Australian Government
Department of Health and Aged Care
Office of the Gene Technology Regulator

Risk Assessment and Risk Management Plan (Consultation version) for

DIR 192

Clinical trial of a genetically modified (GM) chimeric Orthopoxvirus (CF33-hNIS) as a cancer treatment

Applicant: Medpace Australia Pty Ltd

14 July 2022

This RARMP is open for consultation until 18 August 2022.

Written comments on the risks to human health and safety and the environment posed by this proposed clinical trial of the GM chimeric Orthopoxvirus treatment are invited. You may make your submission

via mail to: The Office of the Gene Technology Regulator, MDP 54 GPO Box 9848, Canberra ACT 2601

or

via email to: ogtr@health.gov.au.

Please note that issues regarding patient safety and the quality of the GM chimeric Orthopoxvirus treatment **do not** fall within the scope of these evaluations as they are the responsibilities of other agencies and authorities.

PAGE INTENTIONALLY LEFT BLANK

Summary of the Risk Assessment and Risk Management Plan

(Consultation Version) for

Licence Application DIR 192

Introduction

The Gene Technology Regulator (the Regulator) has received a licence application to conduct a clinical trial using a genetically modified organism (GMO). It qualifies as Dealings involving the Intentional Release (DIR) of genetically modified organisms into the Australian environment under the *Gene Technology Act 2000*.

The applicant, Medpace Australia Pty Ltd (Medpace) proposes to conduct a clinical trial to evaluate the safety and efficacy of a genetically modified (GM) chimeric Orthopoxvirus (CF33-hNIS), alone or in combination with an existing cancer therapy (Pembrolizumab), for the treatment of Australian patients with metastatic or advanced solid cancerous tumours.

The GMO was modified from the oncolytic virus CF33, a chimeric Orthopoxvirus (OPXV) strain which has been shown to target cancer cells. The genetic modifications lead to higher levels of viral replication in cancer cells compared to normal cells. Additionally, the genetic modifications facilitate the visualisation of the GMO after administration to patients by medical imaging.

The GMO would be manufactured overseas and imported into Australia. It would be administered by intratumoural injection or by intravenous infusion in up to 18 Australian patients at clinical trial sites and hospitals in Australia.

Clinical trials in Australia are conducted in accordance with requirements of the *Therapeutic Goods Act 1989*, which is administered by the Therapeutic Goods Administration (TGA). Therefore, in addition to approval by the Regulator, Medpace would also require authorisation from TGA before the trial commences. Clinical trials conducted in Australia must also be conducted in accordance with the *National Statement on Ethical Conduct in Human Research* and with the *Guidelines for Good Clinical Practice* of the International Council for Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use. Medpace would also require approval from the Department of Agriculture, Fisheries and Forestry for import of the GMO.

The Regulator has prepared a Risk Assessment and Risk Management Plan (RARMP) for this application, which concludes that the proposed clinical trial poses negligible risks to human health and safety and the environment. Licence conditions have been drafted for the proposed clinical trial. The Regulator invites submissions on the RARMP, including draft licence conditions, to inform the decision on whether to issue a licence.

The application

Project Title	Clinical trial of a genetically modified (GM) chimeric Orthopoxvirus (CF33-hNIS) as a cancer treatment [1]
Parent organism	Chimeric Orthopoxvirus (CF33)
Genetic modifications	• Deletion of *J2R* gene (viral thymidine kinase) – leading to preferential viral multiplication in cancer cells. • Insertion of the human sodium-iodide symporter (hNIS) gene – to facilitate the visualisation of the virus by medical imaging.
Principal purpose	The proposed trial is a Phase 1 study designed to evaluate the safety and efficacy of a GM chimeric Orthopoxvirus, (known as CF33-hNIS; VAXinia; HOV2), alone and in combination with an existing cancer therapy (Pembrolizumab), for the treatment of Australian patients with metastatic or advanced solid cancerous tumours.
Previous clinical trials	The proposed study is the first clinical trial to be conducted with CF33-hNIS (the GMO).
Proposed limits and controls	
Proposed duration	5 years
Proposed release size	Up to 18 participants would be enrolled in the trial in Australia
Proposed locations	This clinical trial would be conducted within clinical trial sites and hospitals in Australia. The number of sites and specific locations are yet to be determined.
Proposed controls	• The GMO would be administered to trial participants within a suitable medical facility. • Staff preparing and administering the GMO would use personal protective equipment. • Import, transport and storage of the GMO would be carried out according to the OGTR *Guidelines for the* T*ransport, Storage and Disposal of* GMOs. • Waste that may contain the GMO would be disposed of as infectious material (e.g. via the clinical waste stream).

[1] The title of the project as supplied by the applicant is "A Phase I, Dose Escalation Safety and Tolerability Study of VAXINIA (CF33-hNIS), Administered Intratumorally or Intravenously as a Monotherapy or in Combination with Pembrolizumab in Adult Patients with Metastatic or Advanced Solid Tumors".

Risk assessment

The risk assessment concludes that risks to the health and safety of people or the environment from the proposed clinical trial are negligible. No specific risk treatment measures are required to manage these negligible risks.

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

Credible pathways to potential harm that were considered include the; potential exposure of people and animals to the GMO; and the potential for the GMO to transfer or acquire genetic material from other viruses. The potential for the GMO to be released into the environment and its effects were also considered.

Important factors in reaching the conclusions of the risk assessment included that the GMO is designed to preferentially replicate in cancer cells, and unintended exposure to the GMOs would be minimised by the limits and controls.

As risks to the health and safety of people, or the environment, from the proposed trial of the GMO treatment have been assessed as negligible, the Regulator considers that the dealings involved do not pose a significant risk to either people or the environment.

Risk management

The risk management plan describes measures to protect the health and safety of people and to protect the environment by controlling or mitigating risk. The risk management plan is given effect through licence conditions. Draft licence conditions are detailed in Chapter 4 of the RARMP.

As the level of risk is considered negligible, specific risk treatment is not required. However, since this is a clinical trial, the draft licence includes limits on the number of trial participants, types of facilities used and duration of the trial, limits on the duration of the trial, as well as a range of controls to minimise the potential for the GMO to spread in the environment. In addition, there are several general conditions relating to ongoing licence holder suitability, auditing and monitoring, and reporting requirements which include an obligation to report any unintended effects.

Table of contents

Abbreviations

AS	Ankara strain of Vaccinia virus
AICIS	Australian Industrial Chemicals Introduction Scheme
APVMA	Australian Pesticides and Veterinary Medicines Authority
CL	Calf-Lymph strain of *Vaccinia virus*
CDC	Centers for Disease Control and Prevention
CTN	Clinical Trial Notification Scheme
CT	Computed Tomography
DIR	Dealings involving Intentional Release
DNA	Deoxyribonucleic acid
dTTP	deoxythymidine triphosphate
EU	European Union
FSANZ	Food Standards Australia New Zealand
GTTAC	Gene Technology Technical Advisory Committee
GM	Genetically modified
GMO	Genetically modified organism
MV-NIS	GM Measles virus expressing sodium iodide symporter
ICH-GCP	*Guidelines for Good Clinical Practice* of the International Council for Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use
HREC	Human Research Ethics Committee
hNIS	Human Sodium Iodide Symporter
IBC	Institutional Biosafety Committee
IATA	International Air Transport Association
IHD	International Health Department strain of *Vaccinia virus*
i.t.	Intratumoural
i.v.	Intravenous
LC	Lederle-Chorioallantoic strain of *Vaccinia virus*
MTD	Maximum Tolerated Dose
ml	Milli litre
min	Minute
MPXV	*Monkeypox virus*
NHMRC	National Health and Medical Research Council
NYCBOH	New York City Board of Health strain of *Vaccinia virus*
OGTR	Office of the Gene Technology Regulator
OPXV	Orthopoxvirus
PPE	Personal Protective Equipment
PFU	Plaque Forming Units
PCR	Polymerase chain reaction

PET	Positron emission tomography
RPXV	Rabbitpox virus
RAF	*Risk Analysis Framework*
RARMP	Risk Assessment and Risk Management Plan
SPECT	Single-photon Emission Computed Tomography
SOP	Standard Operating Procedure
the Act	The *Gene Technology Act 2000*
the Regulations	The Gene Technology Regulations 2001
the Regulator	The Gene Technology Regulator
TGA	Therapeutic Goods Administration
TK	Thymidine kinase
USA	United States of America
VIG	Vaccinia immune globulin
VACV	*Vaccinia virus*
VARV	*Variola virus*
WR	Western Reserve strain of *Vaccinia virus*
WHO	World Health Organization

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 and the Gene Technology Regulations 2001 (the Regulations), together with corresponding State and Territory legislation, 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. Section 50 of the Act requires that the Gene Technology Regulator (the Regulator) must prepare a Risk Assessment and Risk Management Plan (RARMP) in response to an application for release of GMOs into the Australian environment. Sections 50, 50A and 51 of the Act and sections 9 and 10 of the Regulations outline the matters which the Regulator must take into account and who must be consulted when preparing the RARMP.

4. The *Risk Analysis Framework* (RAF) (OGTR, 2013) explains the Regulator's approach to the preparation of RARMPs in accordance with the Act and the Regulations. The Regulator has also developed operational policies and guidelines that are relevant to DIR licences. These documents are available from the Office of the Gene Technology Regulator (OGTR website).

5. Figure 1 shows the information that is considered, within the regulatory framework above, in establishing the risk assessment context. This information is specific for each application. Risks to the health and safety of people or the environment posed by the proposed supply are assessed within this context. Chapter 1 describes the risk assessment context for this application.

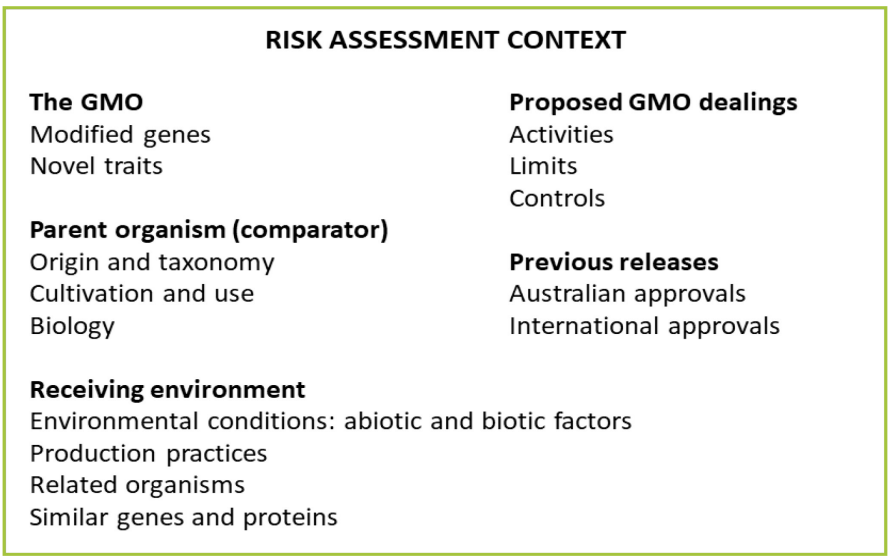

Figure 1. Summary of parameters used to establish the risk assessment context, within the legislative requirements, operational policies and guidelines of the OGTR and the RAF.

6. In accordance with Section 50A of the Act, this application is considered to be a limited and controlled release application, as the Regulator was satisfied that it meets the criteria prescribed by the Act. Therefore, the Regulator was not required to consult with prescribed experts, agencies and authorities before preparation of the RARMP.

7. Section 52 of the Act requires the Regulator to seek comment on the consultation RARMP from agencies - the Gene Technology Technical Advisory Committee (GTTAC), State and Territory Governments, Australian Government authorities or agencies prescribed in the Regulations, Australian local councils and the Minister for the Environment - and from the public.

1.1 Interface with other regulatory schemes

8. Gene technology legislation operates in conjunction with other regulatory schemes in Australia. The GMOs and any proposed dealings conducted under a licence issued by the Regulator may also be subject to regulation by other Australian government agencies that regulate GMOs or GM products, including Food Standards Australia New Zealand (FSANZ), the Australian Pesticides and Veterinary Medicines Authority (APVMA), the Therapeutic Goods Administration (TGA), the Australian Industrial Chemicals Introduction Scheme (AICIS) and the Department of Agriculture, Fisheries and Forestry.

9. Medicines and other therapeutic goods for use in Australia are required to be assessed for quality, safety and efficacy under the *Therapeutic Goods Act 1989* and must be included in the Australian Register of Therapeutic Goods. The 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 Approval (CTA) scheme or the Clinical Trial Notification (CTN) scheme.

10. For clinical trials, the TGA has regulatory responsibility for the supply of unapproved therapeutic products. In terms of risk to individuals participating in a clinical trial, the 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 vaccine, and risks associated with import, transport and disposal of the GMO.

11. 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 (ICH 1996). The guideline was developed with consideration of the current good clinical practices of the European Union (EU), Japan, and the United States of America (USA), as well as those of Australia, Canada, the Nordic countries and the World Health Organization (WHO). The TGA has adopted the ICH-GCP in principle as Note for Guidance on Good Clinical Practice (designated CPMP/ICH/135/95) (Therapeutic Goods Administration 2000), which provides overarching guidance for conducting clinical trials in Australia which fall under TGA regulation.

12. The National Health and Medical Research Council (NHMRC) has issued the *National Statement on Ethical Conduct in Human Research* (National Health and Medical Research Council et al., 2018). 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.

13. Approval by a Human Research Ethics Committee (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.

14. The Department of Agriculture, Fisheries and Forestry administers Australian biosecurity conditions for the importation of biological products under the *Biosecurity Act 2015*. Biological products include animal or microbial derived products such as foods, therapeutics, laboratory materials and vaccines (including GM vaccines). Import of GM vaccine is subject to regulation by the Department of Agriculture, Fisheries and Forestry and the Regulator.

15. All clinical trial sites would be located at medical facilities including out-patient settings, hospitals and associated pharmacies. Analysis of biological samples collected from trial participants administered with the GMO would occur at clinical trial sites, or at pathology laboratories. These facilities are regulated by State and Territory governments and adhere to professional standards for safety (NSQHS), disease control (Australian Guidelines for the Prevention and Control of Infection in Healthcare (2019) and handling of pathology samples (NPAAC).

16. The state and territory governments regulate hospitals and other medical facilities in Australia. All public and private hospitals and day procedure services need to be accredited to the National Safety and Quality Health Service (NSQHS) Standards developed by the Australian Commission on Safety and Quality in Healthcare (the Commission) and endorsed by the state and territory Health Ministers. The Commission coordinates accreditation processes via the Australian Health Service Safety and Quality Accreditation (AHSSQA) scheme. The NSQHS Standards provide a quality assurance mechanism that tests whether relevant systems are in place to ensure that the minimum standards of safety and quality are met. The safety aspects addressed by the NSQHS Standards include the safe use of sharps, disinfection, sterilisation and appropriate handling of potentially infectious substances. Additionally, the Commission has developed the National Model Clinical Guidance Framework, which is based on, and builds on NSQHS Standards to ensure that clinical governance systems are implemented effectively and to support better care for patients and consumers.

17. The National Pathology Accreditation Advisory Council (NPAAC) advises Commonwealth, State and Territory health ministers on matters relating to the accreditation of pathology laboratories. 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. While compliance with NPAAC standards and guidelines is not mandatory, there is a strong motivation for pathology services to comply, as Medicare benefits are only payable for pathology services if conducted in an appropriate Accredited Pathology Laboratory (APL) category, by an Approved Pathology Practitioner (APP) employed by an Approved Pathology Authority (Anandasabapathy et al., 2015). Accreditation of pathology services is overseen by Services Australia (formerly Department of Human Services), and currently, the only endorsed assessing body for pathology accreditation is the National Association of Testing Authorities (NATA).

18. Hospitals and pathology laboratories, including their workers, managers and executives, all have a role in making the workplace safe and managing the risks associated with handling potentially infectious substances including the proposed GMO. There are minimum infection prevention practices that apply to all health care in any setting where health care is provided. These prevention practices were initially developed by the Centers for Disease Control and Prevention (CDC), and are known as the standard precautions for working with potentially infectious material. The standard precautions are described in the Australian Guidelines for the Prevention and Control of Infection in Healthcare (2019).

Section 2 The proposed dealings

19. Medpace Australia Ltd is seeking authorisation to carry out a clinical trial to assess the safety and efficacy of a genetically modified (GM) chimeric Orthopoxivus (CF33-hNIS) alone and in combination with an existing cancer therapy (Pembrolizumab), for the treatment of Australian

patients with metastatic or advanced solid cancerous tumours. The proposed study is a multi-centre clinical trial to be conducted in Australia and United States of America (USA). The product sponsor is Imugene Limited and the applicant Medpace Australia Ltd, will act as clinical research organisation and licence holder.

20. The dealings involved in the proposed clinical trial are:

(a) import the GMO;

(b) conduct the following with the GMO:

i. prepare the GMO for administration to trial participants;

ii. administer the GMO to clinical trial participants by intratumoural injection (i.t.). or by intravenous infusion (i.v.);

iii. collect samples from trial participants;

iv. analyse the samples

v. prepare samples for export;

(c) transport the GMO;

(d) dispose the GMO;

and the possession (including storage), supply and use the GMO for the purposes of, or in the course of, any of these dealings.

2.1 The proposed limits of the trial (duration, scale, location, people)

21. The clinical trial is proposed to take place over a five-year period from the date of issue of the licence. Up to 18 patients in Australia would receive multiple doses of the GMO over a period of 24 months.

22. The clinical trial would take place at clinical trial sites and hospitals in Australia, these clinical sites have not yet been identified.

23. Only trained and authorised staff would conduct dealings with the GMO. Administration of the GMO in trial participants would be conducted by highly trained medical staff.

2.2 The proposed controls to restrict the spread and persistence of the GMOs in the environment

24. The applicant has proposed a number of controls to minimise exposure to the GMO, and to restrict the spread and persistence of the GMOs in the environment. These include:

- The GMO would be administered to trial participants within a suitable medical facility;
- Staff preparing and administering the GMO would use personal protective equipment (PPE);
- Immunocompromised and pregnant medical staff would be excluded from handling the GMO;
- Transport, storage and disposal of the GMO and any contaminated waste generated at a clinical trial site would be disposed in accordance with the current version of the Regulator's Guidelines for the Transport, Storage and Disposal of GMOs.

25. The applicant has proposed detailed control measures to minimise the exposure of close contacts and animals to the GMO. Trial participants would be instructed to follow these measures for

at least 24 h after the GMO admistration and in the event pustules/skin lesions[2] develop, as this may be one of the symptoms of the GM virus. These include:

- Injection sites must be covered with a dressing for at least 24 hours after injection or until completely dry. If the dressing applied after treatment falls off before the site is completely dry, a new dressing must be immediately applied;
- Trial participants or caregivers should wear disposable protective gloves when removing or changing dressings;
- Used dressings, gloves and any cleaning materials must be disposed of in the biohazard bags provided by the study site staff and returned to the study in the next follow-up visit;
- Avoid accidental autoinoculation by direct contact with the injection site and other parts of the body. In case of accidental exposure, the affected area should be thoroughly cleaned with soap and water and/or a disinfectant. If the eye was touched or exposed, it should be flushed with water for 15 minutes;
- Clean any household areas that may have been exposed to the GMO with a 10% bleach solution;
- Contaminated clothing and bedding should be washed with disinfectants such as bleach.

2.3 Details of the proposed dealings

2.3.1 *Manufacturing of the GMO*

26. The GMO will be manufactured overseas in accordance with Good Manufacturing Practice (GMP) guidelines. The final product would be packaged into polypropylene cryogenic vials as a 1.1 ml aqueous suspension of the GMO and stored at -80°C. Each CF33-hNIS vial would be individually packaged in a carton and clearly labelled with product name, titre, name of the Sponsor and clinical trial details (i.e. clinical trial site and patient number).

27. The GMO would be shipped from USA to Singapore and stored at Catalent´s Singapore depot until shipment to clinical trial sites in Australia. The applicant has estimated that up to 666 vials would be imported for this trial.

2.3.2 *Import, transport and storage of the GMO*

28. The GMO would be imported into Australia by specialist courier companies such as World Courier. The frozen GMO would be packaged and labelled for transport in accordance with the packaging and labelling requirements of the International Air Transport Association (IATA) code UN 3373 (Biological Substance, Category B). Briefly, GMO vials would be shipped in one-vial cartons within a container containing dry ice and a temperature monitoring device.

29. The GMO would be shipped to clinical trial sites in Australia upon approval of patient enrolment in the clinical trial and re-supplied as needed.

30. Transport within Australia to the clinical trial sites would be conducted in accordance with the Regulator's *Guidelines for the Transport, Storage and Disposal of GMOs* by a suitable courier company. Upon arrival at trial sites, the GMO would be unpacked and inspected for damage by trained staff. The GMO would then be stored in a freezer at -80°C ±10°C.

31. Storage of the GMO would be within the clinical trial site in a secure freezer with access restricted to appropriately trained and authorised personnel.

[2] For the purposes of this RARMP, the term pustule and lesion are interchangeably used.

32. Samples collected from trial participants would be transported to analytical facilities within the clinical trial site and to third party analytical facilities located within Australia or prepared for export. All samples would be treated as though they contain the GMO and transported in accordance with the Regulator's *Guidelines for the Transport, Storage and Disposal of GMOs.*

33. Waste that may contain GMOs would be destroyed at the clinical trial sites or transported for disposal as per institutional standard operating procedures (SOP) for disposal of risk group 2 infectious material.

2.3.3 *Clinical trial sites*

34. The clinical trial would be carried out at clinical trial sites and hospitals, which are yet to be confirmed. Clinical trial sites would be assessed by the applicant for their experience in cancer research and treatment.

2.3.4 *The clinical trial*

35. The proposed clinical trial is a Phase 1, multi-centre, open-label, dose-escalation study that would evaluate the safety and efficacy of the GMO (CF33-hNIS) as a treatment for metastatic or advanced solid cancerous tumours. The GMO would be administered via i.t. injection or i.v. infusion, alone and in combination with an existing cancer therapy (Pembrolizumab).

36. The GMO would be administered on days 1, 8 and 22 and subsequently every 21 days (treatment cycle) for up to 24 months. As the clinical trial's secondary aim is to identify the Maximum Tolerated Dose (MTD) of the GMO, up to 4 dose levels (8.6×10^5, 9.4×10^6, 3×10^7 and 1.1×10^8 plaque forming units (PFU)) would be tested. The first patient cohort would receive the lowest dose of the GMO alone via i.t. injection, with i.v. infusion and/or higher doses only administered once this is shown to be safe. If the first cohort cannot tolerate the 8.6×10^5 PFU dose, the next cohort would receive a reduced dose of the GMO (de-escalation dose to be determined).

37. The administration of the GMO in combination with pembrolizumab would only occur if the administration of the next dose level of the GMO alone is shown to be safe (e.g. 8.6×10^5 PFU of the GMO + pembrolizumab would be administered only if the GMO dose level of 9.4×10^6 PFU alone is shown to be safe). Pembrolizumab would be administered intravenously after CF33-hNIS treatment, beginning on day 22.

2.3.5 *Selection of trial participants*

38. Relevant inclusion criteria proposed by the applicant include that participants must:

- be adults aged 18 years or older;
- have a locally advanced or metastatic solid tumour;
- be willing and able to comply with scheduled visits, treatment plan, laboratory tests, and other study procedures;
- refrain from egg or sperm donation throughout the study and for at least 60 days after receiving the last dose of the GMO;
- agree to use contraceptives to prevent pregnancy throughout the study and for at least 60 days after receiving the last dose of the GMO.

39. Relevant exclusion criteria proposed by the applicant include:

- prior treatment with an oncolytic virus;
- evidence of an active infection, or immunosuppressive disorder;
- pregnancy or breastfeeding;

- have received a vaccine within 4 weeks of study treatment;
- in addition, participants may be excluded for any reason that, in the opinion of the clinical trial investigator, makes the participant unsuitable for the study.

2.3.6 *Preparation and administration of the GMO for administration*

40. The GMO vial would be removed from the freezer and prepared by trained staff following the institution SOPs for infectious material. The GMO must be administered within 2 hours of completion of thawing.

41. The GMO would be prepared in a biosafety cabinet. Briefly, a needle attached to a syringe would be used to withdraw the GMO suspension from the vial. The same needle/syringe would be used to withdraw an appropriated volume of saline as per Medpace pharmacy manual. For i.t. administration, the syringe would be recapped using the scoop technique. The needle used during the GMO preparation would be removed and replaced with a new capped administration needle. For the i.v. infusion, the GMO would be injected into a saline i.v. infusion bag.

42. The syringe and/or saline bag containing the GMO would be transported from preparation rooms to administration sites in a suitable container appropriate for transportation of biohazard material.

43. The i.v. infusion would be performed for at least 30 min. The infusion line would be flushed with sterile normal saline.

44. The volume of the GMO used for i.t. injection would be determined based on the size and number of the tumours to be treated, but not exceeding 4 ml. Each tumour would receive 5 injections (4 quadrants and 1 central). The GMO would be administered either under direct visual guidance (skin), or into lymph node, with or without the aid of ultrasound or computed tomography (CT) guidance.

45. The injection site would be cleaned with alcohol and covered with dressing.

2.3.7 *Sample collection and analysis*

46. Biological samples would be collected for clinical monitoring of participants and for analyses of the presence of the GMO genome or viable viral particles. Samples of serum, urine, oral and rectal swabs would be collected on days 1, 2, 8, 9 and 22 and subsequently on the first day of every second treatment cycle. On dosing days, samples would be collected prior and 1 hour after GMO administration.

47. Swab samples from injection/infusion sites would be collected on days 2 and 9 then on day 1 of subsequent treatment cycles. Samples would be collected from the previous administration site and from any other skin pustules should they occur.

48. Dressings used to cover the administration site or pustules would be returned by the trial participant on follow-up visits on days 2 and 9 of the first treatment cycle and then on day 1 of subsequent treatment cycles.

49. Biological samples would be analysed at 360 Biolabs facility in Melbourne or prepared for export and shipped to USA for analysis.

2.3.8 *Decontamination and disposal of the GMO*

50. Waste generated during preparation and/or administration of the GMOs (e.g. needles, syringes, dressings) would be destroyed at the clinical trial sites or transported for disposal as per clinical trial site procedures.

51. Reusable items, work surfaces, the administration room and dedicated bathroom facilities for patients would be decontaminated with chemical disinfectant (e.g. sodium hypochlorite (0.5-10%), isopropyl alcohol (50%) and ethanol (70%)) as per clinical trial site SOPs.

52. Empty, partially used and unused vials of GMO would be destroyed at the clinical trial site as per clinical trial site SOPs. If the clinical trial site is unable to destroy the GMO vials, the vials would be properly stored until a suitable method of disposal can be arranged by the Sponsor. Disposal or destruction of the GMO would be documented.

2.3.9 ***Training***

53. The applicant's IBC declared that the training and experience of individuals involved in these dealings is satisfactory. The applicant stated that the GMO would be handled, prepared, and administered in medical settings by qualified and trained staff. Staff would be trained on the licence conditions and its variations and on how to safely conduct dealings with the GMO by Medpace and/or Imugene representatives. Training of staff that did not attend or commenced work after the initial training would be carried out by existing trained staff.

2.3.10 ***Accountability and Monitoring***

54. A log detailing the dates and quantities of the GMO used would be maintained by site staff and would be verified by the Medpace Clinical Research Associate during site visits.

55. All documentation (e.g. receipts, authorisation for use, dispensing, destruction, temperature monitoring) would be filed on-site and available for inspection by the Clinical Research Associate.

2.3.11 ***Contingency plans***

56. Immunocompromised and pregnant medical staff would be excluded from handling the GMO.

57. In the event of inadvertent exposure due to needle-stick, sharps exposure, mucosa or broken skin exposure, persons who have had accidental direct contact with the GMO would be instructed to:

- bleed from the skin wound;
- wash the area with soap and water for at least 15 min, and seek medical attention;
- notify the appropriate institutional contacts as per institutional procedures, principal investigator and primary study coordinator of the exposure;
- seek medical attention for treatment and/or surveillance.

58. Staff would be trained in appropriate procedures in spill management as per institutional guidelines. As an example, the applicant has described the following procedure for treating a spill:

- quarantine the area;
- remove contaminated clothing and place it in a biohazard bag;
- personnel cleaning up the spill must wear personal protective equipment (e.g. disposable gown, shoe covers, gloves, face mask and eye protection);
- cover spill with absorbent material;
- decontaminate the area by applying an appropriate chemical disinfectant against the GMO using spill kit and procedures developed for clinical material spills;
- wait for at least 10 minutes before removing the material for disposal;
- if sharps are present, they should be removed using tongs and/or a broom and dustpan;
- the licence holder must be notified of the spill as soon as reasonably possible.

Section 3 Parent organism

3.1 Origin

59. The parent organism, known as CF33, is a chimeric oncolytic Orthopoxvirus (OPXV) originated through homologous recombination among Rabbitpox virus (RPXV) and 6 strains of *Vaccinia virus* (VACV). Briefly, mammalian cells cultured in laboratory were infected simultaneously with 9 strains of OPXVs, including RPXV, *Cowpox virus*, *Raccoonpox virus* and 6 VACV strains. After a period of time, resulting viruses were purified and tested for their oncolytic activity in human cancer cells cultured in the laboratory. CF33 was then selected for further studies due to its ability to preferentially replicate in and destroy cancer cells (O'Leary et al., 2018).

60. Rabbitpox virus (RPXV) was first isolated in 1932 after of a series of outbreaks in laboratories conducting work with VACV in rabbits at the Rockefeller Institute (New York, USA). Rabbitpox was highly lethal in rabbits and was shown to be transmitted by direct contact or aerosols (respiratory route). In 1941, a similar disease outbreak among rabbits at the University of Utrecht (the Netherlands) led to isolation of the RPXV strain Utrecht (RPXV-Utrecht) (Nalca and Nichols, 2011). It is important to note that all reported rabbitpox outbreaks occurred in laboratory colonies of rabbits, with the latest one reported in the 1960s. RPXV-Utrecht is currently used in laboratory studies as a model for smallpox and was included in the generation of CF33 (O'Leary et al., 2018). It is genetically similar to VACV, with the exception of three genes associated with virulence of other poxviruses (Martinez-Pomares et al., 1995; Li et al., 2005). These three genes are not present in CF33 (Chaurasiya et al., 2022).

61. VACV was first identified in 1939 (Downie, 1939) and is considered to have originated from mutation or recombination involving cowpox virus, *Variola virus* (VARV, causative agent of smallpox) and other related OPXV ancestors. VACV strains were used globally as a vaccine against smallpox prior to the latter's declared eradication in 1980 (WHO, 2022b).

62. CF33 is derived from VACV strains and the close relative RPXV-Utrecht. As previously mentioned, genes related to the increased virulence of RPXV-Utrecht virus in rabbits are not present in the CF33 genome. Therefore, for the purpose of this RARMP, when data is not available for CF33, VACV will be used as the non-GM parent organism to provide a baseline for comparing the potential for harm associated with dealings with the GMO. Additionally, as the biology of VACV has been described in detail in the RARMPs for DIR-116, and DIR-140 (clinical trials with GM *Vaccinia viruses*), RARMP for DIR-170 (trial with GM *Vaccinia viruses* in horses) and more recently in the RARMP for DIR-179 (trial with GM *Vaccinia viruses* for cancer treatment), a summary is presented in this section.

3.2 Classification and genome characteristics

63. OPXVs belong to the genus Orthopoxvirus, family Poxviridae and subfamily Chordopoxvirinae. The genus includes the human pathogen *Variola virus*, *Vaccinia virus*, Cowpox virus, *Horsepox virus*, *Monkeypox virus* and others (International Committee on Taxonomy of Viruses 2022).

64. As an OPXV, the CF33 is a large, enveloped virus containing a linear double-stranded DNA genome of about 189 kilobases (kb), encoding 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 (Babkin et al., 2022).

65. CF33 genome is derived from 7 out of 9 OPXVs used in its construction (see paragraph 59). It contains DNA fragments from RPXV-Utrecht and 6 strains of VACV (Western Reserve (WR), International Health Department (IHD), Lister, Lederle-Chorioallantoic (LC), Calf-Lymph (CL) and Ankara (AS)) (Chaurasiya et al., 2022).

66. CF33 is highly homologous to VACV strains. The majority of CF33 pathogenesis-related genes (i.e. replication, cell attachment and entry genes) are derived from the IHD, Lister and WR VACV strains. Together, these 3 VACV strains account for 60% of the CF33 genome (Chaurasiya et al.,

2022). An overview of VACV strains and RPXV-Utrecht collaboration in the CF33 genome is shown in Figure 2.

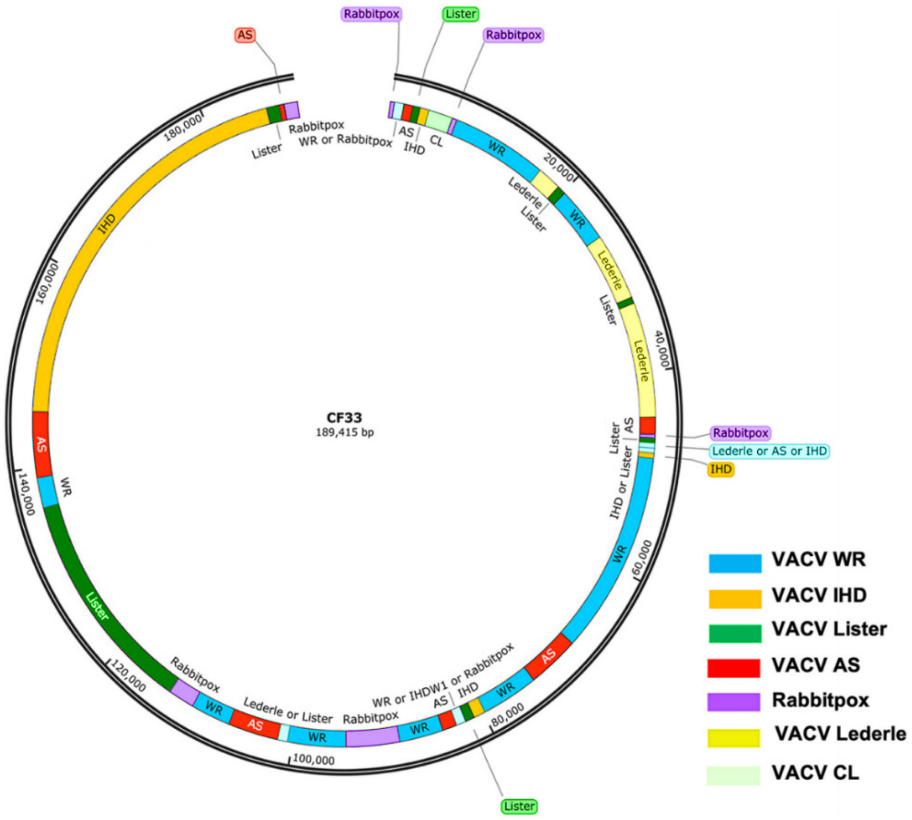

Figure 2. Map of CF33 genome showing components of parental viruses (adapted from Chaurasiya et al., 2022).

3.3 Lifecycle

67. 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 (Liu et al., 2014).

68. VACV does not integrate into the host genome and its entire life cycle takes place within the cytoplasm (Liu et al., 2014). Consequently, VACVs are unable to use host replication enzymes and their genomes encode enzymes required for both DNA replication and gene transcription (Schramm and Locker, 2005).

69. VACV genes are expressed in three temporal stages, i.e. expression of early, intermediate and late genes (Yang et al., 2011). Proteins required for the process cytoplasmic DNA replication are expressed in the early stages. DNA replication begins after ~2 hours post-infection and initiates the transcription and expression of genes required for the expression of the intermediate and late genes (i.e. proteins involved in the assembly of new virus particles) (Shors et al., 1999; Tolonen et al., 2001).

70. After DNA replication and late protein expression, VACV goes through an assembly process. This involves the formation of immature virions, consisting of a membrane enclosing a nucleoprotein mass (a complex of DNA and proteins), which are then enveloped to form the mature infectious virions (Liu et al., 2014). Approximately 10,000 copies of the viral genome are made within 12 hours of infection; half of these are incorporated into mature virions and released upon host cell lysis (Tolonen et al., 2001)

71. CF33 showed efficient replication in healthy human cells cultured in the laboratory, with viral titres comparable to IHD and WR strains of VACV (~1000 plaque-forming unit/cell (PFU/cell)) after 48 and 72 hours of infection. In healthy mouse cells, CF33 showed reduced replication (~10-100 pfu/cell) when compared to VACV strain IHD (~1000-10 000 pfu/cell) or WR (~100-1000 pfu/cell) (Chaurasiya et al., 2022). These results suggest that CF33 replicates poorly in healthy mouse cells when compared to healthy human cells.

3.4 Pathology

72. Generally, VACV is considered a mild pathogen in people. As an OPXV, VACV preferentially infects and replicates in epithelial cells and tends to produce skin pustules (Moussatche et al., 2008). As the infection progresses, immune cells such as macrophages and other antigen presenting cells are recruited to the infection site to contain the infection. However, VACV can infect and manipulate some of these immune cells and use them to spread to other parts of the host body through the blood stream (Smith et al., 2013; El-Jesr et al., 2020).

73. When administered as a vaccine, the vaccine recipients normally develop a single lesion at the site of exposure around 3-4 days post vaccination, it indicates a successful vaccination and generally heals over 2-3 weeks (Fulginiti et al., 2003a; CDC, 2022d). This is often accompanied by flu-like symptoms (fever, malaise, headache, nausea and muscle aches), swelling and redness around the vaccination, and swelling and tenderness of the draining lymph node. In healthy people, these reactions resolve spontaneously and require only observation and symptomatic treatment (Cono et al., 2003; Fulginiti et al., 2003b; Maurer et al., 2003).

74. Serious adverse reactions associated with VACV such as post-vaccinia encephalitis or death are rare, strain dependent, and particularly affect those with underlying risk factors such as atopic dermatitis or who are immunocompromised, as in the case of HIV infection (Cono et al., 2003). More information regarding severe adverse reactions can be found in Section 3.8.1 of this Chapter.

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

3.5 Epidemiology

3.5.1 *Geographic distribution and host range*

76. OPXVs can infect a wide range of organisms, from humans to domestic and wild animals (Silva et al., 2020). The natural host of VACV is not known, but in the environment and in laboratories, VACV is able to infect a variety of species and cause disease in humans, several monkey species, a variety of rodents and marsupials, buffalo, dairy cattle, sheep, horses, rabbits, and domestic cats and dogs (Robinson and Mercer, 1988; Bennett et al., 1989; Brochier et al., 1989; Artois et al., 1990; Dumbell and Richardson, 1993; Adams et al., 2007; Abrahão et al., 2010; Felipetto Cargnelutti et al., 2012; Riyesh et al., 2014; Miranda et al., 2017). Birds are not known to be a host for VACV, but a study of a GM VACV-based rabies vaccine demonstrated sufficient viral replication in several Canadian bird species to permit seroconversion. Furthermore, poxvirus infections (often novel and not well characterised) have been reported in native Australian mammals, birds and reptiles (Wildlife Health Australia, 2012, 2019a, b; Sarker et al., 2021).

77. Infections with VACV or close relatives have been documented in South America, India, Indonesia, Egypt and other countries. In Brazil, outbreaks of zoonotic disease caused by VACV-like viruses affected dairy cattle and rural workers, and VACV infections were found in remote Amazonian wildlife (Silva et al., 2020).

78. A multi-country outbreak of monkeypox is ongoing since early May 2022. As of 8 June 2022, WHO has registered a total of 1285 confirmed cases reported between 13 May to 8 June 2022 in 28 countries where monkeypox virus is not endemic (WHO, 2022a).

3.5.2 *VACV shedding and transmission*

Shedding from infected hosts

79. VACV vaccination normally leads to the development of a single pustule at the injection site (CDC, 2022d). Individuals vaccinated with VACV can shed viral particles from the site of injection from day 1 to day 21 post-vaccination – from the time the pustule develops until the scab drops off, and possibly longer. Maximal shedding occurs between days 4 and 13, and peak titres of 10^7 plaque forming units (pfu)/ml have been detected in swabs taken from the vaccination site (Cooney et al., 1991; Wharton et al., 2003; Cummings et al., 2008).

80. In the context of non-human hosts, VACV was found in milk and faeces of experimentally infected dairy cattle, suggesting a systemic infection (de Oliveira et al., 2015; Matos et al., 2018). The virus continued to be shed even after lesions on the teats and udders had healed. VACV of both high and low pathogenic strains was also found in faeces and urine of experimentally infected mice (Ferreira et al., 2008).

Transmission between humans

81. The most common route of VACV transmission between humans is through direct contact with lesions caused by the virus or contact with a contaminated fomite (e.g. dressing, clothing, sheets and towels). An vaccinated/infected person may also spread VACV from the initial infection site by touching other body parts or people with contaminated hands (Cono et al., 2003; Egan et al., 2004; Oliveira et al., 2014; Webber et al., 2014). Transmission of VACV via aerosols (airborne droplets) has never been clearly documented in people when used as a vaccine and is considered unlikely (Lane and Fulginiti, 2003).

82. Reported cases predominantly involved transmission between family members or other close contacts or transmission in hospital settings. 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)

Transmission to and between animals

83. 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 from infected cows, and further transmission to cows from 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).

84. Faecal shedding from experimentally infected cows has been demonstrated (Matos et al., 2018), 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 (Abrahão et al., 2009). These data suggest that horizontal transmission via contaminated faeces is possible, and that faeces could provide a means for viral dissemination into the environment (Abrahão et al., 2009; D'Anunciacao et al., 2012).

85. VACV infection has been documented in domestic dogs and wild opossums after a VACV outbreak in Sao Paulo, Brazil. It has been suggested 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).

86. In the context of smallpox vaccination, the US Centres for Disease Control and Prevention (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 material or surfaces contaminated with fluid from a vaccination site is recommended (CDC, 2022c).

Laboratory-related VACV infections

87. Laboratory-acquired VACV infections are rare and typically involve unvaccinated individuals working with a non-attenuated VACV strain. During 2005-2008, 15 cases of VACV exposure via needlestick injures (9/15), spill (1/15) or eye splash (5/15) with the concentrated virus were reported to CDC. Six out of 9 individuals exposed via needlestick injuries developed VACV infection and 4 cases resulted in hospitalisation. None of the work practices in the laboratories where work was conducted had met the Advisory Committee on Immunization Practices (ACIP) vaccination recommendation for working with non-highly attenuated VACV or other Orthopoxviruses (e.g., monkeypox, cowpox). Exposure via spill or eye splash did not result in infection (CDC, 2008).

3.6 Recombination

88. Homologous recombination requires both viruses to be present and replicating within the same infected host cell. Recombination of OPXVs in cell cultures in a laboratory setting are easily produced and have been documented between other vaccinia strains (Fenner and Comben, 1958), between related poxviruses such as rabbit Fibroma and Myxoma viruses (Woodroofe and Fenner, 1960) and between *Variola virus, Cowpox* and *Rabbitpox* viruses (Bedson and Dumbell, 1964a, b). As described in section 3.1, CF33 is a chimeric virus originated in laboratory cell culture through homologous recombination among 7 strains of OPXVs.

89. Examples of natural OPXV recombinants which have clearly occurred between co-infecting viruses are nearly non-existent (Gershon et al., 1989), with very few examples of potential recombination events between poxviruses in co-infected animals (Strayer et al., 1983). Although replicating poxviruses can recombine very efficiently under certain circumstances, there are physical constraints within a cell that limit recombination between co-infecting viruses. VACV transcription, translation and replication takes place in the cytoplasm but within membrane-bound cytoplasmic structures known as viral factories or virosomes (Katsafanas and Moss, 2007; Lin and Evans, 2010; Paszkowski et al., 2016), thus, compartmentalising and preventing the mixing of their nucleic acid with other viruses in the same cell (Paszkowski et al., 2016).

3.7 Environmental stability and methods of decontamination for VACV

90. Poxviruses are well known for their ability to persist in the environment, and they are more resistant to drying and increased temperature than other enveloped viruses. VACV stability is determined by temperature, relative humidity and the materials on which VACV is introduced into the environment (Wood et al., 2013). Dried VACV can be kept for more than 35 weeks at 4°C with no loss of infectivity (Rheinbaben et al., 2007). Murine faeces exposed to environmental conditions retained infectious VACV particles for at least 20 days (Abrahão et al., 2009). Clothes, bedding and personal effects from smallpox (not VACV) patients remained contagious after several years of storage or use.

91. VACV can be inactivated within 1 minute by chemical disinfectants such as 0.5% sodium hypochlorite, 30% isopropanol, 40-70% ethanol, 0.5% sodium hypochlorite, 0.02% glutaraldehyde, 0.01% benzalkonium chloride, 30% Sanytex and 0.12% ortho phenylphenol. The virus can also be inactivated by dry heat treatment at 95°C for 2 hours (Sauerbrei and Wutzler, 2009) and autoclaving (Espy et al., 2002; Canada, 2011).

92. 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).

3.8 VACV as a vaccine

93. Several strains of VACV were used for human immunisation against VARV, the causative agent of smallpox. VACV vaccination provides cross-protection against VARV and was a key factor for the smallpox eradication in the late 1970s (Jacobs et al., 2009).

94. Historical studies of smallpox vaccination indicate that approximately 40-47% of individuals receiving a VACV vaccine reported mild pain at the site of inoculation and 2–3% reported a severe pain. Mild fever was a common side effect reported by approximately 5–12% of vaccinees. Other side effects reported included headache, myalgia, chills, nausea and fatigue. Moderate to severe complications occurred in approximately 1 to 250 individuals per million primary vaccines (Rotz et al., 2001) and included generalised vaccinia, progressive vaccinia, myopericarditis and post-vaccinial encephalitis (PVE) and in rare cases death (CDC, 2022c).

95. Rates of severe post-vaccination effects varied greatly depending on the strain (Kretzschmar et al., 2006; Jacobs et al., 2009). The Bern strain caused by far the highest rates of severe adverse effects and, based on a mathematical model, it was estimated to cause nearly 45 cases of PVE and 55 deaths per million of primary vaccinations. The Copenhagen strain led to intermediate/high rates of adverse events with 33 estimated cases of PVE and 31 deaths per million vaccinations. The NYCBH strain was the most benign, with less than 3 estimated cases of PVE and 1 or 2 deaths per million vaccinations (Kretzschmar et al., 2006).

96. Because non-attenuated vaccinia strains present a greater risk, especially to immunocompromised people, they have been replaced for vaccination by highly attenuated strains where replication either cannot occur or is severely reduced. These attenuated strains were generated through sequential passage in tissue culture cells from alternative hosts, and more recently, through genetic engineering (Jacobs et al., 2009).

97. Currently, VACV is considered well-suited as a viral vector to create a new generation of safer GM vaccines and treatments (Nagata et al., 2018). Some of the features of VACV viral vectors that make them suitable for GM treatment applications include their ability to induce strong humoral and cell-mediated immune responses that enhance the immune response to the target antigens, absence of oncogenic potential and no evidence of integration into the host genome.

3.8.1 *VACV vaccine adverse events*

98. VACV vaccines are generally safe and effective, but some people do experience side effects and adverse reactions. Severe adverse reactions are more common in people who are being vaccinated for the first time and in young children (≤5 years of age) (CDC, 2022c). Adverse events following VACV vaccination include:

Unintentional Transfer of Vaccinia Virus

- Inadvertent inoculation: unintentional transfer of VACV from the vaccination site to another place on the vaccinee's body. The most common sites are the eye and surrounding orbit (ocular vaccinia), followed by the face, nose, mouth, lips, genitalia, and anus (Maurer et al., 2003; Wharton et al., 2003; CDC, 2022c).
- Contact transmission: spread of the VACV from the vaccination site (or other lesions distant from the vaccination site) to close contacts through direct contact or through other vectors such as clothing, bedding, or dressing contaminated by vaccinia virus.
- Ocular vaccinia: inflammation of the eyelid, conjunctivitis, keratitis iritis, or combinations thereof. Infections can be clinically mild to severe and can lead to vision loss.

Diffuse Dermatologic Complications

- Generalised vaccinia: disseminated vesicular or pustular rash in locations distant from the vaccination site and sometimes covering the entire body. Individuals receiving VACV vaccine for the first time or those with underlying immunodeficiency are at higher risk for generalised vaccinia.
- Eczema vaccinatum: localised or systemic spread of VACV. It occurs most often in individuals who have a history of atopic dermatitis. The rash is often accompanied by fever and swollen lymph nodes, and affected persons are frequently systemically ill.

Progressive vaccinia

- Progressive vaccinia is rare, severe, and often fatal. It occurs when a vaccination site fails to heal. VACV replication persists, spreads to secondary sites and leads to necrosis and ulceration. Lesions can become susceptible to concomitant bacterial infection. Progressive vaccinia typically occurs in immunocompromised individuals.

Rare Adverse Reactions

- Foetal vaccinia: a rare complication, with only 50 cases reported in the literature (Cono et al., 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 foetuses or pregnant women have been identified.
- Post-vaccinial encephalitis or encephalomyelitis: Most common among infants aged less than 12 months, symptoms develop 6-10 days following vaccination. Symptoms reflect cerebral or cerebellar dysfunction with headache, fever, vomiting, altered mental status, lethargy, seizures, and coma. No clinical criteria, radiologic findings, or laboratory tests that are diagnostic for these adverse reactions exist.

Cardiac Adverse Events

- Myo/pericarditis: characterised by the inflammation of the myocardium, pericardium, or both and typically associated with the New York City Board of Health (NYCBOH) VACV strain. Clinical presentation may include chest pain, shortness of breath, and palpitations ranging from subtle to severe.

3.8.2 *Contraindications for use of VACV*

99. The CDC advises that, in the absence of a smallpox outbreak, VACV should not be given to individuals with specific conditions associated with the adverse reactions (CDC, 2022b). Individuals 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 (i.e. burns, impetigo, severe acne, etc.);
- with conditions associated with immunosuppression (i.e. HIV/AIDS, leukemia, lymphoma, etc.);
- who have undergoing therapy with alkylating agents, antimetabolites, radiation, tumor necrosis factor (TNF) inhibitors or high doses of corticosteroids;
- who have underlying heart disease;
- who are pregnant or breastfeeding;
- who are aged less than one year;
- who have a serious allergy to any component of the vaccine.

3.8.3 *Treatment of VACV adverse events*

100. Treatment of VACV infections is mainly supportive and include hydration, nutritional supplementation, and prevention of secondary infections. In the case of an severe adverse event, Vaccinia immune globulin (VIG) is recommended as the first line of treatment. Antivirals such as Tecovirimat, Brincidofovir and Cidofovir are available from the US CDC in limited quantity and under an Investigational New Drug (IND) protocol for treatment of specific smallpox vaccine reactions (CDC, 2021). These antivirals are used as a second line of defence. Cidofovir is available in Australia but is not approved for the treatment of vaccinia-related complications; off-label use would thus be required.

3.9 Risk group of VACV

101. The Australian Standard 2243.3:2010 Safety in Laboratories Part 3: Microbiological safety and containment (Standards Australia/New Zealand, 2010) classifies VACV as a risk group 2 organism, and the Australian Immunisation Handbook recommends vaccination of people working with a repeated risk of exposure to, or working with large quantities or concentrations of, *Vaccinia virus* cultures (Australian Technical Advisory Group on Immunisation (ATAGI), 2018).

Section 4 The GMO - nature and effect of the genetic modification

102. Oncolytic viral therapy is a relatively new strategy that uses viruses as a treatment for cancer. Oncolytic viruses can occur naturally or consist of a genetically modified virus that can preferentially infect, replicate in, and destroy cancer cells. Oncolytic viruses can also stimulate the immune system, which is often suppressed within tumours, to aid in the clearance of the tumours (Dyer et al., 2019; Santos Apolonio et al., 2021).

103. The GMO (CF33-hNIS) was modified from the oncolytic Orthopoxvirus CF33, and is designed to preferentially target cancer cells. It is also known as VAXinia and HOV2. Details of CF33-hNIS genetic modification are discussed in the following section.

4.1 The genetic modifications and effects

104. The GMO was produced by homologous recombination into the viral J2R gene, disrupting and partially deleting the *J2R* gene sequence and introducing the human sodium iodide symporter (hNIS). Briefly, the coding DNA sequence for the hNIS under the control of the VACV H5 synthetic early promotor (S/E) was cloned into a plasmid flanked by VACV *J2R* sequences. Next, the plasmid was transfected into cultured mammalian cells already infected with the parent virus (CF33) allowing the hNIS gene to integrate into the viral *J2R* gene through homologous recombination (Figure 3). After a period of incubation, the viruses were selected, purified and tested for the presence and expression of hNIS. The insertion of the hNIS transgene resulted in deletion of >80% sequence of J2R gene causing complete inactivation of the gene.

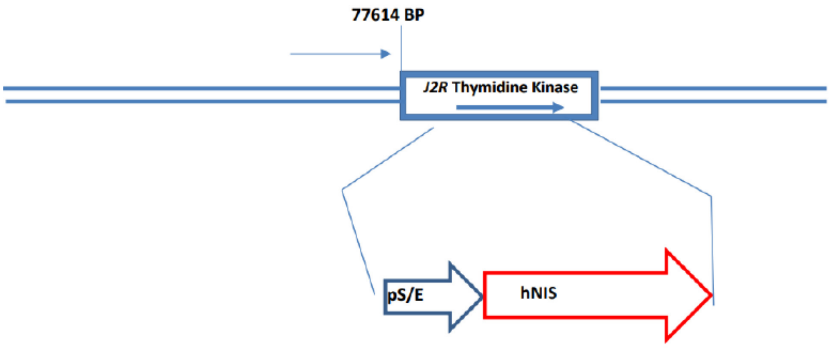

Figure 3. hNIS transgene into the J2R locus of CF33

4.1.1 *The genetic modifications*

The J2R gene and thymidine kinase protein

105. The J2R gene encodes for the viral thymidine kinase (TK) protein. TK is an enzyme found in most living cells and some viruses. It is responsible for recycling and regenerating thymidine for DNA synthesis. TK catalyses the transfer of the terminal phosphoryl moiety from adenosine triphosphate (ATP) to deoxythymidine (dT), yielding deoxythymidine monophosphate (dTMP). Following further phosphorylation by cellular enzymes, this pathway results in deoxythymidine triphosphate (dTTP), one of the four nucleotides that make up the DNA molecule (El Omari et al. 2006, Mortimer 2015).

106. TK is expressed in healthy cells that are preparing to divide, but is absent in resting cells (Hengstschlager et al., 1994). Its activity is also increased in the foetus and can be detected in foetal cells circulating in maternal blood (Hengstschlager and Bernaschek, 1997). TK levels are elevated in many types of cancer cells, including lung, colon, breast, and prostate (Bitter et al., 2020). *In vitro* studies suggest that TK activity is at least 5-fold higher in cells transformed with DNA tumour viruses than in healthy replicating cells (Hengstschlager et al., 1994).

107. In viruses, such as VACV, the role of TK is to generate sufficient dTTP for synthesis of viral DNA in host cells that are not actively dividing.

Effect of deleting the TK protein

108. The insertion of the hNIS transgene resulted in loss of the TK protein. Consequently, the GMO depends on dTTP produced by the host cells to replicate (Zeh and Bartlett, 2002). As mentioned in paragraph 106, this nucleotide is present in dividing cells and in higher levels in cancer cells. Accordingly, the GMO is expected to replicate preferentially in these cell types (Warner et al., 2019).

109. TK is not essential for VACV replication in cells cultured in the laboratory (Mackett et al., 1982). However, VACV lacking the TK protein showed reduced replication when injected in healthy mice (Lee et al., 1992). The absence of the TK protein did not affect the GMO replication in cells cultured in laboratory (Warner et al., 2019).

110. The disruption of TK has been employed in the design of other oncolytic viruses, for example, the GM VACV approved by the Regulator for a Phase 3 clinical trial (DIR-140) and the herpes simplex virus 1 (known as Talimogene laherparepvec), approved for commercial supply (DIR-132) and included on the Australian Register of Therapeutic Goods as a cancer therapy by the TGA.

The human sodium iodide symporter

111. Sodium Iodide Symporter (NIS) is a cell-surface protein responsible for iodide uptake in mammalian cells and normally expressed in thyroid cells. The human sodium iodide symporter is composed of 13 transmembrane helices and 643 amino acid residues. hNIS is responsible for the transport of iodide from the bloodstream into the thyroid gland and makes an essential contribution to thyroid hormone synthesis. Its expression level and localisation are regulated by intracellular levels of iodide and thyroid-stimulating hormone (Darrouzet et al., 2014). hNIS is also expressed in other cells/tissues, such as salivary gland ductal cells, gastric mucosa, mammary gland during lactation and ciliary body of the eye, choroid plexus (Dohan et al., 2003), and testicular cells (Russo et al., 2011). The functional role of the protein in these tissues remains unclear (Dohan et al., 2003). NIS homologous proteins are present in other animals, such as fish and birds (Concilio et al., 2020)

112. The ability of the thyroid gland to accumulate iodine via hNIS provided the basis for diagnostic imaging and treatment of thyroid cancer by using radioactive iodine (Radioiodine). Radioiodine scintigraphy combined with SPECT/CT (single-photon emission computed tomography/ computed tomography) has become a powerful diagnostic imaging tool for identification of regional and distant metastases in thyroid cancer (Avram, 2012). High doses of

radioiodine can destroy thyroid cells, including cancer cells, and has been used to treat some types of thyroid cancer with little effect on the rest of the body.

hNIS and autoimmune disease

113. In the late 1990s, it was hypothesised that anti-hNIS antibodies could induce autoimmune diseases of the thyroid, such as Hashimoto's thyroiditis and Graves' disease. Further investigation showed that anti-hNIS antibodies occur with low frequency among a large sample of patients with autoimmune thyroid diseases (Seissler et al., 2000). Additionally, sera collected from such patients did not interfere with the activity of hNIS in cells cultured in the laboratory (Tonacchera et al., 2001). Therefore, hNIS does not appear to be a relevant antigen in autoimmune thyroid diseases.

hNIS and gene therapy

114. In 1997, Shimura and colleagues generated cancer cells expressing the transgene NIS. When injected into rats, these cells induced tumours that, upon treatment, accumulated radioiodine and could be imaged by autoradiography (Shimura et al., 1997). Since then, NIS has been extensively studied for imaging and/or therapeutic purposes. NIS-expressing GMOs investigated in preclinical studies include: a replication-defective adenovirus (Spitzweg et al., 2000); an oncolytic herpes simplex virus type 1 (Li et al., 2010), VACV and others (Ravera et al., 2017).

115. MV-NIS has been evaluated in clinical trials for treatment of patients with relapsing drug-refractory myeloma and multiple glucose-avid plasmacytomas (Russell et al., 2014) and ovarian cancer (Galanis et al., 2015). In the latter, the expression of NIS was detected in 3 out of 15 patients treated. Symptoms associated with the treatment included fever, fatigue, and neutropenia mostly likely associated with the measles virus. Currently, 6 clinical trials in which MV-NIS is being administered to treat different types of cancer are listed on the website of the US National Institutes of Health (www.clinicaltrial.gov, accessed on 18 May 20 22).

Effect of inserting the hNIS transgene into the GMO

116. The insertion of the hNIS gene into the GMO leads to hNIS expression on the surface of cells infected with the GMO. This facilitates non-invasive imaging of tumours using PET/ CT via the accumulation of radioiodine within tumours. The expression of hNIS by the GMO does not appear to cause cytotoxicity, as it showed similar oncolytic activity in human cancer cells when compared to CF33 and a GM CF33 strain expressing green-fluorescent protein (CF33-GFP) (Warner et al., 2019)

117. hNIS is a human gene naturally expressed in a range of cells (see paragraph 111). It has been investigated in a variety of pre-clinical and clinical trials studies and its expression has not been associate with autoimmune disease or cytotoxic effects (Portulano et al., 2014; Russell et al., 2014; Galanis et al., 2015).

4.2 Stability of the GMO during *in vitro* passage

118. CF33-hNIS will be manufactured according to Good Manufacturing Practice (GMP) as a biological medicinal product for investigational use in humans. To determine the genetic stability of CF33-hNIS, the GMO was serially propagated for up to 5 passages (~96 hours each) in cultured A549 cells. Genome sequencing of each passage showed 2 to 4 point mutations raised per passage in the inverted terminal repeats (ITR), an untranslated region at both ends of the GMO genome. Due to the repetitive nature of these regions, it is unclear whether these variants are due to errors in the DNA replication or an artifact of the sequencing alignment. No mutations were observed in the protein-coding regions among all the 5 passages. This suggests that the GMO is stable and has low-level capacity for random mutation during viral replication.

4.3 Biodistribution and shedding of the GMO

119. Biodistribution and shedding of the GMO have been evaluated in immunocompetent mice with xenografts tumours. The GMO treatment was administered as single or multiple doses, alone or in

combination with anti-PD1 (immune checkpoint inhibitor). Biological samples that were positive for the presence of the GMO genome in a polymerase chain reaction (PCR) test were then evaluated for the presence of viable GMO particles. These studies are briefly described below.

- **Single dose of the GMO via intratumoural injection:** GMO genome was found in 11/20 tumour samples at day 8, and 11/19 tumour samples at day 19. Additionally, GMO genome was detected in 2 ovary and 1 bladder sample at day 8. GM infectious viral particles were isolated from 12/17 tumour samples at day 8 and 5/5 tumour samples at 19 and 1/2 ovary samples at day 8.
- **6 doses of the GMO (every 2 days) via intratumoural injection:** GMO genome was found in 18/20 tumour samples at day 12 (24 h after the last treatment), and 13/19 tumour samples at day 19. Additionally, GMO genome was detected in 1/20 spleen samples. GM infectious viral particles were isolated from 17/19 tumour samples at day 12, and 10/18 tumour samples at day 19 and 1 spleen sample at day 12.
- **Single dose of the GMO administered via intravenous infusion:** GMO genome was found in 15/20 injection site (tail) samples at day 8, and 7/19 injection site samples at day 19. Besides injection site, the GMO genome was detected in 1/20 ovary, 1/20 lung, 1/20 bladder and 1 tumour samples at day 8. GM infectious viral particles were isolated from 14/15 injection site samples at day 8 and 7/7 injection site samples at day 19.
- **6 doses of the GMO (every 2 days) via intravenous infusion:** GMO genome was found in 20/20 injection site samples at day 12, and 18/20 injection site samples at day 19. Besides injection site, the GMO genome was detected in 1/20 ovary, 1/20 lung, 1/20 bladder and 3/20 spleen samples at day 12. GM infectious viral particles were isolated from 15/20 injection site samples at day 12, and 6/18 injection site samples at day 19.

120. All the other tested samples, including muscle, brain, intestine, heart, kidney, liver, testes, saliva, urine, and whole blood were negative for the presence of the GMO. No skin lesions or pustule formation were observed in the treated mice.

121. Overall, the biodistribution studies showed minimal or no GMO detection except at the site of injection (i.t. or i.v.) and was more frequent in mice receiving 6 doses of the GMO, suggesting that the dissemination of the GMO would be limited to direct contact with the injection site even after multiple doses of treatment. However, it should be noted that the biodistribution data provided by the applicant is limited as the samples were not taken until day 8 for single dose and day 12 for repeat dose experiments and there is no data available regarding the GMO shedding during the first few days post-administration. Additionally, there is uncertainty as to whether the findings from these studies performed in mouse models would be transferrable when the GMO is administered in humans, as the GMO has been shown to replicate poorly in healthy mice tissue compare to human tissue.

122. In clinical trials, the administration of GM oncolytic VACVs via i.t. injection or i.v. infusion led to detection of GM VACV DNA in blood 15 minutes post-administration. Viraemia was shown to be dose-dependent and decreased over time (Heo et al., 2013; Zeh et al., 2015). Viral genome was detected in blood of 3/15 patients at day 5 post-treatment via i.t. injection (Zeh et al., 2015), and in 3/29 patients at days 15-36 post-treatment via i.v. infusion (Heo et al., 2013). Similar viraemia levels were observed for 1, 2 or 3 doses of the treatment (Heo et al., 2013). No GM VACV DNA or infectious particles were detected in urine or saliva at any time point (Zeh et al., 2015).

4.4 Transmissibility of the GMO

123. The GMO is expected to infect the same range of hosts and cells within a host, as the unmodified VACV. Based on VACV tropism, infected cells would likely include epithelial cells and antigen-presenting cells (see section 3.4). However, as discussed in section 4.1.1, GMO replication

relies on dTTP molecules produced by the infected host cell. Therefore, GMO replication is expected to occur preferentially in cancer cells and, to a lesser extent, in other infected dividing cells.

124. There is limited data describing the transmissibility of the GMO to other animals or humans. Immunocompetent mice with established tumours were treated with the GMO (i.t. or i.v.) and mixed with untreated mice on the following day. Results suggested that the GMO was not transmitted from GM-infected mice to untreated mice, when housed together for up to 3 weeks. A similar result was shown in mice treated with CF33-hNIS-antiPDL1 (Zhang et al., 2021), a similar GMO based on the same parent organism. However, these studies did not provide data regarding eventual GMO transmission by contact with the injection site in the first 24 h post-treatment.

4.4.1 *Stability in the environment and decontamination*

125. The stability of CF33-hNIS in the environment (surfaces, water types and sediments) has not been tested. However, as mentioned in Section 3.7, VACV can persist for long periods in the environment. Therefore, it is expected that the survival of the GMO in the environment would be similar.

126. Methods of decontamination effective against VACV are expected to be equally effective against the GMO (see Section 3.7).

4.4.2 *Pre-clinical studies using CF33-hNIS*

127. The oncolytic effect of CF33-hNIS, and other GMOs derived from the same parent organism CF33, have been evaluated in cancer cells cultured in laboratory and mouse models of cancer, including colon cancer, lung, TNBC, ovarian cancer and pancreatic cancer.

128. CF33-hNIS was able to replicate in and destroy human cancer cells cultured in laboratory in a dose-dependent manner (Warner et al., 2019). In addition to the cell lysis induced by replication, it has been suggested that the GMO kills cancer cells via a mechanism called *Immunogenic cell death* (ICD), a type of cell death that induces an immune response and can aid tumour clearance (Warner et al., 2019).

129. In a xenograft model of colorectal cancer in immunodeficient nude mice, the treatment with CF33-hNIS via i.t. injection allowed tumour imaging via I-124 PET/CT and promoted tumour regression in some of the treated mice. Additionally, the combination of CF33-hNIS treatment with I-131 radiotherapy was shown to have a synergistic oncolytic activity against the induced tumours (Warner et al., 2019).

4.4.3 *Clinical trials using CF33-hNIS*

130. The proposed study is the first clinical trial to be conducted with CF33-hNIS in humans and has been approved in the USA (NCT05346484). The clinical trial sponsor announced that a trial participant in the USA received the first dose of the GMO on the 17 May 2022 (Imugene, 2022). A Phase I study to evaluate a similar GMO, CF33-hNIS-antiPDL1, has been also approved in the USA (NCT05081492) and is currently recruiting patients.

131. Other GM strains of VACV designed to selectively target cancer cells have been evaluated in Phase I and Phase II clinical trials and were generally safe and well-tolerate when administered via i.t. injection or i.v. infusion. Viral genome was found in blood 15 min post-administration (i.t. or i.v.) and was shown to be dose-dependent and decreased over time. The most common treatment-related adverse events included nausea, chills, fever, fatigue, pain, myalgia, anaemia, and headache (Hwang et al., 2011; Downs-Canner et al., 2016). Several clinical trials with GM VACV are currently on-ongoing (www.clinicaltrials.gov accessed on 02 June 2022)

4.5 Relevant information relating to Pembrolizumab

132. The applicant proposed to administer the GMO alone or in combination with a cancer immunotherapy known as Pembrolizumab. Pembrolizumab targets and blocks a protein called PD-1

on the surface of certain immune cells called T cells. Blocking PD-1 triggers the T cells to find and kill cancer cells. Of relevance to this DIR application are Pembrolizumab side-effects that can occur in more than 10% of treated participants, which include nausea and vomiting, diarrhoea, and skin changes (dryness, itching and rashes similar to acne, severe reactions can lead to skin blistering) (UK, 2022). These reactions could increase the potential of GMO shedding.

Section 5 The receiving environment

133. The receiving environment forms part of the context for assessing risks associated with dealings with GM vaccine (OGTR, 2013). It informs the consideration of potential exposure pathways, including the likelihood of the GMO spreading or persisting outside the site of release.

5.1 Clinical trial sites

134. The intended primary receiving environment would be solid tumours within the clinical trial participants. As stated in Chapter 1, Section 2.1 , each patient would receive multiples doses of the GMO via i.t. injection or i.v. infusion over the period of 24 months.

135. The secondary receiving environment would be the clinical trial site and hospital where the GMO would be prepared, administered and the waste disposed of. These exact sites are yet to be identified. All clinical sites involved in the study would be equipped to handle infectious agents and procedures would be conducted in accordance with institutional policies based on standard precautions for handling potentially infectious substances and in accordance with the *Australian Guidelines for the Prevention and Control of Infection in Healthcare* (National Health and Medical Research Council, 2019).

136. The principal route by which the GMO could enter the wider environment is via shedding from the inoculated trial participants once they leave the hospital and return home.

5.2 Relevant environmental factors

137. 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, and the presence of competent vector species.

138. The parental organism CF33 has never been used in clinical trials and it is not expected to be found in the environment. However, VACV was used worldwide as a vaccine to protect against smallpox infection and many individuals aged over 40 years old are expected to have been vaccinated, either in Australia or overseas if they have emigrated. People vaccinated many years ago may be less susceptible to VACV infection, or infection may be asymptomatic or produce less severe symptoms (Cohen, 2001). VACV vaccination is used only for occasional vaccination of laboratory personnel who are required to work with replication competent poxviruses.

139. Immunocompromised individuals and those with specific conditions associated with the adverse reactions listed in Section 3.8.2, are more are susceptible to VACV infection and could come into contact with trial participants during a potential shedding period. Those are more likely to include individuals living in the same household, partners and carers.

140. 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. patient's homes). Such animals are most likely to include domestic pets and, potentially, livestock. Additional natural hosts for VACV were discussed in section 3.5.1.

5.2.1 ***Related viral species in the receiving environment***

141. The presence of related viral species may offer an opportunity for introduced genetic material to transfer from the GMOs to other organisms in the receiving environment. As poxviruses 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.

142. *Molluscum contagiosum virus* (MCV) is a relatively common poxvirus adapted specifically to humans and present in Australia. It is classified as a member of the family *Poxviridae* but has no close relatives and is the only member of the Molluscipoxvirus genus. Molluscum contagiosum infections are more common during childhood and typically resolve without complication. However, it is more severe and persistent in immunosuppressed patients, particularly in those with HIV/AIDS (Healthdirect Australia, 2022).

143. Poxviruses are known to infect many native Australian animals, including mammals, birds and reptiles. Aside from an outbreak in common ringtail possums attributed to an OPXV, many of the poxviruses infecting mammals have not been characterised (Wildlife Health Australia, 2019b). In birds, Avian pox is caused by 13 viruses of the genus Avipoxvirus (Wildlife Health Australia, 2012). Pox disease caused by *Crocodilepox virus* (genus Crocodylidpoxvirus) has been reported in crocodile farms and may be associated with stressors such as relocation, handling and inappropriate water temperature (Wildlife Health Australia, 2019a). In addition, a recent study has identified a novel poxvirus in green sea turtles (Sarker et al., 2021).

144. *Myxoma virus* (family *Poxviridae*, genus *Leporipoxvirus)* was introduced in Australia in 1950 to reduce pest rabbit numbers (Kerr et al., 2013). It specifically infects rabbits and hares (Wang et al., 2004), causing lethal disease in some species.

145. As of 8 June 2022, Australia has registered 5 cases of monkeypox virus disease, all identified in travellers returning from United Kingdom and Europe. It is a rare zoonotic disease and can spread when a person comes into contact with the virus from an infected animal, or contaminated material. Transmission from human-to-human can occur through contact with respiratory secretions, skin lesions of an infected person or recently contaminated objects (Australian Government Department of Health, 2022). Disease related symptoms include fever, headache, muscle aches, swollen lymph nodes and pustules formation, and lasts for 2-4 weeks. Severe cases can occur among children and immunocompromised individuals (CDC, 2022a). As described in paragraph 78, WHO reported a multi-country monkeypox outbreak, with confirmed cases of monkeypox reported by 28 countries where monkeypox virus is not endemic (WHO, 2022a).

Section 6 Previous authorisations

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

147. However, the Regulator has issued limited and controlled DIR licences (DIR-116, DIR-140 and DIR-179) utilising VACV for clinical trials in humans. The clinical trial for DIR-116, no longer ongoing, involved dealings with a GM VACV and GM Fowlpox virus. The purpose of the clinical trial was to evaluate the efficacy of these GMOs in treating prostate cancer. The purpose of DIR-140 and DIR-179 is to evaluate the efficacy of GM VACVs for treatment of different types of solid cancers.

148. The Regulator has also issued a limited and controlled DIR licence (DIR-170) utilising GM VACV as a vaccine to protect horses against *Ross River virus* infection. This licence has been surrendered upon the applicant's request.

Chapter 2 Risk assessment

Section 1 Introduction

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

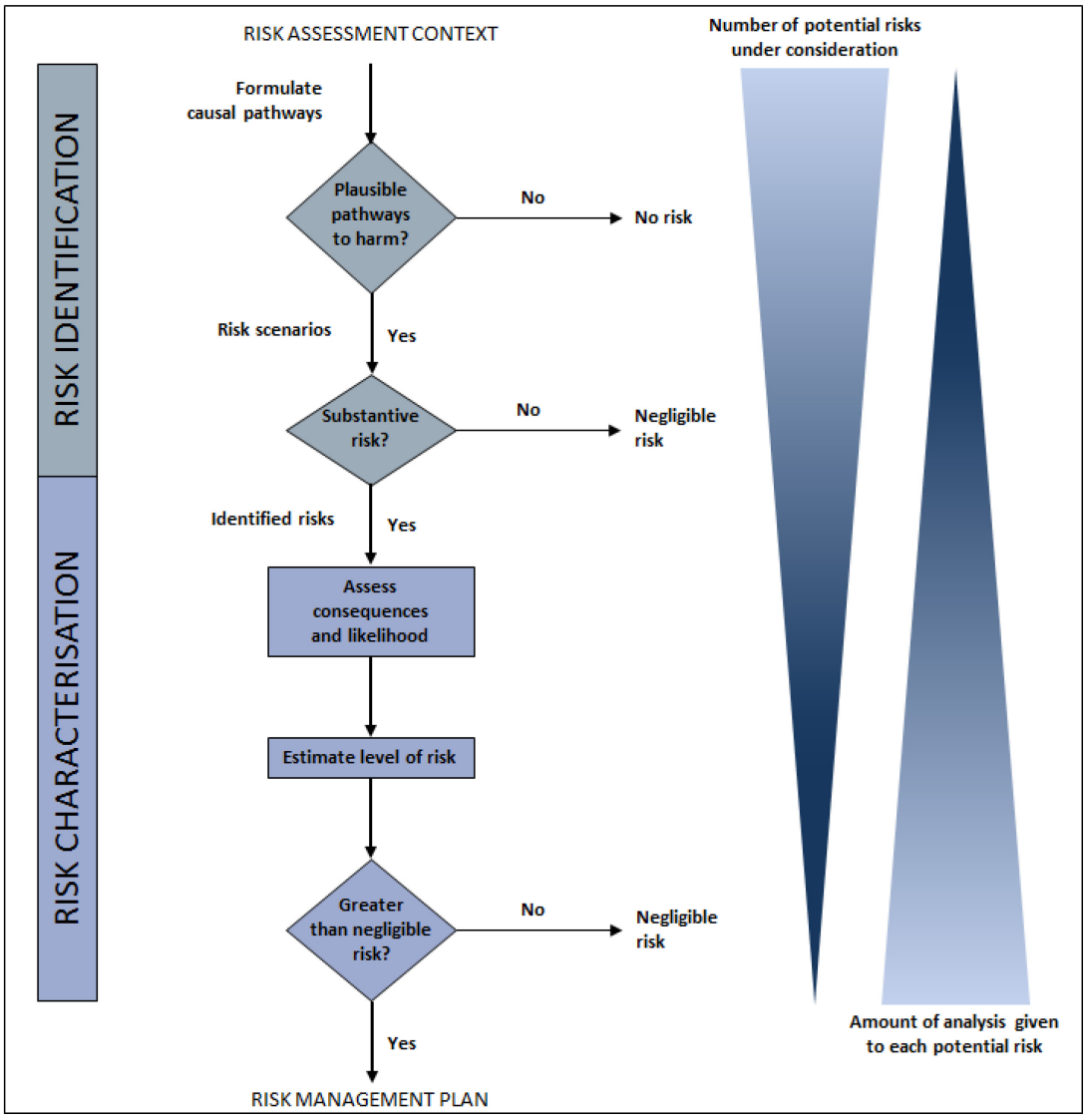

Figure 4: The risk assessment process

150. The Regulator uses a number of techniques to identify risks, including checklists, brainstorming, previous agency experience, reported international experience and consultation (OGTR, 2013).

151. Risk identification first considers a wide range of circumstances in which the GMO, or the introduced genetic material, could come into contact with people or the environment. This leads to postulating causal pathways that may give rise to harm for people or the environment from dealings with a GMO. These are called risk scenarios.

152. Risk scenarios are screened to identify substantive risks, which are risk scenarios that are considered to have some reasonable chance of causing harm. Risk scenarios that could not plausibly occur, or do not lead to harm in the short and long term, do not advance in the risk assessment process (Figure 4), i.e. the risk is considered no greater than negligible.

153. Risk scenarios identified as substantive risks are further characterised in terms of the potential seriousness of harm (Consequence assessment) and the likelihood of harm (Likelihood assessment). The consequence and likelihood assessments are combined to estimate the level of risk and determine whether risk treatment measures are required. The potential for interactions between risks is also considered.

Section 2 Risk identification

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

i. The source of potential harm (risk source)

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

iii. Potential harm to people or the environment.

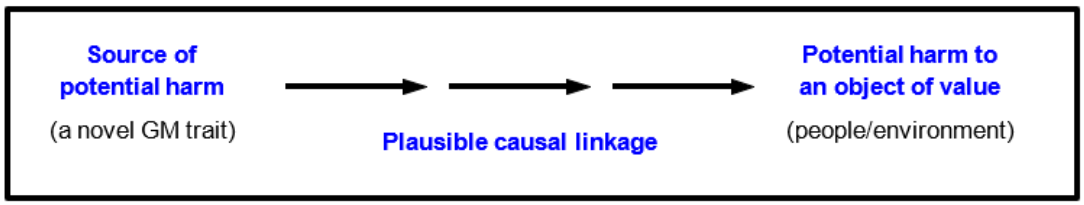

Figure 5: Components of a risk scenario

155. When postulating relevant risk scenarios, the risk context is taken into account, including the following factors detailed in Chapter 1:

- the proposed dealings
- the proposed limits including the extent and scale of the proposed dealings
- the proposed controls to limit the spread and persistence of the GMO and
- the characteristics of the parent organism(s).

2.1 Risk source

156. The parent organism of the GMO is CF33, a chimeric Orthopoxvirus closely related to VACV. Details on the pathogenicity and transmissibility of VACV is discussed in Chapter 1, Section 3. Vaccination with VACV tends to produce a pustule at the inoculation site. Transmission of VACV from patients receiving the GMO to other people and susceptible hosts, such as domestic pets, could occur from this site through direct contact or through other vectors such as clothing, bedding, or dressing contaminated by vaccinia virus.

157. The sources of potential harm can be the intended novel GM traits associated with one or more introduced genetic elements, with deletion of genetic elements from the GMO, or unintended effects arising from the use of gene technology.

158. As discussed in Chapter 1, Section 4.1, the GMO has been modified by deleting the *J2R* gene and inserting the hNIS gene to increase virus replication in cancer cells and facilitate non-invasive imaging. These modified genes are considered further as a potential source of risk.

159. The expression of the introduced gene is controlled by poxviral regulatory sequences. Regulatory sequences are naturally present in all organisms and the introduced/endogenous sequences are expected to operate in similar ways to endogenous sequences. The regulatory

sequences are DNA that is not expressed as a protein; they are poxvirus specific and do not present a risk in the absence of poxvirus cellular machinery. Hence, potential harms from the regulatory sequences will not be further assessed for this application.

160. Infection with VACV does not result in latent infection or integration into the host genome, and this will not be considered further.

161. The current assessment focusses on risks posed to people or the environment, including long term persistence of the GMOs, which might arise from the import, transport, storage or disposal of CF33-hNIS.

2.2 Causal pathway

162. The following factors are taken into account when postulating plausible causal pathways to potential harm:

- the proposed dealings;
- proposed limits, including the extent and scale of the proposed dealings;
- characteristics of the parent organism;
- routes of exposure to the GMOs, the introduced gene(s) and gene product(s);
- potential effects of the introduced gene(s) and gene product(s) on the properties of the organism;
- potential exposure of other organisms to the introduced gene(s) and gene product(s) from other sources in the environment;
- potential exposure of other organisms to the GMOs in the environment;
- the release environment;
- spread and persistence of the GMOs (e.g. dispersal pathways and establishment potential);
- environmental stability of the organism (tolerance to temperature, UV irradiation and humidity);
- unauthorised activities; and
- practices before and after administration of the GMO.

163. Although these factors are taken into account, many are not included in the risk scenarios below as they do not lead to a plausible pathway to harm.

164. As discussed in Chapter 1, Section 1.1, the TGA, the trial sponsor, the Investigators and HREC all have roles in ensuring the safety of trial participants under the *Therapeutic Goods Act 1989*, and human clinical trials must be conducted in accordance with the *National Statement on Ethical Conduct in Human Research* (National Health and Medical Research Council et al., 2018). Therefore, risk scenarios in the current assessment focus primarily on risks posed to people other than the intended treatment recipient, and to the environment.

165. *Vaccinia virus* is transmitted through direct contact. Aerosol transmission is not considered as a viable route of infection for the GMO (see Paragraph 81). Therefore, aerosol transmission will not be considered further.

166. Proposed transport, storage and disposal of the GMO are consistent with the Regulator's *Guidelines for the Transport, Storage and Disposal of GMOs*. These are standard protocols to minimise exposure to GMOs during these activities, so risks associated with such transport, storage, and disposal will not be further assessed.

167. The Act provides for substantial penalties for unauthorised dealings with GMOs or non-compliance with licence conditions, and also requires the Regulator to have regard to the suitability

of an applicant to hold a licence prior to the issuing of the licence. These legislative provisions are considered sufficient to minimise risks from unauthorised activities. Therefore, unauthorised activities will not be considered further.

2.3 Potential harms

168. The following factors are taken into account when postulating relevant risk scenarios for this licence application:

- harm to the health of people or desirable organisms, including disease in humans or animals or adverse immune response to the GMO
- the potential for establishment of a novel virus that could cause harm to people or the environment.

2.4 Postulated risk scenarios

169. Three risk scenarios were postulated and screened to identify substantive risk. These hypothetical scenarios are summarised in Table 1 and discussed in depth in Sections 2.4.1-2.4.2 (this chapter).

170. In the context of the activities proposed by the applicant and considering both the short and long term, none of the three risk scenarios gave rise to any substantive risks that could be greater than negligible.

Table 1 Summary of hypothetical risk scenarios from dealings with GM vaccine

Risk scenario	Risk source	Possible causal pathway	Potential harm	Substantive risk	Reason
1	GMO	Exposure of people undertaking dealings in clinical trial facilities to GMO via: (a) needle stick/ sharps injury/ eye splash during GMO preparation, administration, or sample collection and analysis (b) Contact with injection site and/or skin lesions (c) Contact with GMO contaminated material ⬇ Infection of host cells ⬇	Clinical symptoms ranging from mild to severe (e.g. flu-like illness, formation of lesions, severe adverse reactions)	No	• Only trained and experienced personnel would conduct dealings with the GMO. Staff preparing and administering the GMO would also be experienced in the use and disposal of sharps. • Staff handling the GMO would wear appropriate PPE (e.g. gown, gloves and eye protection), minimising the potential for exposure to staff handling the GMO. • High-risk personnel are excluded from handling the GMO. • Sample testing would be conducted by qualified personnel in pathology or other testing laboratories. • Accidental exposure of personnel to the GMO would be documented and the person would receive medical attention

Risk scenario	Risk source	Possible causal pathway	Potential harm	Substantive risk	Reason
		Replication of the GMO and expression of the hNIS transgene ⬇ Further transmission to people or animals			and would be monitored for symptoms. • Personnel would be instructed to cover pustules should they occur and avoid contact with high-risk groups and animals. • The GMO is designed to preferentially replicate in cancer cells. It is expected to be cleared by the immune response in healthy individuals and animals. The introduced gene has not been associated with toxicity in pre-clinical and clinical trials studies.
2	GMO	Treatment of trial participant with the GMO ⬇ GMO is shed at the injection sites or in body fluids (such as blood, urine, semen and vaginal secretions) ⬇ Exposure of people (e.g. carers or household contacts), or animals (e.g. domestic pets or wildlife) through contact with trial participant or contaminated items (e.g. contaminated dressings) outside the clinical/hospital setting ⬇ Infection of host cells ⬇ Replication of the GMO and expression of the hNIS transgene	Clinical symptoms ranging from mild to severe (e.g. flu-like illness, formation of lesions, severe adverse reactions) Establishment of VACV infection in animals	No	In addition to the reasons described in scenario 1: • High-risk trial participants, including immunocompromised persons and those who have a history of significant skin disease who may develop more pustules, would be excluded from the trial. • Residual inoculum GMO is unlikely to be present at the site of administration as injection/infusion sites would be cleaned with alcohol and covered with a dressing. • Trial participants would be instructed to wear gloves when removing/changing dressings. Contaminated dressings would be disposed of in a primary container (e.g. plastic bag) and stored in a biohazard container provided by the trial site. The biohazard bin would be returned to the clinical trial site for disposal. • In the event a pustule forms, trial participants

Risk scenario	Risk source	Possible causal pathway	Potential harm	Substantive risk	Reason
		⬇ Further transmission to people or animals			would be instructed to cover pustules, appropriately dispose any bandages, and avoid contact with animals and immunocompromised individuals. • Trial participants would be instructed to use barrier contraception to prevent pregnancy and transmission during the treatment and at least 60 days after the last GMO treatment. • If exposure occurred, it is likely to be at a low dose. Additionally, there was no report of transmission from people who have received a VACV vaccine to other people in more recent vaccination programmes.
3	GMO	Treatment of trial participant with the GMO ⬇ Trial participant becomes or is already infected with another compatible virus ⬇ The GMO recombines with another virus in the host ⬇ Produces a replication competent recombinant virus ⬇ Recombinant virus is shed ⬇	Establishment of novel virus with unknown pathogenicity in the environment	No	• VAVC is not present in the Australian environment. Additionally, there is limited opportunity for the GMO to come into contact with other related poxviruses. • For recombination to occur, the GMO and other poxviruses need to be present in the same cell at the same time. Poxviral recombination does not occur frequently in nature. • Viral factory compartmentalisation adds a further barrier for recombination. • As noted in Scenario 1, the GM virus is expected to preferentially replicate in cancer cells, further limiting the likelihood of encountering another poxvirus within the host cell.

Risk scenario	Risk source	Possible causal pathway	Potential harm	Substantive risk	Reason
		Recombinant virus infects other hosts			

2.4.1 ***Risk scenario 1***

Risk source	GMO
Causal pathway	Exposure of people undertaking dealings in clinical trial facilities to GMO via: (a) needle stick/ sharps injury and/or eye splash during GMO preparation, administration, or sample collection and analysis (b) Contact with injection site and/or skin lesions (c) Contact with GMO contaminated material ⬇ Infection of host cells ⬇ Replication of the GMO and expression of the hNIS transgene ⬇ Further transmission to people or animals
Potential harm	Clinical symptoms ranging from mild to severe (e.g. flu-like illness, formation of lesions, severe adverse reactions)

Risk source

171. The source of potential harm for this postulated risk scenario is the GMO.

Causal Pathway

172. There are a number of ways that people may be exposed to the GMOs while undertaking the dealings as part of this trial.

Exposure via needle stick, sharps injury, and/or eye splash

173. There is potential for exposure of people other than the trial participant to the GMO during the preparation and administration of the GMO and collection of biological samples via needle stick or sharps injury and eye splash.

174. As discussed in Chapter 1, Section 2.1, the preparation and administration of the GMO and sample collection would be carried out in clinical trial sites by authorised, experienced, and trained health professionals. All personnel working in settings where healthcare is provided are required to comply with the standard precautions for working with potentially infectious material, as described in the *Australian Guidelines for the Prevention and Control of Infection in Healthcare* (2019).

175. Controls proposed by the applicant, including appropriate training and use of PPE (e.g. gown, gloves and eye protection) by clinical trial staff would minimise the potential exposure of people to the GMOs via needle stick, sharps injury and/or eye splash.

Exposure via contact with injection site and/or skin lesions

176. As mentioned in Chapter 1, Section 3.5.2, VACV is transmitted through direct contact between infected and non-infected people or animals. If people in clinical trial facilities come in contact with the administration site after patient treatment, skin lesions or directly with the GMO, they could be exposed to the GMOs.

177. The injection/infusion site would be cleaned with alcohol and covered by an occlusive dressing. This would limit the spread of the GMO from the injection/infusion site.

178. The trial participant would be instructed to cover any skin lesions should they occur. Collection of biological samples would be performed by trained personal, wearing appropriate PPE.

Exposure by contact with contaminated materials

179. As discussed in Chapter 1, Section 3.7, VACV can remain viable for extended periods under certain circumstances. The applicant has stated that GMO waste and materials contaminated with the GMO would be disposed according to infectious medical waste management procedures (Chapter 1, Section 2.3.8).

180. The applicant stated that staff exposed to the GMO would receive medical attention and would be monitored for symptoms. The staff would be instructed to cover pustules should they occur and to avoid contact with high-risk groups and animals. This measure would minimise the potential transmission of the GMO to other people and animals.

Potential harm

181. If people are exposed to the GMOs, they could develop symptoms of VACV infection, such as fever, fatigue and skin lesion formation. On rare occasions, they could develop severe adverse reactions (see section Chapter 1, Section 3.8.1). However, exposure is unlikely to cause harm because:

- the dose received in case of accidental exposure to the GMO is likely to be far lower than the GMO dose intentionally administered to trial participants ($8.6 \times 10^5 – 1.1. \times 10^8$);
- the GMO has been modified to preferentially replicate in cancer cells and it is expected to be cleared by the immune system if it infects healthy cells;
- pre-clinical studies with the GMO in immunocompromised mice did not cause severe disease;
- staff in high risk groups (immunocompromised and pregnant individuals) would be excluded from handling the GMO;
- the expression of the transgene hNIS has not been associated with toxicity or allergy in people or animals.

Conclusion

182. The potential for an unintentional exposure of people undertaking dealings in clinical trial sites to the GMO resulting in ill health in humans and animals is not identified as a risk that could be greater than negligible. Therefore, it does not warrant further detailed assessment.

2.4.2 *Risk Scenario 2*

Risk source	GMO
Causal pathway	Treatment of trial participant with the GMO ⬇ GMO is shed at the injection sites or in body fluids (such as blood, urine, semen and vaginal secretions) ⬇ Exposure of people (e.g. carers or household contacts), or animals (e.g. domestic pets) through contact with trial participant, body fluids or contaminated items (e.g. contaminated dressings) outside the clinical/hospital setting ⬇ Infection of host cells ⬇ Replication of the GMO and expression of the hNIS transgene ⬇ Further transmission to people or animals
Potential harm	Clinical symptoms ranging from mild to severe (e.g. flu-like illness, formation of lesions, severe adverse reactions)

Risk source

183. The source of potential harm for this postulated risk scenario is the GMO as a treatment.

Causal Pathway

184. Following GMO administration, the GMO could be dispersed by direct contact with skin lesions, contact with contaminated material and surfaces and/or shed from the trial participant.

Exposure via contact with blood or body fluids

185. As described in Paragraph 80, VACV can shed in milk and faeces of experimentally infected cattle and in the faeces and urine of experimentally infected mice. In humans, the development of viremia (viral presence in the blood) and viruria (viral presence in urine) is uncommon and usually associated with other medical conditions (Lane and Fulginiti, 2003; Cummings et al., 2004). As mentioned in Chapter 1, Section 3.8.1, immunocompromised individuals and those who have a history of significant skin disease are at higher risk of developing adverse reactions that could lead to increase shedding of the GMO such as generalised and/or progressive vaccinia and eczema vaccinatum. These individuals are excluded from the clinical trial.

186. There is limited data available regarding shedding of the GMO in body fluids. In pre-clinical studies conducted in mice, the GMO was found mainly in the injection sites (i.t. or i.v.). The presence of the GMO was more frequent in mice receiving multiple doses of treatment and was shown to be dose dependent (see section Chapter 1, Section 4.3). The GMO was also found in ovary, spleen, lung and bladder of treated mice. In clinical trials, the administration of GM oncolytic VACVs via i.t. injection or i.v. infusion led to detection of viral DNA in blood 15 minutes post-administration. Viraemia was shown to be dose-dependent, decreased over time and was detected in blood of a few participants at day 5-36 post-administration. No viral DNA or infectious particles were detected in urine or saliva at any time point (Zeh et al., 2015). Similar results would be expected following administration of the GMO. As stated in Paragraph 181, the GMO is expected to be cleared by the immune system if it infects healthy cells. Furthermore, as per VACV vaccination, the administration

of the GMO is expected to induce immunological responses capable of protecting against future infections. This acquired immune response would limit the presence of the GMO in the bloodstream following multiple doses of treatment. Additionally, trial participants would be instructed to use barrier contraception should they be sexually active during the treatment and at least 60 days after the last GMO treatment. This measure would minimise the potential of transmission in the event of GMO shedding in body fluids such as semen and vaginal secretion.

187. The risk of exposure of healthcare staff during collection or analysis of biological samples was addressed in risk scenario 1.

Exposure via direct or indirect contact should trial participants develop pustules

188. As described in Chapter 1, Section 3.5.2. VACV can be transmitted by contact with skin lesions and/or contaminated material. The GMO has been modified to preferentially replicate in cancer cells and no skin lesions were observed in pre-clinical studies conducted in mice. However, pustule formation could still occur. Trial participants would be instructed to follow the pustule management plan as described in paragraph 25. The pustule management plan would be part of the trial participants guidance provided to them during the initial screening as part of the informed consent form. Trial participants would also be expected to seal contaminated disposable items in a provided primary container (e.g. press-sealed bag) and then place this into a provided secondary container (biohazard bin). At each visit, trial participants would return the used biohazard bin to the clinical trial site for disposal as clinical waste. Participants would also be instructed to launder contaminated fabrics with disinfectants, wear gloves when changing/replacing dressings, and limit contact with any pets, other animals, or higher-risk individuals (see paragraph 25). If an animal or other person develops a suspicious rash, the trial participant would be instructed to report to the clinical trial investigator. Together, these measures would minimise potential transmission of shed GMO and GMO products to other people and animals.

189. There was no report of VACV transmission from people who have received a VACV vaccine to other people in more recent vaccination programmes, as described in paragraph 82. The limited number of clinical trial participants combined with the guidelines on pustule management is likely to reduce potential transmission.

190. As described in paragraph 132, side effects of Pembrolizumab include skin reactions, which may increase the likelihood of pustule formation in trial participants. If so, this may increase the potential of shedding the GMO. As stated in Paragraph 188, a pustule management plan would be employed in this scenario.

191. As described in DIR-140, 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 (see Chapter 1, Section 3.5.1). Livestock such as cattle and horses are also known to be susceptible to VACV and if infected, there is potential for dissemination via contaminated faeces (see Chapter 1, Section 3.5.2). As described in paragraph 143, poxviruses infect many native Australian animals, including mammals, reptiles and birds. It can be hypothesised that these animals would also be susceptible to infection with the GMO. Given the urbanisation of Australia's population, and that the trial would be likely be conducted in major cities, participants are more likely to come into contact with domestic pets than with livestock or native animals, but the latter cannot be ruled out. As mentioned in paragraph 25 and 188, trial participants would be instructed to manage pustules and contaminated waste properly and avoid close contact with pets and other animals. These measures should minimise the likelihood of GMO transmission to animals.

Potential harm

192. If people or animals (pets, wildlife, livestock) are exposed to the GMO, a range of outcomes are possible. People exposed to the GMO could develop adverse immune responses and vaccinia-like diseases and reactions as described in Risk scenario 1. If an animal is exposed to the GMO, it could lead to infection, shedding of the GMO in the environment via faeces or urine (see paragraph 80) and exposure of other animals and people. However, exposure is unlikely to cause harm as it would involve a low number of viral particles, the GMO was designed to preferentially replicate in cancer cells and it is expected to be cleared by the immune system if it infects healthy cells. Additionally, studies conducted in cells cultured in laboratory and in mice suggest that the GMO replicate poorly in non-human cells (see paragraphs 71 and 121).

Conclusion

193. Risk scenario 2 is not identified as a substantive risk because potential exposure would be limited by the proposed limits and controls (including pustule management guidelines), and the GMO is designed to preferentially replicate in cancer cells. Therefore, this risk could not be greater than negligible and does not warrant further detailed assessment.

2.4.3 ***Risk Scenario 3***

Risk source	GMO
Causal pathway	Treatment of trial participant with the GMO ⬇ Trial participant becomes, or is already, infected with another compatible virus ⬇ The GMO recombines with another virus in the host ⬇ Produces a replication competent recombinant virus ⬇ Recombinant virus is shed ⬇ Recombinant virus infects other hosts
Potential harm	Establishment of novel virus with unknown pathogenicity in the environment

Risk source

194. The source of potential harm for this postulated risk scenario is the GMO as a treatment.

Causal Pathway

195. Recombination between two viruses may occur if they simultaneously infect the same cell. Recombination is more likely to occur between related viruses, for example the GMO is more likely to recombine with another poxvirus than with an unrelated virus.

Previous oncolytic virus treatment or vaccination

196. The applicant proposed to exclude individuals who have received previous oncolytic virus treatment or live vaccine within 4 weeks prior to the first dose of the GMO (see paragraph 39). Previous treatment with other oncolytic viruses or vaccination are unlikely to result in chronic

infection, viral particles are expected to be cleared by the immune system within days or weeks and are unlikely to allow co-infection with the GMO. If co-infection is established, it is highly unlikely that the two viruses would co-infect the same cell at the same time. As mentioned in paragraph 195, recombination is more likely to occur between closely related viruses. Therefore, it is highly unlikely that previous treatment with other oncolytic virus or administration of live vaccines would result in recombination between unrelated viral groups.

Recombination with VACV or other poxviruses

197. Recombination is more likely to occur between closely related viruses (see paragraph 195). As mentioned in Chapter 1, Section 5.2, VACV is not present in the Australian environment and VACV vaccination is not recommended on the National Immunisation Program. Therefore, it is highly unlikely that trial participants would come in contact with other VACV strains either in the environment or via vaccination. In the case of vaccination, VACV vaccine would be administered by the percutaneous route using a multiple puncture technique (skin scarification), while the GMO is proposed to be administered via i.t. injection or i.v. infusion. The spatial and temporal separation between administration would minimise the likelihood of both viruses co-infecting the same host cell.

198. As described in Chapter 1, Section 5.2.1, poxviruses such as *Molluscum contagiosum virus* (MCV), *Monkeypox virus* (MPXV), *Myxoma virus* and others, can be found in Australia. MCV is expected to be more prevalent in children than in adults. Therefore, an eventual co-infection would be more likely to occur in a secondary recipient exposed to the GMO than in an adult trial participant. Additionally, as described in the RARMP for DIR-140, there are no reports on the ability of MCV to recombine with other poxviruses, 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.

199. MPXV has been identified in travellers returning to Australia. As the virus spreads through close contact, the likelihood of spreading to the environment is very low. Additionally, VACV vaccination offers an 85% protection against monkeypox disease (WHO, 2022b). It is likely that a trial participant receiving single or multiple doses of the GMO would develop an immune response against the MPXV and in the event of exposure would clear the virus before an infection is established. Similarly, an individual infected with MPVX is likely to develop immunity against the GMO.

200. *Myxoma virus* is likely to be found in rabbits, as discussed in paragraph 60. However, genes related to increase virulence of Rabbitpox in rabbits are not represented in the GMO, and co-infection with the GMO and *Myxoma virus* is unlikely. Other uncharacterised poxviruses are found in Australian wildlife (see paragraph 143) but wildlife is unlikely to come into contact with trial participants.

201. As mentioned in paragraphs 87 and 194, for the recombination to occur both viruses must co-infect the same host cell at the same time. Trial participants would be instructed to avoid contact with high-risk individuals and animals. In addition, GMO contaminated material would be handled and disposed properly. These measures would reduce the likelihood of GMO dispersal in the environment and co-infection in people and animals. If a co-infection is established, the intracellular compartmentalisation during viral replication would prevent the recombination between viruses (see paragraph 88).

Potential harm

202. In the event of recombination between the GMO and another virus, it could restore the *J2R* locus and re-establish the GMO ability to replicate in healthy resting cells. This recombination is unlikely to generate a virus that is more pathogenic than the parent organism.

203. An eventual recombination between viruses could also lead to the introduction of the hNIS transgene into the genome of another poxvirus. This gene is derived from humans and has not been associated with harm to people or other organisms. It is not expected to provide any advantage to the receiving virus. This recombination would likely result in the disruption of the J2R locus in the receiving virus, resulting in preferential replication in cancer cells, and to a lesser extent, in replicating epithelial cells or antigen-presenting cells (see paragraph 123). This would minimise the spread of the virus in the environment. Therefore, the resulting virus is not expected to be more virulent than the unmodified poxvirus and would not pose additional harm.

Conclusion

204. The potential for an adverse outcome as a result of recombination between viruses is not identified as a substantive risk that could be greater than negligible. Therefore, it does not warrant further assessment.

Section 3 Uncertainty

205. Uncertainty is an intrinsic part of risk analysis[3]. There can be uncertainty in identifying the risk source, the causal linkage to harm, the type and degree of harm, the likelihood of harm or the level of risk. In relation to risk management, there can be uncertainty about the effectiveness, efficiency and practicality of controls.

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

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

207. Uncertainty is addressed by approaches such as balance of evidence, conservative assumptions, and applying risk management measures that reduce the potential for risk scenarios involving uncertainty to lead to harm. If there is residual uncertainty that is important to estimating the level of risk, the Regulator will take this uncertainty into account in making decisions.

208. As clinical trials are designed to gather data, there are generally data gaps when assessing the risks of a clinical trial application involving GMOs. However, proposed clinical trials are required to have limits and controls. Even if there is uncertainty about the characteristics of a GMO, limits and controls restrict exposure to the GMO and thus decrease the likelihood of harm.

209. For DIR-192, uncertainty is noted in relation to the preferential replication in cancer cells over healthy cells, and biodistribution and shedding of the GMO in humans. Pre-clinical data indicates that GMO replication is limited to injection sites (i.t. or i.v.), ovary, lung, spleen and bladder. There is no

[3] 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.

evidence of skin lesion formation or shedding of the GMO in mouse models. However, there is uncertainty as to whether the data gathered in mouse models would be transferrable to humans.

210. Although, the GMO is derived from a novel chimeric Orthopoxvirus closely related to VACV, there is limited data comparing the GMO virulence with other VACV strains. Again, studies conducted in mouse models suggest that the GMO is safe and well tolerated.

211. Overall, the level of uncertainty in this risk assessment is considered low and does not impact on the overall estimate of risk.

Section 4 Risk evaluation

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

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

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

214. Three risk scenarios were identified whereby the proposed dealings might give rise to harm to people or the environment. In the context of the limits and controls proposed by the applicant, and considering both the short and long term, none of these scenarios were identified as substantive risks. The principal reasons for this include:

- the GMO has been designed to preferentially replicate in cancer cells
- the transgene present in the GMO has not been associated with toxicity
- suitability of limits and controls proposed by the applicant.

215. Therefore, any risks to the health and safety of people, or the environment, from the proposed clinical trial using the GMO are considered to be negligible. The *Risk Analysis Framework* (OGTR 2013), which guides the risk assessment and risk management process, defines negligible risks as insubstantial with no present need to invoke actions for their mitigation. No controls are required to treat these negligible risks. Hence, the Regulator considers that the dealings involved in this proposed release do not pose a significant risk to either people or the environment[4]

[4] As none of the proposed dealings are considered to pose a significant risk to people or the environment, Section 52(2)(d)(ii) of the Act mandates a minimum period of 30 days for consultation on the RARMP.

Chapter 3 Risk management plan

Section 1 Background

216. 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 and considers limits and controls proposed by the applicant, as well as general risk management measures. The risk management plan informs the Regulator's decision-making process and is given effect through proposed licence conditions.

217. 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.

218. 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.

219. 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. In addition, the Regulator has extensive powers to monitor compliance with licence conditions under Section 152 of the Act.

Section 2 Risk treatment measures for substantive risks

220. The risk assessment of risk scenarios listed in Chapter 2 concluded that there are negligible risks to people and the environment from the proposed clinical trial with the GMO. These risk scenarios were considered in the context of the scale of the proposed clinical trial (Chapter 1, Section 2.3.3), the proposed controls (Chapter 1, Section 2.1), the proposed receiving environment (Chapter 1, Section 5), and considering both the short and long term effects of the GMO. Limits and controls proposed by the applicant and other general risk management measures are discussed below.

Section 3 General risk management

221. The limits and controls proposed in the application were important in establishing the context for the risk assessment and in reaching the conclusion that the risks posed to people and the environment are negligible. Therefore, to maintain the risk context, draft licence conditions have been proposed to limit the number of trial participants, location limited to hospitals and clinical trial sites, limits on the duration of the trial, as well as a range of controls to restrict the spread and persistence of the GMOs and their genetic material in the environment. The conditions are discussed and summarised in this Chapter and listed in detail in the draft licence.

3.1 Limits and controls on the clinical trial

222. Sections 2.1 and 2.3 in Chapter 1 list the limits and controls proposed by Medpace. Many of these are discussed in the risk scenarios considered in Chapter 2. The appropriateness of the limits and controls is considered further in the following sections.

3.1.1 *Consideration of limits and controls*

223. The proposed clinical trial would involve a maximum of 18 participants within Australia, and most dealings with the GMOs would take place in medical facilities such as clinical trial units and hospitals. Activities that would occur outside of medical facilities include transport, storage and disposal of the GMO. The applicant has proposed that the trial will be completed within 5 years of commencement. Conditions maintaining the risk context and proposed limits of the trial, such as the maximum number of trial participants and duration of the study, have been included in the draft licence.

224. The applicant proposed that import and transport of the GMO and waste containing the GMO would be in accordance with IATA and the Regulator's *Guidelines for the Transport, Storage and Disposal of GMOs* respectively. These are standard protocols for the handling and minimising exposure to the GMOs. Once at the clinical trial site, access to the GMO would be restricted to appropriately trained personnel. The proposed transport conditions are suitable for the GMO. Therefore, the draft licence details the minimum requirements for packaging and labelling the GMO and waste contaminated with the GMO for transport and storage within a clinical trial site, as well as transport of the samples that may contain the GMO for analysis. These measures would limit the exposure of people and the environment to the GMOs.

225. There are proposed inclusion and exclusion criteria for both trial participants (see paragraphs 38 and 39) and staff (paragraph 56). The inclusion and exclusion criteria for trial participants would be subject to approval by a HREC, who would consider the safety of the individuals involved in the trial. There is limited data regarding exposure of pregnant women, young children and immunocompromised individuals to VACV. While some studies suggest that VACV vaccination does not increased the overall risk of negative pregnancy outcomes (Badell et al., 2015), the CDC advises that VACV vaccines should not be administered to pregnant women in the absence of smallpox exposure. Additionally, as described in paragraph 68, severe adverse events are strain dependent and more common in immunocompromised individuals, children under 12 months of age and those with skin disease (see Chapter 1, Section 3.8.1); such groups are also excluded from VACV vaccination (Chapter 1, Section 3.8.2). The GMO is a novel chimeric Orthopoxvirus, highly homologous to VACV and designed to preferentially replicate in cancer cells. However, its effects in the risk groups described above are not known. Therefore, as a precaution, the licence requires that trial participants and staff who are immunocompromised, suffer from severe skin disease, and women who are pregnant or breastfeeding are excluded from participating in the trial. When VACV is used to vaccinate against smallpox, potential skin lesions/ pustule formation is likely to occur within seven days. Given this, licence conditions are proposed to exclude clinical trial staff for whom exclusions apply from engaging in the care of trial participants for at least seven days after each GMO administration or any time pustules are present.

226. The applicant proposed that trial participants should refrain from donating blood, organs, sperm and eggs during the clinical trial and for at least 60 days after the last treatment dose. There is limited data regarding persistence and shedding of the GMO after treatment. Therefore, a condition has been included in the draft licence to reflect this.

227. The applicant proposed that trial participants would be instructed to use contraceptives to avoid pregnancy during the clinical trial treatment and for at least 60 days after the last treatment dose. As mentioned in paragraph 226, there is limited data regarding the shedding of the GMO. Therefore, barrier contraceptive is recommended to avoid exposure to the GMO via shedding in body fluids such as sperm and vaginal secretion. A condition has been included in the draft licence to reflect this.

228. The applicant has proposed to exclude individuals who have received previous oncolytic virus treatment or live vaccines within 4 weeks prior to the first dose of the GMO. As discussed in Risk

Scenario 3, it is highly unlikely that previous treatment or vaccination would result in recombination. Therefore, this condition is not included in the draft licence.

229. The applicant advised that the GMO would be administered to trial participants via either i.t. injection or i.v. infusion by clinical staff at clinical trial sites. The applicant has also proposed that clinical staff would wear PPE including gown, gloves and eye protection. These practices would minimise exposure of people handling and administering the GMOs (Risk scenario 1) and have been proposed as licence conditions.

230. Conditions are included in the draft licence requiring the licence holder to ensure that all GMOs, including material or waste that has been in contact with the GMO, within the clinical trial site, are decontaminated by autoclaving, chemical treatment or by high-temperature incineration. Draft licence conditions require that the licence holder must ensure that the GMO, or material or waste that has been in contact with the GMO, that is to be destroyed by external service providers, is through a clinical waste stream. This is considered satisfactory, provided that the licence holder is only permitted to engage persons who can adhere to appropriate standards to conduct the dealings, as described in paragraph 231.

231. 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, 2010). The clinical waste stream typically involves destruction of infectious waste by incineration or autoclaving, which are considered appropriate for disposal of the GMO. Given that VACV can persist in the environment (Chapter 1, Section 3.7) and compatible hosts such as rodents, marsupials and others as listed in paragraph 76 would be present in the Australian environment, disposal measures such as burial or maceration would not ensure containment. Therefore, licence conditions are proposed, which requires waste disposal by external service providers to be by autoclaving or high-temperature incineration. These measures would limit the exposure of people animals or birds to the GMOs.

232. The applicant has proposed to provide patients with treatment instructions, including instructions should suspicious skin pustules develop, and provide instructions to patients of good hand hygiene. They would also provide trial participants a pustule kit, press sealed bags and a biohazard bin, as described in paragraph 25. Together, these instructions, pustule kit and biohazard bin would limit the exposure of people, other animals or birds to the GMOs should pustules develop. A condition has been included in the draft licence to reflect this.

233. Part of the pustule management plan is for the trial participants to avoid high-risk individuals (paragraph 25). As such, draft licence conditions include that trial participants would avoid direct physical contact with excluded persons and children under 12 months, for at least 7 days after each treatment or any time lesions are present.

234. A standard condition is included in the draft licence requiring the licence holder to ensure that dealings are conducted to ensure containment of the GMO, not compromise the health and safety of people and minimise unintentional exposure to the GMO. A note written under the condition explains that compliance may be achieved by only engaging persons who are required to adhere to appropriate standards to conduct the dealings.

235. Other conditions included in the draft licence are standard conditions that state that only people authorised by the licence holder are covered by the licence, and that the licence holder must inform all people dealing with the GMOs, other than external service providers, of applicable licence conditions.

236. Further conditions to be implemented in the draft licence are to ensure that a compliance management plan is in place for each clinical trial site before administration of the GMOs commences at that site. The compliance management plan must detail how the licence holder intends to comply with the licence conditions, including listing persons responsible for site

management, proposed reporting structures, staff training procedures and transport and disposal processes.

3.1.2 *Summary of licence conditions to be implemented to limit and control the clinical trial*

237. A number of licence conditions have been drafted to limit and control the proposed clinical trial, based on the above considerations. These include requirements to:

- limit the trial to 18 trial participants, which are to be conducted at clinical trial sites;
- restrict access to the GMO;
- ensure personnel involved in the trial are appropriately trained and follow appropriate behavioural requirements;
- ensure appropriate PPE is used;
- restrict personnel permitted to administer the GMO;
- requiring decontamination of the GMO and materials and equipment that have been in contact with the GMO at clinical trial sites using effective disinfectants or disposal using a certified waste contractor in accordance with standard clinical waste disposal practices, as required by the relevant Australian and state legislation;
- transport and storage of the GMO and samples from GMO-treated participants in accordance with the minimum requirements for packaging, and labelling as detailed in the draft licence and import in accordance with IATA shipping classification UN 3373;
- clinical waste stream to be used by external service providers to destroy untreated GMO and GMO-related waste.

3.2 Other risk management considerations

238. 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
- contingency plans
- identification of the persons or classes of persons covered by the licence
- reporting requirements
- access for the purpose of monitoring for compliance.

3.2.1 *Applicant suitability*

239. 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
- 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.

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

241. In addition, the applicant organisation must have access to an IBC and be an accredited organisation under the Act.

3.2.2 *Contingency plans*

242. Should a licence be issued, Medpace is required to submit a contingency plan to the Regulator before commencing dealings with the GMOs. This plan will detail measures to be undertaken in the event of:

- the unintended release of the GMOs, including spills
- exposure of, or transmission to persons other than trial participants
- a person exposed to the GMOs developing a serious adverse response.

3.2.3 *Identification of the persons or classes of persons covered by the licence*

243. If 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 dealings with the GMOs, Medpace is required to provide a list of people and organisations that are covered by the licence, or the function or position where names are not known at the time.

3.2.4 *Reporting requirements*

244. If issued, the licence would require the licence holder to immediately report any of the following to the Regulator:

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

245. A number of written notices are also required under the licence regarding dealings with the GMO, to assist the Regulator in designing and implementing a monitoring program for all licensed dealings. The notices include:

- identification of the clinical trial sites where administration of the GMO to trial participants would take place
- expected date of administration with the GMOs for each clinical trial site
- cease of administration with the GMOs for each clinical trial site.

3.2.5 *Monitoring for compliance*

246. 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.

247. 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.

248. 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 the health and safety of people or the environment could result.

Section 4 Issues to be addressed for future releases

249. Additional information has been identified that may be required to assess an application for a commercial release of the GMO, or to justify a reduction in limits and controls. This includes:

- data regarding the preferential replication in cancer cells, biodistribution and shedding of the GMOs in inoculated trial participants
- data regarding the virulence of the GMO compared to other VACV strains.

Section 5 Conclusions of the consultation RARMP

250. The risk assessment concludes that the proposed clinical trial of the GMOs poses negligible risks to the health and safety of people or the environment as a result of gene technology. These negligible risks do not require specific risk treatment measures.

251. If a licence is issued, conditions would be imposed to limit the trial to the proposed scale, location and duration, and to restrict the spread and persistence of the GMOs and its genetic material in the environment, as these were important considerations in establishing the context for assessing the risks.

Chapter 4 Draft licence conditions

Section 1 Interpretations and Definitions

1. In this licence:

   (a) unless defined otherwise, words and phrases used in this licence have the same meaning as they do in the Act and the Gene Technology Regulations 2001;

   (b) words importing a gender include every other gender;

   (c) words in the singular number include the plural and words in the plural number include the singular;

   (d) expressions used to denote persons generally (such as “person”, “party”, “someone”, “anyone”, “no-one”, “one”, “another” and “whoever”), include a body politic or corporate as well as an individual;

   (e) references to any statute or other legislation (whether primary or subordinate) are a reference to a statute or other legislation of the Commonwealth of Australia as amended or replaced from time to time and equivalent provisions, if any, in corresponding State law, unless the contrary intention appears;

   (f) where a word or phrase is given a particular meaning, other grammatical forms of that word or phrase have corresponding meanings;

   (g) specific conditions prevail over general conditions to the extent of any inconsistency.

2. In this licence:

   ‘Act’ means the *Gene Technology Act 2000* (Commonwealth) or the corresponding State law under which this licence is issued.

   ‘Analytical facility’ means a laboratory in Australia accredited to undertake testing of human diagnostic Samples, such as a medical testing laboratory accredited by the National Pathology Accreditation Advisory Council (NPAAC).

   ‘Clinical trial site’ means a medical facility in Australia such as a clinical trial facility and associated Pharmacy, which are notified in writing to the Regulator for the purposes of conducting this clinical trial.

   ‘Decontaminate’ (or ***‘Decontamination’***) means, as the case requires, kill the GMOs by one or more of the following methods:

   (a) chemical treatment;

   (b) autoclaving;

   (c) high-temperature incineration; or

   (d) a method approved in writing by the Regulator.

   Note: 'As the case requires' has the effect that, depending on the circumstances, one or more of these techniques may not be appropriate.

   ‘Excluded persons’ means:

   - persons who display any evidence of an active infection or any immunosuppressive disorder, including HIV infection;
   - women who are breastfeeding or who are pregnant; and
   - persons who have a history of significant skin disease, such as atopic dermatitis.

'External service provider' means a person engaged by the licence holder solely in relation to transport, storage and/or disposal of the GMOs, and who is not undertaking any dealings with the GMOs that are not for those purposes.

'GM' means genetically modified.

'*GMO'* means the genetically modified organisms that are the subject of the dealings authorised by this licence.

'NLRD' is a Notifiable low risk dealing. Dealings conducted as an NLRD must be assessed by an institutional biosafety committee (IBC) before commencement and must comply with the requirements of the Gene Technology Regulations 2001.

'OGTR' means the Office of the Gene Technology Regulator.

'Personal information' has the same meaning as in the *Privacy Act 1988*. Personal information means information or an opinion about an identified individual, or an individual who is reasonably identifiable:

(a) whether the information or opinion is true or not; and

(b) whether the information or opinion is recorded in a material form or not.

'Pharmacy' means a location within the Clinical trial site, where authorised staff store, prepare, and dispense medications in a medical environment.

'Regulations' means the Gene Technology Regulations 2001 (Commonwealth) or the corresponding State law under which this licence is issued.

'Regulator' means the Gene Technology Regulator.

'Risk group 2 organism' means an organism that satisfies the criteria in AS/NZS 2243.3:2010 for classification as Risk Group 2.

'Sample' means any biological material collected from a treated trial participant for analysis as part of the trial.

'Serious adverse event' means any untoward medical occurrence that at any dose:

- results in death;
- is life-threatening;
- requires inpatient hospitalisation or prolongation of existing hospitalisation;
- results in persistent or significant disability/incapacity;
- is a congenital anomaly/birth defect; or
- is a medically important event or reaction.

Section 2 General conditions and obligations

Holder of licence

3. The licence holder is Medpace Australia Pty Ltd.

Remaining an Accredited Organisation

4. The licence holder must, at all times, remain an accredited organisation.

Validity of licence

5. This licence remains in force until it is suspended, cancelled or surrendered. No dealings with the GMO are authorised during any period of suspension, or after the licence has been cancelled or surrendered.

Note: Although this licence has no expiry date, the duration of preparation and administration of the GMOs is restricted in accordance with Condition 23.

Persons covered by this licence

6. The persons covered by this licence are:

   (a) the licence holder, and any employees, agents or External service providers of the licence holder; and

   (b) the project supervisor(s); and

   (c) other persons who are, or have been, engaged or otherwise authorised by the licence holder or the project supervisor to conduct any of the dealings authorised by this licence.

7. To the extent that any activity by a trial participant may be considered to be a dealing with the GMO as described in Attachment A for purposes of the Act, that dealing is authorised by this licence.

8. The licence holder must keep a record of all persons covered by this licence, and must keep a record of the contact details of the project supervisor(s) for the licence.

   Note: Where External service providers are used, it is sufficient to record the company name and the position or job title of the person(s) conducting the dealing.

9. The licence holder must provide information related to the persons covered by the licence when requested to do so in writing by the Regulator and must provide the information within a time period stipulated by the Regulator.

Description of GMOs covered

10. The licence authorises specified dealings in respect of the GMOs identified and described in **Attachment A**.

Dealings authorised by this licence

11. The licence holder and persons covered by this licence may conduct the following dealings with the GMOs:

   (a) import the GMO;

   (b) conduct the following experiments with the GMOs:

      i) prepare the GMO for administration to clinical trial participants;

      ii) administer the GMO to clinical trial participants by intratumoural injection or by intravenous infusion;

      iii) collect samples from trial participants;

      iv) analyse the samples described in 11(b)iii);

      v) prepare samples described in 11(b)iii) for export;

   (c) transport the GMOs;

   (d) dispose of the GMOs;

   and may possess, supply, use or store the GMO for the purposes of, or in the course of, any of these dealings.

12. Supply of the GMOs for the purposes of dealings to any other person or organisation not covered by this licence is only authorised by this licence if the Regulator provides prior written approval to the licence holder.

Note: For approval to be granted, the receiving person or organisation must have an appropriate authorisation to conduct dealings with the GMOs. This is likely to be an NLRD or a licence issued by the Regulator.

Conditions imposed by the Act

Note: The Act mandates the following 3 conditions.

Informing people of licence conditions (section 63)

13. The licence holder must inform any person covered by the licence, to whom a particular condition of the licence applies, of the following:

    (a) the particular condition, including any variations of it; and

    (b) the cancellation or suspension of the licence; and

    (c) the surrender of the licence.

Note: No particular conditions of this licence apply to trial participants; therefore, Condition 13 does not apply to trial participants.

Monitoring and audits (section 64)

14. If a person is authorised by this licence to deal with the GMOs and a particular condition of this licence applies to the dealing by that person, the person must allow the Regulator, or a person authorised by the Regulator, to enter premises where the dealing is being undertaken, for the purposes of auditing or monitoring the dealing.

Additional information to be given to the Regulator (section 65)

15. The licence holder must inform the Regulator, if they become aware of:

    (a) additional information about any risks to the health and safety of people, or to the environment, associated with the dealings authorised by the licence; or

    (b) any contraventions of the licence by a person covered by the licence; or

    (c) any unintended effects of the dealings authorised by the licence.

Note 1: For the purposes of this condition:

(a) The licence holder is taken to have become aware of additional information if they were reckless as to whether such information existed; and

(b) The licence holder is taken to have become aware of contraventions, or unintended effects, if they were reckless as to whether such contraventions had occurred, or such unintended effects existed.

Note 2: Contraventions of the licence may occur through the action or inaction of a person.

Note 3: Additional information includes any changes at a Clinical trial site, which might increase the likelihood of unintentional exposure of people or release of the GMO into the environment.

Informing the Regulator of any material changes of circumstance

16. The licence holder must immediately, by notice in writing, inform the Regulator of:

    (a) any relevant conviction of the licence holder occurring after the commencement of this licence;

(b) any revocation or suspension after the commencement of this licence, of a licence or permit held by the licence holder under a law of the Commonwealth, a State or a foreign country, being a law relating to the health and safety of people or the environment;

(c) any event or circumstances occurring after the commencement of this licence that would affect the capacity of the licence holder to meet the conditions in it.

17. The licence holder must provide information related to the licence holder's ongoing suitability to hold a licence when requested to do so in writing by the Regulator, and must provide the information within a time period stipulated by the Regulator.

Further conditions with respect to informing persons covered by the licence

18. If a particular condition, including any variation of it, applies to an External service provider covered by this licence, the licence holder must not permit that person to conduct any dealings unless the person has been informed of the condition, including any variation of it.

Note: Information required under Condition 18 may be provided to External service providers who are engaged solely for storage and transport of the GMO through labelling of the outermost container of the GMOs in accordance with Condition 37(a).

19. If a particular condition, including any variation of it, applies to a person with respect to any dealing, other than to an External service provider, the licence holder must not permit a person covered by this licence to conduct that dealing unless:

(a) the licence holder has obtained from the person a signed and dated statement that the person:

i) has been informed by the licence holder of the condition and, when applicable, its variation; and

ii) has understood and agreed to be bound by the condition, or its variation; and

iii) has been trained in accordance with sub-condition 19(b) below; and

(b) the licence holder has trained that person in a manner which enables them to conduct the dealings in accordance with the conditions of this licence.

20. The licence holder must notify all persons covered by the licence, from whom Personal information relevant to the administration and/or enforcement of the licence is collected by the licence holder, that such Personal information may be disclosed to the Regulator.

21. The licence holder must ensure that a copy of the licence is readily available to all persons covered by the licence, other than External service providers, who are conducting dealings with the GMO.

Note: The licence may be made available electronically.

Section 3 Limits and control measures

22. The GMO may be administered to a maximum of 18 trial participants.

23. The preparation and administration of the GMO must be completed within 5 years from the date of issuing of the licence.

Preparation and administration of the GMOs

24. Administration of the GMO to trial participants must not commence prior to approval by a Human Research Ethics Committee.

25. The following activities must occur within a Clinical trial site:

(a) preparation of the GMO for administration to trial participants; and

(b) administration of the GMO to trial participants.

Note: Before any of these activities take place, the details of each Clinical trial site must have been notified to the Regulator in accordance with Condition 42(a).

26. The licence holder must ensure all trial participants, from the time of GMO administration, are provided with a pustule management kit, including disposable waterproof dressing, disposable gloves, press-sealed bags, alcohol swabs, gauze and an unbreakable secondary container appropriate for transporting waste back to the Clinical trial site. The secondary container must be labelled to indicate the contact details for the Clinical trial site; that it contains GMOs; and that it must be destroyed by autoclaving, chemical treatment or high-temperature incineration.

Note: Unbreakable means able to withstand all reasonably expected conditions of storage and transport such as: the forces, shocks and impacts expected during handling; or changes of temperature, humidity or air pressure.

Conditions relating to trial participants

27. The licence holder must notify each trial participant, from whom Personal information relevant to the administration and/or enforcement of the licence is collected by the licence holder, that such Personal information may be disclosed to the Regulator.

28. The licence holder must ensure that exclusion criteria used in selecting trial participants include (though are not limited to) the excluded persons as defined in this licence.

29. Before inoculating any trial participant with the GMOs, the licence holder must obtain written agreement from the trial participant that they would:

    (a) not donate blood, sperm, ova, tissues or organs while participating in the trial and for 60 days after their last treatment with the GMO; and

    (b) agree to use barrier contraceptive during the treatment and for at least 60 days after their last treatment with the GMO.

30. Before inoculating a trial participant with the GMOs, the licence holder must also obtain the trial participant's written agreement that, while undergoing treatment with the GMO:

    (a) they will implement hygiene measures intended to prevent transmission of the GMO to other people and to animals (such as pets, wildlife, birds and livestock), including:

        i) frequent hand washing with soap or hand disinfectant;

        ii) cleaning household surfaces potentially exposed to the GMO; and

        iii) washing contaminated clothing and bedding with disinfectants (e.g. bleach) as per instructions provided by the licence holder.

    (b) they will avoid direct physical contact with children under 12 months of age and Excluded persons as defined in this licence for at least 7 days after each treatment or any time lesions are present;

    (c) should they develop skin lesions, they will follow the instructions provided by the licence holder for pustule management, until the lesions have healed. This includes, but is not limited to:

        i) ensuring persons caring for lesions, wear disposable gloves and wash or disinfect their hands immediately afterwards; and

        ii) sealing used dressings and other materials used in caring for the lesion in a primary container (e.g. a press-sealed bag), placing these within a secondary container (e.g. a biohazard bin) provided by the licence holder, and storing the secondary container such

that it is inaccessible to children and animals until it is returned to the Clinical trial site; and

iii) returning the secondary container referred to above, and its contents, to the Clinical trial site for disposal as clinical waste during the subsequent follow-up visit; and

(d) they will inform the Clinical trial site as soon as reasonably possible if they suspect that transmission, such as physical contact of a lesion, to another person or to an animal may have occurred.

Conditions related to the conduct of the dealings

31. Conditions that apply to dealings with GMOs do not apply to Samples collected from trial participants, or other materials or waste, that are reasonably expected not to contain the GMO. The licence holder must provide to the Regulator upon request, a written justification for this expectation.

32. The licence holder must ensure that dealings are only conducted in a manner which:

(a) does not compromise the health and safety of people; and

(b) minimises the exposure of persons conducting the dealings to the GMO, other than intended exposure of trial participants.

Note: The licence holder may achieve this by only engaging or otherwise authorising persons to conduct dealings who are required to adhere to appropriate standards and guidelines. For example, standards developed by the National Pathology Accreditation Advisory Council for pathology practices, the Australian Guidelines for the Prevention and Control of Infection in Healthcare, Guidelines for Good Clinical Practice and the National Safety and Quality Health Service (NSQHS) Standards.

33. The licence holder must ensure that procedures are in place to account for the GMO from import to destruction/export, and records must be made available to the Regulator on request.

Work practices at Clinical trial sites

34. For the purposes of Condition 32, the licence holder must ensure that the work practices and behaviours within a Clinical trial site must include, but are not limited to, the following:

(a) Excluded persons as defined in this licence must not conduct dealings with the GMO. In addition, these persons must not be engaged in the care of the trial participants for at least 7 days after each treatment or any time lesions are present;

(b) persons conducting dealings with the GMOs must wear personal protective equipment (PPE), including gowns, gloves and eye protection;

(c) any broken skin (e.g. cuts, scratches, dermatitis) of persons conducting dealings not covered by PPE or clothing must be covered with a waterproof dressing;

(d) all work surfaces must be decontaminated before and after they have been used for conducting dealings authorised by this licence;

(e) equipment used for dealings with the GMOs must be decontaminated after use;

(f) preparation and administration of the GMO must be conducted by suitably qualified and trained staff; and

(g) the inoculation site must be covered with an occlusive dressing following administration of the GMO.

Transport, storage and disposal of the GMOs

35. The licence holder must ensure that transport of the GMOs is conducted only for the purposes of, or in the course of, another dealing permitted by this licence, for supply in accordance with Condition 12, or for export.

36. For the purposes of import or export, and transport between the border and a Clinical trial site, the licence holder must ensure the GMO is packaged, labelled, stored and transported consistent with IATA shipping classification UN 3373, Category B.

37. The licence holder must ensure that transport and storage of the GMOs within the Clinical trial site, transport of Samples to an Analytical facility and, unless conducted according to condition 36, follows these sub-conditions:

    (a) GMOs must be contained within sealed, unbreakable primary and secondary containers, with the outer packaging labelled to indicate at least:

        i) that it contains GMOs; and

        ii) that it contains biohazardous material as designated by a biohazard label; and

        iii) the contact details for the licence holder; and

        iv) instructions to notify the licence holder in case of loss or spill of the GMOs; and

    (b) the external surface of the primary and secondary container must be decontaminated prior to and after transport; and

    (c) procedures must be in place to ensure that GMOs are accounted for and that a loss of GMOs during transport or storage or failure of delivery can be detected; and

    (d) access to the GMOs is restricted to authorised persons for whom Condition 18 has been met (i.e. the GMOs are within a locked unit or an area which has restricted access). This includes situations where containers are left for collection in a holding area, or left unattended prior to decontamination; and

    Note: All stored GMOs remain the responsibility of the licence holder.

    (e) if the GMO is being transported or stored with a coolant (e.g. dry ice, liquid nitrogen or any other coolant) which will release a gas, a mechanism to allow the escape of the gas must be included. If water ice is used as a coolant then the outer packaging should be constructed so as to prevent any leakage. All containers must be able to withstand the temperatures to which they will be subjected; and

    Note: When transporting and storing with coolants, it is preferable for coolants to be used outside of the secondary container.

    (f) a consolidated record of all GMOs being stored under this condition is maintained and made available to the Regulator upon request.

    (g) For the purposes of transport entirely within a building, and the GMOs are accompanied by authorised persons for whom Condition 18 has been met, Conditions 37(a)iii), 37(a)iv) and 37(c) do not apply.

38. The licence holder must ensure that all GMOs and waste reasonably expected to contain the GMOs are Decontaminated:

    (a) prior to disposal, unless the method of disposal is also a method of Decontamination; and

    (b) before or upon suspension, cancellation or surrender of the licence, unless covered by another authorisation under the Act, or exported; and

(c) by autoclaving, chemical treatment or high-temperature incineration.

39. Where transport is conducted by External service providers for the purpose of destruction, the licence holder must ensure that the GMO, or waste reasonably expected to contain the GMO, enters the clinical waste stream for decontamination via autoclaving or high-temperature incineration.

Note: In the event of a spill during transport by an External service provider, compliance with relevant State or Territory legislation and regulations to manage clinical or biohazardous spills is sufficient.

Contingency plans

40. The licence holder must ensure that any person (other than a trial participant) exposed to the GMOs is offered prompt medical attention. The clinician must be provided with any relevant information about the GMO, including advice on monitoring for symptoms and hygiene practices to minimise the risk of the spread of the GMO.

41. If there is a spill or an unintentional release of the GMOs at a Clinical trial site, the following measures must be implemented:

(a) the GMOs must be contained to prevent further dispersal; and

(b) persons cleaning up the GMO must wear protective clothing; and

(c) the exposed area must be decontaminated with an appropriate chemical disinfectant effective against the GMOs, such as sodium hypochlorite (0.5-10%), isopropyl alcohol (50%) or ethanol (70%); and

(d) any material used to clean up the spill or personal protective clothing worn during clean-up of the spill must be decontaminated; and

(e) the licence holder must be notified as soon as reasonably possible.

Section 4 Reporting and Documentation

Note: The following licence conditions are imposed to demonstrate compliance with other conditions and facilitate monitoring of compliance by staff of the OGTR. Notices and reports may be emailed to OGTR.M&C@health.gov.au. A summary of notification and reporting requirements is provided at ***Attachment B****.*

42. At least 14 days prior to first administering the GMO at each Clinical trial site, or a timeframe agreed to in writing by the Regulator, the licence holder must provide the Regulator with a Compliance Management Plan for that Clinical trial site, specifying:

(a) the name, address and description of the Clinical trial site, including any associated Pharmacies/ Analytical facilities;

(b) the key persons responsible for the management of the trial at the site;

(c) that the Institutional Biosafety Committee (IBC) associated with the site (if any) has been notified of the trial and have been consulted regarding site specific procedures;

(d) the proposed reporting structure for the trial at the site and how the reporting structure enables the licence holder to become aware of any self-reported incidents for the purposes of Condition 44;

(e) details of how the persons covered by the licence (for that type of dealing) will be informed of licence conditions applicable to them and how they will be trained to safely conduct the dealings;

(f) the person(s) or class of persons administering the GMO;

(g) where, within the site, the GMO is expected to be administered; and

(h) the expected date of first administration.

Note: For the purpose of finding out whether the Act has been complied with, an OGTR inspector may, if entry is at a reasonable time, enter a facility occupied by the licence holder or a person covered by the licence and exercise monitoring powers.

43. The licence holder must notify the Regulator, in writing, of the final GMO administration to the last trial participant at each Clinical trial site, within 30 days of the last GMO administration.

44. The licence holder must inform the Regulator as soon as reasonably possible:

(a) in the event of a trial participant experiencing a Serious adverse event which may be related to the GMO;

(b) if they are notified of, or otherwise become aware of, a loss or spill of the GMO;

(c) if they are notified, or otherwise become aware of the exposure of a person other than a trial participant or animals, to the GMO; and

(d) if they become aware that a trial participant has not followed the procedures described in the instructions provided by the licence holder.

45. Upon request from the Regulator, the licence holder must provide any signed records or documentation collected under a condition of this licence, within a time period stipulated by the Regulator.

Attachment A

DIR No: 192

Title: Clinical trial of a genetically modified (GM) chimeric Orthopoxvirus (CF33-hNIS) as a cancer treatment

Organisation Details

Postal address: Medpace Australia Pty Ltd
Office B0804, Level 8 Como Tower
644 Chapel Street
South Yarra
Victoria, 3141

Phone No: (03) 9092 5500

GMO Description

GMOs covered by this licence:

Chimeric Orthopoxvirus CF33 genetically modified by introduction or deletion of only the genes or genetic elements listed in Table 1 below.

Parent Organisms:

Common Name: CF33

Scientific Name: Chimeric Orthopoxvirus CF33

Modified traits:

Categories: Human therapeutic

Description: The GMO, known as CF33-hNIS, Vaxinia or HOV2, is a live Orthopoxvirus treatment derived from the Chimeric Orthopoxvirus CF33, modified to preferentially replicate in cancerous cells and to facilitate non-invasive imaging. Modified genes are listed in Table 1.

Table 1. Nucleic acid responsible for conferring the modified traits

Genetic modifications	
Source, identity, nature of modification	**Modified trait description**
• Deletion of *J2R* gene (viral Thymidine kinase)	Preferential viral replication in cancer cells.
• Insertion of the Human sodium/iodide symporter (hNIS) gene	Protein expression, facilitates non-invasive imaging.

Purpose of the dealings with the GMOs:

To conduct clinical trials assessing the safety, tolerability and efficacy of a genetically modified chimeric Orthopoxvirus as a cancer treatment.

Trial participants and route of administration of the GMOs

Intratumoural or intravenous administration to adult humans in patients with advanced or metastatic solid tumours.

Attachment B – Summary of reporting requirements*

Prior to the commencement of the trial	Condition	Timeframe for reporting
A written Compliance Management Plan for each Clinical trial site: (a) the name, address and description of the Clinical trial site, including any associated Pharmacies/Analytical facilities; (b) the key persons responsible for the management of the trial at the site; (c) that the Institutional Biosafety Committee (IBC) associated with the site (if any) has been notified of the trial and have been consulted regarding site specific procedures; (d) the proposed reporting structure for the trial at the site and how the reporting structure enables the licence holder to become aware of any self-reported incidents for the purposes of Condition 44; (e) details of how the persons covered by the licence (for that type of dealing) will be informed of licence conditions applicable to them and how they will be trained to safely conduct the dealings; (f) the person(s) or class of persons administering the GMO; (g) where, within the site, the GMO is expected to be administered; (h) expected date of first administration;	42	At least 14 days prior to the first administration of the GMO at each Clinical trial site, or a timeframe agreed to in writing by the Regulator
Information to be provided at any time during the clinical trial	**Condition**	**Timeframe for reporting**
Any additional information related to the health and safety of people and the environment associated with the dealings covered by the licence, or any unintended effects of the dealings authorised by the licence	15(a), (c)	As soon as the licence holder becomes aware
Information related to any contravention of the licence by a person covered by the licence	15(b)	As soon as the licence holder becomes aware
Any relevant conviction of the licence holder	16(a)	Immediately
Any revocation or suspension of a licence or permit held by the licence holder under a law of the Commonwealth, a State or a foreign country	16(b)	Immediately
Any event or circumstances that would impact the licence holder capacity to meet the licence conditions	16(c)	Immediately
Provide notification to the Regulator, in writing, of the final GMO administration of the last trial participant at each Clinical trial site	43	Within 30 days of the decision to cease GMO administration at that particular Clinical trial site.
Any Serious adverse event which may be related to the GMO	44(a)	As soon as reasonably possible
Any loss or spill of the GMO, or exposure of a person other than the trial participant to the GMO	44(a), (c)	As soon as reasonably possible after becoming aware of the event

Any event where a trial participant has not followed the procedures described in the instruction provided by the licence holder	44(d)	As soon as reasonably possible after becoming aware of the event
Information to be provided on request by the Regulator		
Information related to the persons covered by the licence	9	Within a timeframe stipulated by the Regulator
Information related to the licence holder's ongoing suitability to hold a licence	17	Within a timeframe stipulated by the Regulator
Copies of signed and dated statements and training records	19	Within a timeframe stipulated by the Regulator
A consolidated record of all GMOs being stored	37(f)	Within a timeframe stipulated by the Regulator
Any signed records or documentation collected under a condition of this licence	45	Within a timeframe stipulated by the Regulator

***** Notifications and documents to be sent to OGTR.M&C@health.gov.au

References

Abrahão, J.S., de Souza Trindade, G., Siqueira Ferreira, J.M., Campos, R.K., Bonjardim, C.A., Peregrino Ferreira, P.C., and Kroon, E.G. (2009). Long-lasting stability of *Vaccinia virus* strains in murine feces: implications for virus circulation and environmental maintenance. Archives of Virology *154*, 1551-1553.

Abrahão, J.S., Silva-Fernandes, A.T., Lima, L.S., Campos, R.K., Guedes, M.I.M.C., Cota, M.M.G., Assis, F.L*., et al.* (2010). *Vaccinia virus* infection in monkeys, Brazilian Amazon. Emerging Infectious Diseases *16*, 976-979.

Adams, M.M., Rice, A.D., and Moyer, R.W. (2007). *Rabbitpox virus* and *Vaccinia virus* infection of rabbits as a model for human smallpox. Journal of Virology *81*, 11084-11095.

Anandasabapathy, N., Breton, G., Hurley, A., Caskey, M., Trumpfheller, C., Sarma, P., Pring, J*., et al.* (2015). Efficacy and safety of CDX-301, recombinant human Flt3L, at expanding dendritic cells and hematopoietic stem cells in healthy human volunteers. Bone Marrow Transplantation *50*, 924-930.

Artois, M., Charlton, K.M., Tolson, N.D., Casey, G.A., Knowles, M.K., and Campbell, J.B. (1990). Vaccinia recombinant virus expressing the rabies virus glycoprotein: safety and efficacy trials in Canadian wildlife. Canadian Journal of Veterinary Research *54*, 504-507.

Australian Government Department of Health (2022). Monkeypox. Accessed: 10 June 2022.

Australian Technical Advisory Group on Immunisation (ATAGI) (2018). Australian Immunisation Handbook, Vol 1, 10 edn (Australian Government Department of Health, Canberra, Australia: Australian Government National Health and Medical Research Council).

Avram, A.M. (2012). Radioiodine scintigraphy with SPECT/CT: an important diagnostic tool for thyroid cancer staging and risk stratification. J Nucl Med *53*, 754-764.

Babkin, I.V., Babkina, I.N., and Tikunova, N.V. (2022). An Update of Orthopoxvirus Molecular Evolution. Viruses *14*.

Badell, M.L., Meaney-Delman, D., Tuuli, M.G., Rasmussen, S.A., Petersen, B.W., Sheffield, J.S., Beigi, R.H*., et al.* (2015). Risks Associated With Smallpox Vaccination in Pregnancy: A Systematic Review and Meta-analysis. Obstet Gynecol *125*, 1439-1451.

Bammer, G., and Smithson, M. (2008). Uncertainty and risk: Multidisciplinary perspectives (London: Earthscan).

Bedson, H.S., and Dumbell, K.R. (1964a). Hybrids derived from the viruses of alastrim and rabbit pox The Journal of Hygeine (London) *62*, 141-146.

Bedson, H.S., and Dumbell, K.R. (1964b). Hybrids derived from the viruses of variola major and cowpox. The Journal of Hygiene *62*, 147-158.

Bennett, M., Gaskell, R.M., Gaskell, C.J., Baxby, D., and Kelly, D.F. (1989). Studies on poxvirus infection in cats. Archives of Virology *104*, 19-33.

Biohazard Waste Industry (2010). Industry Code of Practice for the Management of Clinical and Related Wastes, 6 edn.

Bitter, E.E., Townsend, M.H., Erickson, R., Allen, C., and O'Neill, K.L. (2020). Thymidine kinase 1 through the ages: a comprehensive review. Cell Biosci *10*, 138.

Brochier, B., Blancou, J., Thomas, I., Languet, B., Artois, M., Kieny, M.P., Lecocq, J.P.*, et al.* (1989). Use of recombinant vaccinia-rabies glycoprotein virus for oral vaccination of wildlife against rabies: innocuity to several non-target bait consuming species. Journal of Wildlife Diseases *25*, 540-547.

Buller, R.M., and Palumbo, G.J. (1991). Poxvirus pathogenesis. Microbiological Reviews *55*, 80-122.

Canada, P.H.A.o. (2011). Pathogen Safety Data Sheets: Infectious Substances – Vaccinia Virus. Accessed: 26 May 2022.

Casey, C.G., Iskander, J.K., Roper, M.H., Mast, E.E., Wen, X.-J., Török, T.J., Chapman, L.E.*, et al.* (2005). Adverse events associated with smallpox vaccination in the United States, January-October 2003. JAMA *294*, 2734-2743.

Centers for Disease Control and Prevention (2008). Laboratory-Acquired Vaccinia Exposures and Infections --- United States, 2005--2007.

Centers for Disease Control and Prevention (2021). Smallpox treatment. Accessed: 20 May 2022.

Centers for Disease Control and Prevention (2022a). Monkeypox - Signs and Symptoms. Accessed: 10 June 2022.

Centers for Disease Control and Prevention (2022b). Nonemergency Use of Smallpox Vaccine. Accessed: 16 May 2022.

Centers for Disease Control and Prevention (2022c). Smallpox Vaccine. Accessed: 02 June 2022.

Centers for Disease Control and Prevention (2022d). Vaccine “Take” Evaluation. Accessed: 09 June 2022.

Chaurasiya, S., Yang, A., Zhang, Z., Lu, J., Valencia, H., Kim, S.I., Woo, Y.*, et al.* (2022). A comprehensive preclinical study supporting clinical trial of oncolytic chimeric poxvirus CF33-hNIS-anti-PD-L1 to treat breast cancer. Mol Ther Methods Clin Dev *24*, 102-116.

Clark, A.J., and Brinkley, T. (2001). Risk management: for climate, agriculture and policy. (Canberra: Commonwealth of Australia).

Cohen, J. (2001). Bioterrorism. Smallpox vaccinations: how much protection remains? Science *294*, 985.

Concilio, S.C., Zhekova, H.R., Noskov, S.Y., and Russell, S.J. (2020). Inter-species variation in monovalent anion substrate selectivity and inhibitor sensitivity in the sodium iodide symporter (NIS). PLoS One *15*, e0229085.

Cono, J., Casey, C.G., and Bell, D.M. (2003). Smallpox vaccination and adverse reactions. Guidance for clinicians. MMWR Recommendations and Reports *52*, 1-28.

Cooney, E.L., Collier, A.C., Greenberg, P.D., Coombs, R.W., Zarling, J., Arditti, D.E., Hoffman, M.C.*, et al.* (1991). Safety of and immunological response to a recombinant *Vaccinia virus* vaccine expressing HIV envelope glycoprotein. The Lancet *337*, 567-572.

Cummings, J.F., Polhemus, M.E., Hawkes, C., Klote, M., Ludwig, G.V., and Wortmann, G. (2004). Lack of vaccinia viremia after smallpox vaccination. Clin Infect Dis *38*, 456-458.

Cummings, J.F., Polhemus, M.E., Hawkes, C., Klote, M., Ludwig, G.V., and Wortmann, G. (2008). Persistence of vaccinia at the site of smallpox vaccination. Clinical Infectious Diseases *46*, 101-102.

D'Anunciacao, L., Guedes, M.I., Oliveira, T.L., Rehfeld, I., Bonjardim, C.A., Ferreira, P.P., Trindade Gde, S.*, et al.* (2012). Filling one more gap: experimental evidence of horizontal transmission of Vaccinia virus between bovines and rodents. Vector Borne Zoonotic Dis *12*, 61-64.

Darrouzet, E., Lindenthal, S., Marcellin, D., Pellequer, J.L., and Pourcher, T. (2014). The sodium/iodide symporter: state of the art of its molecular characterization. Biochim Biophys Acta *1838*, 244-253.

de Oliveira, T.M., Guedes, M.I., Rehfeld, I.S., Matos, A.C., Rivetti, A.V., Jr., Alves, P.A., Galinari, G.C.*, et al.* (2015). Detection of Vaccinia Virus in Milk: Evidence of a Systemic and Persistent Infection in Experimentally Infected Cows. Foodborne Pathog Dis *12*, 898-903.

Dohan, O., De la Vieja, A., Paroder, V., Riedel, C., Artani, M., Reed, M., Ginter, C.S.*, et al.* (2003). The sodium/iodide Symporter (NIS): characterization, regulation, and medical significance. Endocr Rev *24*, 48-77.

Downie, A.W. (1939). A study of the lesions produced experimentally by cowpox virus. The Journal of Pathology and Bacteriology *48*, 361-379.

Downs-Canner, S., Guo, Z.S., Ravindranathan, R., Breitbach, C.J., O'Malley, M.E., Jones, H.L., Moon, A.*, et al.* (2016). Phase 1 Study of Intravenous Oncolytic Poxvirus (vvDD) in Patients With Advanced Solid Cancers. Mol Ther *24*, 1492-1501.

Dumbell, K., and Richardson, M. (1993). Virological investigations of specimens from buffaloes affected by buffalopox in Maharashtra State, India between 1985 and 1987. Archives of Virology *128*, 257-267.

Dyer, A., Baugh, R., Chia, S.L., Frost, S., Iris, Jacobus, E.J., Khalique, H.*, et al.* (2019). Turning cold tumours hot: oncolytic virotherapy gets up close and personal with other therapeutics at the 11th Oncolytic Virus Conference. Cancer Gene Therapy *26*, 59-73.

Egan, C., Kelly, C.D., Rush-Wilson, K., Davis, S.W., Samsonoff, W.A., Pfeiffer, H., Miller, J.*, et al.* (2004). Laboratory-confirmed transmission of *Vaccinia virus* infection through sexual contact with a military vaccinee. Journal of Clinical Microbiology *42*, 5409-5411.

El-Jesr, M., Teir, M., and Maluquer de Motes, C. (2020). Vaccinia Virus Activation and Antagonism of Cytosolic DNA Sensing. Front Immunol *11*, 568412.

Espy, M.J., Uhl, J.R., Sloan, L.M., Rosenblatt, J.E., Cockerill, F.R., and Smith, T.F. (2002). Detection of vaccinia virus, herpes simplex virus, varicella-zoster virus, and *Bacillus anthracis* DNA by LightCycler polymerase chain reaction after autoclaving: Implications for biosafety of bioterrorism agents. Mayo Clinic Proceedings *77*, 624-628.

Felipetto Cargnelutti, J., Schmidt, C., Masuda, E.K., Braum, L.D., Weiblen, R., and Furtado Flores, E. (2012). *Vaccinia viruses* isolated from cutaneous disease in horses are highly virulent for rabbits. Microbial Pathogenesis *52*, 192-199.

Fenner, F., and Comben, B.M. (1958). Genetic studies with mammalian poxviruses. I. Demonstration of recombination between two strains of vaccinia virus. Virology *5*, 530-548.

Ferreira, J.M.S., Abrahao, J.S., Drumond, B.P., Oliveira, F.M., Alves, P.A., Pascoal-Xavier, M.A., Lobato, Z.I.P.*, et al.* (2008). Vaccinia virus: shedding and horizontal transmission in a murine model. J Gen Virol *89*, 2986-2991.

Fulginiti, V.A., Papier, A., Lane, J.M., Neff, J.M., and Henderson, D.A. (2003a). Smallpox vaccination: A review, part I. Background, vaccination technique, normal vaccination and revaccination, and expected normal reactions. Clinical Infectious Diseases *37*, 241-250.

Fulginiti, V.A., Papier, A., Lane, J.M., Neff, J.M., and Henderson, D.A. (2003b). Smallpox vaccination: A review, part II. Adverse events. Clinical Infectious Diseases *37*, 251-271.

Galanis, E., Atherton, P.J., Maurer, M.J., Knutson, K.L., Dowdy, S.C., Cliby, W.A., Haluska, P., Jr.*, et al.* (2015). Oncolytic measles virus expressing the sodium iodide symporter to treat drug-resistant ovarian cancer. Cancer Res *75*, 22-30.

Gershon, P.D., Kitching, R.P., Hammond, J.M., and Black, D.N. (1989). Poxvirus genetic recombination during natural virus transmission. Journal of General Virology *70 (Pt 2)*, 485-489.

Hayes, K.R. (2004). Ecological implications of GMOs: robust methodologies for ecological risk assessment. Best practice and current practice in ecological risk assessment for genetically modified organisms. (Tasmania: CSIRO Division of Marine Research).

Healthdirect Australia (2022). Molluscum contagiosum.

Hengstschlager, M., and Bernaschek, G. (1997). Fetal cells in the peripheral blood of pregnant women express thymidine kinase: a new marker for detection. FEBS Lett *404*, 299-302.

Hengstschlager, M., Mullner, E., and Wawra, E. (1994). Thymidine kinase is expressed differently in transformed versus normal-cells - a novel test for malignancy. Int J Oncol *4*, 207-210.

Heo, J., Reid, T., Ruo, L., Breitbach, C.J., Rose, S., Bloomston, M., Cho, M.*, et al.* (2013). Randomized dose-finding clinical trial of oncolytic immunotherapeutic vaccinia JX-594 in liver cancer. Nat Med *19*, 329-336.

Hwang, T.H., Moon, A., Burke, J., Ribas, A., Stephenson, J., Breitbach, C.J., Daneshmand, M.*, et al.* (2011). A mechanistic proof-of-concept clinical trial with JX-594, a targeted multi-mechanistic oncolytic poxvirus, in patients with metastatic melanoma. Mol Ther *19*, 1913-1922.

Imugene (2022). Imugene and City of Hope announce first patient dosed in Phase 1 trial to test cancer-killing oncolytic virus against solid tumours Accessed: 07 June 2022.

International Committee on Taxonomy of Viruses (2022). Orthopoxvirus. Accessed: 05 May 2022.

Jacobs, B.L., Langland, J.O., Kibler, K.V., Denzler, K.L., White, S.D., Holechek, S.A., Wong, S.*, et al.* (2009). *Vaccinia virus* vaccines: past, present and future. Antiviral Research *84*, 1-13.

Katsafanas, G.C., and Moss, B. (2007). Colocalization of transcription and translation within cytoplasmic poxvirus factories coordinates viral expression and subjugates host functions. Cell Host & Microbe *2*, 221-228.

Kretzschmar, M., Wallinga, J., Teunis, P., Xing, S., and Mikolajczyk, R. (2006). Frequency of adverse events after vaccination with different *Vaccinia* strains. PLOS Medicine *3*, e272.

Lane, J.M., and Fulginiti, V.A. (2003). Transmission of *Vaccinia virus* and rationale for measures for prevention. Clinical Infectious Diseases *37*, 281-284.

Lee, M.S., Roos, J.M., McGuigan, L.C., Smith, K.A., Cormier, N., Cohen, L.K., Roberts, B.E.*, et al.* (1992). Molecular attenuation of vaccinia virus: mutant generation and animal characterization. J Virol *66*, 2617-2630.

Li, G., Chen, N., Roper, R.L., Feng, Z., Hunter, A., Danila, M., Lefkowitz, E.J.*, et al.* (2005). Complete coding sequences of the rabbitpox virus genome. J Gen Virol *86*, 2969-2977.

Li, H., Peng, K.W., Dingli, D., Kratzke, R.A., and Russell, S.J. (2010). Oncolytic measles viruses encoding interferon beta and the thyroidal sodium iodide symporter gene for mesothelioma virotherapy. Cancer Gene Ther *17*, 550-558.

Lin, Y.-C.J., and Evans, D.H. (2010). *Vaccinia virus* particles mix inefficiently, and in a way that would restrict viral recombination, in coinfected cells. Journal of Virology *84*, 2432-2443.

Liu, L., Cooper, T., Howley, P.M., and Hayball, J.D. (2014). From crescent to mature virion: *Vaccinia virus* assembly and maturation. Viruses *6*, 3787-3808.

Mackett, M., Smith, G.L., and Moss, B. (1982). Vaccinia virus: a selectable eukaryotic cloning and expression vector. Proc Natl Acad Sci U S A *79*, 7415-7419.

Martinez-Pomares, L., Thompson, J.P., and Moyer, R.W. (1995). Mapping and investigation of the role in pathogenesis of the major unique secreted 35-kDa protein of rabbitpox virus. Virology *206*, 591-600.

Matos, A.C.D., Rehfeld, I.S., Guedes, M., and Lobato, Z.I.P. (2018). Bovine Vaccinia: Insights into the Disease in Cattle. Viruses *10*.

Maurer, D.M., Harrington, B., and Lane, J.M. (2003). Smallpox vaccine: contraindications, administration, and adverse reactions. American Family Physician *68*, 889-896.

Miranda, J.B., Borges, I.A., Campos, S.P.S., Vieira, F.N., de Azara, T.M.F., Marques, F.A., Costa, G.B.*, et al.* (2017). Serologic and molecular evidence of *Vaccinia virus* circulation among small mammals from different biomes, Brazil. Emerging Infectious Diseases *23*, 931-938.

Moussatche, N., Damaso, C.R., and McFadden, G. (2008). When good vaccines go wild: Feral orthopoxvirus in developing countries and beyond. The Journal of Infection in Developing Countries *2*, 156-173.

Nagata, L.P., Irwin, C.R., Hu, W.G., and Evans, D.H. (2018). Vaccinia-based vaccines to biothreat and emerging viruses. Biotechnology and Genetic Engineering Reviews *34*, 107-121.

Nalca, A., and Nichols, D.K. (2011). Rabbitpox: a model of airborne transmission of smallpox. J Gen Virol *92*, 31-35.

National Health and Medical Research Council (2019). Australian Guidelines for the Prevention and Control of Infection in Healthcare. (Canberra: Commonwealth of Australia, available online

https://www.nhmrc.gov.au/about-us/publications/australian-guidelines-prevention-and-control-infection-healthcare-2019#block-views-block-file-attachments-content-block-1).

National Health and Medical Research Council, Australian Research Council, and Universities Australia (2018). National Statement on Ethical Conduct in Human Research 2007 (Updated 2018). (Canberra: Commonwealth of Australia, available online https://www.nhmrc.gov.au/about-us/publications/national-statement-ethical-conduct-human-research-2007-updated-2018).

O'Leary, M.P., Choi, A.H., Kim, S.I., Chaurasiya, S., Lu, J., Park, A.K., Woo, Y.*, et al.* (2018). Novel oncolytic chimeric orthopoxvirus causes regression of pancreatic cancer xenografts and exhibits abscopal effect at a single low dose. J Transl Med *16*, 110.

OGTR (2013). Risk Analysis Framework 2013. (Office of the Gene Technology Regulator) Accessed: July 2020.

Oliveira, G.P., Silva-Fernandes, A.T., Assis, F.L., Alves, P.A., Franco-Luiz, A.P.M., Figueiredo, L.B., de Almeida, C.M.C.*, et al.* (2014). Intrafamilial transmission of *Vaccinia virus* during a bovine vaccinia outbreak in Brazil: a new insight in viral transmission chain. American Journal of Tropical Medicine and Hygeine *90*, 1021-1023.

Paszkowski, P., Noyce, R.S., and Evans, D.H. (2016). Live-cell imaging of *Vaccinia virus* recombination. PLOS Pathogens *12*, e1005824.

Peres, M.G., Bacchiega, T.S., Appolinario, C.M., Vicente, A.F., Allendorf, S.D., Antunes, J.M., Moreira, S.A.*, et al.* (2013). Serological study of vaccinia virus reservoirs in areas with and without official reports of outbreaks in cattle and humans in Sao Paulo, Brazil. Arch Virol *158*, 2433-2441.

Peres, M.G., Barros, C.B., Appolinario, C.M., Antunes, J.M., Mioni, M.S., Bacchiega, T.S., Allendorf, S.D.*, et al.* (2016). Dogs and Opossums Positive for Vaccinia Virus during Outbreak Affecting Cattle and Humans, Sao Paulo State, Brazil. Emerg Infect Dis *22*, 271-273.

Portulano, C., Paroder-Belenitsky, M., and Carrasco, N. (2014). The Na+/I- symporter (NIS): mechanism and medical impact. Endocr Rev *35*, 106-149.

Ravera, S., Reyna-Neyra, A., Ferrandino, G., Amzel, L.M., and Carrasco, N. (2017). The Sodium/Iodide Symporter (NIS): Molecular Physiology and Preclinical and Clinical Applications. Annu Rev Physiol *79*, 261-289.

Rheinbaben, F.v., Gebel, J., Exner, M., and Schmidt, A. (2007). Environmental resistance, disinfection, and sterilization of poxviruses. In Poxviruses, A.A. Mercer, A. Schmidt, and O. Weber, eds. (Basel: Birkhäuser Basel), pp. 397-405.

Riyesh, T., Karuppusamy, S., Bera, B.C., Barua, S., Virmani, N., Yadav, S., Vaid, R.K.*, et al.* (2014). Laboratory-acquired buffalopox virus infection, India. Emerging Infectious Diseases *20*, 324-326.

Robinson, A.J., and Mercer, A.A. (1988). Orf virus and vaccinia virus do not cross-protect sheep. Archives of Virology *101*, 255-259.

Rotz, L.D., Dotson, D.A., Damon, I.K., Becher, J.A., and Advisory Committee on Immunization, P. (2001). Vaccinia (smallpox) vaccine: recommendations of the Advisory Committee on Immunization Practices (ACIP), 2001. MMWR Recomm Rep *50*, 1-25; quiz CE21-27.

Russell, S.J., Federspiel, M.J., Peng, K.W., Tong, C., Dingli, D., Morice, W.G., Lowe, V.*, et al.* (2014). Remission of disseminated cancer after systemic oncolytic virotherapy. Mayo Clin Proc *89*, 926-933.

Russo, D., Scipioni, A., Durante, C., Ferretti, E., Gandini, L., Maggisano, V., Paoli, D.*, et al.* (2011). Expression and localization of the sodium/iodide symporter (NIS) in testicular cells. Endocrine *40*, 35-40.

Santos Apolonio, J., Lima de Souza Goncalves, V., Cordeiro Santos, M.L., Silva Luz, M., Silva Souza, J.V., Rocha Pinheiro, S.L., de Souza, W.R.*, et al.* (2021). Oncolytic virus therapy in cancer: A current review. World J Virol *10*, 229-255.

Sarker, S., Hannon, C., Athukorala, A., and Bielefeldt-Ohmann, H. (2021). Emergence of a Novel Pathogenic Poxvirus Infection in the Endangered Green Sea Turtle (Chelonia mydas) Highlights a Key Threatening Process. Viruses *13*.

Sauerbrei, A., and Wutzler, P. (2009). Testing thermal resistance of viruses. Archives of Virology *154*, 115-119.

Schramm, B., and Locker, J.K. (2005). Cytoplasmic organization of poxvirus DNA replication. Traffic *6*, 839-846.

Seissler, J., Wagner, S., Schott, M., Lettmann, M., Feldkamp, J., Scherbaum, W.A., and Morgenthaler, N.G. (2000). Low frequency of autoantibodies to the human Na(+)/I(-) symporter in patients with autoimmune thyroid disease. J Clin Endocrinol Metab *85*, 4630-4634.

Sepkowitz, K.A. (2003). How contagious is vaccinia? New England Journal of Medicine *348*, 439-446.

Shimura, H., Haraguchi, K., Miyazaki, A., Endo, T., and Onaya, T. (1997). Iodide uptake and experimental 131I therapy in transplanted undifferentiated thyroid cancer cells expressing the Na+/I- symporter gene. Endocrinology *138*, 4493-4496.

Shors, T., Keck, J.G., and Moss, B. (1999). Down regulation of gene expression by the *Vaccinia virus* D10 protein. Journal of Virology *73*, 791-796.

Silva, N.I.O., de Oliveira, J.S., Kroon, E.G., Trindade, G.S., and Drumond, B.P. (2020). Here, There, and Everywhere: The Wide Host Range and Geographic Distribution of Zoonotic Orthopoxviruses. Viruses *13*.

Smith, G.L., Benfield, C.T.O., Maluquer de Motes, C., Mazzon, M., Ember, S.W.J., Ferguson, B.J., and Sumner, R.P. (2013). Vaccinia virus immune evasion: mechanisms, virulence and immunogenicity. J Gen Virol *94*, 2367-2392.

Spitzweg, C., O'Connor, M.K., Bergert, E.R., Tindall, D.J., Young, C.Y., and Morris, J.C. (2000). Treatment of prostate cancer by radioiodine therapy after tissue-specific expression of the sodium iodide symporter. Cancer Res *60*, 6526-6530.

Standards Australia/New Zealand (2010). Safety in laboratories Part 3: Microbiological safety and containment AS/NZS 2243.3:2010.

Strayer, D.S., Skaletsky, E., Cabirac, G.F., Sharp, P.A., Corbeil, L.B., Sell, S., and Leibowitz, J.L. (1983). Malignant rabbit fibroma virus causes secondary immunosuppression in rabbits. The Journal of Immunology *130*, 399-404.

Tolonen, N., Doglio, L., Schleich, S., and Krijnse Locker, J. (2001). Vaccinia virus DNA replication occurs in endoplasmic reticulum-enclosed cytoplasmic mini-nuclei. Mol Biol Cell *12*, 2031-2046.

Tonacchera, M., Agretti, P., Ceccarini, G., Lenza, R., Refetoff, S., Santini, F., Pinchera, A.*, et al.* (2001). Autoantibodies from patients with autoimmune thyroid disease do not interfere with the activity of the human iodide symporter gene stably transfected in CHO cells. Eur J Endocrinol *144*, 611-618.

UK, C.R. (2022). Pembrolizumab (Keytruda). Accessed: 10 June 2022.

Warner, S.G., Kim, S.I., Chaurasiya, S., O'Leary, M.P., Lu, J., Sivanandam, V., Woo, Y.*, et al.* (2019). A Novel Chimeric Poxvirus Encoding hNIS Is Tumor-Tropic, Imageable, and Synergistic with Radioiodine to Sustain Colon Cancer Regression. Mol Ther Oncolytics *13*, 82-92.

Webber, B.J., Montgomery, J.R., Markelz, A.E., Allen, K.C., Hunninghake, J.C., Ritchie, S.A., Pawlak, M.T.*, et al.* (2014). Spread of vaccinia virus through shaving during military training, Joint Base San Antonio-Lackland, TX, June 2014. Medical Surveillance Monthly Report *21*, 2-6.

Wharton, M., Strikas, R.A., Harpaz, R., Rotz, L.D., Schwartz, B., Casey, C.G., Pearson, M.L.*, et al.* (2003). Recommendations for using smallpox vaccine in a pre-event vaccination program. Supplemental recommendations of the Advisory Committee on Immunization Practices (ACIP) and the Healthcare Infection Control Practices Advisory Committee (HICPAC). MMWR Recommendations and Reports *52*, 1-16.

Wildlife Health Australia (2012). Poxviruses and Australian wild birds.

Wildlife Health Australia (2019a). Diseases of concernin wild Australian crocodiles.

Wildlife Health Australia (2019b). Poxviruses and Australian mammals. Accessed: 25 May 2022.

Wood, J.P., Choi, Y.W., Wendling, M.Q., Rogers, J.V., and Chappie, D.J. (2013). Environmental persistence of *Vaccinia virus* on materials. Letters in Applied Microbiology *57*, 399-404.

Woodroofe, G.M., and Fenner, F. (1960). Genetic studies with mammalian poxviruses. IV. Hybridization between several different poxviruses. Virology *12*, 272-282.

World Health Organization (2022a). Multi-country monkeypox outbreak in non-endemic countries. Accessed: 10 June 2026.

World Health Organization (2022b). Smallpox. Accessed: 10 May 2022.

Yang, Z., Reynolds, S.E., Martens, C.A., Bruno, D.P., Porcella, S.F., and Moss, B. (2011). Expression profiling of the intermediate and late stages of poxvirus replication. Journal of Virology *85*, 9899-9908.

Zeh, H.J., and Bartlett, D.L. (2002). Development of a replication-selective, oncolytic poxvirus for the treatment of human cancers. Cancer Gene Ther *9*, 1001-1012.

Zeh, H.J., Downs-Canner, S., McCart, J.A., Guo, Z.S., Rao, U.N.M., Ramalingam, L., Thorne, S.H.*, et al.* (2015). First-in-man study of western reserve strain oncolytic *Vaccinia virus*: Safety, systemic spread, and antitumor activity. Molecular Therapy *23*, 202-214.

Zhang, Z., Yang, A., Chaurasiya, S., Park, A.K., Lu, J., Kim, S.I., Warner, S.G*., et al.* (2021). CF33-hNIS-antiPDL1 virus primes pancreatic ductal adenocarcinoma for enhanced anti-PD-L1 therapy. Cancer Gene Ther.