast, development of a T_h2 response, which promotes antibody production, may actually suppress viral clearance.

102. To counter host immunity, VACV deploys a range of immune evasion strategies that suppress innate immunity and the T_h1 immune response. These include interference with antigen presentation to T cells, inhibiting complement activation and suppressing apoptosis of infected cells. These tactics allow VACV to infect its hosts and replicate with high efficiency (Owen et al. 2013; Shen & Nemunaitis 2005; Thorne et al. 2005).

4.9   Adverse reactions to VACV infection

103. Although smallpox vaccination using VACV was generally safe and effective, it is well-documented that it caused serious adverse reactions at a higher rate than any other type of vaccine. Most complications occurred in the vaccinated individuals, but as VACV is transmissible, serious and even fatal reactions sometimes developed in others. Several types of adverse event occurred in healthy people, while others were associated with specific risk factors (Cono et al. 2003; Fulginiti et al. 2003; Lane & Goldstein 2003a; Lane & Goldstein 2003b; Maurer et al. 2003; Neff et al. 2002; Wittek 2006).

104. *Accidental implantation* (or self-inoculation) of a body part other than the vaccination site is the most common complication, with the face, eyelid, nose, lips, genitalia and anus most often affected. Transmission to another non-immune person can also occur by this route. The best preventative measure as recommended by the US Center for Disease Control (CDC) *Advisory Committee on Immunization Practices* (ACIP) is consistent hand hygiene using anti-microbial soap and water, or alcohol-based hand disinfectant, after contact with the vaccination site or with materials that have come into contact with it (Maurer et al. 2003; Wharton et al. 2003). No specific treatment is needed if there are only a few implanted lesions. However, multiple or confluent lesions warrant treatment with vaccinia immunoglobulin (VIG).

105. About 6% of patients with vaccinia in the eye develop *vaccinial keratitis*. This may occur where there is injury to the cornea or conjunctiva, allowing viral replication. Ulceration and scarring as the lesion heals can lead to permanent impairment of vision (Fulginiti et al. 2003).

106. Generalised vaccinia is a rare condition associated with viraemic spread of virus from the vaccination site in presumably healthy individuals. Systemic infection enables skin lesions to form in locations distant from the vaccination site and sometimes covering the entire body. Onset is typically within a week of vaccination and, while visually distressing, the condition usually resolves in 1-2 weeks. Extensive or recurrent disease is treatable with VIG.

107. *Post-vaccinial central nervous system (CNS) disease* – post-vaccinial encephalopathy most often affected children under age two and developed 6-10 days after vaccination, while postvaccinial encephalitis usually affected those older than two years and developed 11-15 days post-vaccination. The conditions were characterised by headache, fever, vomiting, seizures and coma and up to one third of cases were fatal. In addition, up to half of the survivors had permanent neuralgic problems.

108. *Congenital* or *fetal vaccinia* was a rare complication, with only 50 cases reported in the literature (Cono, 2003). It results from maternal exposure to VACV during pregnancy or shortly before conception and often led to stillbirth or neonatal death. Due to its rarity, specific risk factors have not been determined. No other specific risks to fetuses or pregnant women have been identified.

109. *Progressive vaccinia* (or *vaccinia necrosum*) is life-threatening and the most severe complication of smallpox vaccination. It is defined as a spreading necrosis at the site of inoculation, with or without metastatic necrotic lesions occurring elsewhere on the body (Neff, 2002). It occurred only in immune-compromised individuals whose defective immunity left

them unable to resolve the infection. The condition involves unchecked viral replication, with slow but progressive necrosis leading to severe viraemia, shock and death.

110. Eczema vaccinatum (EV) is a localized or generalized pustular rash which can occur anywhere on the body but displays a preference for areas of previous atopic dermatitis lesions as the disrupted skin allows viral implantation. People with a history of atopic dermatitis are at greatest risk of this complication, and more reported cases were due to contact transmission than to primary vaccination. EV lesions follow a similar course to the normal vaccination lesion but confluent lesions may occur. Fever and lymph node involvement are common and patients are systemically ill. Without treatment, the condition can be fatal.

111. A number of retrospective studies have estimated the frequency of these complications. Findings vary and are limited by the accuracy of reporting, variation in definitions used at the time, and availability of clinical data. Aggregate data from four studies of US data are shown in Table 1. It is generally acknowledged that populations today include more immunosuppressed people, more people with no immunity to VACV, and higher rates of atopic dermatitis than when VACV was widely used for smallpox vaccination (Cono et al. 2003; Engler et al. 2002; Fulginiti et al. 2003). However, medical interventions are also more advanced.

Table 1 Estimated rates of occurrence of adverse events associated with smallpox (vaccinia virus) vaccination. From (Fulginiti et al. 2003).

Type of adverse event	Rate of adverse events occurring after vaccination at indicated age, no. of events/ 1 million primary vaccinees			
	<1 year	1–4 years	5–19 years	≥20 years
Death (all causes)	5	0.5	0.5	5[a]
Postvaccinial encephalitis	6	2	3	4
Vaccinia necrosum	1	0.5	1	>10[a]
Eczema vaccinatum	14	44	35	30
Generalized rashes[b]	400	9600	140	250
Accidental implantation	507	577	371	606

NOTE. Serious reactions are rare, numbering 3–5 events/1 million primary vaccinations. The small numbers given in this table, divided by age group, may exaggerate the actual frequency. Data are from [6–9].

[a] These nos. are "modern" estimates based on the change in population.
[b] See "Erythema Multiforme."

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4.9.1 Treatment of adverse reactions

112. Vaccinia immunoglobulin (VIG) is made from the plasma of recently vaccinated people and has been successfully used to treat certain complications of vaccinia infection. It is recommended for treating severe cases of accidental implantation, severe generalised vaccinia, eczema vaccinatum and severe progressive vaccinia. It is not recommended for mild instances of accidental implantation, mild or limited generalised vaccinia, and post-vaccinial CNS disease. VIG is contraindicated in patients with vaccinia keratitis (Centers for Disease Control and Prevention 2003; Cono et al. 2003; Enserink 2002; Maurer et al. 2003).

113. VIG has not been tested in controlled clinical trials. It is available from the US CDC in limited quantity and under an Investigational New Drug (IND) protocol for treatment of specific smallpox vaccine reactions. The applicant has advised that sufficient VIG to treat three patients has been stockpiled in Singapore and could be shipped to Australia within 24 hours. More VIG can be provided to the Singapore depot as needed.

114. The antiviral drug Cidofovir may be considered as a second line treatment for the adverse reactions listed in paragraph 112. While Cidofovir has shown anti-poxviral activity *in vitro* and in mice, it has not been used to treat VACV infection in humans (Maurer et al. 2003; Wittek

2006). It can also have severe side effects, including irreversible renal toxicity (Enserink 2002). The US CDC recommends its use only where all VIG supplies are exhausted, a patient fails to improve with VIG treatment, or for patients near death (Centers for Disease Control and Prevention 2003). Cidofovir is available in Australia but is not approved for the treatment of vaccinia-related complications; off-label use would thus be required. The applicant has advised that it could be included on the Clinical Trial Notification as a rescue medication for this study.

4.9.2   Contraindications for use of smallpox vaccine (VACV)

115.   The specific conditions associated with the adverse reactions described above are contra-indications for smallpox vaccination. People who should not be exposed to VACV are those:

- with a history or presence of eczema or atopic dermatitis;
- with other acute, chronic or exfoliative skin conditions;
- with conditions associated with immunosuppression;
- who are pregnant or breast-feeding;
- who are aged less than one year; or
- who have a serious allergy to any component of the vaccine (Wharton et al. 2003)

4.10 Recombination with other poxviruses

116.   Homologous recombination between vaccinia strains (Fenner & COMBEN 1958), and between vaccinia and other poxviral species (WOODROOFE & Fenner 1960), has been demonstrated *in vitro*, and requires both viruses to be present and replicating within the same infected host cell. The ability to recombine with cytoplasmic nucleic acid forms the basis of the molecular biology techniques used in constructing the GM virus described in this application (Nakano et al. 1982; Weir et al. 1982).

117.   Genetic recombination appears to have occurred in nature between other orthopoxviruses (Gershon et al. 1989). Several examples that suggest horizontal gene transfer (HGT) into VACV have also been reported: two *cowpox virus*-like genes are present in VACV Lister strain (Garcel et al. 2007) and a small *horsepox virus*-like region was identified in a Dryvax subclone (Qin et al. 2011). It was once common to periodically cocultivate smallpox vaccine strains with other orthopoxviruses so as to 'refresh' their efficacy - a practice that could well have produced recombinants (Qin et al. 2011).

118.   Although replicating poxviruses can recombine very efficiently under certain circumstances, there are physical constraints within a cell that limit recombination between co-infecting viruses. Poxvirus replication and virion assembly takes place in membrane-wrapped intracellular structures (virosomes), which interact only to a limited extent and appear to limit the mixing of different viral DNAs (Lin & Evans 2010).

119.   *In vitro* studies have demonstrated recombination between GM VACV (the attenuated modified vaccinia virus Ankara (MVA) strain carrying an inserted gene) and naturally-circulating *cowpox virus* in co-infected cells. Hybrid progeny viruses displayed phenotypic differences relative to both parents, and the transgene was readily lost. Other genetic changes were not characterised (Hansen et al. 2004; Okeke et al. 2009). *In vitro* co-infection of different VACV strains yielded recombinant progeny with a patchwork of genomic exchanges (Qin & Evans 2014).

120.   Despite concern regarding potential recombination between the VACV-based rabies vaccine V-RG® and poxviruses circulating in wildlife (Boulanger et al. 1996; Sandvik et al. 1998), no reports assessing whether or not this has occurred were found.

4.11 Susceptibility of VACV to disinfectants

121. Purified VACV is inactivated within 1 minute by a range of common chemical disinfectants including 70% ethanol, 50% isopropyl alcohol and 0.5% sodium hypochlorite (Chambers et al. 2009).

122. Vaccinia samples on hands were disinfected by 2-5 minutes contact with 1.5% chloramine T or 70% isopropylalcohol (Schumann & Grossgebauer 1977), and showed 99.99% reduction in titre from a 30 second hand wash in disinfectants containing greater than 75% ethanol (Kampf et al. 2007).

123. The US CDC *Advisory Committee on Immunization Practices* (ACIP) and Healthcare Infection Control Practices Advisory Committee (HICPAC) recommend that appropriate hand hygiene after contact with items that may be contaminated with VACV includes washing with antimicrobial soap and water or an approved alcohol-based hand-rub containing 60% alcohol or more (Wharton et al. 2003).

4.12 Survival of VACV in the environment

124. Poxviruses are well known for their ability to persist in the environment. They are highly resistant to drying and, historically bedding, clothes and personal effects belonging to smallpox patients remained contagious for several years. Their stability is enhanced by the materials in which they are introduced into the environment: scabs, serum, blood and other proteinaceous secretions (Rheinbaben et al. 2007; Wood et al. 2013).

125. Poxviruses are more tolerant of increased temperature than other enveloped viruses and their environmental resistance at ambient temperature is high. VACV shed in mouse faeces can remain viable for 20 days or more (Abrahão et al. 2009), and VACV in scabs remains viable for over eight weeks at 35°C. Virus from patients is commonly more resistant than virus isolated from cell culture. Cell-bound cultured virus is in turn more resistant than purified virus preparations isolated from culture supernatants (Rheinbaben et al. 2007).

126. At lower temperatures, poxviral stability is even higher. Dried VACV particles can be stored at 4°C for over 35 weeks with no loss of infectivity, and VACV in storm water remained viable at 4.5°C for close to 6 months. When stored frozen (-20°C), one in one thousand virus particles remained viable after 15 years (Essbauer et al. 2007; Rheinbaben et al. 2007).

Section 5   The GMO – nature and effect of the genetic modification

5.1   Introduction to the GMO

127. The GMO was first described by Mastrangelo and colleagues as a vehicle for intra-tumoural expression of human granulocyte-macrophage colony-stimulating factor (hGM-CSF). Their objective was to facilitate the presentation of tumour antigens to the immune system in an environment favourable for developing systemic anti-tumour immunity (Mastrangelo et al. 2000a; Mastrangelo et al. 1998).

128. VACV was used as the vector due to its high infection efficiency and broad cell tropism, which offered potential for therapeutic use against tumours of diverse origin. In addition, its ability to replicate in and lyse cells, releasing infectious progeny, was expected to amplify its effectiveness over that of the initial dose alone. GM-CSF was selected from amongst several immunogenic cytokines as earlier work showed that a tumour vaccine expressing GM-CSF conferred anti-tumour immunity more effectively than vaccines expressing other immunogenic cytokines (Dranoff 2003). Furthermore, GM-CSF had been used clinically in cancer patients and was considered safe and effective in this context.

129. The GMO was produced by homologous recombination into the viral *TK* gene (a commonly used locus), disrupting the TK sequence and introducing the *hGM-CSF* and *E. coli*

lacZ genes. The latter, encoding the β-galactosidase enzyme, was included as a marker to enable infected cells to be detected histochemically and immunologically. On the basis of prior clinical experience with both Dryvax[TM] VACV and GM-CSF, the GM virus was tested directly in patients with metastatic melanoma. No prior *in vitro* or animal studies have been published (Mastrangelo et al. 1998).

130. The GMO has subsequently been investigated as an oncolytic virus (OV) on the basis of preferential replication in and destruction of tumour cells, while also stimulating a systemic anti-tumoral immune response through the expression of the introduced *hGM-CSF* gene (reviewed in (Breitbach et al. 2012; Breitbach et al. 2015; Merrick et al. 2009).

5.2   The genetic modifications and their associated effects

131. The GMO was constructed using standard molecular biology techniques. Briefly, the *hGM-CSF* and *lacZ* genes, flanked by VACV *TK* sequences, were cloned into a plasmid. The plasmid was transfected into cultured mammalian cells already infected with the parent virus preparation (Dryvax[TM]), allowing the two foreign genes to integrate into the viral *TK* gene through homologous recombination (Figure 4). A single virus clone containing the desired genetic change was isolated by plaque purification.

Figure 4.   Structure of the modified region of the JX-594 genome (from (Mastrangelo et al. 1998)).

5.2.1   *Disruption of Thymidine Kinase gene*

The thymidine kinase gene

132. Thymidine kinase (TK), encoded by the *TK* gene, is an enzyme found in most living cells and some viruses. It catalyses the transfer of the terminal phosphoryl moiety from adenosine triphosphate (ATP) to deoxythymidine (dT), yielding deoxythymidine monophosphate (dTMP) (El Omari et al. 2006). This is a necessary step in the production of deoxythymidine triphosphate (dTTP), one of the four nucleotides that make up the DNA molecule.

133. Normal cells express TK when preparing to divide: it is low or absent in resting cells, starts to appear in late G_1 phase, increases in S phase and disappears during mitosis (Welin et al. 2004). The role of viral TK is to generate sufficient dTTP for viral DNA synthesis in host cells that are not actively dividing.

134. The VACV TK gene encodes a 19 kDa polypeptide of 177 amino acids (Weir, 1983) which folds into a protein with two domains: a larger N-terminal α/β-domain and a smaller zinc-containing domain. Binding of the substrates (ATP and dT) involves both domains (El Omari et al. 2006; Welin et al. 2004). The functional enzyme is a homotetramer of TK monomers.

The genetic modification

135. No TK sequence has been deleted in the GM virus. The introduced genes disrupt the *TK* gene partway through the region encoding the N-terminal domain. Mastrangelo et al. (2000) reported that at least part of the viral gene is still transcribed (Mastrangelo et al. 2000a).

However, a partial TK polypeptide sequence, if translated, is unlikely to fold correctly and would be degraded by the cell.

136. Loss of viral *TK* function leaves the virus dependent on host cell nucleotides, which are typically found in dividing cells and in greatest abundance in tumours (Autio et al. 2014; Zeh & Bartlett 2002). Accordingly, the GM virus is expected to replicate preferentially in these cell types.

137. This strategy has been employed in the design of other oncolytic viruses, for example, the GM *herpes simplex virus 1* (known as Talimogene laherparepvec), recently approved by the Regulator for commercial supply (DIR 132) and included on the Australian Register of Therapeutic Goods as a cancer therapy by the TGA.

Effect of disrupting the thymidine kinase gene

138. No *in vivo* data directly comparing the GM virus with unmodified VACV (either the Dryvax® preparation or the specific parental clone) is available, either in tumour models or healthy subjects. However, in the context of other VACV strains, constructs with a disrupted or deleted TK gene generally show attenuation relative to the parent virus. Some examples are discussed below.

139. Buller and colleagues were the first to show that disruption of the VACV *TK* gene during homologous recombination significantly attenuated the recombinant virus. However, the route of exposure influenced the outcome. TK⁻ recombinants were less pathogenic than their parent virus when introduced intracerebrally or intraperitoneally into mice but, when introduced intradermally, *TK* disruption did not affect viral replication (Buller et al. 1986).

140. In contrast, Naik and coworkers found that TK-disruption attenuated the highly virulent Western Reserve (WR) strain of VACV when introduced intradermally into non-human primates (rhesus macaques). The area of necrosis produced by the virus was greatly reduced by TK disruption, although a significant lesion still formed. The less pathogenic Wyeth strain produced only a small lesion that was, in fact, slightly larger in response to the TK⁻ form (Naik et al. 2006).

Animal exposures to TK- VACV constructs

141. The safety of a *TK*-disrupted GM VACV (Copenhagen strain) towards a wide range of animal and bird species has been extensively studied in the context of the rabies vaccine Raboral V-RG® (V-RG) – a GM VACV in which the rabies glycoprotein gene was inserted within the viral *TK* gene. Intended for oral vaccination of wild animals, it has been distributed widely in the environment in Europe and North America since 1987 and 1995, respectively.

142. V-RG and the parental VACV Copenhagen strain displayed similar pathogenicity towards non-human primates after intradermal inoculation. Both viruses caused lesions of similar size at the inoculation site which resolved within three weeks, but no signs of systemic illness and no oral or fecal shedding (Rupprecht et al. 1992).

143. V-RG replicates sufficiently well in target species (red foxes, raccoons and striped skunks) to induce high level immunity to rabies, but no vaccinia-related pathogenicity has been observed at any dose or by any route of administration tested (oral, intramuscular, intraduodenal, subcutaneous, intradermal, conjunctival and intranasal). When administered intradermally to foxes, the cutaneous reaction was significantly reduced relative to the reaction to unmodified VACV (Copenhagen strain). When administered orally, unmodified and TK⁻ viruses behaved similarly to one another, but neither spread beyond the oral cavity or persisted beyond 48 hours, and viral titres were very low (Blancou et al. 1986; Brochier et al. 1989; Pastoret & Brochier 1996; Rupprecht et al. 1988; Thomas et al. 1990).

144. The safety of orally-administered V-RG has also been demonstrated in non-target species including wild boars, badgers, bats, coyotes, and a range of rodent and bird species. Sufficient viral replication for seroconversion took place, however there were no vaccine-related pox lesions or other clinical signs. Apathogenicity of orally delivered V-RG has also been demonstrated in domestic non-target species including laboratory mice, rabbits, ferrets, cattle, cats, dogs and sheep (Artois et al. 1990; Brochier et al. 1989).

Human exposures to TK⁻ VACV constructs

145. A laboratory worker who handled a TK-disrupted form of the Western Reserve VACV strain developed a large (15 mm) inflammatory lesion on one finger, and a smaller lesion on the other hand. The worker had been vaccinated against smallpox 28 years previously and may have retained partial immunity. The infection did not spread, there were no signs of systemic disease and the lesions resolved in under three weeks (Mempel et al. 2003).

146. TK⁻ forms of VACV may affect immune-compromised individuals more seriously. From a small number of human exposures to V-RG[6], two case reports of serious human infection involving at-risk individuals have been published. Both cases involved cutaneous exposure and resulted from handling a ruptured bait sachet found by their dog. The first case involved a pregnant woman with a chronic skin condition who did not wash the site of exposure and developed necrotic lesions, redness and swelling of the affected limb and enlargement of the draining lymph nodes, followed by generalized erythroderma[7] and sloughing of skin from her face, neck, palms and soles. She was hospitalised but not initially diagnosed with vaccinia infection. The lesions were surgically removed and she recovered 34 days post- exposure (Rupprecht et al. 2001). In a second case, a woman taking immunosuppressive medication developed a spreading pustular infection, required two weeks hospitalization and was treated with VIG due to concerns about progressive vaccinia (Dato et al. 2009). In the laboratory setting, experiments with immunodeficient mice showed that orally delivered V-RG was non-pathogenic but parenteral (transdermal, intramuscular and intraperitoneal) exposure led to systemic and progressive infection, albeit less severe than caused by unmodified VACV (Hanlon, 1997).

5.2.2 Introduction of the hGM-CSF gene

147. The gene encoding hGM-CSF has been inserted within the VACV TK gene. GM-CSF is a haematopoietic growth factor produced by a wide range of cell types in response to specific signals. It plays a redundant role in stimulating the development and differentiation of myeloid cells. In addition, it has a range of pro-inflammatory effects on mature haematopoietic cells and

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[6] In a 2001 report on exposures to environmentally-distributed Raboral V-RG® vaccine in Ohio during 1990-2000, 160 instances of human contact with baits containing the vaccine (2ml liquid in a plastic sachet) were cited. In twenty cases, humans were potentially exposed to vaccine due to rupture of the sachet. Two exposed people listed a contraindicated health condition – one being the first case discussed in paragraph 146. The second (a pregnant woman) washed her hands after touching the bait and no adverse consequences were reported (Rupprecht et al. 2001). The second case discussed above was associated with a US wildlife vaccination program running from 2003-2009. In 2008, 291 instances of human or domestic animal contact with baits were reported. The article does not indicate how many of these involved human exposure to the vaccine or contraindicated conditions. The patient described was the only documented case of vaccinia infection associated with this baiting program.

[7] *Erythroderma* is an inflammatory skin disorder characterised by intense redness and scaling, and typically involves almost the whole body surface. Severe cases can be fatal even when properly managed due to metabolic burden and complications (Okoduwa et al. 2009).

is involved in the development of inflammatory and autoimmune diseases (reviewed in (Shiomi & Usui 2015)).

148. *hGM-CSF* gene expression is driven by the non-coding synthetic early-late VACV promoter (Chakrabarti et al. 1997). This promoter has been widely used to direct gene expression throughout the full VACV lifecycle (Baur et al. 2010). Promoter activation is stronger and more sustained in the later stages of the viral lifecycle, thus tying high expression of the transgene to viral replication (Hwang et al. 2011).

149. Expression of hGM-CSF within the inoculated tumour is intended to attract and activate dendritic cells. These are antigen presenting cells which can take up and present tumour antigens released by viral lysis of tumour cells, thus stimulating a specific anti-tumour immune response. hGM-CSF was selected over other cytokines for two reasons: (1) it was the most potent stimulator of anti-tumour immunity of a number tested; and (2) prior clinical experience with its use in cancer patients (Dranoff 2003; Mastrangelo et al. 2000a).

150. hGM-CSF exhibits a degree of species-specificity. It is inactive in mice (Kaushansky et al. 1989; Lee et al. 1985), however is active on canine cells and displays limited cross reactivity in cats (Dunham & Bruce 2004; Mayer et al. 1990). The activity of hGM-CSF in other species is not known.

151. Purified recombinant hGM-CSF(Leukine®) is a United States Food and Drug Administration-approved pharmaceutical used to reconstitute depleted myeloid cells in certain types of cancer patients and after bone marrow transplants. It is viewed as safe for use in cancer patients, but is associated with a number of side effects.

152. At clinically relevant doses (5-10 µg/kg/day), mild-moderate adverse reactions occur in 20-30% of patients and commonly include fever, myalgia, malaise, bone pain, rash and local reaction at the injection site. Skin reactions usually resolve within a few days of discontinuing GM-CSF. Severe reactions are rare (less than a few percent). Early clinical trials using doses above 10 µg/kg were associated with a greater incidence of mild-moderate effects and also the presentation of serious adverse reactions (Stern & Jones 1992).

153. Serum levels of hGM-CSF following therapeutic doses of the cytokine exceed 1 ng/ml (Cebon et al. 1992). In clinical trials of the GMO, detectable levels of circulating hGM-CSF protein have been generated in many patients, with a small percentage reaching systemic levels similar to those associated with therapeutic doses (Park et al. 2015; Park et al. 2008).

154. *In vivo*, GM-CSF inoculation is associated with a local influx of immature dividing monocytes, granulocytes and activated lymphocytes to the inoculation site. In the tumour context, the immune response is mainly active against cancerous tissue, however a small antiviral response was observed in another recombinant viral system (Grossardt et al. 2013).

155. In a preclinical study of the GM virus, high doses of JX-594 and a similar construct expressing murine rather than human GM-CSF (mJX-594) were inoculated intracerebrally into rats and mice. Both viruses were well tolerated in mice, but rats responded poorly to mJX-594. They exhibited poorer grooming and were less active 3-5 days after inoculation, though regained health thereafter. mJX-594 induced more extensive inflammation and tissue necrosis than JX-594, suggesting this was due to GM-CSF expression rather than to the viral infection (Lun et al. 2010).

156. hGM-CSF has been introduced into several other recombinant oncolytic viruses intended for cancer treatment, including adenovirus, Newcastle disease virus, herpes simplex virus

(HSV) and measles virus (Grossardt et al. 2013). The Regulator recently approved the commercial supply in Australia of GM *herpes simplex virus 1* (known as Talimogene laherparepvec) expressing hGM-CSF and intended as a prescription-only cancer therapeutic[8]. Therapeutic use of Talimogene laherparepvec is subject to approval by the Therapeutic Goods Administration.

5.2.3   Introduction of E. coli LacZ gene

157.   The bacterial *lacZ* gene encodes the enzyme β-D-galactoside galactohydrolase (β-galactosidase), which hydrolyses b-galactosidic bonds in sugars such as lactose. It is part of the *lac* operon (lactose operon) found in *E. coli* and many other enteric bacteria. While glucose is the preferred carbon source for most bacteria, the *lac* operon allows for digestion of lactose when glucose is not available.

158.   The *lacZ* gene has a long history as a marker or reporter of promoter activity, as the β-galactosidase enzyme can be detected using a simple biochemical test. It has been expressed both ubiquitously and in selected tissues in transgenic mice, and does not cause obvious phenotypic changes or toxicity (Beddington et al. 1989; Suemori et al. 1990; Zambrowicz et al. 1997; Savatier et al. 1990).

159.   The *lacZ* gene has been expressed in a variety of cell types in different species, including cotton rats and non-human primates. Cells expressing this reporter gene have also been safely introduced into humans (Puumalainen et al. 1998).

160.   In the GM virus, expression of the *lacZ* gene is driven by the p7.5 VACV promoter. As this is a constitutive early/late promoter, the LacZ gene product would be expressed both early and late in the virus life cycle (Baur et al. 2010).

5.2.4   Toxicity or adverse response associated with the genetic modifications

161.   The GM virus is intended for clinical use as a therapeutic agent for patients with, in this clinical trial, advanced HCC. It is cytotoxic for infected tumour cells as the viral replication cycle ends in cell lysis. In addition, the GM virus is intended to stimulate a systemic anti-tumour immune response, directed at both infected and non-infected tumour cells.

162.   Should the residual *TK* gene be translated, it is not expected to fold correctly and would likely be degraded within the cell (see Section 5.2.1). Therefore, it is not expected to give rise to a toxin or allergen.

163.   hGM-CSF is an unmodified human protein that is an FDA-approved therapeutic product for cancer patients. As such, it is not a toxin or allergen. However, it is a pro-inflammatory cytokine and when present systemically at sufficient concentration, leads to the type of adverse reaction described in Section 5.2.2. When delivered locally, it stimulates localised inflammation (Lun et al. 2010). It displays limited cross-reactivity with other species (Section 5.2.2).

5.3   Characterisation of the GM virus

5.3.1   Genotype stability and molecular characterisation of the GM virus

164.   As no sequence was deleted from the *TK* gene in constructing the GM virus, it is possible that JX-594 could revert to the wild-type VACV sequence by eliminating the entire inserted expression cassette. However, genetic stability studies have not detected spontaneous

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[8] The Risk Assessment and Risk Management Plan for DIR-132 is available at
http://www.ogtr.gov.au/internet/ogtr/publishing.nsf/Content/DIR132

reversion. The applicant has stated that each batch of GM virus produced is tested for retention of the *hGM-CSF/LacZ* insert by real-time PCR using a primer pair that detects TK[+] viral genomes. To date, five lots have been tested and all have been negative for TK[+] virus.

165. β-galactosidase enzyme activity is also used as a marker of genomic integrity. Over 500 viral plaques from two clinical lots of JX-594 have been assessed, yielding 99.86% and 100% positive plaques.

5.3.2 Transmissibility of the GM Virus

166. There is little restriction on VACV entry into cells – permissiveness is at the level of survival and replication once inside the cell. Disruption of the viral *TK* gene renders the GM virus dependent for replication on cellular deoxythymidine. The GMO is therefore expected to replicate most effectively in cells with a high intracellular nucleoside pool. These are most likely to be cancer cells, followed by normally dividing cells. The GMO is expected to infect the same range of cells as the unmodified virus, and at the species level, the host range is unlikely to differ from that of unmodified VACV.

167. There are no studies describing the transmissibility of the GMO from humans to humans, humans to animals, or between animals. In previous clinical trials, measures were taken to minimise the opportunity for interpersonal transmission and no transmission to clinical staff or household contacts was reported.

168. In the context of the TK-disrupted VACV V-RG® (see paragraph 141), no horizontal transmission from vaccinated to unvaccinated animals kept in close proximity was detected in the case of foxes, wild boars, badgers or mice. In a small study, limited transmission between pairs of raccoons housed together did occur but was limited to male/female pairings. Suckling racoons replaced with their mother immediately after she received an oral dose of V-RG seroconverted and developed immunity to rabies. The route of transmission (e.g. lactation, grooming) was not determined (Blancou et al. 1986; Brochier et al. 1989; Rupprecht et al. 1988).

5.3.3 Non-clinical studies of the GMO

169. A safety study was performed in New Zealand White rabbits by intravenous infusion of one or three weekly doses of JX-594 (Kim et al. 2006). Doses of 4×10^8 pfu/kg were well tolerated; the only overt clinical sign observed over a 92 day period was a small initial weight loss that was regained over the next four weeks. There were no major toxicological findings. Mild inflammation in the liver and lungs, and mild follicular hyperplasia in the spleen were observed at day 4, when viral replication was expected to be maximal. Lymphoid hyperplasia increased over time but resolved by the end of the study (92 days). These findings are consistent with mild VACV infection (inflammation) and with expression of the hGM-CSF transgene (lymphoid hyperplasia).

170. As blood cells will come into contact with the GM virus, either via IV infusion or during secondary spread following *in vivo* replication, the GMO has been assessed for its ability to infect them. Parato and colleagues demonstrated that JX-594 replicates in neither resting nor activated peripheral blood mononuclear cells (PBMCs) isolated from human blood (Parato et al. 2012).

171. To examine the tumour selectivity of the GM virus, the *ex vivo* sensitivity of human tumour biopsy material and companion normal tissue were compared. Tumour tissue was more sensitive to JX-594 infection than the normal tissue counterpart (Breitbach et al. 2011; Parato et al. 2012). Tissue selectivity was also assessed in a transgenic mouse model of ovarian cancer. When mice were treated intraperitoneally with a version of the GM virus in which the *lacZ* gene was replaced by the luciferase marker gene, luciferase expression was concentrated in the cancerous region (Parato et al. 2012).

172. To examine the efficacy of the GM virus, JX-594 was tested in a rabbit liver cancer model. A single dose of the GMO was delivered either by direct injection into the liver tumour or by intravenous infusion to target both primary tumour and lung metastases. JX-594 delivered by either method slowed tumour progression and increased survival time significantly, and completely prevented the appearance of metastases (Kim et al. 2006).

173. JX-594 was further tested for efficacy against carcinogen-induced liver tumours in a rat model. Intravenously delivered JX-594 caused complete tumour regression in 5 of 6 animals, whereas tumours in control animals progressed to a point where the animals were sacrificed for ethical reasons (Kim et al. 2006).

5.3.4 Clinical trials of the GMO

174. A total of thirteen clinical studies have been or are currently being carried out in North America, Asia and Europe. Approximately 300 patients have received the GMO in 1200 treatments of varying dose. Seven trials have been completed and published, and relevant information is summarised in Table 2.

Table 2 Summary of published clinical trials.

No.	Study Design	Patients	Comments	Reference
1	**Phase I** open-label uncontrolled study investigating the safety and efficacy of JX-594 delivered by intratumoural (IT) injection. Twice weekly escalating dose treatments ($1\times10^4 - 8\times10^7$ pfu) for at least six weeks. Treatment of patients with unresectable stage 3-4 malignant melanoma. Trial ID: BB-IND 6486	7	Mild flu-like symptoms developed within 4-6 hours of higher dose treatments ($>=4\times10^7$ pfu) and resolved within 24 hours. Local toxicity: dose-dependent inflammation of injected tumours. Lesions: not discussed.	(Mastrangelo et al. 2000b)
2	**Phase I/II** open-label, multi-centre study investigating the safety and mechanism of action of JX-594 delivered by IT injection. Six treatments at weekly intervals. Single dose (1×10^8 pfu). Treatment of patients with unresectable stage 3-4 malignant melanoma. Trial ID: NCT00429312; JX594-IT-HEP001	10	All patients experienced at least one adverse event. Mild-moderate flu-like symptoms were most common. One case each of severe hypoglycaemia, fever and anaemia. All were transient. Circulating viral genomes detected in 50% of patients 5-7 days after initial inoculation. Lesions: not discussed.	(Hwang et al. 2011)
3	**Phase I** open-label study to determine maximum tolerated dose and assess the safety of JX-594 delivered by IT injection. Two to four treatments at three week intervals. Four doses (range $1\times10^8 - 3\times10^9$). Treatment of patients with unresectable primary or metastatic tumours in the liver. Trial ID: NCT00629759; JX594-MEL005	14	All patients experienced mild-moderate flu-like symptoms 4-16 hours after treatment. Dose-limiting toxicities at highest dose (3×10^9 pfu) (see paragraph 175). Circulating viral genomes detected in 12/14 patients between days 3 and 22 post-inoculation. Lesions: not discussed. Shedding: no virus detected in urine or throat swabs.	(Park et al. 2008) (Liu et al. 2008) (patients with HCC only)

No.	Study Design	Patients	Comments	Reference
4	**Phase I** open-label multi-centre study to determine the maximum tolerated dose of JX-594 delivered by intravenous (IV) infusion. Single treatment at one of six dose levels ($1\times10^5 - 3\times10^7$ pfu/kg). Treatment of patients with advanced/metastatic solid tumours of various origin, refractory to standard therapy. Trial ID: NCT00625456; JX594-IV-011	23	Most common adverse events were mild flu-like symptoms lasting up to 24 hours. No dose-limiting toxicities. Viral replication in tumours but not surrounding tissue. <u>Lesions</u>: a single skin lesion developed in each of two patients one week after infusion and resolved without sequelae. Treatment dose not reported. <u>Shedding</u>: see Section 5.3.6.	(Breitbach et al. 2011) (Breitbach et al. 2013)
5	**Phase I** open-label study to determine the maximum tolerated dose and investigate the safety of JX-594 delivered by IT injection. 1-2 treatments at one of two doses (1×10^6/kg and 1×10^7/kg). Treatment of pediatric patients (age 4-21 years) with advanced or metastatic, unresectable solid tumours of various origin. Trial ID: NCT01169584; JX594-IT-P009	6	Mild-moderate flu-like symptoms beginning at 6-10 hours and resolving by 24 hours. <u>Lesions</u>: developed in all three patients receiving the higher dose (ages 10, 18, 21 years). Developed within a week and resolved after 3-4 weeks.	(Cripe et al. 2015)
6	**Phase IB** open-label study to determine the maximum tolerated dose and investigate the safety of JX-594 delivered by IV infusion. 2-4 treatments at two week intervals, at one of three doses (range $1\times10^6 - 3\times10^7$ pfu/kg). Treatment of patients with advanced, refractory colorectal carcinoma. Trial ID: NCT01469611; SMC IRB 2009-06-055	15	All patients experienced mild flu-like symptoms, generally lasting less than 24 hours. No dose-limiting toxicities. <u>Lesions</u>: developed in 7/9 patients receiving the highest dose and none at the two lower doses. Appeared on days 3-7 of first cycle and resolved within 5-26 days. Locations included palms, soles, oral mucosa, lips. <u>Shedding</u>: no virus detected in urine. Throat swabs positive from days 5-8 in 1/6 lower dose patients and 5/9 at the highest dose. High dose patients with positive throat swabs had active oral or lip lesions at the time.	(Park et al. 2015)
7	**Phase IIA** open-label study investigating the safety, tolerability and efficacy of JX-594 delivered by IT injection. Three treatments at fortnightly intervals, at low or high dose (1×10^8 or 1×10^9 pfu). Treatment of patients with unresectable primary hepatocellular carcinoma. Trial ID: NCT00554372; JX594-IT-HEP007	30	Mild flu-like symptoms in all patients after 12-24 hours. One serious adverse event (high dose) – nausea and vomiting with prolonged hospitalisation. Circulating viral genomes detected in 3/30 patients between days 15-36 post-inoculation. <u>Lesions</u>: 1/16 high dose patients developed 8-10 skin lesions on extremities, face and trunk. Developed 4 days after treatment and resolved within 6 weeks.	(Heo et al. 2013)

175. The maximum tolerated dose was 1×10^9 pfu (the dose to be administered in the proposed Phase 3 trial). Patients receiving a higher dose experienced dose-limiting toxicities of severe abdominal pain, loss of appetite and tissue obstruction due to tumour swelling (Park et al. 2008).

176. At lower doses of the GMO, mild-moderate flu-like symptoms (e.g. fever, chills, fatigue, headache, myalgia, nausea, vomiting, anorexia , tachycardia, hypotension and hypertension) lasting up to 24 hours were common (Mastrangelo et al. 2000b; Cripe et al. 2015; Heo et al. 2013; Breitbach et al. 2011; Park et al. 2015; Liu et al. 2008; Park et al. 2008). Other adverse effects include transient decreases in lymphocytes, platelets and red blood cells (Park et al. 2008).

177. Circulating viral genomes were observed immediately and for some hours after intratumoural inoculation. They reappeared in the blood at later time points, consistent with viral replication in and shedding from tumours (Heo et al. 2013; Hwang et al. 2011; Liu et al. 2008; Park et al. 2008).

5.3.5 Lesion formation in the clinical studies

178. Thirteen (12.4%) of the 105 patients participating in the published clinical trials developed one or more skin or oral lesions within 3-7 days of the first treatment (Figure 3). Locations included the soles, palms, arms, fingers, torso, face, oral mucosa and lips. All lesions resolved within six weeks or less. Reportedly, skin lesions were not bothersome to the patients and healed without scarring, however oral/lip lesions did cause pain or other discomfort (Cripe et al. 2015; Heo et al. 2013; Breitbach et al. 2011; Park et al. 2015).

Fig. 4. Examples of skin lesions forming after intratumoural (A) and intravenous (B) treatment with JX-594 (from (Cripe et al. 2015) and (Park et al. 2015)).

179. Lesions developed after both intravenous and intratumoural inoculation of the GMO, and were reported in four of the seven published trials. In two of the trials where they were reported, inoculation was intravenous (Park, 2015), and in the other two, intratumoural (Cripe et al. 2015). Lesions formed more frequently after intravenous inoculation (9/38 patients) versus intratumoural (4/38 patients). In two of the four trials, their appearance was dose-dependent. Cripe and coworkers observed lesions in all three patients receiving the higher of two IT doses (1×10^7 pfu/kg), and in none of the patients who received 1×10^6 pfu/kg (Cripe et al. 2015). Likewise, Park at al. reported lesions in 7/9 patients receiving the highest IV dose (3×10^7 pfu/kg) and none at either of the lower doses (1×10^6 and 1×10^7 pfu/kg) (Park et al. 2015). These higher doses are comparable with the dose that patients in the proposed trial will

receive. It should be noted that in the study by Heo at al., only one of 16 patients receiving $1x10^9$ pfu IT developed lesions (Heo et al. 2013), so this reaction has varied markedly between studies.

5.3.6 Shedding of the GM virus in the clinical studies

180. Viral shedding from inoculated patients was monitored in the Phase I study summarised in row 4 of Table 2. In this trial, the GM virus was administered by intravenous (IV) infusion to patients with advanced, metastatic solid tumours (Breitbach et al. 2011). Each patient received a single treatment at one of six dose levels between $1x10^5$ pfu/kg body weight and $1x10^9$ pfu in total. Dose-related viral presence and replication was evident in tumour biopsies 8-10 days after treatment.

181. Viral shedding to the environment was monitored by collecting urine, blood and throat swab samples from all 23 patients at multiple time points over the 28 days post-treatment and testing for the presence of GM virus. No urine samples were confirmed positive for virus, while blood samples were still being assessed at the time of publication. In 25% of patients, all of whom had tumours contacting the upper aerodigestive tract, GM virus was detected in throat swabs at one or two time points during the first 2 weeks after treatment. The remaining 75% of patients tested negative at all times. No correlation with viral dose was reported.

182. Two patients each developed a single skin lesion, less than 5 mm in diameter, within a week of treatment. Fluid was obtained by either lifting the intact scab or lancing the intact pustule and swabbing. Both samples tested positive for GM virus by plaque-forming assay (which detects live virus) and PCR.

183. The applicant has also provided a data summary compiled from multiple clinical trials. Fluid drawn from intact GMO-related skin lesions contained live GM virus. Throat swabs revealed low levels of the GM virus in 30 of 93 patients 4-8 days after IV infusion of the GMO. In patients treated intratumourally, however, all throat swabs were negative. No GMO was detected in urine samples collected from a total of 62 patients at various times after treatment with JX-594.

Section 6 The receiving environment

6.1 Sites of release

184. The intended primary receiving environment would be internally-located (liver) tumours within the clinical trial participants. Each patient is to receive a total dose of $1x10^9$ pfu distributed between several tumours, administered via image-guided intratumoural injection.

185. The secondary receiving environment would be the hospital where the GMO is dispensed, administered and waste disposed of. All clinical sites involved in the study would be equipped to handle infectious agents and procedures would be conducted in accordance with Universal Standard Precautions (World Health Organisation 2007) and the *Australian Guidelines for the Prevention and Control of Infection in Healthcare* (National Health and Medical Research Council 2010).

186. The principal route by which the GM virus could enter the wider environment is by shedding from inoculated trial participants once they leave the hospital and return home. The tertiary receiving environment includes the trial participants' homes and any places they visit during the period when the GM virus is replicating and shedding. Furthermore, as the applicant has proposed that soiled dressings and cleaning materials used in caring for lesions be disposed of with general household waste, landfill sites also form part of the receiving environment.

6.2 Relevant environmental factors

187. Environmental factors relevant to the potential persistence or spread of the GMO, or the harm it may cause, include the presence of susceptible hosts and any physical conditions that may aid or restrict transmission to these hosts.

188. Smallpox vaccination was never mandatory in Australia, and ceased worldwide in 1980. Forty seven percent of Australian residents are under 35 and so would have no pre-existing immunity to VACV. Of Australian residents aged 35 and over, 57% were born in Australia and it is unknown how many would have received the smallpox vaccine. However, the 43% born overseas could have been vaccinated in their country of birth. People vaccinated many years ago may be less susceptible to VACV infection, or infection may be asymptomatic or produce less severe symptoms (Cohen 2001; Hatakeyama et al. 2005).

189. It is widely acknowledged that people for whom smallpox vaccination is contraindicated are more prevalent in the population today that during the era of mass smallpox vaccination. For example, 23% of Australian 6-7 year old children have a history of atopic dermatitis (Gold & Kemp 2005). There are also likely to be significant numbers taking immunosuppressive drugs for disease control (e.g for autoimmune inflammatory conditions), organ transplant recipients and people with HIV-AIDS.

190. Animals that can or may be infected with the GMO may be present in environments where it could be shed by trial participants (e.g. patients' homes). Such animals are most likely to include domestic pets and, potentially, livestock.

6.3 Related viral species in the receiving environment

191. The presence of related viral species may offer an opportunity for the horizontal transfer of introduced material from the GMO to other organisms in the receiving environment. As orthopoxviruses replicate in the cytoplasm and do not integrate into the genome of infected cells, horizontal transfer of introduced DNA would most likely be to another poxvirus.

192. Vaccinia virus is not endemic in Australia and is used only for occasional vaccination of laboratory personnel who are required to work with poxviruses. Clinical staff involved in the study will not be vaccinated. It is therefore not expected that trial participants or materials they dispose of would come into contact with unmodified VACV.

193. *Molluscum contagiosum virus* (MCV) is a relatively common poxvirus adapted specifically to humans. It is classified as a member of the family *Poxviridae,* but has no close relatives and is the only member of the *Molluscipoxvirus* genus. Since the eradication of smallpox, it has been the principal poxvirus cause of human disease. MCV causes localised infections in the epidermal layer of the skin, and rarely mucous membranes (Chen et al. 2013; Senkevich et al. 1997).

194. A study published in 1999 found that 23% of people in a random sample of Australian residents were seropositive for MCV, indicating a current or prior infection. While the disease can develop at any age, it is most common in children and young adults. Clinical infection is characterised by small benign skin lesions that persist for many months, with only a weak immune response and minimal inflammation. In immune-compromised individuals, skin lesions can become extensive, and MCV is an opportunistic infection of AIDS patients (Konya & Thompson 1999; Senkevich et al. 1997).

195. Poxviruses of the family *Poxviridae*, subfamily *Chordopoxvirinae* infect many native Australian mammals and reptiles. Aside from an outbreak in common ringtail possums attributed to an orthopoxvirus, these poxviruses have not been characterised. It is considered likely that all mammal and reptile species are susceptible to these viruses (Australian Wildlife Health Network 2012b). Avian poxviruses are also present in native bird populations (Australian Wildlife Health Network 2012a).

196.   The *myxoma virus* (family *Poxviridae*, genus *Leporipoxvirus*) specifically infects rabbits and hares (Wang et al. 2004), causing lethal disease in some species. It was introduced into Australia in the 1950s in an attempt to control the feral rabbit population (Kerr et al. 2013).

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

197.   The *hGM-CSF* gene encodes the protein human Granulocyte-Macrophage Colony-Stimulating Factor (hGM-CSF). GM-CSF homologues are found in all mammalian species, but display a degree of species specificity. For example, hGM-CSF is not active on mouse cells, and mouse GM-CSF is not active on human cells (Kaushansky et al. 1989; Lee et al. 1985). However, hGM-CSF is active on dog cells and weakly active on bovine cells, indicating that it does not exhibit absolute species specificity (Maliszewski et al. 1988; Mayer et al. 1990).

6.5   Presence of the *LacZ* gene and related genes in the environment

198.   The bacterial *lacZ* gene is found in *E. coli* and other enteric bacteria that reside in the mammalian gut and possess the ability to metabolise lactose.

199.   A related β-galactosidase enzyme is also ubiquitously expressed in mammals. The protein localises to the lysosomes and is distinguished from the bacterial enzyme by a lower pH optimum.

Section 7   Relevant Australian and international approvals

7.1   Australian approvals

7.1.1   Previous approvals by the Gene Technology Regulator

200.   The Regulator has not previously approved any DIR or DNIR licences for dealings with the proposed GMO.

201.   The Regulator recently issued a DIR licence (DIR-132) for the commercial supply of cancer therapeutic *Talimogene laherparepvec*, a tumour-selective GM virus based on *herpes simplex virus 1*. *Talimogene laherparepvec* also contains an introduced hGM-CSF gene.

202.   The Regulator has also issued a Limited and Controlled DIR licence (DIR-116) for a clinical trial involving a GM VACV and GM fowlpox virus. The purpose of the trial is to evaluate the efficacy of these GMOs in treating prostate cancer.

7.1.2   Approvals by other government agencies

203.   The proposed Phase 3 clinical trial will be notified to the TGA under the Clinical Trials Notification scheme. An import permit from the Department of Agriculture will also be required.

7.2   International approvals

204.   The GMO has been or is currently being evaluated in thirteen clinical trials in multiple countries, including the USA, Canada, France, Germany, The Republic of Korea, China and Taiwan. In April 2015, the US Food and Drug Administration agreed to a Special Protocol Assessment (SPA) of the current Phase 3 clinical trial. Import and controlled release of the GMO for the same trial was approved by the New Zealand Environmental Protection Authority on 27 October 2015. The GMO has not been approved for commercial use in any country.

Chapter 2   Risk assessment

Section 1   Introduction

205. 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 5). 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.
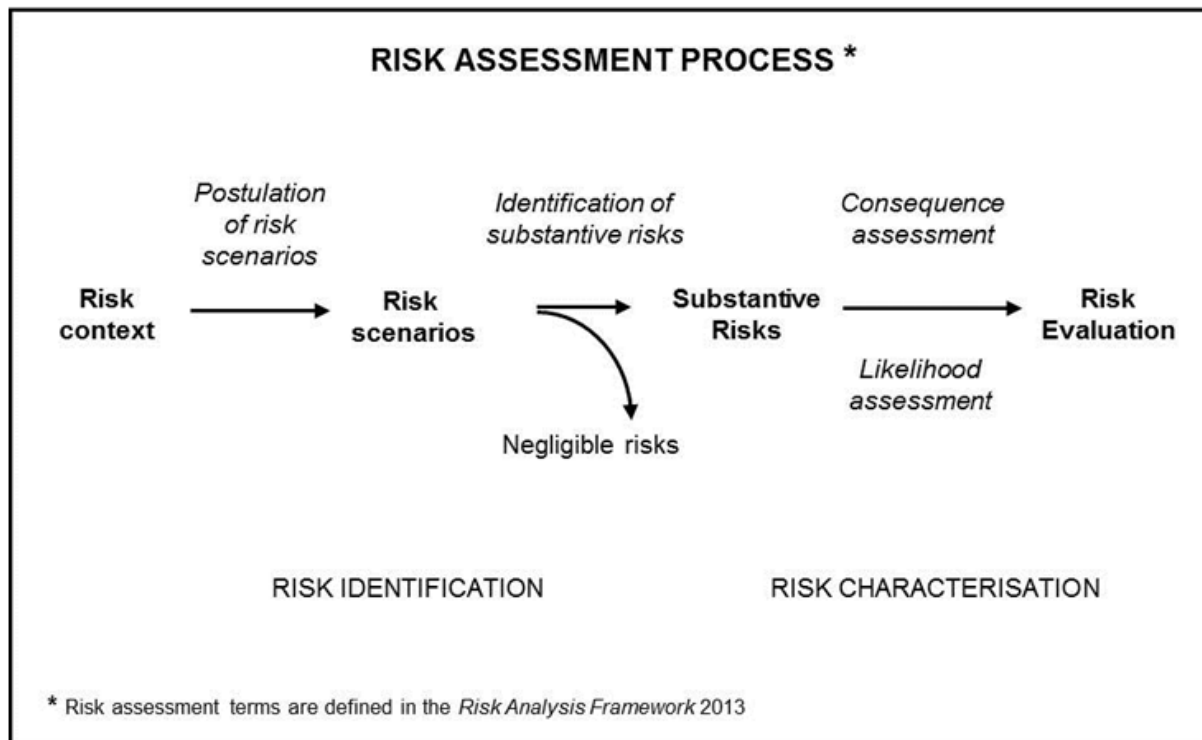

Figure 5.   The risk assessment process

206. 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.

207. 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.

208. 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.

209. 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

210. Postulated risk scenarios incorporate three components (Figure 5):

i.   The source of potential harm (risk source)

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

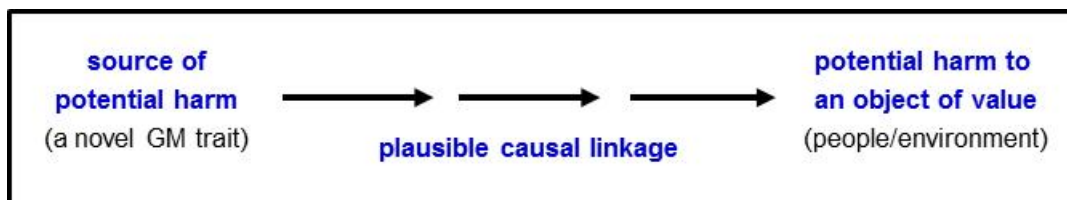iii.   Potential harm to an object of value, people or the environment.

Figure 6.   Risk scenario

211. 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, conduct experiments with, transport or dispose of the GMOs and the possession (including storage), supply and use of the GMOs in the course of any of these dealings;

- the proposed limits, including the extent and scale of the proposed dealings;

- the proposed controls to restrict the spread and persistence of the GMO;

- characteristics of the parent organism;

- routes of exposure to the GMOs, the introduced genes and gene products;

- potential effects of the introduced genes and gene products expressed in the GMOs;

- potential exposure to the introduced genes and gene products from other sources in the environment; and

- the environment at the site(s) of release.

212. As discussed in Chapter 1, Section 2, the TGA, the trial sponsor, the investigators and the Human Research Ethics Committee (HREC) all have roles in ensuring the safety of participants under the *Therapeutic Goods Act 1989,* and the use of a therapeutic good in a clinical trial must be in accordance with the *National Statement on the Ethical Conduct in Research Involving Humans* (National Health and Medical Research Council 2013). Therefore, risk scenarios in the current assessment focus primarily on risks posed to people other than those participating in the clinical trial, and to the environment.

213. Nine risk scenarios were postulated and screened to identify substantive risks. 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 control measures proposed by the applicant, two of the risk scenarios were identified as posing substantive risks which warranted further assessment. More detail on the scenarios not identified as substantive risks is provided later in this Section, while the substantive risks are characterised in Section 3 of this chapter.

Table 3   Summary of risk scenarios from dealings with the GMO

	Risk Scenario			Substantive risk?	Reasons
#	Risk source	Causal pathway	Potential harm		
Section 2.1. Increased disease burden					
(a) clinical symptoms following direct exposure to the GM virus					
1	GM virus	i. Exposure of persons dispensing or administering the GMO in a hospital via: - needle stick/sharps injury; - contact with abraded skin or mucous membranes (esp. eyes) ⬇ ii. Establishment of viral infection.	Clinical symptoms ranging from mild to severe (e.g. flu-like illness, formation of lesions, severe adverse reactions).	No	• The GM virus would be dispensed in a Class II BSC by staff wearing appropriate PPE and in accordance with approved clinical site procedures. • The GMO would be administered by trained medical staff wearing appropriate PPE and in accordance with Standard Universal Precautions and national guidelines. • The dose received through accidental exposure would be far less than that administered to trial participants. • Excluding high-risk individuals from handling or administering the GMO would minimise the possibility of a severe adverse reaction. • The GM virus is likely to be attenuated with respect to its ability to replicate efficiently in non-dividing cells. • Neither the disrupted gene nor the introduced genes have previously been associated with toxicity in people or animals. • The introduced *LacZ* gene encodes the *E. coli* β-galactosidase enzyme, which has a history of safe use. • The introduced *hGM-CSF* gene is a natural human protein. It may stimulate local inflammation if expressed at high levels but symptoms cease once expression ends. • Inadvertent exposures documented to date did not lead to clinically significant symptoms or require treatment beyond first aid and observation.

	Risk Scenario			Substantive risk?	Reasons
#	Risk source	Causal pathway	Potential harm		
2	GM virus	i. Unused GMO or waste containing the GMO disposed of from clinical site ⬇ ii. Exposure of persons handling waste to the GMO. ⬇ iii. Establishment of viral infection.	Clinical symptoms ranging from mild to severe (e.g. flu-like illness, formation of lesions, severe adverse reactions).	No	• Contaminated waste will be placed in appropriately labelled clinical waste containers and disposed of as infectious clinical waste. • As noted in Scenario 1, inadvertent exposure to the GMO is likely to involve a low dose, the GMO is likely to be attenuated; the introduced genes have not been associated with toxicity; any local inflammation which may be caused by expression of the introduced *hGM-CSF* gene would cease once expression ends; and previous inadvertent exposures have not lead to clinically significant outcomes.
3	GM virus	i. Exposure of people or animals to the GM virus due to unintentional release during transport or storage ⬇ ii. Establishment of viral infection	Clinical symptoms ranging from mild to severe (e.g. flu-like illness, formation of lesions, severe adverse reactions)	No	• Transport to clinical sites will follow appropriate standards for medical products. • The GMO will be double-contained for internal transport within clinical sites and a spills procedure will be in place. • Storage will be at secure storage or clinical facilities. • All stocks of the GMO will be accounted for, and all unused GMO will be destroyed. • As noted in Scenario 1, inadvertent exposure to the GMO is likely to involve a low dose, the GMO is likely to be attenuated; the introduced genes have not been associated with toxicity; any local inflammation which may be caused by expression of the introduced *hGM-CSF* gene would cease once expression ends; and previous inadvertent exposures have not lead to clinically significant outcomes.
4	GM virus	i. Treatment of trial participant with the GMO ⬇ ii. Samples containing GMO collected from trial participant ⬇ iii. Laboratory staff exposed to GMO during analysis ⬇ iv. Establishment of viral infection	Clinical symptoms ranging from mild to severe (e.g. flu-like illness, formation of lesions, severe adverse reactions).	No	• Sample testing would be conducted by qualified personnel in pathology or other testing laboratories, which are required to adhere to national standards for handling of infectious substances. • As noted in Scenario 1, inadvertent exposure to the GMO is likely to involve a low dose, the GM virus is likely to be attenuated; the introduced genes have not been associated with toxicity; any local inflammation which may be caused by expression of the introduced *hGM-CSF* gene would cease once expression ends; and previous inadvertent exposures have not lead to clinically significant outcomes.

Risk Scenario				Substantive risk?	Reasons
#	Risk source	Causal pathway	Potential harm		
(b) clinical symptoms following exposure to GM virus shed from trial participants					
5	GM virus	i. Treatment of trial participant with the GMO ii. Trial participant develops lesions and sheds the GMO iii. Exposure of clinical/hospital staff or other patients to trial participant shedding the GMO iv. Establishment of viral infection	Clinical symptoms ranging from mild to severe (e.g. flu-like illness, formation of lesions, severe adverse reactions)	No	• Patient management would be by qualified clinical staff in accordance with Standard Universal Precautions (where no lesions are present), with the addition (where lesions develop) of Contact Precautions appropriate for each clinical site. • Clinical sites will be expected to develop and implement appropriate precautions to minimise contact between study participants and other patients. • Excluding high-risk individuals from caring for patients with lesions would minimise the possibility of a severe adverse reaction. • As noted in Scenario 1 the GM virus is likely to be attenuated; the introduced genes have not been associated with toxicity; any local inflammation which may be caused by expression of the introduced *hGM-CSF* gene would cease once expression ends; and previous inadvertent exposures have not lead to clinically significant outcomes.
6	GM virus	i. Treatment of trial participant with the GMO ii. Trial participant develops lesions and sheds the GMO iii. Exposure of people (e.g. carers or household contacts), other than at-risk people, or animals (e.g. domestic pets) through contact with trial participant or contaminated items (e.g. contaminated dressings) outside the clinical/hospital setting iv. Establishment of viral infection (apparent or subclinical). v. Further transmission to people or animals e.g. due to primary infection going unrecognised.	Clinical symptoms ranging from mild to severe (e.g. flu-like illness, formation of lesions, severe adverse reactions).	No	• Trial participants will be instructed in well-established hygiene practices known to minimise inadvertent transmission of the parent virus (VACV). • When changing dressings at home, patients will be instructed to seal contaminated waste in a primary container (e.g. plastic bag), store it in a biohazard container provided by the treating hospital, and return the container and contents to the hospital at each clinical visit. • As noted in Scenario 1, the GM virus is likely to be attenuated; the introduced genes have not been associated with toxicity; any local inflammation which may be caused by expression of the introduced *hGM-CSF* gene would cease once expression ends; and previous inadvertent exposures have not lead to clinically significant outcomes. • Transmission to human non-trial participants not reported in previous clinical trials (involving over 300 patients and 1200 administration events).

Risk Scenario				Substantive risk?	Reasons
#	Risk source	Causal pathway	Potential harm		
7	GM virus	i. Treatment of trial participant with the GMO ⬇ ii. Trial participant develops lesions and sheds the GMO ⬇ iii. Exposure of at-risk people (such as pregnant women, infants, those with a severe inflammatory skin condition or a history of eczema, and the immunocompromised; e.g. carers or household contacts) through contact with trial participant or contaminated items (e.g. contaminated dressings) outside the clinical/hospital setting ⬇ iv. Establishment of viral infection	Marginal to severe clinical symptoms	Yes	• Some trial participants may develop lesions, leading to shedding of the GM virus. • If trial participants don't immediately recognise these lesions, there is potential for any at-risk people with whom they have close contact to be exposed to the GMO. • There is uncertainty about the consequences for at-risk people exposed to the GM virus. See Section 3.1 for risk characterisation.
Section 2.2: Horizontal transfer of genes or genetic elements					
8	GM virus	i. Exposure of people or animals to the GM virus leading to infection (see risk Scenarios 1-8) ⬇ ii. Person or animal also infected with another related virus ⬇ iii. Both viruses infect and replicate in the same host cell ⬇ iv. Recombination between viral genomes takes place ⬇ v. Recombinant virus infects other hosts	Disease in humans or animals. Establishment of novel virus in environment.	No	• There is no reservoir of VACV in the Australian environment and limited opportunity for the GMO to come into contact with other related poxviruses. • Poxviral recombination does not occur frequently in nature. • even if HGT were to occur, expression of introduced genes is not expected to increase disease burden.

2.1 Increased disease burden to the GM virus

214. Baseline information on the characteristics of *Vaccinia virus* is discussed in Chapter 1, Section 4. Briefly, VACV is not known to occur naturally in Australia. It was previously used as a vaccine for smallpox, however vaccination was never mandatory in Australia. Therefore, only a percentage of Australian residents, including those born and vaccinated overseas, would have had prior exposure to VACV. An even smaller percentage would retain significant immunity after three or more decades (Combadiere et al. 2004).

215. VACV can be transmitted to people and animals by direct contact with the purified virus or with lesions that form on people infected with the virus, and by contact with items contaminated by contact with lesions (e.g. bandages, towels, sheets and clothing). The virus can persist for extended periods of time when in contact with organic material (e.g. pustular material or scabs).

216. Trial participants will be intentionally exposed to the GM virus. However, a range of other people and animals may be inadvertently exposed – either directly to purified GMO supplied for use in the study, or to GM virus shed by trial participants.

217. As unmodified VACV is available in Australia on a very limited basis and there is currently *no* disease burden due to this virus, any clinically significant symptoms in people, or disease in animals, due to infection with the GMO can be considered an increased disease burden.

218. Pathways that could lead to an increased disease burden from the GM virus include:

- exposure of medical staff administering the GM virus, and other hospital staff or contractors handling it during preparation, transport, storage, disposal or analysis of patient samples;
- exposure of people to GM virus shed from trial participants who develop lesions. Such people could include clinical staff caring for participants on return visits to the hospital, other patients, home carers and close household contacts;
- exposure of animals such as domestic pets to GM virus shed from trial participants who develop lesions; and
- exposure of people or animals in the environment to GM virus disposed of via the general waste stream.

These pathways are discussed below.

2.1.1 Risk scenario 1 – Exposure of persons dispensing and administering the GMO

Risk source	Causal pathway	Potential harm
GM virus	i. Exposure of persons dispensing or administering the GMO in a hospital via: • needle stick/sharps injury; • contact with abraded skin or mucous membranes (esp. eyes) ⬇ ii. Establishment of viral infection	Clinical symptoms ranging from mild to severe (e.g. flu-like illness, formation of lesions, severe adverse reactions)

Causal pathway

219. Pharmacy or laboratory staff dispensing the GMO and medical staff administering it to trial participants could be exposed via a needle stick injury, a splash to the eyes or mouth, or through contact with contaminated items.

220.   The clinical trial will be conducted by appropriately qualified pharmacy and medical staff who have been specifically trained in the requirements of the study and appropriate precautions (discussed in Chapter 1, Section 3.1.5).

221.   Staff dispensing the GMO (i.e. diluting virus stock and loading syringes) will be required to handle the GMO in its most concentrated form, and could receive a high viral dose if exposed. They will work in a Class II biological safety cabinet, follow PC2 work practices and wear protective clothing that includes a laboratory gown, disposable gloves, eye protection and surgical masks (see paragraphs 46 and 47). The use of protective clothing while handling concentrated virus, plus the use of a biological safety cabinet and PC2 work practices minimise the likelihood of exposure. Eye protection and surgical masks will provide splash protection to the eyes and mouth while gloves will limit exposure via direct contact with the virus solution.

222.   Medical staff inoculating patients with the GMO will wear the same PPE, which would provide similar protection from exposure to the diluted virus solution.

223.   Sharps will be used while both dispensing and administering the GM virus (see paragraph 42). Depending on equipment preferred at individual study sites, staff dispensing the GMO may need to remove contaminated needles after loading diluted GMO solution into syringes. All sharps handling will follow institutional procedures, which would be in accordance with Universal Standard Precautions (World Health Organisation 2007) and the Australian Guidelines for the Prevention and Control of Infection in Healthcare (2010).

224.   Sharps will also be used for intratumoural injection (see paragraph 43), but the needle will first be inserted into the patient, then the syringe attached via flexible tubing. The injection apparatus will be flushed with saline before the needle is withdrawn to minimise contamination of the injection site when the needle is withdrawn. Both procedures will minimise handling of contaminated sharps, and therefore the potential for exposure to the GMO via sharps injury. Immediate disposal of needles into appropriate sharps containers will also minimise the risk of exposure by this route.

Potential harm

225.   Any dose received by accidental exposure to purified virus while dispensing or administering the GMO is likely to be far lower than the dose intentionally administered to trial participants ($1x10^9$ pfu).

226.   Even if exposure occurred, the GMO is likely to be attenuated in non-dividing cells and the site of exposure would not contain the density of rapidly dividing cells found in tumours. It is expected that viral replication would be limited in immune-competent people, and the GMO rapidly cleared by the immune system.

227.   Excluding at-risk individuals (including pregnant women, those with a severe inflammatory skin condition or a history of eczema, and the immunocompromised; see paragraph 45) from handling or administering the GMO would minimise the possibility of a severe adverse reaction. Should disease nonetheless eventuate, access to the available treatments for some forms of severe VACV infection has been arranged (see Chapter 1, Section 4.9.1).

228.   Neither the *TK* gene nor the introduced genes have previously been associated with toxicity or allergy in people or animals. *hGM-CSF* is of human origin, has not been modified, and there is no reason to think it would be toxic or allergenic when expressed in an infected cell. The *lacZ* gene and the β-galactosidase enzyme it encodes are widespread in the environment and have a long history of safe use in the laboratory. *LacZ* has also been expressed *in vivo* in humans and animals without signs of toxicity (Chapter 1, Section 5.2.3).

229. hGM-CSF is a pro-inflammatory cytokine which can, in a concentration-dependent manner, stimulate localized inflammation or flu-like symptoms if present systemically. The latter are similar to reported side effects of the GMO (paragraph 176), and dissipate once the cytokine is removed. In low-risk individuals in whom viral replication is likely to be limited (paragraph 226), it is expected that any production of hGM-CSF would be low level and transient.

230. Four inadvertent exposures (three needlesticks and one contact exposure) to the GMO have been documented in association with previous clinical trials and pre-clinical studies. These did not cause clinical symptoms or require any treatment beyond immediate first aid and observation.

Conclusion

231. Risk scenario 1 is not identified as a substantive risk because the potential for exposure would be minimised by standard handling procedures and specific precautions proposed by the applicant, the GMO is likely to be attenuated, at-risk people will be excluded from handling the GMO and treatment for some adverse reactions is available in case of serious infection. Therefore, this risk could not be greater than negligible and does not warrant further detailed assessment.

2.1.2   Risk Scenario 2 – Exposure of persons to GMO waste in clinical facilities

Risk source	Causal pathway	Potential harm
GM virus	i. Unused GMO or waste containing the GMO disposed of from clinical site ⬇ ii. Exposure of persons handling waste to the GMO ⬇ iii. Establishment of viral infection	Clinical symptoms ranging from mild to severe (e.g. flu-like illness, formation of lesions, severe adverse reactions)

Causal pathway

232. Hospital staff (who may or may not be involved in the dealings) may come into contact with unused GMO or waste contaminated with the GM virus.

233. All contaminated waste (including needles, syringes, tubing, gloves, swabs etc) will promptly be discarded into appropriately-labelled biological waste containers, which would minimise exposure to material contaminated with the GM virus once it has been discarded.

234. Contaminated waste will be disposed of by each clinical site following standard clinical waste disposal methods (Australian Capital Territory 1991; 2011; 2012; EPA Victoria 2009; New South Wales 1997; Northern Territory 2009; Queensland 2000; South Australia 2009; Victoria 2000; West Australia 2004). The Industry Code of Practice for the Management of Clinical and Related Wastes details requirements for clinical waste including waste segregation, packaging, labelling, storage, transport and accountability (Biohazard Waste Industry Australia and New Zealand (BWI) 2010). The clinical waste stream typically involves destruction of infectious waste by incineration, autoclaving or chemical decontamination, which are considered appropriate for disposal of the GMO.

Potential harm

235. As discussed in Risk Scenario 1, inadvertent exposure to the GMO is likely to involve a low dose, the GM virus is likely to be attenuated, and it is expected that any viral replication would be limited and the GMO rapidly cleared by the immune system. Low level expression of

hGM-CSF and β-galactosidase have not previously been associated with adverse effects. Finally, reported exposures to the GMO have not resulted in clinical symptoms or required specific treatment.

Conclusion

236. Risk scenario 2 is not identified as a substantive risk because the potential for exposure would be minimised by discarding of contaminated waste into appropriate biological waste containers followed by disposal via the clinical waste stream, the GMO is likely to be attenuated and inadvertent exposure would involve small quantities only. Therefore, this risk could not be greater than negligible and does not warrant further detailed assessment.

2.1.3 Risk Scenario 3 – Exposure of people or animals to the GMO due to unintentional release during transport or storage

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 viral infection	Clinical symptoms ranging from mild to severe (e.g. flu-like illness, formation of lesions, severe adverse reactions)

Causal pathway

237. Staff at clinical or storage sites (who may or may not be involved in the dealings), and people or animals outside of these sites, may come into contact with the GMO due to a spill during transport or storage.

238. As described in Chapter 1, Section 3.1.4, the GM virus will be supplied in small volumes and securely packaged in sealed vials (the primary container), individually packaged in labelled cardboard boxes (the clinical product pack), and sealed for transport within leak-proof secondary packaging (a plastic bag).

239. Transport for the purpose of import and distribution within Australia will be by commercial courier companies experienced in the transport of pharmaceutical products, and in accordance with IATA Dangerous Goods Regulations and, within Australia, the *Australian Dangerous Goods Code*.

240. For transport within clinical sites, the GMO will be double-contained in accordance with the Regulator's *Guidelines for the Transport, Storage and Disposal of GMOs*.

241. Storage at the central depot and clinical sites will be in the primary packaging supplied, with a secure freezer providing secondary containment. Access will be restricted to staff working in the pharmacy/laboratory or storage facility, the vial and box label will clearly indicate the contents, the product will be stored only with other therapeutic agents and not with laboratory samples, and it will be dispensed only with written authorization from the lead investigator at the site.

242. Any spills occurring in a clinical setting would be disinfected and cleaned in accordance with the Australian Guidelines for the Prevention and Control of Infection in Healthcare (2010). Spills outside of clinical facilities would be disinfected and contained according to the requirements of the Regulator's *Guidelines for the Transport, Storage and Disposal of GMOs*. In addition, the GMO is supplied as purified virus particles which have reduced capacity to survive in the environment compared with VACV derived from patients and found in scabs and other biological specimens (Chapter 1, Section 4.12). Therefore there is little potential for exposure of humans or other animals to the GMO.

243. When the study at each clinical site is complete, any unused GMO will be destroyed. Records of all GMO received, dispensed and destroyed will be maintained and verified during regular site visits by the CRO and licence holder.

Potential harm

244. As discussed in Risk Scenario 1, inadvertent exposure to the GMO is likely to involve a low dose, the GM virus is likely to be attenuated, and it is expected that any viral replication would be limited and the GMO rapidly cleared by the immune system of people or animals. Low level expression of hGM-CSF and β-galactosidase have not previously been associated with adverse effects. Finally, reported exposures of people to the GMO have not resulted in clinical symptoms or required specific treatment.

Conclusion

245. Risk scenario 3 is not identified as a substantive risk because the potential for exposure would be minimised by appropriate packaging and containment during transport and storage, ensuring spills procedures are in place, and disposing of unused GMO at the end of the study. In addition, the GMO is likely to be attenuated and inadvertent exposure would involve small quantities only. Therefore, this risk could not be greater than negligible and does not warrant further detailed assessment.

2.1.4 Risk Scenario 4 – Exposure of persons analysing patient samples that contain the GMO

Risk source	Causal pathway	Potential harm
GM virus	i. Treatment of trial participant with the GMO ⬇ ii. Samples containing GMO collected from trial participant ⬇ iii. Laboratory staff exposed to GMO during analysis ⬇ iv. Establishment of viral infection	Clinical symptoms ranging from mild to severe (e.g. flu-like illness, formation of lesions, severe adverse reactions).

Causal pathway

246. Blood and tissue specimens collected from patients will routinely be exported for analysis overseas, but could if required be analysed in Australia. Such samples may contain low levels of the GMO (Hwang, 2011; Park, 2008). Laboratory staff in Australian facilities could be exposed by contact with abraded skin, eyes or mouth.

247. The applicant has advised that analytical laboratory staff would follow institutional Standard Operating Procedures in place for the safe handling and disposal of clinical and diagnostic specimens. The National Pathology Accreditation Advisory Council (NPAAC) plays a key role in ensuring the quality of Australian pathology services and is responsible for the development and maintenance of standards and guidelines for pathology practices. The standards include safety precautions to protect the safety of workers from exposure to infectious microorganisms in pathology laboratories. Australian pathology laboratories conform to *AS/NZS 2243.3:2010 Safety in Laboratories Part 3: Microbiological Safety and Containment*, which stipulates that human clinical and diagnostic specimens be handled in PC2 containment as a minimum standard. As unmodified VACV is classified as a Risk Group 2 organism in Australia, this would provide sufficient protection from exposure to the GM virus.

Potential harm

248. As discussed in Risk Scenario 1, inadvertent exposure to the GMO is likely to involve a low dose, the GM virus is likely to be attenuated, and it is expected that any viral replication would be limited and the GMO rapidly cleared by the immune system. Low level expression of hGM-CSF and β-galactosidase have not previously been associated with adverse effects. Finally, reported exposures to the GMO have not resulted in clinical symptoms or required specific treatment.

Conclusion

249. Risk scenario 4 is not identified as a substantive risk because the potential for exposure would be minimised by handling the GMO according to national standards that require PC2 containment and PC2 work practices. In addition, the GMO is likely to be attenuated and inadvertent exposure would involve small quantities only. Therefore, this risk could not be greater than negligible and does not warrant further detailed assessment.

2.1.5   Risk Scenario 5 – Exposure of nursing staff and other patients to the GMO

Risk source	Causal pathway	Potential harm
GM virus	i. Treatment of trial participant with the GMO ⬇ ii. Trial participant develops pustular lesions and sheds the GMO ⬇ iii. Exposure of hospital staff or other patients to trial participant shedding the GMO ⬇ iv. Establishment of viral infection	Clinical symptoms ranging from mild to severe (e.g. flu-like illness, formation of lesions, severe adverse reactions)

Causal pathway

250. Patients inoculated with the GMO will be required to return to the hospital for additional treatment and follow-up tests. Patients inoculated by the intratumoral route have been found to shed virus only via pustular lesions that sometimes develop after the first round of treatment (Chapter 1, Section 5.3.6). Should a patient develop GMO-related lesions, clinical staff caring for them or collecting samples, and other hospital patients, could be exposed to GMO shed by the participant.

251. Trial participants who develop lesions will be required to follow well-established hygiene practices known to minimise interpersonal transmission of the virus (see paragraphs 28 and 29). These practices will be explained to prospective participants during initial screening and anyone unwilling or unable to comply will not be enrolled.

252. Care of and samples collection from inoculated patients will be in accordance with Universal Standard Precautions at a minimum (see paragraph 48). Where returning patients present with GMO-related lesions, additional risk-based 'Contact Precautions' are recommended (see paragraph 49) to minimise opportunities for transmission to staff and other patients. It should be noted that two inadvertent exposures have been documented in association with previous clinical trials[9]. Provided suitable precautions, based on risk

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[9] Four exposures (three needlesticks and one contact exposure) have been documented by the international trial sponsor. Two of these occurred in the course of clinical trials that have involved approximately 1200 patient inoculations (or one exposure per 600 inoculations). The proposed trial in Australia will involve up to 150 patient inoculations.

assessment and documented in Standard Operating Procedures (SOPs) are in place and followed at each study site, exposure of clinical staff and other patients to the GMO should be minimised.

Potential harm

253.   As discussed in Risk Scenario 1, the GM virus is likely to be attenuated, and it is expected that any viral replication would be limited and the GMO rapidly cleared by the immune system. Low level expression of hGM-CSF and β-galactosidase have not previously been associated with adverse effects. Finally, reported exposures to the GMO have not resulted in clinical symptoms or required specific treatment (see paragraphs 225-226 and 228-230).

254.   The applicant has proposed to exclude at-risk individuals (including pregnant women, those with a severe inflammatory skin condition or a history of eczema, and the immunocompromised; see paragraph 45) from caring for patient with lesions or sharing a hospital room with them, which would minimise the possibility of a severe adverse reaction in an exposed person. Should disease nonetheless eventuate, access to the available treatments for some forms of severe VACV infection has been arranged (see Chapter 1, Section 4.9.1).

Conclusion

255.   Risk scenario 5 is not identified as a substantive risk because the potential for exposure would be minimised by implementing risk-based precautions at each clinical site and requiring that trial participants who develop lesions follow clearly-defined hygiene practices, the GMO is likely to be attenuated, at-risk people will be excluded from caring for patients with lesions or sharing a hospital room with them, and treatment for some adverse reactions is available in case of serious infection. Therefore, this risk could not be greater than negligible and does not warrant further detailed assessment.

2.1.6   Risk Scenario 6 – Exposure of people or animals in the home environment to the GMO

Risk source	Causal pathway	Potential harm
GM virus	i. Treatment of trial participant with the GMO ⬇ ii. Trial participant develops pustular lesions and sheds the GMO ⬇ iii. Exposure of people (e.g. carers or household contacts), other than at-risk people, or animals (e.g. domestic pets), through contact with trial participant or contaminated items (e.g. contaminated dressings) outside the clinical/hospital setting. ⬇ iv. Establishment of viral infection (apparent or subclinical). ⬇ v. Further transmission to people or animals e.g. due to unrecognised primary infection.	Clinical symptoms ranging from mild to severe (e.g. flu-like illness, formation of lesions, severe adverse reactions)

Causal pathway

256.   Patients inoculated with the GMO will reside in their homes over the course of the trial. Should they develop GMO-related lesions after the first round of treatment, household contacts such as carers or family members, and animals such as domestic pets or livestock, could be exposed to the GM virus.

257.   A wide range of animal species are susceptible to infection with VACV, although information about development of clinical disease in species other than cattle, mice and rabbits is limited. Household pets are most likely to be exposed, directly or indirectly, to GMO shed

by trial participants. Dogs and cats – the most common domestic pets in Australia – can both be infected with VACV (Chapter 1, Section 4.2 and Chapter 1, Section 4.6.2). It has been suggested (although not proven) that dogs with asymptomatic VACV infection could spread the virus to other hosts (Chapter 1, Section 4.6.2). Livestock such as cattle and horses are known to be susceptible to VACV (Chapter 1, Section 4.4.3), and if infected, the potential for dissemination via contaminated faeces has been demonstrated (Chapter 1, Section 4.6.2). Given the urbanisation of Australia's population, and that the trial would be conducted in major cities, participants are more likely to come into contact with domestic pets than with livestock, but the latter cannot be ruled out.

258.   Trial participants who develop lesions will be instructed to follow well-established hygiene practices known to minimise transmission of unmodified VACV (see paragraphs 28 - 29). These practices will be explained to prospective participants during initial screening and anyone unwilling or unable to comply will not be enrolled in the trial. Participants will also be expected to seal contaminated disposable items in a primary container (e.g zip-loc bag) and store these during the interval between clinical visits in a secondary (biohazard) container provided by the clinical site. At each visit, patients will return the filled container to the hospital for disposal as clinical waste. Participants will also be advised that any items contacting the outer surface of a dressing should be considered contaminated, and instructed to launder contaminated fabrics in hot water with detergent and/or bleach (see paragraphs 30 and 52). These measures would limit the opportunity for household contacts to come into contact with shed GMO.

259.   The applicant has not directly addressed the issue of contact with household pets or livestock. However, the measures proposed to minimise transmission to human contacts would also minimise transmission to animal contacts, provided they are applied with equal attention.

Potential harm

260.   As discussed in Risk Scenario 1, the GM virus is likely to be attenuated, and it is expected that any viral replication would be limited and the GMO rapidly cleared by the immune system. Low level expression of hGM-CSF and β-galactosidase have not previously been associated with adverse effects (see paragraphs 225-226 and 228-230.

261.   Trial participants will be instructed to inform clinical staff should they suspect transmission to a human or animal contact. Any reports will be promptly investigated by a medical or veterinary professional, as appropriate, following a documented protocol.

262.   With similar risk management practices in place, the applicant has advised that no discernible transmission to human non-participants has been reported in previous clinical trials, involving over 300 patients and 1200 inoculations with the GM virus.

Conclusion

263.   Risk scenario 6 is not identified as a substantive risk because the potential for exposure would be minimised by ensuring that trial participants who develop lesions are willing and able to comply with well-established hygiene practices, storing contaminated waste within two layers of containment before returning it to the treating hospital for disposal as clinical waste, and correctly laundering contaminated textiles. The GMO is also likely to be attenuated, a protocol is in place to ensure medical or veterinary follow-up for any potential transmission event, no previous transmission events have been reported, and treatment for some adverse reactions is available in case of serious infection. Therefore, this risk could not be greater than negligible and does not warrant further detailed assessment.

2.2 Horizontal transfer of genes or genetic elements to other organisms

264. Horizontal gene transfer (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 in expression of endogenous genes. The novel trait may result in negative, neutral or positive effects.

265. Information on the presence of the introduced genes in the environment is provided in Sections 5.4 and 5.5. They are derived from humans and bacteria (*E. coli*) and are already available for HGT from these natural sources. Thus their transfer to organisms beyond other viruses will not be assessed further, and the risk assessment will address potential HGT only between the GMO and other viruses.

2.2.1 Risk Scenario 8 – Presence of the introduced genetic material in other organisms as a result of horizontal gene transfer

Risk source	Causal pathway		Potential harm
GM virus	i.	Exposure of people or animals to the GM virus leading to infection (see risk Scenarios 1-8)	Disease in humans or animals.
		⬇	Establishment of a novel virus in the environment.
	ii.	Person or animal also infected with another compatible virus	
		⬇	
	iii.	Both viruses infect and replicate in the same host cell	
		⬇	
	iv.	Recombination between viral genomes takes place	
		⬇	
	v.	Recombinant virus infects other hosts	

Causal pathway

266. Recombination between two viruses may occur if they simultaneously infect the same cell. Recombination can occur within and between viral types (DeFillipis & Villarreal 2001), meaning that introduced genes could potentially be transferred to other viruses, or an intact thymidine kinase gene could be restored to the GMO. While recombination between different classes of virus can occur, its frequency decreases with decreasing relationship between viruses – meaning that the GM virus is more likely to recombine with another poxvirus than with an unrelated virus.

267. There is no reservoir of vaccinia virus in the Australian environment that would allow recombination between GM and wild-type VACV.

268. *Molluscum contagiosum virus* (MCV) is likely to be present in the Australian population. As MCV infection is more prevalent in children than in adults (Konya & Thompson 1999), co-infection is more likely to occur in a secondary recipient of the virus than in an (adult) trial participant. Measures to minimise transmission of the GMO to other people will be in place (see Risk Scenarios 1-5), which would limit the opportunity for co-infection.

269. Immunocompetent individuals tend to develop only a small number of MCV lesions (generally fewer than twenty); these have an average diameter of 3-5 mm (Chen et al. 2013). Likewise, a maximum of ten lesions per person have been observed in patients treated with the GM virus in previous clinical trials (Heo et al. 2013). Should a person with an active MCV infection also become infected with the GM virus, it is unlikely that the small number of

lesions attributable to each virus would co-locate and provide an opportunity for viral recombination.

270. Finally, while no reports on the ability of MCV to recombine with other poxviruses were located, MCV has co-existed with *variola virus* (the causative agent for smallpox) for thousands of years, and with VACV for over 150 years, without evidence of recombinants forming and persisting in the human population.

271. Other uncharacterised poxviruses are found in Australian wildlife; their prevalence is unknown but wildlife is unlikely to come into contact with trial participants. Contact between scavenging animals and participants' discarded waste is discussed further in Section 3.2.2 and considered highly unlikely due to dilution within the general waste stream.

272. Again, should animals be infected with two different poxviruses, the same cell would need to be infected for there to be any potential for recombination, and the GM virus is expected to be attenuated with respect to its ability to replicate efficiently in non-dividing cells.

273. Finally, poxviral recombination does not occur readily. Physical constraints around the replication and assembly processes limit the mixing of viral genomes (see Chapter 1, Section 4.8), and few examples have been documented.

Potential harm

274. Should the GM virus recombine with MCV in co-infected humans, MCV has been shown to lack a thymidine kinase gene so could not restore this to GM VACV (Senkevich et al. 1997).

275. A key consideration in the risk assessment process should be the safety of the protein product resulting from expression of the introduced genes rather than horizontal gene transfer per se (Keese 2008). If the introduced genes or their end products are not associated with harm to people or other organisms then even in the unlikely event of HGT occurring, they should not pose risks to humans, animals or the environment. The introduced genes are derived from humans and bacteria (*E. coli*) and have not been associated with toxic effects. They are not expected to provide any advantage to a receiving virus, nor cause adverse effects in a host organism. In fact, should the hGM-CSF gene be transferred to MCV through recombination, it would potentially increase the cell-mediated immune response to the virus, enhancing the host's ability to clear the infection.

Conclusion

276. The potential for an adverse outcome as a result of horizontal gene transfer is not identified as a substantive risk. Therefore, this risk could not be greater than negligible and does not warrant further detailed assessment.

Section 3   Risk characterisation

277. Eight 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 control measures proposed by the applicant, two of the risk scenarios were identified as posing substantive risks which warranted further assessment. More detail on the evaluation of these scenarios is provided in Section 3 of this chapter.

3.1 Risk Scenario 7 – Exposure of at-risk people in the home environment to GM virus shed by trial participants

Risk source	Causal pathway	Potential harm
GM virus	i. Treatment of trial participant with the GMO ⬇ ii. Trial participant develops pustular lesions and sheds the GMO ⬇ iii. Exposure of at-risk people (such as infants, pregnant women, those with a severe inflammatory skin condition or a history of eczema, and the immunocompromised; e.g. carers or household contacts) through contact with trial participant or contaminated items (e.g. contaminated dressings) outside the clinical/hospital setting ⬇ iv. Establishment of viral infection	Marginal to severe clinical symptoms.

278. Once patients return home after treatment, home carers and close contacts (e.g. partners and other family members) could be exposed if patients shed the GM virus. Certain individuals are likely to suffer more severe responses to the GM virus than the general population.

3.1.1 Likelihood assessment

279. The extent to which trial participants shed the GMO is an important factor in assessing its potential for transmission. As discussed in Chapter 1, Section 5.3.5, patients inoculated by the intratumoral route have been found to shed virus only via pustular lesions that sometimes develop. Lesions have typically formed within 3-7 days of the first treatment cycle (but not subsequent cycles), and in diverse locations including the soles, palms, arms, fingers, torso, face, oral mucosa and lips (Breitbach, 2011; Cripe, 2015; Heo, 2013; Park, 2015). While they have occurred inconsistently, a recent publication reported lesions in 100% of patients (3/3) receiving an intratumoral GMO dose comparable to that proposed for this study (Cripe, 2015). The potential therefore exists for any or all of the trial participants to develop lesions. The number of lesions, however, is usually small (1-10).

280. Any trial participants who develop lesions will be required to follow well-established hygiene practices known to minimise interpersonal transmission of unmodified VACV (see paragraphs 28 and 29). These practices will be explained to prospective participants during initial screening and anyone unwilling or unable to comply will not be enrolled in the trial. Participants will also be advised that any items contacting the outer surface of a dressing should be considered contaminated, and instructed to launder contaminated textiles in hot water with detergent and/or bleach, and store contaminated disposable items within two layers of containment before returning them to the treating hospital (see paragraphs 30 and 52).These measures would limit the opportunity for household contacts to come into contact with the GMO.

281. Participants will also be instructed that, should they develop a lesion they must avoid any direct physical contact with 'Excluded Individuals' and children under 12 months of age (paragraph 29 (e)), who are at highest risk of developing severe disease. Again, prospective participants unable or unwilling to comply will not be enrolled in the trial.

282. Given that lesions are initially small and do not form in a consistent location (such as the inoculation site), there is potential for patients to be unaware of a lesion in its early stages. These lesions have previously developed within 3-7 days of the initial treatment, and no follow up visits to the hospital – when the patient could be assessed – are scheduled within this time frame. Viral shedding reportedly occurs from the papule stage (Chapter 1, Section 4.5). It is

possible therefore, that at-risk individuals among a patient's close associates could be exposed to the GMO before the patient is aware that they should avoid direct physical contact with that person.

283. This scenario is considered **highly unlikely** to occur as the number of lesions and quantity of virus shed at an early stage would be low, patients would be following established hygiene practices and the at-risk person would need to be exposed to the GM virus in a manner that leads to infection. Historically, reports of interpersonal transmission have been infrequent (Chapter 1, Section 4.5).

3.1.2   Consequence assessment

284. The effect of the GM virus on at-risk individuals is unknown as exposure of such individuals has not been reported. The consequences of infection may be less severe than those described for unmodified VACV in Chapter 1, Section 4.4.3 due to the expected effect of the genetic modification, restricting viral replication.

285. Experience with the Raboral V-RG$^®$ rabies vaccine discussed in Chapter 1, Section 5.2.1 has, however, shown that inadvertent exposure to a modified VACV possessing a disrupted *TK* gene can cause illness in at-risk individuals, which has required hospitalisation and specialised treatment (paragraph 146). Information about the total number of exposures is limited to a single vaccination program (Ohio 1990 – 2000) (Rupprecht et al. 2001) but in this example, one of the two contraindicated (at-risk) people exposed to the vaccine suffered an adverse reaction.

286. The Raboral V-RG$^®$ rabies vaccine is derived from the Copenhagen vaccinia strain. Historically, the NYCBH vaccinia 'strain', as used in the Dryvax$^®$ vaccine on which the GMO is based, was associated with a lower rate of adverse reactions than other vaccine strains (including Copenhagen) (Kretzschmar et al. 2006). Therefore, the GM VACV proposed for trial in this application, JX-594, may produce less severe adverse reactions in at-risk people than Raboral V-RG$^®$. However, the Dryvax$^®$ vaccine preparation is a heterogenous pool of vaccinia strains which are known to vary in virulence (Osborne et al. 2007; Li et al. 2006; Nalca & Zumbrun 2010; Qin et al. 2011). The GMO is derived from a single VACV clone from this pool, isolated by plaque purification after carrying out the genetic modification. This clone has not been characterised and so its unique properties are not known. It may be more or less virulent than the phenotypic 'average' displayed by the Dryvax$^®$ preparation as a whole.

287. Given the uncertainty regarding the ability of the GMO to induce an adverse reaction in at-risk people, the potential harm to this group may therefore be considered **marginal** (minimal or no increase in illness/injury to people) to **intermediate** (significant increase in illness/injury to people that requires specialised treatment).

3.1.3   Risk estimate

288. The risk estimate is based on a combination of the likelihood and consequence assessments, using the Risk Estimate Matrix (see Chapter 2, Section 1), as described in the Regulator's Risk Analysis Framework (OGTR 2013).

289. The consequences of exposure via this pathway and infection with the GMO are considered **marginal** to **intermediate** but **highly unlikely** to occur. The risk is therefore estimated to be **negligible** (risk is of no discernible concern and there is no present need to invoke actions for mitigation) to **low** (risk is of minimal concern, but may invoke actions for mitigation beyond standard practices). Consideration of the need for treatment of this low risk is made in Section 5, below.

Section 4   Uncertainty

290.   Uncertainty is an intrinsic part of risk analysis[10]. 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.

291.   For clinical trials, which involve research, some knowledge gaps are inevitable. This is one reason they are conducted under specific limits and controls intended to minimise exposure to the GMO and thus decrease the likelihood of harm.

292.   For DIR 140, uncertainty is noted particularly in relation to the degree of attenuation of the GMO relative to unmodified VACV, its likely effect on at-risk people, its capacity for transmission from an infected individual, and its effect on animals.

293.   There is little data comparing the GMO with the parent organism on which to base a robust assessment of its attenuation. There is also little information about its behaviour in people with normal levels of dividing cells (i.e. people who do not have cancer). Therefore information on unmodified vaccinia viruses and other vaccinia virus strains modified using a similar *TK*-disruption strategy were used in the risk assessment.

294.   The uncertainty about the GMO's ability to cause adverse reactions in at-risk people has been addressed in Scenario 7 by considering a range of possible consequences should such individuals be exposed to the GM virus. Accommodating this uncertainty resulted in an estimate of risk of negligible to low.

295.   The uncertainty regarding the effect of the GMO on animals, and its capacity for transmission, has been addressed in the Risk Context by reference to another GM VACV with a similar genetic modification (Raboral V-RG®), as well as considering the properties of unmodified VACV.

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[10] 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 5   Risk Evaluation

296.   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 whether additional information is needed.

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

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

298.   Eight risk scenarios were postulated whereby the proposed dealings could give rise to harm to people or the environment. This included consideration of the potential for: expression of the introduced genes and genetic modifications to impact on the disease burden caused by the GM virus; infection of at-risk individuals; and infection of animals. The opportunity for gene transfer to other organisms, and its effects if it were to occur, was also considered.

299.   A risk is only identified as substantive when a risk scenario is considered to have some chance of causing harm. 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. In the context of the control measures proposed by the applicant, one of the eight risk scenarios was identified as a substantive risk requiring further assessment.

300.   The likelihood and consequences of the substantive risk was characterised (Chapter 2, Section 3), and the level of risk estimated using the Risk Estimate Matrix, as described in the Regulator's Risk Analysis Framework (OGTR 2013) (see Chapter 2, Section 1).

301.   The risk from exposure of at-risk individuals through contact with trial participants who are shedding the GMO was estimated as posing negligible to low risk to human health and safety.

302.   The applicant has proposed some control measures related to this risk. Additional treatment measures to mitigate the identified negligible to low risk should be applied. Treatment measures to reduce the level of this risk are considered in Chapter 3.

303.   Given that the substantive risk is assessed as negligible to low, the Regulator considers that the dealings involved in this proposed release do not pose a significant risk to either people or the environment.

Chapter 3 Risk management

Section 1 Background

304. 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 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.

305. 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.

306. 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.

307. 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.

308. Licence conditions are discussed and summarised in this chapter and listed in detail in the licence).

Section 2 Risk treatment measures for substantive risks evaluated as requiring treatment

309. The risk identification process (Chapter 2, Section 2) led to identification of one substantive risk, relating to exposure of at-risk individuals to GM virus shed by trial participants outside the clinical setting. This risk was characterised in Chapter 2, Section 3, and risk evaluation proposed that this risk should be treated.

310. The applicant has proposed that patients who have GMO-related lesions avoid contact with at-risk people but has not proposed measures to minimise the potential for exposure due to unrecognised GMO-related lesions. The risk posed by transmission to at-risk individuals from an unrecognised lesion was assessed as negligible to low. To manage this risk, the licence holder could instruct *all* trial participants to avoid direct physical contact with individuals at risk of severe adverse reactions to VACV (including pregnant women, infants, those with a severe inflammatory skin condition or a history of eczema, and the immunocompromised) after the first round of treatment with the GMO until the first follow-up visit to the clinic that takes place on or after day 8 post-inoculation. During the follow-up visit, the presence or absence of GMO-related lesions should be assessed. This treatment measure is considered to be practical and effective and has been imposed as a licence condition.

2.1 Summary of licence conditions proposed to manage identified risks

311. Licence conditions have been imposed to reduce the potential for transmission of the GMO from trial participants to at-risk individuals. These include requirements that all trial participants be instructed to avoid direct physical contact with at-risk individuals for at least 7

days after the first round of treatment with the GMO, and until the presence or absence of GMO-related lesions has been assessed by their clinician.

Section 3   General risk management

312. The limits and controls proposed in the application were important in establishing the context for the risk assessment and in reaching the conclusion about the risks posed to people and the environment. Therefore, to maintain the risk context, licence conditions have been imposed to limit the release in scale and duration, and to restrict the spread and persistence of the GMO and its genetic material in the environment.

3.1   Consideration of the limits and controls proposed by Clinical Network Services (CNS) Pty Ltd

313. Chapter 1, Sections 2.2 and 2.3 provide details of the limits and controls proposed by CNS, which are discussed in the risk scenarios considered in Chapter 2. The appropriateness of these limits and controls is considered further below.

314. The proposed clinical trial will involve a maximum of 50 participants within Australia, and most activities will take place in hospitals providing specialised cancer-treatment services. The applicant has proposed to complete the trial within five years of commencement. These limits would limit the exposure of people and animals to the GM viruses. To provide some flexibility in patient enrolment, licence conditions allow for administration of the GMO in hospitals providing specialised cancer-treatment services to up to75 participants, over a 5 year period.

315. Excluding individuals at risk of severe adverse effects from exposure to the GM virus from participating in the trial, and from handling the GM virus and caring for patients who present with GMO-related lesions, would reduce the possibility of complications requiring medical treatment and excessive shedding of the GMO into the environment. These include people who have previously experienced an adverse reaction to VACV vaccination (excluded from trial participation only, not from handling the GMO or caring for patients in the hospital setting), people with immunodeficiencies, people with inflammatory skin conditions or a history of eczema, and women who are pregnant or breastfeeding. These exclusion criteria have been imposed as licence conditions.

316. The GMO will be administered by intratumoural inoculation. This route is associated with reduced viral shedding compared to intravenous infusion (see Chapter 1, Section 5.3.6), so would limit the opportunity for interpersonal transmission of the GMO and has been imposed as a licence condition.

317. Participants will be inoculated and cared for by trained clinical staff at hospital facilities in accordance with the *World Health Organisation Standard Precautions in Health Care* (World Health Organisation 2007) and the *International Conference on Harmonisation Good Clinical Practice Guidelines* (ICH 1996). The WHO standard precautions detail appropriate hygiene, personal protective equipment and decontamination procedures to prevent direct contact with infectious agents. These practices would minimise exposure of people handling the GMO and caring for patients. Licence conditions require administration in hospitals providing specialised cancer-treatment services and adherence to ICH-GCP and TGA GCP Guidelines and WHO Universal Standard Precautions.

318. When handling the GMO, the applicant has indicated that PPE including a gown, gloves, eye protection and a surgical mask must be worn. Additionally, while dispensing the GMO, a Class II biological safety cabinet (BSC) and PC2 work practices would be used. These practices would further reduce exposure of people handling the GMO, and have been imposed as licence conditions.

319. As patients returning to the hospital for further treatment or follow-up may present with GMO-related lesions, the applicant has proposed that clinical sites conduct a risk assessment and develop 'Contact Precautions' appropriate to their facility that minimise the possibility of GMO transmission to clinical staff and other patients, including patients in at-risk categories. Such precautions would further reduce the risk of exposure of clinical staff and patients to the GMO. Licence conditions require the licence holder to ensure that suitable precautions are developed through risk assessment, documented in Standard Operating Procedures (SOPs) and implemented at each study site.

320. The applicant will require trial participants to take precautions intended to minimise transmission of the GMO, which would reduce the opportunity for exposure of other people and animals to the GMO or shedding of the GMO into the environment. These measures include covering lesions, wearing a surgical mask where oral lesions occur, and avoiding all physical contact with lesions (by self and others). Licence conditions require the licence holder to:

- educate trial participants about the potential for transmission of the GMO to other people and to animals, about possible adverse effects, and how to recognise that transmission may have occurred;

- instruct trial participants in precautions to minimise spread of the GMO; and

- exclude people from the trial if they are unwilling or unable to comply with these precautions.

321. The applicant would require trial participants who develop GMO-related lesions to avoid all direct physical contact with individuals at risk of severe adverse reactions to VACV, which would further reduce the opportunity for transmission to people at greatest risk of harm from exposure to the GMO. Again, a requirement for trial participants to be informed of this measure and excluded from the trial if unwilling or unable to comply has been imposed as a licence condition. The risk related to transmission to at-risk individuals from an unrecognised lesion was assessed as negligible to low, and is addressed in Section 2, above.

322. The applicant will instruct participants that should they suspect transmission of the GM virus to another person or to an animal, they are to inform staff at the clinical site. Any such reports will be investigated by a medical or veterinary professional, as appropriate, to determine whether transmission has occurred. Requirements for participants to be advised of this requirement, and for protocols for prompt medical or veterinary investigation of suspected transmission events to be in place, have been imposed as licence conditions.

323. In the event of a clinically-significant reaction to the GMO, the applicant has advised that study sites are to seek infectious disease expertise and immediately inform the international trial sponsor. SillaJen, Inc has made arrangements for VIG (recommended for treating some adverse reactions to VACV) to be provided to study sites in Australia within 24 hours of request. A requirement for appropriate medical treatment to be provided to persons who are found to have been accidentally infected with the GMO has been imposed as a licence condition.

324. Storage and transport, including of waste containing the GM virus, will be in accordance with relevant International Air Transport Association requirements and/or the Regulator's guidelines. This will minimise exposure of other people and the environment to the GM virus and has been included as licence conditions.

325. All waste generated at the clinical sites will be disposed of in accordance with standard clinical waste disposal practices. This would also minimise exposure of people and the environment to the GMO and has been imposed as a licence condition.

326. The applicant has proposed that trial participants dispose of dressings and other items which may be contaminated with the GMO by sealing them in a container or plastic bag, and storing these in a biohazard container provided by the clinical site. Patients would return these containers to the treating hospital at each clinical visit, at which time they would be replaced. Hospitals would be responsible for tracking containers and ensuring their return, and disposing of them via the clinical waste stream. Double containment is consistent with the Regulator's guidelines for the *Transport, Storage and Disposal of GMOs*, and would minimise exposure of people and the environment to the GMO. Provided they are labeled in accordance with these guidelines, the proposed biohazard containers are suitable for storage and transport of home-generated waste to clinical sites.

327. As it would not be known in advance who would develop GMO-related lesions, all trial participants should be provided with a container at the time of inoculation. Between visits to the hospital, participants who do develop lesions should store the container such that the waste is inaccessible to children and animals. Licence conditions have been included requiring that the licence holder provide all trial participants with appropriate waste containers and instruction in handling, storing and returning waste, as well as implement a system for tracking the dispensation, return and destruction of the containers.

328. Maintaining records of all GMO received, dispensed and destroyed will ensure all vials of the GMO are accounted for. Destroying all GMO remaining when the study at each clinical site is complete will ensure it is not inadvertently released at a later time. These practices have been imposed as licence conditions.

3.2 Summary of draft licence conditions proposed to limit and control the release

329. A number of licence conditions have been imposed to limit and control the proposed release, based on the considerations discussed in subsection 3.1 above. These include requirements that:

- limit the release to a maximum of 75 trial participants inoculated with the GM viruses at designated clinical facilities over a 5 year period

- restrict the method of administration of the GMO to intratumoural inoculation

- restrict exposure of at-risk individuals (both patients and staff) by specific exclusion criteria

- the GMO be administered, and patients cared for, by trained clinical staff at hospital facilities in accordance with Universal Standard Precautions and ICH-GCP[11], and that appropriate protective equipment is worn and used;

- patients be educated about the potential for transmission of the GMO and instructed in precautions to minimise its spread to other people and to animals;

- additional precautions be developed and implemented to prevent interpersonal spread of the GMO within the clinical sites;

- patients who develop GMO-related lesions be requested to store potentially-contaminated waste under two levels of containment and return it to the hospital for disposal;

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[11] The international conference on harmonisation of technical requirements for registration of pharmaceuticals for human use, guidelines for good clinical practice (ICH 1996)

- all patients be provided with suitable containers for disposal of home-generated waste, from the time of inoculation with the GMO, and a system for tracking and ensuring the return of these containers implemented;

- transport and storage of the GMO be in accordance with relevant regulations and guidelines[12];

- all waste generated at clinical sites and returned by trial participants be disposed of in accordance with standard disposal practices for infectious clinical waste;

- contingency plans be in place to detect and manage any exposure to the GM virus, and treat any vaccinia-related illness that may eventuate; and

- all unused GMO be destroyed on completion of the study, and records of all GMO received, dispensed and destroyed be maintained.

Section 4   Other risk management considerations

330. 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, including a requirement to inform the Regulator is the applicant becomes aware of any additional information about risks to the health and safety of people or the environment; and

- a requirement that the applicant allow access to the trial sites by the Regulator, or persons authorised by the Regulator, for purpose of monitoring or auditing.

4.1 Applicant suitability

331. 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

332. The Regulator considered the suitability of the applicant when the application was received. The Regulator will reassess the suitability of CNS before making the decision whether or not to issue a licence for this application (DIR 140).

333. If a licence were issued, the conditions would include a requirement for the licence holder to inform the Regulator of any circumstances that would affect their suitability.

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

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[12] The Gene Technology Regulator's *Guidelines for the Transport, Storage and Disposal of GMOs;* IATA Transportation Regulations

4.2   Contingency plans

335. If a licence is issued, the licence holder would be required to submit a contingency plan to the Regulator prior to conducting any dealings authorised by the licence. This plan must detail measures to be undertaken in the event of:

    a)   the unintended release of the GMO, including spills outside of clinical sites, and exposure of or transmission to persons other than trial participants; and

    b)   a person exposed to the GMO developing a severe adverse response.

336. The licence holder would also be 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 would be required within 30 days of the issue date of the licence.

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

337. If a licence were to be issued, the persons covered by the licence would be the licence holder and employees, agents or contractors of the licence holder and other persons who are, or have been, engaged or otherwise authorised by the licence holder to undertake any activity in connection with the dealings authorised by the licence. Prior to commencing dealings at any clinical site, CNS would also be required to notify the Regulator of the participating organisation, and provide a list of people who will be covered, or the function or position where names are not known at the time.

4.4   Reporting requirements

338. If issued, the licence would oblige 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 trial

    •   any contraventions of the licence by persons covered by the licence

    •   any unintended effects of the trial

339. The licence holder would also be obliged to submit an Annual Report within 90 days of the anniversary of the licence containing any information required by the licence, including the results of inspection activities.

4.5   Monitoring for Compliance

340. 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.

341. If monitoring activities identify changes in the risks associated with the authorised dealings, the Regulator may also vary licence conditions, or if necessary, suspend or cancel the licence.

342. 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 5 Conclusions of the RARMP

343. The risk assessment concludes that the proposed limited and controlled release of GM virus to take place in Australian hospitals, involving up to 75 trial participants and expected to run for up to five years, poses negligible to low risks to the health and safety of people or the environment as a result of gene technology.

344. The risk management plan concludes that the identified negligible to low risks can be managed so as to protect the health and safety of people and the environment by imposing risk treatment measures. Licence conditions are imposed to limit the release in size, locations and duration, and to restrict the spread and persistence of the GMO and its genetic material in the environment, as these were important considerations in establishing the context for assessing the risks. Specific risk treatment measures have also been imposed to manage the identified negligible to low risk.

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Appendix A: Summary of submissions from prescribed experts, agencies and authorities[13]

Advice received by the Regulator from prescribed experts, agencies and authorities on the consultation RARMP is summarised below. 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 informed the Regulator's decision to issue the licence.

Sub. No.	Summary of issues raised	Consideration in RARMP	Comment
1	Agreed with the overall conclusions of the RARMP.		Noted
	The Regulator should consider whether exclusion of patients with at-risk contacts is warranted.	Chapter 2, Section 3.1 (Risk scenario 7) Chapter 3, Section 2.	Given the likely attenuation of the GMO, the small number of participants and measures proposed by the applicant, it is considered that this risk can be managed through licence conditions. Licence conditions require that trial participants be instructed to avoid direct contact with at-risk individuals, keep GMO-related lesions covered and decontaminate or correctly dispose of any potentially contaminated items.
	The Regulator should consider whether exclusion of patients with animal contacts is warranted.	Chapter 1, Section 4.6.2 Chapter 2, Section 2.1.6 (Risk scenario 6)	Additional text has been added to the RARMP. The procedures in place to minimise transmission to human contacts are equivalent to those recommended by the US Centre for Disease control for preventing transmission to animals. Licence conditions have been modified to emphasize that these precautions must be implemented with regard to animal as well as human contacts.
	The Regulator should consider whether occlusive bandages should be used during the trial.	Chapter 1, Section 3.1.3	The applicant's decision to use porous (non-occlusive) dressings is based on US CDC recommendations in the context of vaccination with unmodified VACV in the non-healthcare setting. The applicant has advised that patients will be instructed that anything coming into contact with the outer side of a non-occlusive dressing could potentially be contaminated. Text has been added to Chapter 1, Section 3.1.3 noting this, and that textiles and other items that come into direct contact with the dressing are to be decontaminated as described in Section 3.1.6. This point has also been included in licence conditions.
	The Regulator should further consider recombination with other poxviruses.	Chapter 1, Sections 4.10 and 6.3; Chapter 2, Section 2.2 (Risk scenario 8)	Additional text has been added to the RARMP addressing poxviral recombination (Section 4.10), and consideration of *Molluscum contagiosum virus* (MCV) as a poxviral species present in the Australian environment (Section 6.3). Risk Scenario 9 (relating to horizontal gene transfer) has been expanded to consider the possibility of recombination between the GMO and MCV.

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

Sub. No.	Summary of issues raised	Consideration in RARMP	Comment
2	Council does not generally support use of GMOs in its local government area, however does not formally oppose the application as it relates to a human therapeutic. The need for broad public notification and opportunity to comment on GMO releases was emphasised.		Noted.
3	No objections to issuance of licence or proposed licence conditions outlined in the draft RARMP.		Noted.
	The parent strain of vaccinia virus is not of animal biosecurity concern and the proposed genetic modification does not alter its characteristics in a way that would increase its biosecurity risk.		Noted.
4	Successful risk mitigation depends on the training provided to, and compliance by, those handling the GM virus, contaminated materials and treated patients. Training and compliance by participants and home-carers would be potentially more tenuous than that of hospital staff.	Chapter 1, Sections 3.1.3 and 3.1.7	The applicant has provided additional information regarding education of trial participants about procedures to follow should inadvertent transmission to a third party (human or animal) be suspected, and contingency plans to manage any transmission events should they occur. Additional detail has been included in the RARMP.
	It is unclear how the willingness of patients to comply with risk management requirements would be assessed and standardised across multiple trial sites. The RARMP should address the evaluation of potential participants' willingness to comply with risk management requirements.	Chapter 1, Section 3.1.2	The applicant has provided additional information regarding the assessment of participants' willingness to comply with mandated requirements, and standardisation across different Study Sites. Additional detail has been included in the RARMP.
	Further clarity is needed regarding the management of cutaneous pustules that can appear in treated patients and contain infectious GM virus. The RARMP should address the management of these pustules should they appear.	Chapter 1, Section 3.1.3	Management of GMO-related lesions is described in Chapter 1, Section 3.1.3 of the RARMP. Additional text describing written information that will be provided to trial participants has been included.

Sub. No.	Summary of issues raised	Consideration in RARMP	Comment
5	Supported the conclusion of the RARMP that the proposed dealings pose negligible risk of harm to human health and the environment.	-	Noted.
	Support inclusion of the following measures proposed in the RARMP to manage the identified risks: - all trial participants be instructed to avoid direct physical contact with at-risk individuals for at least 7 days after the first round of treatment with the GMO, and until the presence or absence of GMO-related lesions has been assessed by their clinician. - ensure all contaminated waste generated during home care of GMO-related lesions is returned to the treating hospital for disposal via the clinical waste stream.	Chapter 1, Sections 3.1.6 and 3.1.8. Chapter 3, Section 2.	These conditions have been retained in the finalised licence and further strengthened by inclusion of a requirement for tracking of waste generated during home care. After consultation on the draft RARMP, the applicant amended their proposal for disposing of waste generated in patients' homes. The Risk Context has been updated to reflect the new information and Risk Scenario 8 (Exposure of people or animals to GM virus following disposal of GM waste into landfill) removed from the RARMP. Licence conditions maintain the risk context.
	Transmissibility of this GM virus to other mammals and reptile species is not adequately addressed in the RARMP.	Chapter 1, Sections 4.2, 4.4.3, 4.6.2 and 5.3.2.	Text on the ability of unmodified *vaccinia virus* to cause disease in animals, and transmission to and between animals, has been added to Chapter 1 of the RARMP.

Sub. No.	Summary of issues raised	Consideration in RARMP	Comment
6	Due to the broad host range of vaccinia virus, release into the Australian environment should be avoided.	Chapter 2; Chapter 3	Risks associated with the potential for the GMO to spread in the environment are considered in the RARMP. A range of licence conditions have been imposed to minimise the likelihood of people (other than trial participants) or animals being infected with the GMO.
	Advises that draft licence conditions requiring that trial participants avoid contact with infants and at-risk individuals for at least 8 days post-inoculation and until any lesions that develop have healed, should be extended to exclude direct physical contact with domestic and other animals over the same period.	Chapter 1, Section 4.6.2 Chapter 2, Section 2.1.6 (Risk scenario 6)	Additional text has been added to the RARMP. The procedures in place to minimise transmission to human contacts are equivalent to those recommended by the US Centre for Disease control for preventing to transmission to animals. Licence conditions have been modified to emphasize that these precautions must be implemented with regard to animal as well as human contacts.
	Noted that disposal of contaminated materials generated in the patients' homes into landfill provides a potential exposure route to the environment and Australian native animals. Supports proposed licence conditions requiring that such materials be returned to the hospital for disposal.	Chapter 1, Section 3.1.2, 3.1.6 Chapter 3, Section 3.1	Following consultation on the draft RARMP, the applicant amended the proposed method for disposing of contaminated waste generated during home care of trial participants. Waste will be returned to the clinical site and treated as clinical waste; it will not be discarded into landfill.
	However does not consider that relying on ill patients to follow appropriate disposal procedures for the GMO adequately manages the risk. Advises that the applicant should arrange for waste collection from participants homes, using accepted protocols for transport and disposal of hazardous biological materials.		Licence conditions requiring return of potentially contaminated waste generated during home care have been retained and strengthened by inclusion of a requirement for tracking of this waste. Relevant text has been added to the RARMP. The applicant has also advised that patients selected for the trial will at a minimum be physically capable of light or sedentary work. These measures are considered appropriate to manage risks associated with disposal of potentially contaminated waste.
	Provided licence conditions are adjusted to reflect this advice, agrees with the conclusion of the RARMP that there is negligible to low risk to the environment, based on the limited number of trial participants over a finite trial period, and application of appropriate risk treatment measures by both applicant and trial participants.		Noted.