# Risk Assessment and Risk Management Plan

# for

# DIR 192

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

Applicant: Medpace Australia Pty Ltd

15 September 2022

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# Summary of the Risk Assessment and Risk Management Plan

### for

# Licence Application DIR 192

## *Decision*

The Gene Technology Regulator (the Regulator) has decided to issue a licence application for 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 Act).

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 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. A Risk Assessment and Risk Management Plan (RARMP) for this application has been prepared by the Regulator in accordance with the Act and corresponding state and territory legislation, and finalised following consultation with a wide range of experts, agencies and authorities, and the public. The RARMP concluded that the proposed clinical trial poses negligible risks to human health and safety and the environment and that any risks posed by the dealings can be managed by imposing conditions on the release.

## *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.<br>• 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.<br>• Staff preparing and administering the GMO would use personal protective equipment.<br>• Import, transport and storage of the GMO would be carried out according to the OGTR *Guidelines for the Transport, Storage and Disposal of* GMOs.<br>• Waste that may contain the GMO would be disposed of as infectious material (e.g. via the clinical waste stream). |

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[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.

As the level of risk is considered negligible, specific risk treatment is not required. However, since this is a clinical trial, the licence includes limits on the number of trial participants, types of facilities used and 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.

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# 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 |
| DAFF | Department of Agriculture, Fisheries and Forestry |
| 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 release are assessed within this context. Chapter 1 describes the risk assessment context for this application.
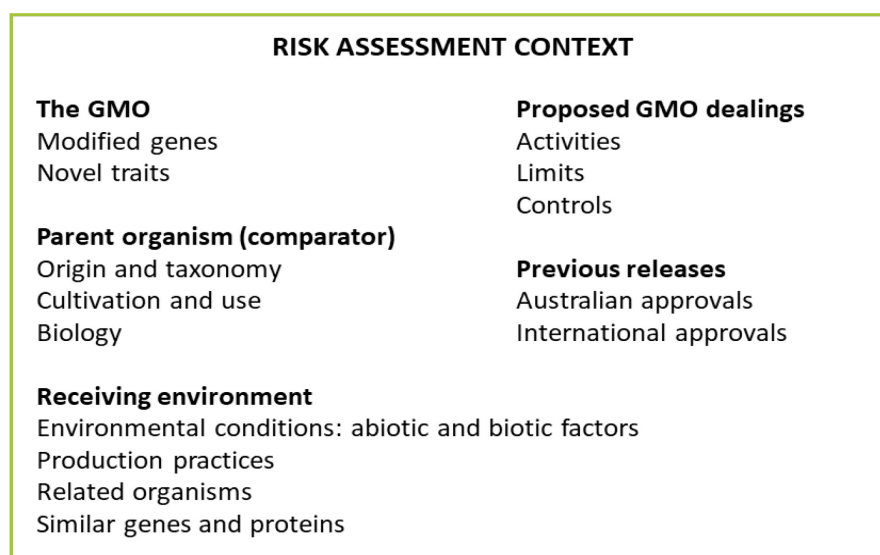


*Figure 1. Summary of parameters used to establish the risk assessment context, within the legislative requirements, operational policies and guidelines of the OGTR and the RAF.*

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. The advice from the prescribed experts, agencies and authorities and how it was taken into account is summarised in Appendix A. One public submission was received, and its consideration is summarised in Appendix B.

## 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 (DAFF).

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 treatment, 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.     DAFF 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, laboratory materials, therapeutics and vaccines (including GM treatments and vaccines). Import of GM treatment is subject to regulation by DAFF 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 orthopoxvirus (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:

- Avoid exposing other people and animals to injection sites and contaminated dressings;

- 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 site 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.

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

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.

32.     Samples collected from trial participants would be transported to analytical facilities within the clinical trial site, 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 ($8.6 \times 10^5$, $9.4 \times 10^6$, $3 \times 10^7$ and $1.1 \times 10^8$ plaque forming units (PFU)) would be tested. The first patient cohort would receive the lowest dose of the GMO alone via i.t. injection. After analysis of the patient safety data, the Cohort Review Committee (CRC) comprised of Investigators, the Sponsor, and the Medical Monitor may decide to administer the GMO via i.v. infusion, increase or reduce the dose of GMO to be administered to the next cohort (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. $8.6 \times 10^5$ PFU of the GMO + pembrolizumab would be administered only if the GMO dose level of $9.4 \times 10^6$ 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 <u>must</u>:

- 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 appropriate 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 potential variations and on how to safely conduct dealings with the GMO by Medpace and/or Imugene representatives. Staff that did not attend the initial training or commenced work after it would be trained 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 between rabbitpox virus (RPXV) and 6 strains of vaccinia virus (VACV). Briefly, mammalian cells cultured in the 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 pathogens 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). It encodes around 200 proteins with roles in viral entry, transcription of viral genes, DNA synthesis, assembly of virus particles, and suppression of the host anti-viral response (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.,

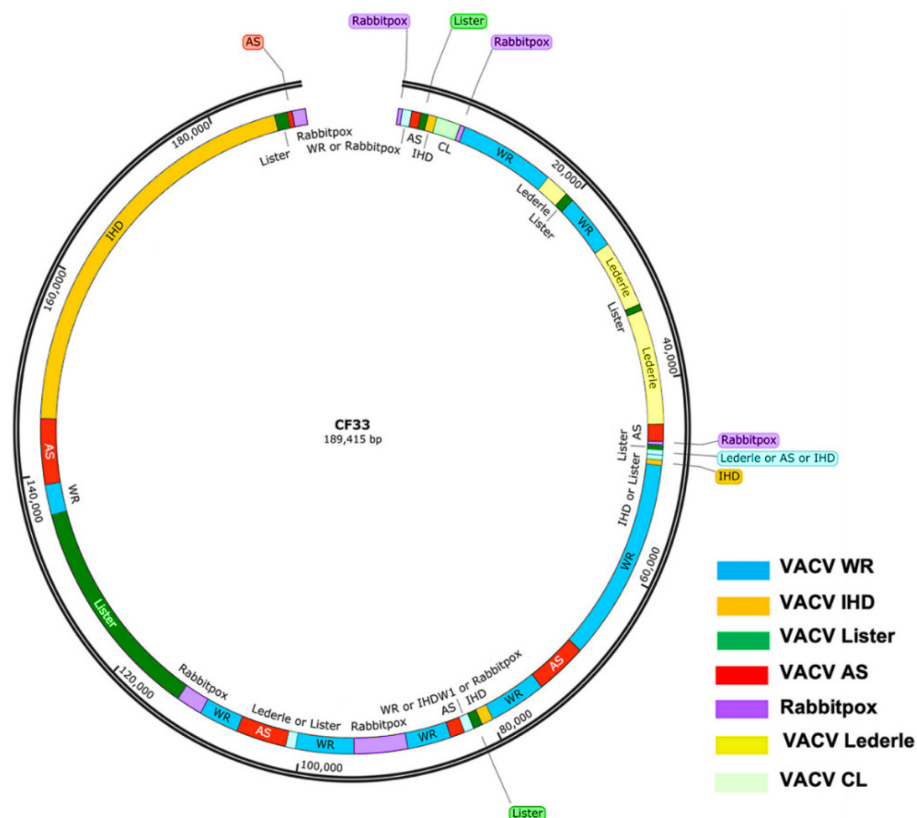2022). An overview of VACV strains and RPXV-Utrecht collaboration in the CF33 genome is shown in Figure 2.



*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 - that is, 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 (for example, 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 bloodstream (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 individuals, 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 in humans has been ongoing since early May 2022. As of 22 August 2022, WHO has registered a total of 41,664 laboratory-confirmed cases and 12 deaths in 96 countries. Although monkeypox virus (MPXV) is endemic to parts of Africa, the recent outbreak has been detected in the UK, Europe, South and North America, Middle East and other areas where MPXV is not endemic (WHO, 2022a). On 23 July 2022, WHO declared the global monkeypox situation a Public Health Emergency of international concern. Australia's Chief Medical Officer, Professor Paul Kelly, declared the monkeypox outbreak a <u>Communicable Disease Incident of National Significance</u>. This means that the response to monkeypox in Australia will have national coordination to assist states and territories to manage the outbreaks within their jurisdictions (Australian Government Department of Health 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 fomites (for example, contaminated dressing, clothing, sheets, towels and surfaces). A 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 (for example, monkeypox and cowpox). Exposure via spill or eye splash did not result in infection (CDC, 2008).

## 3.6  Mutation and recombination

88.    Mutation is an important source of genetic variation in viruses. As described in Section 3.1 of this chapter, VACV is considered to have originated from related OPVX ancestors. Recent analysis of VACV genomes suggested that mutations happened about fourteen times more often than recombination events, and mutation has been a major contribution in the evolution of VACV strains (Molteni et al., 2022). Viral evolution by mutation or self-recombination events may occur over time and usually involve repeated viral transmission to other hosts. For example, genome analysis indicates that Buffalopox virus (BPXV), the causative agent of buffalopox disease, originated from the VACV Lister strain used for inoculating buffalo calves to produce a smallpox vaccine. The virus was first isolated in India in 1967 and has evolved over a period of forty years to be pathogenic to buffalo, cattle, and humans (Eltom et al., 2020).

89.    Homologous recombination between viral strains 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.

90.    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; Sahu et al., 2022). 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

91.    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.

92.    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).

93.     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

94.    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).

95.    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).

96.    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 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).

97.    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).

98.    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*

99.     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 ($\leq$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*

100.  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*

101.  Treatment of VACV infections is mainly supportive and include hydration, nutritional supplementation, and prevention of secondary infections. In the case of a 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

102.  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

103.  Oncolytic viral therapy 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).

104.  The GMO (CF33-hNIS) was modified from the oncolytic orthopoxvirus CF33 and was 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

105.  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.
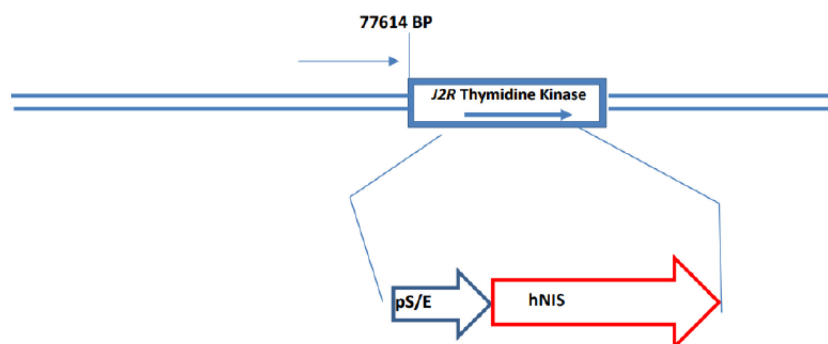


*Figure 3.* hNIS transgene into the J2R locus of CF33

### 4.1.1 *The genetic modifications*

**The J2R gene and thymidine kinase protein**

106.   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).

107.   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).

108.   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*

109.   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 107, 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).

110.   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 into healthy mice (Lee et al., 1992). The absence of the TK protein did not affect the GMO replication in cells cultured in the laboratory (Warner et al., 2019).

111.   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**

112.   Sodium Iodide Symporter (NIS) is a cell-surface protein responsible for iodide uptake in mammalian cells and is normally expressed in thyroid cells. The human sodium iodide symporter (hNIS) 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, 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).

113.   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 have been used to treat some types of thyroid cancer with little effect on the rest of the body.

*hNIS and autoimmune disease*

114.   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*

115.   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).

116.   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 2022).

*Effect of inserting the hNIS transgene into the GMO*

117.   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).

118.   hNIS is a human gene naturally expressed in a range of cells (see paragraph 112). 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

119.   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

120.   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,  5/5 tumour samples at day 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, 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 sample 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.

121.   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 formations were observed in the treated mice.

122.   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 compared to human tissue.

123.   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   Host range of the GMO

124.   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.

125.   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.

## 4.5   Stability in the environment and decontamination

126.   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.

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

## 4.6   Pre-clinical studies using CF33-hNIS

128.   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.

129.   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).

130.   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).

## 4.7   Clinical trials using CF33-hNIS

131.   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 (Imugene) announced that a trial participant in the USA received the first dose of the GMO on 17 May 2022 (Imugene, 2022b). A similar GM cancer treatment, based on the CF33 parent organism and known as CHECKvacc (CF33-hNIS-antiPDL1), is being evaluated in a Phase 1, dose escalation clinical trial in the USA ([NCT05081492](#)). Imugene has reported the dosing of the first patient in cohort 3 of the clinical trial, with no toxicity observed in cohort 1 and 2 (Imugene, 2022a)

132.   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 ongoing ([www.clinicaltrials.gov](#) accessed on 02 June 2022).

## 4.8   Relevant information relating to Pembrolizumab

133.   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. The safety of the administration of Pembrolizumab in trial participants, medical staff or carers is not considered as part of this application as this is not a GMO. As mentioned in Chapter 1, Section 1.1, the TGA and the HREC would assess this aspect of the clinical trial.

# Section 5     The receiving environment

134.   The receiving environment forms part of the context for assessing risks associated with dealings with GMOs (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

135.   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.

136.   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)*.

137.   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

138.   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.

139.   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. Due to the ongoing monkeypox outbreak, VACV vaccination is currently recommended for close contacts of individuals infected with MPXV, high risk exposure groups and healthcare workers (Australian Government Department of Health 2022b).

140.   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.

141.   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*

142.   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.

143.   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).

144.   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).

145.   Orf virus (family *Poxviridae*, genus *Parapoxvirus*) is present in Australia and causes scabby mouth disease in sheep, goats and some other ruminant animals. The disease is characterised by the presence of pustules on the animal's mouth, muzzle, teats, legs or feet. It can be transmitted to humans via direct contact with the virus from lesions present in infected animals or fomites. The lesions typically resolve spontaneously in healthy individuals. However, immunosuppressed individuals exposed to orf virus may develop serious symptoms such as progressive disease (CDC, 2015; NSW Department of Primary Industries, 2016; Farrell and Stewart, 2022) .

146.   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.

147.   As of 23 August 2022, Australia has registered 102 cases of monkeypox infection, most of these cases were identified in travellers returning from overseas, however some infections have been acquired in Australia (Australian Government Department of Health, 2022b; NSW Health, 2022). Monkeypox is a 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, 2022b). 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 reported in many countries where monkeypox virus is not endemic (WHO, 2022a).

## Section 6      Previous authorisations

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

149.   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.

150.   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

151.  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.
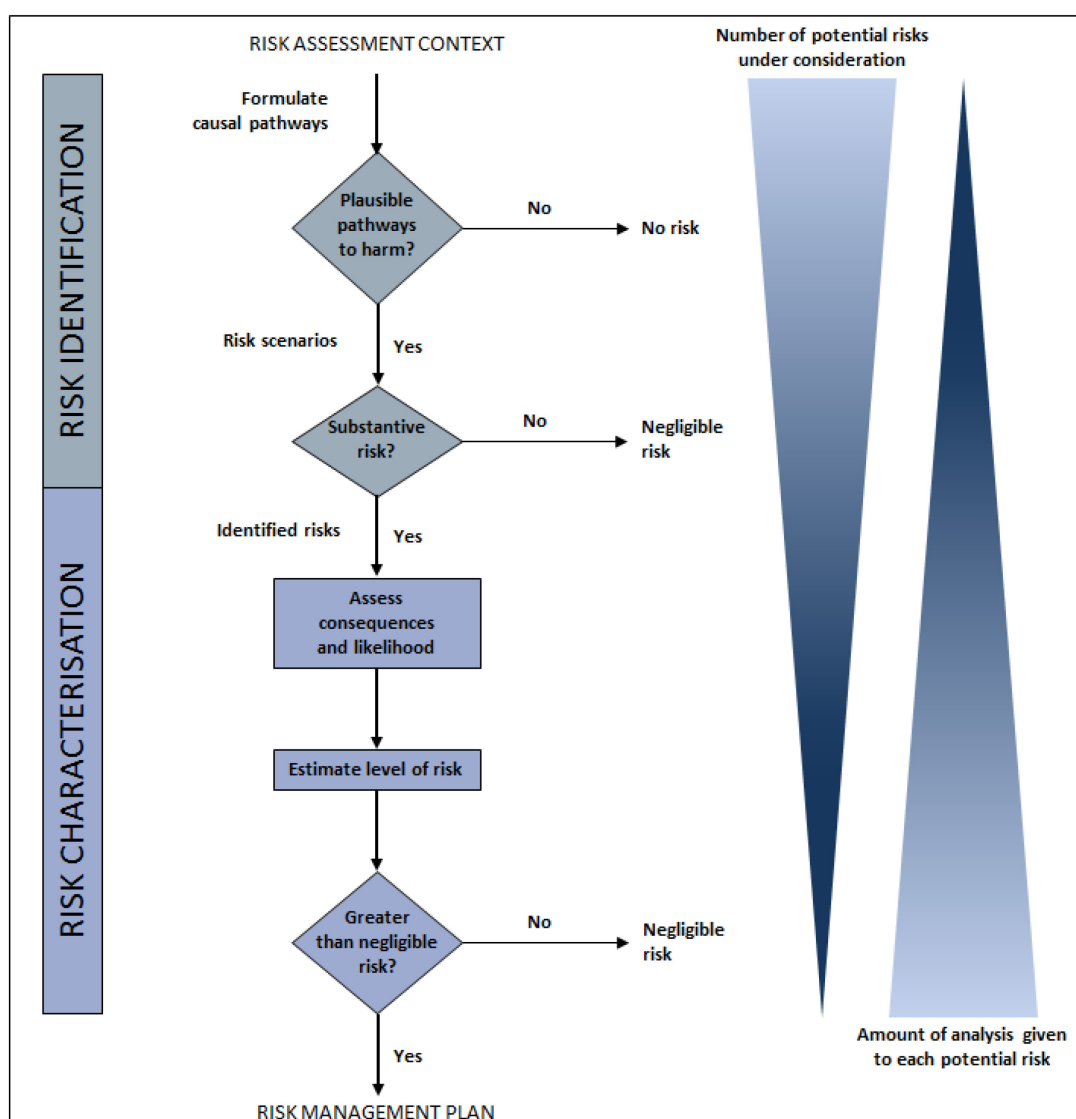


*Figure 4:  The risk assessment process*

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

153.  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.

154.  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.

155.  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

156.  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

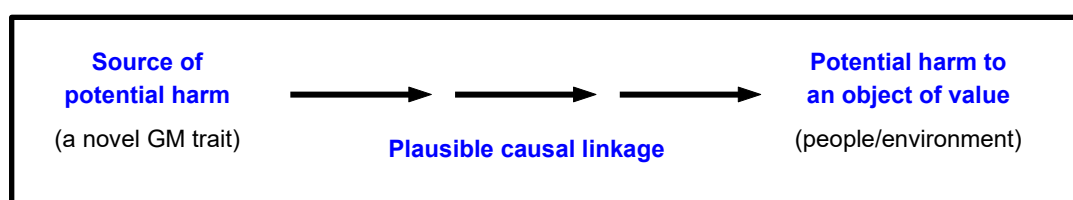  iii.  Potential harm to people or the environment.



*Figure 5: Components of a risk scenario*

157.  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

158.  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.

159.   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.

160.  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.

161.   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.

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

163.   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

164.   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.


165.   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.

166.   As discussed in Chapter 1, Section 1.1, the TGA, the trial sponsor, the Investigators and HRECs 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.

167.   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.

168.   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.

169.   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

170.  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

171.  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 2.4.1-2.4.3 (this chapter).

172.  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 the GM treatment**

| 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:<br><br>(a) needle stick/ sharps injury/ eye splash during GMO preparation, administration, or sample collection, analysis or preparation for export<br><br>(b) Contact with injection site and/or skin lesions<br><br>(c) Contact with GMO contaminated material<br><br>⬇<br><br>Infection of host cells, including healthy cells<br><br>⬇<br><br>Replication of the GMO and expression of the hNIS transgene<br><br>⬇ | 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.<br><br>• 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.<br><br>• High-risk personnel are excluded from handling the GMO.<br><br>• Sample testing would be conducted by qualified personnel in pathology or other testing laboratories. |

| Risk scenario | Risk source | Possible causal pathway | Potential harm | Substantive risk | Reason |
|---|---|---|---|---|---|
| | | Further transmission to people or animals | | | • Accidental exposure of personnel to the GMO would be documented and the person would receive medical attention and would be monitored for symptoms.<br><br>• Personnel would be instructed to cover pustules should they occur and avoid contact with high-risk groups and animals.<br><br>• 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 trial studies. |
| 2 | GMO | Treatment of trial participant with the GMO<br><br>⬇<br><br>GMO is shed at the injection sites or in body fluids (such as blood, urine, semen and vaginal secretions)<br><br>⬇<br><br>Exposure of people (e.g. carers or household contacts), or animals (e.g. domestic pets, livestock and wildlife) through contact with trial participant or contaminated items (e.g. contaminated dressings) outside the clinical/hospital setting<br><br>⬇<br><br>Infection of host cells, including healthy cells | Clinical symptoms ranging from mild to severe (e.g. flu-like illness, formation of lesions, severe adverse reactions)<br><br>Establishment of VACV infection in animals | No | In addition to the reasons described in scenario 1:<br><br>• 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.<br><br>• 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.<br><br>• Trial participants would be instructed to wear gloves when removing/changing |

| Risk scenario | Risk source | Possible causal pathway | Potential harm | Substantive risk | Reason |
|---|---|---|---|---|---|
| | | ⬇<br><br>Replication of the GMO and expression of the hNIS transgene<br><br>⬇<br><br>Further transmission to people or animals | | | 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.<br><br>• In the event of pustule formation, trial participants would be instructed to cover pustules, appropriately dispose any bandages and avoid contact with animals and immunocompromised individuals.<br><br>• 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.<br><br>• If exposure occur, 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<br><br>⬇<br><br>The GMO mutates, self-recombines or recombines with another virus co- | Establishment of novel virus with unknown pathogenicity in the environment | No | • The GMO is expected to be cleared by the immune system within weeks and not spread to other hosts.<br><br>• VACV is not present in the Australian environment. |

| Risk scenario | Risk source | Possible causal pathway | Potential harm | Substantive risk | Reason |
|---|---|---|---|---|---|
| | | infecting the trial participant<br><br>⬇<br><br>Produces a mutant/ recombinant virus strain<br><br>⬇<br><br>Mutant/recombinant virus is shed<br><br>⬇<br><br>Mutant/recombinant virus infects new hosts or is more virulent | | | Additionally, there is limited opportunity for the GMO to come into contact with other related poxviruses.<br><br>• 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.<br><br>• Viral factory compartmentalisation adds a further barrier for recombination.<br><br>• 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. |

### 2.4.1 *Risk scenario 1*

| Risk source | GMO |
|---|---|
| **Causal pathway** | Exposure of people undertaking dealings in clinical trial facilities to GMO via:<br><br>(a)  needle stick/ sharps injury and/or eye splash during GMO preparation, administration, or sample collection and analysis<br><br>(b)  Contact with injection site and/or skin lesions<br><br>(c)  Contact with GMO contaminated material<br><br>⬇<br><br>Infection of host cells, including healthy cells<br><br>⬇<br><br>Replication of the GMO and expression of the hNIS transgene<br><br>⬇<br><br>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**

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

**Causal Pathway**

174.   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*

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

176.   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. Biological samples would be analysed or prepared for export by qualified personnel in clinical trial sites or pathology laboratories. 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).

177.   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*

178.   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.

179.   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.

180.   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*

181.   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).

182.   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**

183.   If people are exposed to the GMO, 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 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**

184.   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<br>⬇<br>GMO is shed at the injection sites or in body fluids (such as blood, urine, semen and vaginal secretions)<br>⬇<br>Exposure of people (e.g. carers or household contacts), or animals (e.g. domestic pets, livestock and wildlife) through contact with trial participant, body fluids or contaminated items (e.g. contaminated dressings) outside the clinical/hospital setting<br>⬇<br>Infection of host cells, including healthy cells<br>⬇<br>Replication of the GMO and expression of the hNIS transgene<br>⬇<br>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**

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

**Causal Pathway**

186.   As discussed in Chapter 1, Section 4.1.1, the GMO has been modified by deleting the TK enzyme and relies on nucleotides (dTTP) produced by the host cells to replicate. TK levels is elevated in many cancer cells. It is also expressed in a range of healthy dividing cells but not in resting cells. Therefore, the GMO lacking TK enzyme is expected to replicate preferentially in cancer cells and to a lesser extent in healthy dividing cells.

187.    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*

188.   As described in Paragraph 80, VACV can be 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 increase biodistribution and shedding of the GMO such as generalised and/or progressive vaccinia and eczema vaccinatum. These individuals are excluded from the clinical trial.

189.   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 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 183, 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. The acquired immune response, developed by the trial participant following multiple doses of the treatment, would limit the presence of the GMO in their body and body fluids. Additionally, trial participants would be instructed to use barrier contraception should they be sexually active during the treatment and for at least 60 days after the last GMO treatment. This measure would minimise the potential of transmission of the GMO via exposure to body fluids such as semen and vaginal secretion.

190.   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*

191.   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 instructions provided to trial participants during the initial screening as part of the informed consent. Trial participants would also be expected to seal contaminated disposable items in the primary container provided (e.g. press-sealed bag) and then place this into the secondary container provided (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.

192.   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.

193.   As described in paragraph 133, 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 191, a pustule management plan would be employed in this scenario.

194.   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 13.5.2). As described in paragraph 144, 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 191, trial participants would be instructed to manage pustules and contaminated waste properly and avoid close contact with pets and other animals. In addition, trial participants will be instructed to inform the clinical trial site and/or study doctor if they suspect that transmission via physical contact with a lesion to another person or to an animal may have occurred. The study doctor will provide instructions to the exposed individual as to whether further medical evaluation is needed. If an animal has been potentially exposed, the trial participant will be instructed to contact a veterinary for further guidance. These measures should minimise the likelihood of GMO transmission to animals.

**Potential harm**

195.  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 replicates poorly in non-human cells (see paragraphs 71 and 122).

**Conclusion**

196.  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 ⬇ The GMO mutates, self-recombines or recombines with another virus co-infecting the trial participant ⬇ Produces a mutant/ recombinant virus strain ⬇ Mutant/recombinant virus is shed ⬇ Mutant/recombinant virus infects new hosts or is more virulent |
| Potential harm | Establishment of novel virus with unknown pathogenicity in the environment |

**Risk source**

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

**Causal Pathway**

198.   Mutation and self-recombination occur over time following repeated transmission of viruses to other hosts. Both events are unlikely to occur in this clinical trial as the GMO is expected to be contained within the trial participant and be cleared by the immune system within weeks following administration.

199.   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*

200.   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 199, 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*

201.   Recombination is more likely to occur between closely related viruses (see paragraph 199). 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.

202.   As described in Chapter 1, Section 5.2.1, poxviruses such as molluscum contagiosum virus (MCV), monkeypox virus (MPXV), orf virus, 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.

203.   There is an ongoing outbreak of MPXV in Australia. As mentioned in paragraph 147, most cases of monkeypox infection in Australia were identified in travellers returning from overseas. Although a few cases of locally acquired monkeypox have been reported, the virus spreads through close contact and the likelihood of spreading to the environment is very low. VACV based vaccines offers about 85% protection against monkeypox disease and can be given before or within 14 days after exposure to the MPXV. Vaccination within 4 days from the exposure date may prevent the onset of the disease, however, if given between 4–14 days after the date of exposure the vaccine may reduce the symptoms of disease but may not prevent the disease (WHO, Australian Government Department of Health 2022b; 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. In addition, natural recombination

between MPXV and other viruses have not been reported (Alakunle et al., 2020). Thus, recombination between MPVX and the GMO is unlikely to occur even if a co-infection is established. In the unlikely event of recombination between the GMO and MPXV, the resulting virus is unlikely be more pathogenic than the parent organism or the MPXV.

204.   Myxoma virus is likely to be found in rabbits. as discussed in paragraph 146. 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. As mentioned in paragraph 145, orf virus can be found in sheep and goats. Recent analysis showed that orf virus is more closely related to viruses belonging to the genus *Parapoxvirus* than to orthopoxviruses. Thus, recombination between orf virus and the GMO is highly unlikely (Sahu et al., 2022). Other uncharacterised poxviruses are found in Australian wildlife (see paragraph 144) but wildlife is unlikely to come into contact with trial participants.

205.   As mentioned in paragraphs 89 and 199, 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 90).

**Potential harm**

206.   Mutation and self-recombination events could lead to viral adaptation to other hosts. These events are unlikely to restore the *J2R* locus and the resulting virus would still depend on an external source of dTTP to replicate. In addition, the resulting virus is unlikely to be more pathogenic than a viral strain resulting from mutation or self-recombination of the parent organism.

207.   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 124). 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**

208.   The potential for an adverse outcome as a result of mutation, self-recombination or 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

209.   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.

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[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.

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

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

211.   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.

212.   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.

213.   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 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.

214.   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.

215.   The uncertainties outline above have been addressed in the risk assessment by conservative assumptions including the possibility that the GMO is able to infect and replicate in healthy cells and assuming that the GMO can be shed by trial participants and infect other people or animals. After taking this uncertainty into account, all risk scenarios were estimated as negligible.

## Section 4     Risk evaluation

216.   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.

217.   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.

218.   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.

219.   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 clinical trial do not pose a significant risk to either people or the environment.

# Chapter 3     Risk management plan

## Section 1     Background

220.  Risk management is used to protect the health and safety of people and to protect the environment by controlling or mitigating risk. The risk management 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.

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

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

223.  The licence is also subject to any conditions imposed by the Regulator. Examples of the matters to which conditions may relate are listed in Section 62 of the Act. Licence conditions can be imposed to limit and control the scope of the dealings. 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

224.  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

225.   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, licence conditions have been imposed to limit the number of trial participants, limit the location to hospitals and clinical trial sites, limit the duration of the trial, as well as a range of controls to restrict the spread and persistence of the GMO and its genetic material in the environment. The conditions are discussed and summarised in this Chapter and listed in detail in the licence.

### 3.1   Limits and controls on the clinical trial

226.   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*

227.   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 licence.

228.   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 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.

229.   There is an ongoing multi-country outbreak of monkeypox virus and local transmission of the virus has been reported. Therefore, as a precaution, the licence requires the licence holder to provide a written methodology to reliably detect the GMO, or the presence of the genetic modifications described in this licence in a person. The written methodology must be provided to the Regulator at least 14 days prior to first administering the GMO and must be capable of differentiating the GMO from the strain(s) of monkeypox virus currently circulating in Australia.

230.   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, 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 imposed 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.

231.   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 licence to reflect this.

232.   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. However, as mentioned in paragraph 231, 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 secretions. A condition has been included in the licence to reflect this.

233.   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 licence.

234.   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 included in the licence.

235.   Conditions are included in the 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. 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 236.

236.   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 imposed, 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 GMO.

237.   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, animals or birds to the GMO should pustules develop. A condition has been included in the licence to reflect this.

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

239.   A standard condition is included in the 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.

240.   Other conditions included in the 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.

241.   Further conditions imposed in the 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*

242.   A number of licence conditions have been imposed 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 licence and import in accordance with IATA shipping classification UN 3373.

## 3.2   Other risk management considerations

243.   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*

244.   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.

245.   On the basis of information submitted by the applicant and records held by the OGTR, The Regulator considers Medpace suitable to hold a licence.  The licence includes a requirement for the licence holder to inform the Regulator of any information that would affect their suitability.

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

### 3.2.2 *Contingency plans*

247. As per licence conditions, 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*

248. 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*

249. 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.

250. 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*

251. 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.

252. 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.

253. 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

254. 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
- data regarding the formation of pustule at the injection site or any other location on the body.

# Section 5    Conclusions of the RARMP

255.   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.

256.   Conditions are imposed to limit the number of trial participants, location and duration, and to minimise the potential 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.

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# Appendix A: Summary of submissions from prescribed experts, agencies and authorities on the consultation RARMP

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

| Submission | Summary of issues raised | Comment |
|---|---|---|
| 1 | The Regulator should further consider: | |
| | • the terminology around replication competency of the GMO and its ability to replicate in non-cancerous cells | In the risk assessment the GMO has been considered as a replication competent virus. The RARMP has been amended to clarify the terminology around the competency of the GMO (Risk Scenario 2). As mentioned in Chapter 2, Section 3 of the RARMP, there are uncertainties regarding the preferential replication of the GMO in cancer cells over healthy cells and the transferability of data gathered in non-clinical studies to humans. These uncertainties were taken into account in the RARMP by considering the possibility that the GMO could infect and replicate in healthy cells and assuming that the GMO could be shed by trial participants and infect other people or animals (Risk Scenario 2). |
| | • the risks posed by resheathing needles | The risk of exposure of people undertaking dealings in clinical trial facilities to the GMO via needle stick injury was addressed in Risk scenario 1. The clinical trial will take place in hospitals and clinical trial sites. Preparation and administration of the GMO will be performed by experienced and trained health professionals. Personel working in these settings 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). These standard precautions would minimise the risk of exposure to the GMO. Licence conditions are imposed to reflect this. In addition, licence conditions exclude high-risk individuals from conducting dealings with the GMO minimising harm in the event of a needle |

| Submission | Summary of issues raised | Comment |
|---|---|---|
| | | stick injury occuring and a person being exposed to small quantity of GMOs. |
| | Agrees: | |
| | • that the proposed limits and controls are appropriate. | Noted |
| | • with the overall conclusion of the RARMP. | Noted |
| 2 | Agrees with main conclusions of the RARMP which states that the proposed clinical trial poses negligible risks to human health and safety and the enviroments. | Noted |
| | Requested amendments to the RARMP to include additional control measures: | |
| | • patients must avoid the exposure of pets, livestock or wildlife to unhealed vaccination sites or to material or surfaces contaminated with fluid from a vaccination site. | Chapter 1, Section 2.2 of the RARMP has been amended to clarify that the applicant proposed that trial participants will be instructed to avoid exposing animals (pets, livestock and wildlife) to injection sites, pustules and fomites. This is part of the pustule management plan (Chapter 3 Section 3.1.1.) and has been included in the licence conditions. |
| | • patients should provide a written declaration following each treatment that they will avoid contact with animals for at least 24 hours after treatment administration or until after any injection site lesions have healed and; they will comply with the proposed hygeine practices to avoid the exposure of other people and animals to the GMO. | Licence conditions require the licence holder to obtain the trial participant's written agreement that, while undergoing treatment, they will implement hygiene measures to limit transmission of the GMO and will avoid contact with animals and high risk individuals for at least 7 days after each treatment or any time pustules are present. This agreement must be signed by the trial participant before receiving the first dose of the GMO. |
| 3 | Raised concerns regarding: | |
| | • the detection of the GMO in assays currently used for detection of monkeypox virus and other orthopoxviruses. Suggested making the sequence of the GMO available through the National Center for Biotechnology Information (NCBI) which could be used to differentiate these two different viruses. | The RARMP has been amended and a licence condition included requiring the licence holder to provide the Regulator with a written methodology to reliably detect and differentiate the GMO from the strain(s) of monkeypox virus currently circulating in Australia at least 14 days prior to administering the GMO to any trial participant. This would ensure there is a detection assay available on request to differentiate the GMO from the |

| Submission | Summary of issues raised | Comment |
|---|---|---|
| | | current monkeypox virus strain circulating in Australia. |
| | • the administration of the GMO combined with Pembrolizumab could lead to increased severity of side effects of autoimmunity. | This assessment has taken into consideration the effect of the co-administration of the GMO with Pembrolizumab (increase shedding of GMO by the trial participant). The assessment from the OGTR, while considering some aspects of the safety of the GMO as a treatment, is conducted by considering the likelihood of exposure of medical staff, carers and animals, and evaluating the harm resulting from this exposure. Risks associated with the safety of trial participants receiving the GMO are assessed by other agencies and authorities. As discussed in Chapter 1, Section 1.1, the TGA, the trial sponsor, the Investigators and HRECs 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). |
| 4 | Raised concerns about the considerable uncertainty and a lack of data regarding this novel GM poxvirus that does not clearly support the conclusion that 'uncertainty is considered low'. The GM virus can replicate in normal cells and preferential replication in cancer cells was demonstrated in mice only and as mentioned in the RARMP this data may not be transferrable to humans. There is limited human and host range data. | The consultation RARMP considered the uncertainties associated with the GMO by considering the GMO as replication competent in cancer cells as well as in healthy cells (Risk Scenario 2). Chapter 2, Section 3 of the RARMP which discusses uncertainty has been amended to clarify the conservative assumptions made in the risk assessment.<br><br>As mentioned in Chapter 1,Section 4.4, the GMO is expected to infect the same range of hosts as the unmodified VACV. Licence conditions are imposed to minimise the risk of exposure of other people and animals to the GMO. |
| | Recommended amendments to: | |
| | • the RARMP to include controls to limit exposure of animals to the GMO by specifically instructing the participants to avoid contact with domesticated and native animals and birds. | Chapter 1, Section 2.2 of the RARMP has been amended to clarify that the applicant proposed that trial participants will be instructed to avoid exposing animals (pets, livestock, birds and wildlife) to injection sites, pustules and fomites. Licence conditions have been amended to include a condition requiring trial participants to agree in writing to avoid contact with all |

| Submission | Summary of issues raised | Comment |
|---|---|---|
| | | animals for at least 7 days after each treatment or any time pustules are present. |
| | • the licence conditions to request the licence holder, prior to administration of the GMO, to collect information from participants about all possible animal contacts and inform participants of the uncertainty and risks to animals posed by the novel GM poxvirus. | Licence conditions require the licence holder to obtain the trial participant's written agreement that while undergoing treatment they will:<br><br>• implement hygiene measures to limit transmission of the GMO to other people and animals;<br><br>• inform the clinical trial site if they suspect that transmission via physical contact with a lesion to another person or to an animal may have occurred.The study doctor will provide instructions to the exposed individual as to whether further medical evaluation is needed. If an animal has been potentially exposed, the trial participant will be instructed to contact a veterinary for further guidance. |
| | • the RARMP to request the applicant to collect data on replication, biodistribution and shedding in humans during the clinical trial and information regarding the virulence and animal host range of the GM poxvirus. | The applicant has proposed to collect biological samples from the clinical trial participants. These samples will be analysed for the presence of the GMO genome or viable viral particles. More details are included in Chapter 1, Section 2.3.7.<br><br>The genetic modifications made to the parent organism are not expected to impact the host range of this virus. However, as part of licence conditions, the licence holder is required to inform the Regulator of any new information that may arise during the trial affecting the risk context established at the time of submission. Any serious adverse event would also be reported to the Regulator, including an increase in virulence of the GMO compared to the parent organism. |
| | Commented about the overall role of recombination events in the evolution of poxviruses and their host range. | Chapter 1, Section 3.6 has been expanded to discuss the role of mutation and recombination events in the evolution of poxviruses. The risks of mutation, self-recombination and recombination between the GMO and other poxviruses were addressed in Risk Scenario 3. |

| Submission | Summary of issues raised | Comment |
|---|---|---|
| 5 | Raised concerns regarding recombination between poxviruses. Suggested the exclusion of individuals diagnosed with monkeypox infection within 4 weeks prior to the first dose of the GMO to minimise the risk of recombination. "The document argues that monkeypox infection is likely to generate immunity against the GMO but this immunity takes time - hence the four week exclusion period". | Recombination events were addressed in Chapter 1, Section 3.6 and Risk Scenario 3. Risk scenario 3 has been expanded to include that natural recombination events have not been reported for monkeypox virus (MPXV) and are unlikely to occur between MPXV and the GMO. |
|  | Inquired whether orf virus (ORFV) was considered in the consultation RARMP. Stated that ORFV causes scabby mouth in sheep and goats, and ecthyma contagiosum in humans and is reasonably common in Australia. | The RARMP has been amended to include the orf virus as a poxvirus present in the Australian environment (Chapter 1, Section 5.2.1), and Risk Scenario 3 amended to include potential recombination between the GMO and this endemic virus. This recombination was determined to be highly unlikely due to the viruses being in diferent genera. However, a licence condition has been included to limit the trial participants contact with animals for at least 7 days after receiving the GMO treatment and any time pustules are present. This measure should minimise the risks of co-infection of the trial participants or animals with the GMO and orf virus. |
| 6 | Raised a number of concerns regarding the safety of trial participants, including:<br><br>• "CF33 virus is largely uncharacterized in humans" and the safety of VACV vaccines may not be applicable to this strains;<br><br>• "many cancer patients are immunosuppressed and may react differently from healthy individuals";<br><br>• "a large part of the CF33 genome is derived from the Vaccinia WR strain which is much less mild than the standard DryVax strain used as the main immunization against lab infections and monkeypox in Australia";<br><br>• the risks of autoimmune disease induced by the GMO transgene. | This assesment has taken into consideration the risks posed by the unintentional exposure of people (other than the trial participant) to the GMO. The GMO and a similar GM treatment based on the CF33 strain are being evaluated in Phase I clinical trials in the USA with no toxicity observed to date (see Chapter 1, Section 4.7). The assessment from the OGTR, while considering some aspects of the safety of the GMO as a treatment, is conducted by considering the likelihood of exposure of medical staff, carers and animal and evaluating the harm resulting from this exposure. Risks associated with the safety of trial participants receiving the GMO are assessed by other agencies and authorities. As discussed in Chapter 1, Section 1.1, the TGA, the trial sponsor, the Investigators and HRECs 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). |

| Submission | Summary of issues raised | Comment |
|---|---|---|
| | Commented on the risk of exposure of close contacts (medical practitioners and family members) to the GMO and the limited data regarding the GMO transmissibility. Stated that "cancer patients are likely to be immunosuppressed and so harbour larger viral loads than, for example, DryVax immunized otherwise healthy individuals. As such there is a small risk of high levels of virus shedding from a subset of patients." | The risk of exposure of medical staff and close contacts to the GMO was addressed in Risk scenario 1 and Risk Scenario 2. Licence conditions are imposed to minimise the risk of GMO transmission to health care personnel, close contacts and animals. |
| | Stated that "In addition to transmission from the patient the other possible source of risk to the environment comes from needle stick injuries of medical practitioners. It is unclear about whether it was proposed to vaccinate those individuals with VACV which would likely be sufficiently cross reactive to provide protection." | The risk of exposure of people undertaking dealings in clinical trial facilities to GMO via needle stick injury was addressed in Risk scenario 1. This risk scenario considers the risk to health care personnel that have not received VACV vaccines. However, the preparation and administration of the GMO will be performed by experienced and trained health professionals. 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). These standard precautions would minimise the risk of exposure to the GMO. Licence conditions are imposed to reflect this. In addition, licence conditions exclude high-risk individuals from conducting dealings with the GMO, minimising harm in the event of a needle stick injury occuring and a person being exposed to small quantity of GMOs. |
| 7 | The members agree that the range of controls to minimise the potential for the GMO to spread in the environment and that unintended exposure to the GMOs would be minimised by the limits and controls proposed in this RARMP. Supports the decision that there is no requirement for specific risk treatment measures with the use of this GMO as proposed in this application. | Noted |
| 8 | Stated that there is no need for comments if Council is not required to support the Clinical trial within their facilities at this stage. | Noted |

# Appendix B: Summary of submissions from the public on the consultation RARMP

The Regulator received 1 submission from the public on the consultation RARMP. The issue raised in the submission is summarised in the table below. All issues that related to risks to the health and safety of people and the environment were considered in the context of currently available scientific evidence in finalising the RARMP that formed the basis of the Regulator's decision to issue the licence.

| Submission | Summary of issues raised | Comment |
| --- | --- | --- |
| 1 | The submitter supports the approval of the clinical trial and stated it could be a game-changing cancer treatment. | Noted |