Australian Government
Department of Health
Office of the Gene Technology Regulator

April 2021

Risk Assessment and Risk Management Plan

for

DIR 179

Clinical trial with a genetically modified *Vaccinia virus* based treatment for solid cancerous tumours

Applicant – Novotech (Australia) Pty Limited

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Summary of the Risk Assessment and Risk Management Plan
for
Licence Application No. DIR 179

Decision

The Gene Technology Regulator (the Regulator) has received a licence application to conduct a clinical trial using a genetically modified organism (GMO). It qualifies as a DIR licence application under the *Gene Technology Act 2000* (the Act).

The applicant, Novotech (Australia) Pty Limited (Novotech), proposes to conduct a clinical trial to evaluate the safety and efficacy of genetically modified (GM) *Vaccinia virus* known as TBio-6517, alone and in combination with an existing cancer therapy (Pembrolizumab), for the treatment of Australian patients with advanced solid cancerous tumours.

Vaccinia virus has been used more extensively for human immunisation than any other vaccine and was employed to provide cross-protection against smallpox, until the disease was declared eradicated in 1980. The proposed GM *Vaccinia virus* has been designed to preferentially multiply in, and kill cancer cells. The GM *Vaccinia virus* would be manufactured overseas and imported into Australia. It would be administered by intratumoural injection or by intravenous infusion in up to 150 Australian patients with advanced solid cancerous tumours at clinical facilities 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, Novotech would 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. Novotech would also require approval from the Department of Agriculture, Water and the Environment for import of the GM treatment.

The Regulator has prepared a Risk Assessment and Risk Management Plan (RARMP) for this application, which concludes that the proposed clinical trials pose 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 clinical trial.

The application

Project Title	Clinical trial with a genetically modified *Vaccinia virus* based treatment for solid cancerous tumours[1]
Parent organism	*Vaccinia virus* (Copenhagen strain)
Principal purpose	The proposed trial is a Phase 1/2a study designed to evaluate the safety and efficacy of genetically modified (GM) *Vaccinia virus*, known as TBio-6517, alone and in combination with Pembrolizumab, an existing cancer therapy, for the treatment of Australian patients with advanced solid cancerous tumours
Genetic modifications	Modified *Vaccinia virus* (Copenhagen strain) Deletion and disruption of multiple genes[2] including virulence factors - leading to improved destruction of human tumour cells Introduction of genes conferring enhanced immune response: • Anti-Cytotoxic T-lymphocyte-Associated protein 4 (anti-CTLA-4) antibody • FMS-like tyrosine kinase 3 ligand (FLT3L) • Membrane-bound interleukin-12 p35 subunit (IL-12p35) Together, these introduced genes stimulate the human immune system allowing improved detection and destruction of cancerous tumour cells
Previous clinical trials	A Phase 1/2a trial is currently being conducted in the United States
Proposed limits and controls	
Proposed duration	5 years
Proposed release size	Up to 150 clinical trial participants in Australia
Proposed locations	The proposed trial would be conducted at a number of hospitals and clinics across Australia but the exact sites are yet to be identified
Proposed controls	• Transport and storage of the GMO that are appropriate for risk group 2 organisms • Require staff handling the GMO to be trained and to use personal protective equipment • Higher-risk staff are excluded from handling the GMO • Destroy waste that may contain GMO according to clinical site procedures appropriate for risk group 2 organisms • Provide patients with detailed instructions regarding the care of any skin-related reactions post-treatment and the use of good hygiene practices

[1] The title of the project as supplied by the applicant is 'Clinical trial with an oncolytic vaccinia virus vaccine (TBio-6517)'.

[2] Confidential Commercial Information: Some details about the genes deleted in GM *Vaccinia virus* have been declared as Confidential Commercial Information (CCI) under Section 185 of the Act. Relevant CCI was made available to the prescribed experts and agencies that were consulted on the RARMP for this application.

Risk assessment

The risk assessment concludes that risks to the health and safety of people and 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, taking into account information in the application (including proposed controls), relevant previous approvals and current scientific/technical knowledge. Both the short and long term impact are considered.

Credible pathways to potential harm that were considered included exposure of people or animals to the GMOs, and whether there is the potential for reassortment with other viruses. Potential harms that were considered in relation to these pathways included ill health and increased disease in people or animals.

Important factors in reaching the conclusions of the risk assessment included that the GM *Vaccinia virus* treatment is designed to selectively 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 GM *Vaccinia virus* 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 plan

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

anti-CTLA-4 antibody	Anti-Cytotoxic T-lymphocyte-associated protein 4 antibody
°C	Degrees Celsius
CCI	Confidential Commercial Information under Section 185 of the *Gene Technology Act 2000*
CDC	Centers for Disease Control and Prevention
DAWE	Department of Agriculture, Water and the Environment
DIR	Dealings involving Intentional Release
DNA	Deoxyribonucleic acid
FLT3L	FMS-like tyrosine kinase 3 ligand
GM	Genetically modified
GMO	Genetically modified organism
IL-12	Interleukin-12
IL-12p35	Membrane-bound version of interleukin-12 with p35 subunit
kb	kilobase
HREC	Human Research Ethics Committee
IATA	International Air Transport Association
IBC	Institutional Biosafety Committee
ICH-GCP	*Guidelines for Good Clinical Practice* of the International Council for Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use
i.t.	Intratumoural
i.v.	Intravenous
NLRD	Notifiable Low Risk Dealings
NYCBH	New York City Board of Health
OGTR	Office of the Gene Technology Regulator
OPV	*Orthopoxvirus*
pfu	pock-forming units; plaque forming units
PPE	Personal protective equipment
RARMP	Risk Assessment and Risk Management Plan
Regulations	Gene Technology Regulations 2001
Regulator	Gene Technology Regulator
SKV	Genetically modified backbone of the Copenhagen strain of *Vaccinia virus*
TBio-6517	GM *Vaccinia virus* proposed to be used as a treatment in patients with solid cancerous tumours
TGA	Therapeutic Goods Administration
the Act	*Gene Technology Act 2000*
TSD	Regulator's Guidelines for the Transport, Storage and Disposal of GMOs
USA	United States of America
VACV	*Vaccinia virus*
VIG	Vaccinia immunoglobulin
WHO	World Health Organisation

Chapter 1 Risk assessment context

Section 1 Background

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

2. The Act 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* (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, in establishing the risk assessment context. This information is specific for each application. Potential risks to the health and safety of people or the environment posed by the proposed release are assessed within this context. Chapter 1 provides the specific information for establishing the risk assessment context for this application.

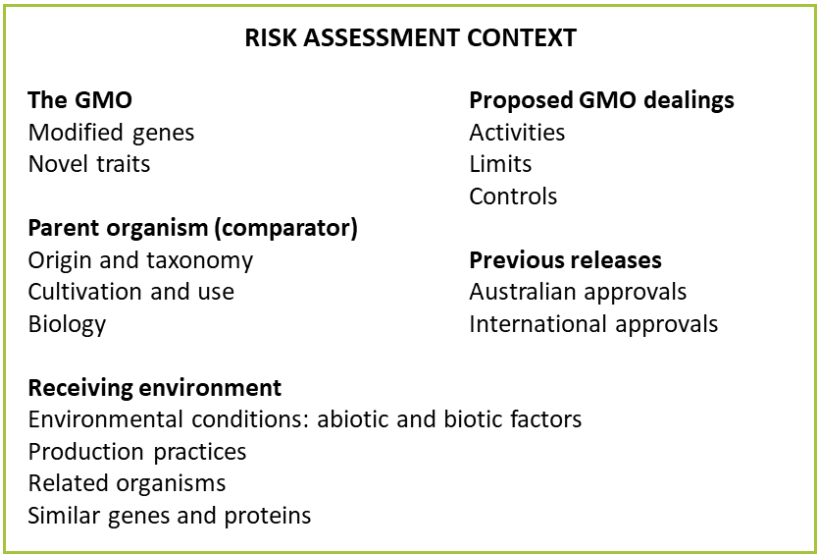

Figure 1. Summary of parameters used to establish the risk assessment context, within the legislative requirements, operational policies and guidelines of the OGTR and the RAF.

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 consultation 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 submissions 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 Chemical Introduction Scheme (AICIS) and the Department of Agriculture, Water and the Environment (DAWE).

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 participant's 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 GMO, 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, 2016). The guideline was developed with consideration of the current good clinical practices of the European Union, 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 *Integrated addendum to ICH E6(R1): Guideline for good clinical practice E6(R2)* (Therapeutic Goods Administration), 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 investigational product accounting and reconciliation.

14. The DAWE regulates products imported into Australia to protect Australia from biosecurity risks. Under the *Biosecurity Act 2015*, the importation of biological material such as live GM vaccines and treatments requires a permit from DAWE.

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.

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 (APA). 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. Novotech (Australia) Pty Limited (Novotech) has proposed Phase1/2a clinical trials of a live GM *Vaccinia virus* (TBio-6517) that has been designed to preferentially replicate in cancer cells. The purpose of the clinical trials is to assess the safety and efficacy of the GM treatment in patients with advanced solid cancerous tumours, alone and in combination with Pembrolizumab, an existing immunotherapy drug.

20. The GM treatment would be manufactured overseas and imported into Australia. It would be administered to patients over 18 years of age with advanced solid cancerous tumours by intratumoural (i.t.) injection or intravenous (i.v.) infusion. Samples that may contain GMOs would be collected from the trial participants for analysis in laboratories within Australia or exported overseas.

21. The dealings involved in the proposed clinical trials are:

(a) import the GMOs;

(b) conduct the following experiments with the GMOs:

i. prepare the GMO for administration to trial participants;

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

iii. collect samples from trial participants;

iv. analyse the samples described in 21(b)iii;

(c) transport the GMOs; and

(d) dispose of the GMOs;

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)

22. The clinical trial is proposed to take place over a five-year period from the date of issue of the licence. Up to 150 patients in Australia would receive four doses over a period of 24 months. Additional booster doses may be administered upon the discretion of the principal investigator.

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

24. Only trained and authorised staff would be permitted to conduct dealings with the GMO. Administration of the GMO in trial participants would be conducted by highly trained medical staff. Staff considered to be at high-risk (see Paragraph 38) are excluded from handling the GMO.

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

25. 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:

- ensuring the GM treatment is administered by authorised, appropriately trained medical staff in clinical facilities;
- requiring that clinical trial staff handling and/or administering the GM treatment wear and use personal protective clothing and equipment;
- transport and storage of the GMO and any contaminated waste generated at a clinical trial site must be accordance with the current version of the Regulator's *Guidelines for the Transport, Storage and Disposal of GMOs;*
- requiring decontamination of materials and equipment that have been in contact with the GMOs 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;
- providing patients with treatment instructions, including instructions should suspicious skin pustules develop, and providing instructions to patients about good hand hygiene and cough etiquette practices.

26. The applicant has proposed detailed control measures should trial participants develop vaccinia-related pustules/skin lesions[3] as this may be one of the symptoms of *Vaccinia virus* administration (Cono et al., 2003). Based on non-GM *Vaccinia virus*, pustules would likely appear around 1 week following the first treatment and would likely resolve within 2 to 4 weeks. If a patient develops a

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

suspicious skin pustule, a swab would be collected within 5 days of the occurrence for analysis and followed until resolution. The following would be implemented to minimise the potential for spread:

- All patients would be educated on the care and management of a pustules and instructed to avoid high-risk individuals (see Paragraph 38), including children under 12 months of age. Pustule management includes:
    - covering the pustule with a dressing (or wearing a mask when around other people if oral lesions are present)
    - avoiding accidental autoinoculation by direct contact with the pustule(s) and other parts of the body (e.g. nose, ears, eyes, or other areas). In particular, not touching the pustule and then touching their eyes until hands are thoroughly washed
    - laundering sheets and clothing in hot soapy water daily.
- All patients upon their first i.t. injection or i.v. infusion would receive a 'pustule' kit with 3 weeks' worth of bandages, gloves, zip-lock bags, alcohol swabs, masks and gauze, as well as a biohazard bin to take home in the event of pustule formation.
- The patient would be instructed to change the dressing privately, unless they require assistance from a caregiver, and limit access to any pets (particularly dogs), animals, or higher-risk individuals (see Paragraph 38). If an animal or other person develops a suspicious rash, this would be reported and may be examined by the clinical trial investigator.
- These pustule kits and biohazard bins are logged in an accountability style log. If trial participants develop a pustule, the participants would put the contaminated dressings into the provided zip-lock bag, and the zip-lock bag into the provided biohazard bin for return to the hospital/clinical trial site for appropriate disposal.
- Patients would be instructed to bring their biohazard bins to every visit until the last visit to the clinical trial site.

2.3 Details of the proposed dealings

2.3.1 Manufacture of the GMO

27. The GMO[4] (TBio-6517) would be manufactured overseas in accordance with applicable Good Manufacturing Practice (GMP) regulations.

2.3.2 Conduct of the clinical trial

28. The international sponsor for the trial is Turnstone Biologics, Corp. (Turnstone), which is headquartered in Canada. Novotech, is applying for authorisation to conduct the proposed clinical trial in Australia and as a clinical research organisation (CRO) for this application, Novotech would also be responsible for ensuring that the licence conditions are met.

29. There are two phases proposed in this clinical trial, dose escalation (Phase 1) and dose expansion (Phase 2a). Each phase would consist of four experimental arms:

- Arm A – i.t. injection with the GMO alone
- Arm B – i.t. injection with the GMO in combination with pembrolizumab (200 mg via i.v. every 3 weeks beginning at Day 8)
- Arm C – i.v. infusion with the GMO alone
- Arm D – i.v. infusion with the GMO in combination with pembrolizumab (200 mg via i.v. every 3 weeks beginning at Day 9)

Trial participants with the following indications are proposed to be treated in this study:

- Microsatellite-stable colorectal carcinoma (MSS-CRC)
- Triple negative breast cancer (TNBC)
- Non-small cell lung adenocarcinoma (NSCLC)

[4] The GMO is also known as TBio-6517 or TAK-605 or RIVAL-01.

- Cervical adenocarcinoma
- Renal cell carcinoma (RCC)
- Mesothelioma

30. For i.t. injection – the TBio-6517 i.t. injection protocol has been declared CCI under Section 185 of the Act. Relevant CCI was made available to the prescribed experts and agencies that were consulted on the RARMP for this application.

31. For i.v. infusion – TBio-6517 would be diluted in approximately 250 mL of sterile normal saline and the infusion would be performed over 60 min (± 5 min). The infusion line would be flushed with sterile normal saline.

32. The applicant has stated that the infusion/injection site would be covered with an occlusive dressing until complete wound healing. The dressing should be changed daily. Contaminated dressings would be placed in a biohazard waste container (provided to the patient) and returned to the clinical trial site at the next scheduled site visit.

33. Trial participants are to receive four doses over a period of 24 months. Additional booster doses may be administered upon the discretion of the international sponsor and principal investigator.

2.3.3 Dose levels

34. The proposed dose levels and dosing schedules have been declared CCI under Section 185 of the Act. Relevant CCI was made available to the prescribed experts and agencies that were consulted on the RARMP for this application.

35. The clinical trial for Arm B would only commence once the maximum tolerable dose (MTD) and maximum feasible dose (MFD) from Arm A has been established; Arm D would only commence once the MTD/MFD from Arm C is established. The dose levels explored in Arm B and Arm D would not exceed the MTD/MFD established in Arm A and Arm C, respectively.

2.3.4 Selection of trial participants and behavioural requirements

36. Relevant inclusion criteria to this assessment proposed by the applicant include that trial participants must:

- be of any gender over 18 years; and
- have a locally advanced or metastatic solid tumour. Disease must be incurable and standard measures either do not exist or are no longer effective; and
- be willing and able to attend protocol-specified visits, complete protocol procedures, and adhere to post-TBio-6517 treatment care instructions within the informed consent to minimise the risk of transmission to caregivers and close contacts; and
- agree to forgo any vaccination with live vaccines while participating in the trial; and
- agree to use barrier contraception during sexual activity, starting from Day 1 through to 6 weeks after the last dose of TBio-6517 to prevent the risk of environmental shedding; and
- agree to use a highly effective contraception method to prevent pregnancy for patients who are able to conceive or father children starting from screening through to 4 months after the last dose of TBio-6517, including with pembrolizumab (Arm B and Arm D).

37. Relevant exclusion criteria include:

- any evidence of an active infection or any immunosuppressive disorder, including HIV infection;
- ongoing severe inflammatory skin condition or history of severe eczema requiring prior medical treatment;
- prior treatment with an oncolytic virus;
- patients who have received live vaccines 30 days prior to the first dose of the GMO;

- prior intolerance to PD-1/PD-L1 targeted antibody therapy for those trial participants intending to receive pembrolizumab (Arm B and Arm D); and
- women who are pregnant or breastfeeding.

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.

38. For the purposes of this RARMP, persons who fit the criteria as listed in the points of relevant exclusion criteria (Paragraph 37) are considered persons at a higher-risk of a serious adverse event when exposed to the GM *Vaccinia virus*.

2.3.5 Transport and storage of the GMO

39. The GMO would be imported according to the packaging and labelling requirements of the International Air Transport Association (IATA) code UN 3373 [Category B].

40. Transport of the GMO from the Australian border would either be to a central depot for distribution to clinical sites, or directly to the clinical sites. Once at a clinical site or storage facility, the GMO would be stored in a freezer, with access restricted to appropriately trained personnel. When the GMO is transported between locations in Australia, the outer container would be labelled to indicate that it contains the GMO vials, the OGTR licence number and the contact details of an appropriate clinical trial staff member in case of loss of containment (who would on-report to Novotech).

41. The proposed method of supply and storage of the GMOs, as advised by the applicant, would be in accordance with the *Regulator's Guidelines for the Transport, Storage and Disposal of GMOs* (TSD).

42. Waste generated at clinical trial sites would be transported from clinical waste bins by waste contractors for destruction by autoclaving or high-temperature incineration.

2.3.6 Sample collection and analysis

43. Samples collected from trial participants after treatment may be analysed in Australia or overseas. These samples are considered likely to contain the GMO and transport and storage of these would be treated as described in Section 2.3.5.

44. The types of samples intended to be collected and analysed have been declared CCI under Section 185 of the Act. Relevant CCI was made available to the prescribed experts and agencies that were consulted on the RARMP for this application.

2.3.7 Personal protective equipment and exclusion criteria

45. The applicant advised that persons handling the GMO (other than during transport and disposal) would be instructed to wear appropriate personal protective equipment (PPE) for the task. For example, clinical trial staff performing dealings with the GMO including preparation and administration of the GMO to trial participants and clean-up of potential spills would wear a gown, gloves, mask and eye protection.

46. The applicant has stated that higher-risk individuals, as described in Paragraph 37 would be excluded from handling or preparing the GMO.

2.3.8 Decontamination and disposal of the GMOs (including waste contaminated with the GMOs)

47. The applicant states that all used and unused vials of GMO and disposable materials used during the administration procedure or for the collection of samples from trial participants and any other contaminated waste (e.g. gloves, needles, tissues) would be disposed according to infectious medical waste management procedures. The outer containment of the waste would be labelled to indicate that it contains GMOs. Commercial waste management contractors would be used. All disposable GMO waste would be destroyed by autoclaving or high-temperature incineration.

48. Waste contractors would be selected based on their experience and capability in disposing of infectious clinical waste and laundering/disposing of linen, which has been contaminated with

infectious substances. Waste contractors would handle GMO contaminated waste using the same safety precautions used for handling infectious waste.

49. Contaminated laundry would be double-bagged and laundered according to institutional procedures that are suitable for inactivating infectious diseases, including viruses and bacteria. However, contaminated laundry, including gowns would be incinerated if direct contact with a spill containing the GMO is made which exceeds 50 mL.

50. GMO spills would be decontaminated using a fresh dilution of bleach (with at least 0.6% of active chlorine), with at least 10 minutes of contact time.

51. Unused vials of the GMO may be returned to the international sponsor (exported from Australia). In this instance, the vials would be transported within Australia in accordance with the TSD guidelines.

2.3.9 Training of clinical trial personnel

52. Novotech would have responsibility for ensuring training of personnel and compliance with licence conditions.

53. Persons handling the GMO during administration (i.e. the principal investigator, the study coordinator and medical staff assisting in administration of the GMO to participants), would be trained in all procedures specific to the GMO including handling, spill procedures, containment and disposal. Records of this training would be kept within the clinical trial master file. A copy of the licence would also be kept in the clinical trial file at the site.

54. The appropriate clinical trial staff member whose contact details are listed on the outer container(s) of GMOs would be trained in the conditions of the licence including the requirement to report loss of containment to the OGTR and the procedure for doing so.

55. External service providers would be informed they are transporting a GMO via the labelling on the outer container. In addition, a copy of the licence would be included in the shipping documentation.

2.3.10 Contingency plans

56. In case of unintentional release of the GMO due to an accidental spill, the spill would be reported to Novotech by clinical trial staff trained in the OGTR reporting requirements. Novotech would on-report to the OGTR. The local Institutional Biosafety Committee (IBC) would also be notified of loss of containment or suspected loss of containment.

57. For spills of the GMO outside a biological safety cabinet, the applicant proposes that entry is restricted to the room for at least 30 minutes to allow aerosols to settle. After which, absorbent material is to be placed carefully over the spill to prevent further aerosolising. Disinfectant (e.g. bleach with at least 0.6% of active chlorine) would then be applied and allowed to remain on the spill for at least 10 minutes before initiating clean-up.

58. In case of exposure of people to the GMO, the applicant has proposed that:

- the affected area be washed immediately with water and soap;
- in case of eye contact, flush with water for at least 3 minutes;
- in the event of exposure to broken skin or needle-stick, clean the affected area thoroughly with soap and water and/or appropriate disinfectant.

2.3.11 Accountability and monitoring

59. The applicant has stated that as per Good Clinical Practice (GCP) requirements, documentation related to GMO shipment, receipt, authorisation for use, dispensing, destruction, temperature monitoring, etc., would be filed in the pharmacy manual and must be available for inspection.

60. As stated in Paragraph 26, pustule kits and biohazard bins provided to trial participants would be accounted for.

61. Severe adverse events that are suspected to be related the GMO, evidence of secondary transmission and any other potential loss of containment would be reported to the OGTR.

Section 3 Parent organism – *Vaccinia virus*

62. The parent organism is the Copenhagen strain of *Vaccinia virus*. The characteristics of the non-GM parent organism provide a baseline for comparing the potential for harm from dealings with GMOs. As such, the relevant biological properties of *Vaccinia virus* (VACV) will be discussed here.

63. VACV was used globally as a vaccine against smallpox prior to the latter's declared eradication in 1980. It is a highly effective vaccine because it is a mild pathogen that stimulates an immune response to the closely related and often lethal smallpox agent *Variola virus* (Middaugh et al., 2016). The biology of VACV has been described in detail in the RARMPs for DIR-116 and DIR-140 (clinical trials with GM *Vaccinia viruses*) and more recently in the RARMP for DIR-170 (trial with GM *Vaccinia viruses* in horses). A summary is presented in this section.

3.1 Classification and genome characteristics

64. VACV is a large enveloped virus belonging to the genus *Orthopoxvirus* (OPV), subfamily *Chordopoxvirinae*, family *Poxviridae* (ICTV, accessed in October 2020). The OPV genus also includes the human pathogen *Variola virus* (causative agent of smallpox), cowpox virus, horsepox virus, monkeypox virus, mousepox virus and others (McLysaght et al., 2003; Tulman et al., 2006). It should be noted that the human disease chickenpox is caused by the *Varicella zoster virus*, which is not a member of family *Poxviridae*.

65. The VACV genome is linear double-stranded DNA, approximately 192 kilobases (kb) in length and encodes around 200 proteins with roles in viral entry, transcription of viral genes, DNA synthesis, assembly of virus particles, and suppression of the host anti-viral response (Liu et al., 2014).

3.2 Origin, geographic distribution and host range

66. VACV was first identified in 1939 (Downie, 1939) and is considered to have originated from mutation or recombination involving cowpox virus, *Variola virus* and other related OPV ancestors at some point during 150 years of propagation and use as a vaccine under a variety of *in vivo* and *in vitro* conditions (Hendrickson et al., 2010; Esparza et al., 2017).

67. Due to their evolution in different parts of the world over 150 years of smallpox vaccination, many strains of VACV exist (e.g. Paris, Copenhagen, Bern, Ankara, Lister and New York City Board of Health (NYCBH) strains). These differ in viral characteristics, host range, pathogenicity and prevalence of adverse reactions to vaccination (see Paragraph 93).

68. Poxviruses can infect a wide range of organisms, with certain poxviruses having a variable host range, while some are restricted to a single host (Oliveira et al., 2017). 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, 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; Oliveira et al., 2015; Miranda et al., 2017; Silva et al., 2021). OPVs, namely VACV, monkeypox and cowpox are responsible for outbreaks of skin diseases around the world both in humans and animals (including native wildlife) and are considered emergent zoonotic diseases (Oliveira et al., 2017). Furthermore, poxvirus infections (often novel and not well characterised) have been reported in native Australian mammals and reptiles (Wildlife Health Australia, 2019).

69. 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 (Artois et al., 1990).

70. The RARMP for DIR-170 describes that naturally occurring infections with VACV or close relatives have been documented in South America, India, Indonesia, Egypt and other countries. In Brazil, outbreaks of zoonotic disease (transferable between animals and people) caused by VACV-like viruses affecting dairy cattle and rural workers, as well as significant levels of VACV infection found in remote Amazonian wildlife have also been described.

3.3 Infection cycle

71. 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 for DNA replication and gene transcription (Schramm and Locker, 2005).

72. VACV genes are expressed in three temporal classes, i.e. early, intermediate and late genes (Yang et al., 2011). Because the infectious virus particles contain the entire transcription machinery, i.e. viral RNA polymerase and early transcription factors, these do not need to be synthesised *de novo* upon entry into a host cell. Expression of intermediate and late genes occurs post-DNA replication and needs *de novo* RNA and protein synthesis (Shors et al., 1999).

73. After DNA replication and late protein expression, poxviruses go through a complicated assembly and maturation process. This involves fabrication of membrane crescents that evolve into spherical, immature virions enclosing a nucleoprotein mass. The immature virions 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 (Zeh and Bartlett, 2002).

3.4 VACV persistence in infected hosts

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

3.5 Pathology of VACV

75. Generally, VACV is considered a mild pathogen in people. OPVs display a tropism for epithelial cells and tend to produce cutaneous[5] lesions (Moussatche et al., 2008). In addition, tropism for VACV are primary hematolymphoid human cells (preferentially antigen presenting cells and activated T cells) (Chahroudi et al., 2005).

76. When administered as a vaccine, the vaccine recipients normally develop a single lesion at site of exposure around 3-4 days post-vaccination, which generally resolves over 2-3 weeks (Fulginiti et al., 2003a). This is often accompanied by flu-like symptoms (fever, malaise, headache, nausea and muscle aches), swelling and redness around the vaccination site, and swelling and tenderness of the draining lymph node (Cono et al., 2003; Public Health Agency of Canada, 2011). In healthy people, these reactions resolve spontaneously and require only observation and symptomatic treatment (Cono et al., 2003; Fulginiti et al., 2003b; Maurer et al., 2003).

77. Serious adverse reactions associated with VACV such as *postvaccinia encephalitis* (PVE) 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 3.10 of this Chapter.

3.6 Transmission and shedding

78. The RARMPs for DIR-140 and DIR-170 describe transmission (both between humans, as well as to and between animals) and shedding of VACV in detail. Importantly, direct physical contact with

[5] Relating to or affecting the skin.

lesions or the vaccine inoculation site or contact with a contaminated object (e.g. bandages, clothing, sheets and towels) can result in transmission. An infected person may also spread VACV from the initial infection site by touching other body parts or people with contaminated hands, or through every day activities such as shaving (Cono et al., 2003; Egan et al., 2004; Oliveira et al., 2014; Webber et al., 2014). Oral transmission via drinking contaminated cattle milk has been observed in humans (Damaso et al., 2000). Aerosol transmission of VACV has never been clearly documented in people when used as a vaccine (Lane and Fulginiti, 2003) and is considered unlikely. More generally, poxvirus infections can occur via all possible routes, and most pathogenic poxviruses infect via the respiratory tract (Buller and Palumbo, 1991; Moussatche et al., 2008).

79. In the context of smallpox vaccination, VACV can be shed from the primary lesion from at least the third to twenty first days post-vaccination – from the time the pustule develops until the scab drops off, and possibly longer. Maximal shedding occurs between days 4 and 13, and peak titres of 10^7 pock-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). In non-human hosts, poxviruses can be shed into the milk and faeces of experimentally infected dairy cattle, reviewed in Matos et al. (2018).

80. The infectious dose is unknown, however, VACV titre used for smallpox vaccination is usually 10^8 pfu/ml. Smallpox vaccine is administered by puncturing the skin multiple times with a bifurcated needle containing a small quantity of vaccine (Belongia and Naleway, 2003; Public Health Agency of Canada, 2011).

81. VACV was used worldwide as a vaccine to protect against smallpox infection. The smallpox vaccination program is no longer ongoing, but the majority of people over forty years of age in Australia are likely to have been vaccinated. In the US, the military resumed the use of VACV as a vaccine for preparedness against use of smallpox as a biological weapon (Grabenstein and Winkenwerder, 2003). In addition, the US military laboratory and health personnel working with the vaccine or other OPVs still receive the vaccine (Public Health Agency of Canada, 2011). Average transmission rates are generally from historical smallpox vaccination campaigns. In the US and UK these are reported as 20-60 per million primary vaccinations (Neff et al., 2002). However, vaccinees were generally young children and the majority of their contacts would have been immune or had previous exposure to vaccinia due to ongoing vaccination campaigns. Therefore, not every exposure would have resulted in an observable infection. While it would be reasonable to expect a higher transmission rate from adults to the predominantly unvaccinated population of today, a report estimates that, among health care workers and military personnel vaccinated between 2003 and 2011, the rate of transmission to non-vaccinees was still 54 per million vaccinees (Wertheimer et al., 2012).

82. Reported cases predominantly involved transmission between family members or other close contacts (e.g. school friends), and transmission in a hospital setting. The latter involved transmission to clusters of patients from recently vaccinated health care workers (HCWs) or patients hospitalised with a vaccine-related complication (Sepkowitz, 2003). In more recent (post-2000) vaccination programmes involving HCWs in Israel and the USA, there were no reports of transmission from ~18,000 vaccinated Israeli HCWs to patients and no transmission in the healthcare setting from ~56,000 vaccinated military and civilian HCWs in the USA. Outside of the healthcare setting, 10 transmissions have been recorded among ~260,000 primary vaccines. The authors of the study state that these transmissions are presumably via sexual contact (Lane and Fulginiti, 2003). Similarly, the rate of contact vaccinia transmission in another study was 4.4 per 100,000 vaccinations (Tack et al., 2013).

83. Environmental samples were collected and tested on three different study days from 43 persons with major vaccinia-related skin reactions following vaccination with replication competent strains, the vaccination site was covered with a dressing. A total of 387 samples were analysed and included: linen from the study participant's bed (approximate location of sleeping area), the middle of his or her bath towel, and the inside area of a shirt sleeve adjacent to the vaccination bandage (before laundering). An additional 129 samples from the palm of the study participant's hand were also analysed. The outside of the bandage covering the vaccination site for each study participant were

also analysed. A swab from the lesion itself was used as a positive control. All 516 environmental samples were negative for live virus as determined by plaque infectivity assay. Only 1 of 129 dressing samples (0.78%) tested on day 7 had measurable titres of vaccinia (Stark et al., 2006). This demonstrates that the waterproof bandage was able to contain the replication-competent VACV strains.

3.6.1 Laboratory acquired infections

84. In a 30 year period until 2014, 18 significant laboratory infections and two other laboratory infections with recombinant VACV were reported (Boston University, 2014). In the USA, 15 laboratory-related vaccinia exposures (4 requiring hospitalisation) were reported to the CDC between 2005-2008 (MacNeil et al., 2009).

3.7 Recombination

85. Recombination between OPVs in cell cultures in a laboratory setting are easily produced and have been described in the RARMP for DIR-170.

86. Examples of natural OPV recombinants which have clearly occurred between co-infecting viruses are nearly non-existent (Gershon et al., 1989), with very few examples of potential recombination events between poxviruses in co-infected animals (Strayer et al., 1983). Although replicating poxviruses can recombine very efficiently under certain circumstances, there are physical constraints within a cell that limit recombination between co-infecting viruses. VAVC 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 from other viruses in the same cell (Paszkowski et al., 2016).

3.8 Environmental stability and methods of decontamination for VACV

87. 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 (fomites) (Wood et al., 2013).

88. VACV survival decreases at high temperatures or high humidity, and is greater at lower temperatures. Dried VACV can be kept for more than 35 weeks at 4°C with no loss of infectivity (Rheinbaben et al., 2007). When frozen (-20°C), 1 in 1000 virus particles remained viable after 15 years (Essbauer et al., 2007; Rheinbaben et al., 2007). Samples of VACV (15 mL) at $10^{7.5}$ $TCID_{50}$/mL (median Tissue Culture Infectious Dose 50%; equivalent to approximately 0.5 x 10^8 pfu) can also remain viable for more than two weeks on food samples in the fridge (4°C) or close to 6 months in storm water at 4.5°C. However, the presence of soil in stormwater decreased survival time of the VACV sample to 6 days at 4.5°C or 3 days at 21.5°C (Essbauer 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.

89. In the context of non-human hosts, mice exposed to bovine faeces containing VACV displayed signs of viral replication (D'Anunciação et al., 2012). VACV of both high and low pathogenic strains can shed into the faeces and urine of experimentally infected mice, and be transmitted to other mice via their excrement (Ferreira et al., 2008). These data suggest that horizontal transmission via contaminated faeces is possible, and that faeces could provide a means for viral dissemination into the environment. In humans, viremia (viral presence in the blood) and viruria (viral presence in urine) is uncommon, although does occur in patients with progressive vaccinia and eczema vaccinatum (Lane and Fulginiti, 2003), see Paragraphs 102-103.

90. Purified samples of poxvirus are less stable than those found in association with host cells and proteins from patients (Rheinbaben et al., 2007; Chambers et al., 2009). Purified samples of VACV are inactivated within 1 minute by a range of common chemical disinfectants including 70% ethanol, 50% isopropyl alcohol and 0.5% sodium hypochlorite (Chambers et al., 2009).

91. VACV is also susceptible to UV irradiation, with less than 10% of the VACV particles remaining resistant to inactivation when dried onto fomites and environmental surfaces (Sagripanti and Lytle, 2011). Additionally, VACV is relatively resistant to iodine, a broad temperature range, drying and pH (Rheinbaben et al., 2007). However, VACV is inactivated by dry heat at 95°C for 2 hours (Sauerbrei and Wutzler, 2009) and by autoclaving (Espy et al., 2002). 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). Further information on *Vaccinia virus* decontamination can be found in the RARMPs for DIR-140 and DIR-170.

3.9 VACV as a vaccine

92. VACV is considered a mild pathogen in humans. However, its infection protects from smallpox, which is lethal in approximately 30% of cases (Belongia and Naleway, 2003). Therefore, various strains of VACV were globally used as vaccines against smallpox during the eradication program (Jacobs et al., 2009).

93. Standard and recombinant vaccinia strains are capable of replicating and causing illness in humans. Historical studies of smallpox vaccination indicate that incidence rates of severe post-vaccination effects, such as *postvaccinal encephalitis* (PVE) and vaccination-related deaths, 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 reactions, with nearly 45 expected cases of PVE and 55 deaths per million primary vaccinations. The Copenhagen strain led to intermediate/high rates of adverse reactions with 33 expected cases of PVE and 31 deaths per million vaccinations. The NYCBH strain was the most benign, with less than 3 expected cases of PVE and 1 or 2 deaths per million vaccinations (Kretzschmar et al., 2006).

94. 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.

95. Currently, VACV is considered well-suited as a viral vector to create a new generation of safer GM vaccines and treatments, such as the ones proposed within this application (Nagata et al., 2018). Some of the features of VACV viral vectors that make them suitable for GM vaccine and treatment applications are:

- thermostability, which allows for a cold-chain independent distribution capacity
- large DNA genome capable of accepting inserts of up to 25 kb
- ability to grow to high titres *in vitro*
- ability to elicit strong humoral and cell-mediated immune responses that enhance the immune response to the target antigens
- absence of oncogenic potential or evidence of integration into the host genome, and
- wide host range.

3.10 Adverse reactions to VACV infections

96. Although smallpox vaccination using VACV during the eradication campaign was generally safe and effective, serious adverse reactions have been well documented. Most complications occurred in the vaccinated individuals, but as VACV is transmissible, serious and even fatal reactions sometimes developed when VACV was passed to others. Several types of adverse reactions have occurred in healthy people, while other reactions have been associated with specific risk factors or underlying conditions (Neff et al., 2002; Cono et al., 2003; Fulginiti et al., 2003b; Lane and Goldstein, 2003b; Lane and Goldstein, 2003a; Maurer et al., 2003; Wittek, 2006).

97. *Accidental implantation* (or self-inoculation) of a body part other than the vaccination site is the most common complication. Transmission to another naïve person can also occur by this route. The best preventative measure is consistent hand hygiene using antimicrobial soap and water, or alcohol-based hand disinfectant, after contact with the vaccination site or with materials that have come into contact with it (Maurer et al., 2003; Wharton et al., 2003). No specific treatment is needed if there are only a few implanted lesions. However, multiple or confluent lesions warrant treatment with vaccinia immunoglobulin (VIG).

98. About 6% of patients with vaccinia infection in the eye go on to develop *vaccinial keratitis*. This may occur where there is injury to the cornea or conjunctiva, allowing viral replication. Ulceration and scarring as the lesion heals can lead to permanent impairment of vision (Lane and Goldstein, 2003b). VIG is contraindicated for use in vaccinial keratitis (Cono et al., 2003).

99. *Generalised vaccinia* is a rare condition associated with viraemic spread of virus from the vaccination site in presumably healthy individuals. Systemic infection enables skin lesions to form in locations distant from the vaccination site and sometimes covers the entire body. Onset is typically within a week of vaccination and, while visually distressing, the condition usually resolves in 1-2 weeks (Cono et al., 2003; Fulginiti et al., 2003b). Extensive or recurrent disease is treatable with VIG (Cono et al., 2003).

100. *Central nervous system disease*, which includes postvaccinal encephalopathy (PVE) and postvaccinal encephalomyelitis (or encephalitis) (PVEM), can occur after smallpox vaccination. PVE is most common in vaccinated children under age two and symptoms develop 6-10 days after vaccination, while PVEM usually affects vaccine recipients older than two years and develops 11-15 days after vaccination. The conditions were characterised by headache, fever, vomiting, seizures and coma, and up to one third of cases have been fatal. In addition, up to half of the survivors have had permanent neurological problems (Cono et al., 2003; Fulginiti et al., 2003b). The rate of PVE varies depending of the vaccinia strain as discussed in Paragraph 93.

101. Congenital or *fetal vaccinia* was a rare complication, with only 50 cases reported in the literature complication during the eradication campaign (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.

102. *Progressive vaccinia* (PV) is life-threatening and the most severe complication of smallpox vaccination. It is defined as a spreading necrosis at the site of inoculation, with or without metastatic necrotic lesions occurring elsewhere on the body, leading to severe viraemia, shock and death (Neff et al., 2002). It occurred only in immunocompromised individuals whose defective immunity left them unable to resolve the infection. Treatment with VIG is recommended, however, immunocompromised persons with PV would have a poor prognosis (Cono et al., 2003).

103. *Eczema vaccinatum* (EV) is a localised or generalised pustular rash, which can occur anywhere on the body but displays a preference for areas of previous atopic dermatitis (eczema) lesions as the disrupted skin allows viral implantation. People with a history of atopic dermatitis are at greatest risk of this complication, and the severity and mortality of EV is reportedly higher in contact transmission cases than among the vaccinated persons (Fulginiti et al., 2003b). EV lesions follow a similar course to the normal vaccination lesion but confluent lesions may occur. Without treatment, the condition can be fatal (Cono et al., 2003).

104. *Myocarditis and/or pericarditis* (inflammation of the heart muscle and lining outside of the heart, respectively) is another serious complication that may follow either primary or revaccination with live VACV, with symptoms occurring at a rate of 5.7 per 1000 primary vaccinations. In clinical trials involving 2983 subjects who received ACAM2000 and 868 subjects who received Dryvax® (both are replication-competent VACV strains derived from NYCBH), 10 cases of suspected myocarditis, 0.2% (7 of 2983) from ACAM2000 subjects and 0.3% (3 of 868) Dryvax® subjects, were identified. The mean time to onset of suspected myocarditis and/or pericarditis from vaccination was 11 days, with a range

of 9 to 20 days. All subjects who experienced these cardiac events were naïve to vaccinia. Of the 10 subjects, 2 were hospitalised. None of the remaining 8 cases required hospitalisation or treatment with medication (Therapeutic Goods Administration, 2020).

3.10.1 Serious adverse reactions in recent vaccination campaigns

105. Due to the threat of smallpox being used in bioterrorism, the United States resumed the vaccination of military and civilian personnel in 2003. Between January 24 and October 31 2003, 38,885 smallpox vaccinations were administered in the civilian population using Dryvax®. The following data is as presented in Casey et al. (2005): A total of 822 (2%) adverse events were reported. There were also 722 non-serious adverse events reported, these included multiple signs and symptoms of mild and self-limited local reactions. Of all reports, 100 were designated as serious, resulting in 85 hospitalisations, 2 permanent disabilities, 10 life-threatening illnesses, and 3 deaths. Among the serious adverse events, 21 cases were classified as myocarditis and/or pericarditis and 10 as ischemic cardiac events that were not anticipated based on historical data. Two cases of generalised vaccinia and 1 case of PVE were detected. No preventable life-threatening adverse reactions, contact transmissions, or adverse reactions that required treatment with VIG were identified. Serious adverse reactions were more common among older re-vaccinees than younger first-time vaccinees.

3.11 Treatment of adverse reactions

106. Vaccinia immunoglobulin (VIG) is made from the plasma of recently vaccinated people and has been successfully used to treat certain complications of VACV infection. It is recommended for treating severe cases of accidental implantation, severe generalised vaccinia, eczema vaccinatum and severe progressive vaccinia. It is not recommended for mild instances of accidental implantation, mild or limited generalised vaccinia, and post-vaccinial CNS disease. VIG is contraindicated in patients with vaccinia keratitis (Enserink, 2002; Cono et al., 2003; Maurer et al., 2003; Centers for Disease Control and Prevention, 2020).

107. In the USA, VIG has been approved as a drug for adverse reactions to the smallpox (vaccinia) vaccine. Antivirals such as Tecovirimat 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 and as a second line of defence (Centers for Disease Control and Prevention, 2020).

108. The antiviral drug Cidofovir may be considered as a second line treatment for the adverse reactions listed in Paragraph 106. While Cidofovir has shown anti-poxviral activity *in vitro* and in mice, there is limited data on its use in humans as a treatment for vaccinia-related adverse events (Maurer et al., 2003; Wittek, 2006; Centers for Disease Control and Prevention, 2020). Cidofovir can also have severe side effects, including irreversible renal toxicity (Enserink, 2002; Centers for Disease Control and Prevention, 2020). More recently, Tecovirimat, which has been used in a small number of individuals with vaccinia-related adverse events, produced fewer side effects than Cidofovir. However, effectiveness data for Tecovirimat in humans is limited (Centers for Disease Control and Prevention, 2020). Cidofovir is available in Australia but is not approved for the treatment of vaccinia-related complications; off-label use would thus be required. The applicant has advised that a supply of Tecovirimat would be maintained to allow for immediate supply in case of a life-threatening replication event. As Tecovirimat is not approved as a therapeutic good in Australia, the applicant has also advised that it will be included as an investigational agent in the clinical trial notification (CTN) to the TGA.

3.12 Risk group of VACV

109. 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

110. Cancer vaccines, oncolytic[6] viruses (OVs) and oncolytic immunotherapy are promising treatment strategies with potential to provide greater clinical benefit to patients with advanced-stage cancer. There are several advantages to OVs, these include (i) preferential replication in tumour cells, (ii) they can be modified to express transgenes that enhance their cytotoxic and immune-stimulatory activities, and (iii) they can modulate the tumour microenvironment to optimise immune-mediated tumour eradication, both at the site of the tumour and systemically. Furthermore, lysis of tumour cells releases tumour-specific antigens that trigger both the innate and adaptive immune systems, which further enhance tumour eradication. Due to their favourable safety profiles, OVs can also be combined with other systemically delivered treatments (Harrington et al., 2019). In particular, GM VACV hold great promise as interventional agents (Guo et al., 2019). Some of these advantages are listed in Paragraph 95.

111. Due to the advances in genetic engineering and molecular virology, the use of OVs in cancer therapy has seen rapid advancements in the last two decades. For example, Talimogene laherparepvec was the first OV approved for the treatment of melanoma in humans. A number of other OV treatments, which are demonstrating excellent safety profiles, are in the preclinical and clinical study phases, reviewed in Chaurasiya et al. (2020).

112. VACV of various strains have also been previously used as OVs. For example, an intratumoural dose escalation clinical trial in 16 patients with advanced solid tumours was well-tolerated in patients and resulted in selective infection of injected and non-injected tumours and demonstrated antitumor activity (Zeh et al., 2015). Another oncolytic VACV, known as Pexa-Vec, has had acceptable safety profiles when administered in Phase 1 and 2 clinical trials in human patients (Heo et al., 2013; Park et al., 2015).

113. The international sponsor, Turnstone Biologics, Corp. (Turnstone) has developed TBio-6517, an oncolytic viral immunotherapy, for the treatment of patients with advanced solid cancerous tumours. TBio-6517 is based on a novel oncolytic vaccinia backbone, termed SKV, and is a derivative of the parental Copenhagen vaccine strain of VACV.

4.1 Genetically modifying the Copenhagen strain to produce SKV backbone

114. Turnstone has used a combination of functional genomics and bio-selection strategies to optimise VACV for an oncolytic treatment. A fitness assay first identified the vaccinia strain with the best ability to replicate in and kill both established cancer cell lines and cancer patient tumour explants. A transposon insertion strategy and deep sequencing of viral populations was then employed to systematically examine the role of each VACV gene in its ability to be an anticancer therapeutic. Ultimately, large regions (25 kb) of the vaccinia genome were deleted. The newly generated vaccinia backbone termed SKV, increases tumour selectivity and the oncolytic activity of the virus (Bell et al., 2019; Dyer et al., 2019). Further information regarding the deleted regions have been declared CCI under Section 185 of the Act. Relevant CCI was made available to the prescribed experts and agencies that were consulted on the RARMP for this application.

4.2 Immunomodulatory genes within TBio-6517

115. Although cancerous tumours possess both innate and adaptive immune cells within its microenvironment, a dynamic immunosuppressive network within the tumour has been suggested to prevent the anti-tumour immune response.

[6] An oncolytic virus is a virus that preferentially multiplies in and kills cancer cells.

116. A potential treatment for these cancers is to infect the tumours with an oncolytic virus, such as VACV, carrying immunomodulatory genes[7]. The GMO, TBio-6517, when delivered to cancer patients would have two primary modes-of-action: (i) infection and lysis[8] of tumour cells rather than healthy cells, due to its selective replication; and (ii) production of multiple immunomodulatory proteins that are expressed directly in the tumour microenvironment, which function to re-program the tumour microenvironment by preventing immunosuppressive networks and re-establishing endogenous anti-tumour immunity.

117. TBio-6517 encodes transgenes for anti-CTLA-4 antibody, FLT3 ligand (FLT3L), and membrane-bound IL-12 (p35 subunit). The immune response induced by these transgenes is envisioned to stimulate tumour-specific cytotoxic T cell[9] responses and generate a long-term immunological memory, which is capable of tumour control and prevention of recurrence.

4.2.1 Introduced anti-CTLA-4 antibody gene

118. Cytotoxic T-lymphocyte-associated protein 4 (CTLA-4) is a protein receptor that functions as an immune checkpoint and downregulates immune responses by inhibiting T cell functions. It is constitutively expressed by regulatory T cells (Tregs) but can also be upregulated by other T cell subsets, especially $CD4^+$ T cells, upon activation.

119. CTLA-4 is homologous to the T cell co-stimulatory protein, CD28, and both can bind the same receptors. As CTLA-4 has a higher affinity for these receptors than CD28, immunosuppression is indirectly mediated through a reduction in CD28 signalling, reviewed in Seidel et al. (2018). In the presence of anti-CTLA-4 antibodies, the number of free CTLA-4 protein decreases significantly resulting in an increase in CD28 binding to the receptor and prolonging T cell activation and proliferation (Tarhini et al., 2010; Snyder et al., 2014).

120. A CTLA-4 inhibitor, Ipilimumab has been shown to have clinical activity as an immunotherapeutic. Toxicities with anti-CTLA-4 antibody treatment (including with Ipilimumab), can result in severe ocular autoimmunity, diarrhoea, hypophysitis, anorexia, fatigue, and increased alanine aminotransferase (Maker et al., 2006; Ansell et al., 2009; Ascierto et al., 2017; Colston et al., 2018).

4.2.2 Introduced FLT3 ligand gene

121. FMS-like tyrosine kinase 3 ligand (FLT3L) is a cytokine[10] involved in the production of stimulatory dendritic cells, namely CD141 in humans. These in turn stimulate cytotoxic T cells and drive immune responses against cancer. For example, production of CD141 in melanoma patients resulted in improved survival (Barry et al., 2018). FLT3L has been used in various oncolytic treatments in non-clinical studies, and has shown eradication of tumour growth in mice (reviewed in Pol et al. (2020); Zhang and Liu (2020)).

4.2.3 Introduced IL-12p35 gene

122. The human *IL-12* gene encodes interleukin-12, a heterodimeric protein composed of p35 and p40 subunits covalently linked by disulfide bonds. IL-12 is a pro-inflammatory cytokine that is secreted in response to infection. IL-12 directly promotes proliferation and cytolytic activity of natural killer cells and T cells. In addition, IL-12 indirectly suppresses angiogenesis[11], which inhibits tumour growth (Del Vecchio et al., 2007; Tugues et al., 2015).

123. Preclinical trials of IL-12 demonstrated potent anti-tumour activity (Del Vecchio et al., 2007; Tugues et al., 2015). Unfortunately, in early human clinical trials, systemic intravenous administration

[7] Immunomodulatory genes are genes which function to modify or stimulate the immune response.
[8] Lysis is the disintegration of a cell by rupture of the cell membrane.
[9] Cytotoxic T cells are a type of white blood cell that kills cancer cells, cells that are infected (particularly with viruses), or cells that are damaged in other ways.
[10] Cytokines are a large group of proteins, peptides or glycoproteins that are secreted by specific cells of the immune system and are important in cell signalling.
[11] Angiogenesis is the formation of new blood vessels.

of IL-12 protein caused severe toxicities, including two deaths. Subsequent clinical trials established maximum tolerated doses for intravenous or subcutaneous administration of IL-12, but had poor treatment response rates at these doses (Atkins et al., 1997; Car et al., 1999; Tugues et al., 2015). Targeted delivery of IL-12 to tumours has a better safety profile and is the focus of current clinical trials of IL-12 as a cancer therapeutic (Tugues et al., 2015; Strauss et al., 2019).

124. A membrane-bound version (IL-12p35) is present in TBio-6517. When expressed by the GMO, IL-12 would mainly be restricted to the tumour environment, reducing the toxicities related to systematically administered IL-12. A similar membrane-associated IL-12 variant of p35 was shown to enhance tumour cell immunogenicity by direct priming of $CD8^+$ T cells (Lim et al., 2010). Membrane-bound versions have also been used in other non-clinical studies, which demonstrate reduced toxicities and reduced circulating levels of IL-12 (Pan et al., 2012; Zhang et al., 2020).

125. Overall, oncolytic viruses, including VACV and the introduced transgenes in TBio-6517 have been previous used in a variety of non-clinical and clinical trials conducted till date. Further information regarding the nature and effect of the genetic modification resulting in the production of TBio-6517 have been declared CCI under Section 185 of the Act. Relevant CCI was made available to the prescribed experts and agencies that were consulted on the RARMP for this application.

4.3 Biodistribution of introduced genes in TBio-6517

126. Turnstone has conducted a number of non-clinical studies in a model species focusing on viral replication, toxicity, shedding and biodistribution. Information regarding these studies have been declared CCI under Section 185 of the Act. Relevant CCI was made available to the prescribed experts and agencies that were consulted on the RARMP for this application. It is to note that there is uncertainty as to whether the findings from these non-clinical studies as performed in the model species would be transferrable when the GMO is administered in humans. Further details around uncertainty is presented in Chapter 2, Section 3.

Section 5 Relevant information relating to Pembrolizumab

127. Arm B and Arm D of the clinical trial are utilising an existing non-GM 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, which include nausea and vomiting, diarrhoea and skin changes (dryness, itching and rashes similar to acne, severe reactions can lead to skin blistering) (Cancer Research UK, 2019). These reactions can increase the potential of GMO shedding.

Section 6 The receiving environment

128. The receiving environment forms part of the context for assessing risks associated with dealings with GMOs. It informs the consideration of potential exposure pathways, including the likelihood of the GMOs spreading or persisting outside the site of release.

6.1 Clinical trial sites

129. The intended primary receiving environment would be solid tumours within the clinical trial participants. As stated in Chapter 1, Section 2.3.2, each patient is to receive four doses of the GM *Vaccinia virus* as a treatment over the period of 24 months. Additional booster doses may be administered at the discretion of the principal investigator. Administration would be via i.t. injection or by i.v. infusion.

130. The secondary receiving environment would be the hospitals and clinics where the GMO would be dispensed, administered and 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 the *Australian Guidelines for the Prevention and Control of Infection in Healthcare* (National Health and Medical Research Council, 2019).

131. The principal route by which the GM VACV as a treatment could enter the wider environment is by shedding from the inoculated trial participants once they leave the hospital and return home. Thus, the tertiary receiving environment includes the trial participant's homes and any places they visit during the period when the GM VACV as a treatment is replicating and shedding.

6.2 Relevant environmental factors

132. 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.

133. The parent organism, VACV, was used worldwide as a vaccine to protect against smallpox infection. The smallpox vaccination program is no longer ongoing, but the majority of people over forty years of age in Australia are likely to have been vaccinated, either in Australia or overseas if they have emigrated. As a result, a proportion of the population has already been exposed to the vaccinia proteins. People vaccinated many years ago may be less susceptible to VACV infection, or infection may be asymptomatic or produce less severe symptoms (Cohen, 2001; Hatakeyama et al., 2005).

134. It is widely acknowledged that people for whom smallpox vaccination is contraindicated are more prevalent in the population today that during the era of mass smallpox vaccination. For example, approximately 17% of the Australian population have a history of atopic dermatitis (Chidwick et al., 2020). There are also likely to be significant numbers taking immunosuppressive drugs for disease control (e.g. for autoimmune inflammatory conditions), organ transplant recipients and people with HIV-AIDS.

135. 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.

6.3 Related viral species in the receiving environment

136. Information on species that are related to VACV in the Australian environment can be found in the RARMPs for DIR-140 and DIR-170.

6.4 Presence of the introduced genes and encoded proteins in the environment

137. As mentioned in Paragraph 133, a proportion of the population has already been exposed to the vaccinia proteins.

138. All three of the introduced genes (anti-CTLA-4 antibody, FLT3 ligand (FLT3L), and membrane-bound IL-12) are derived from human genes. Therefore, humans have already been exposed to the proteins that would be produced by the introduced genes in the GMO. However, the gene sequences have been optimised and modified for the purposes of constructing the GMO.

Section 7 Relevant Australian and international approvals

7.1 Australian approvals

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

140. The Regulator has issued Limited and Controlled DIR licences (DIR-116 and DIR-140) utilising VACV for clinical trials in humans. The clinical trial for DIR-116 is no longer ongoing but it involved dealings with a GM VACV and GM *Fowlpox virus*. The purpose of the trial was to evaluate the efficacy

of these GMOs in treating prostate cancer. The purpose of DIR-140 is to evaluate the efficacy of GM VACV for treatment of liver, kidney and prostate cancer.

141. 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.

7.2 International approvals

142. The GMO is currently being evaluated in a Phase 1/2a clinical trial in the USA, with the intratumoural mode of administration (ClinicalTrials.gov identifier NCT04301011). The applicant has provided preliminary data relating to safety, transgene expression, shedding, and biodistribution of the GMO in a limited number of human trial participants. This information is being sought as CCI under Section 185 of the Act.

Chapter 2 Risk assessment

Section 1 Introduction

143. 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 2). 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.

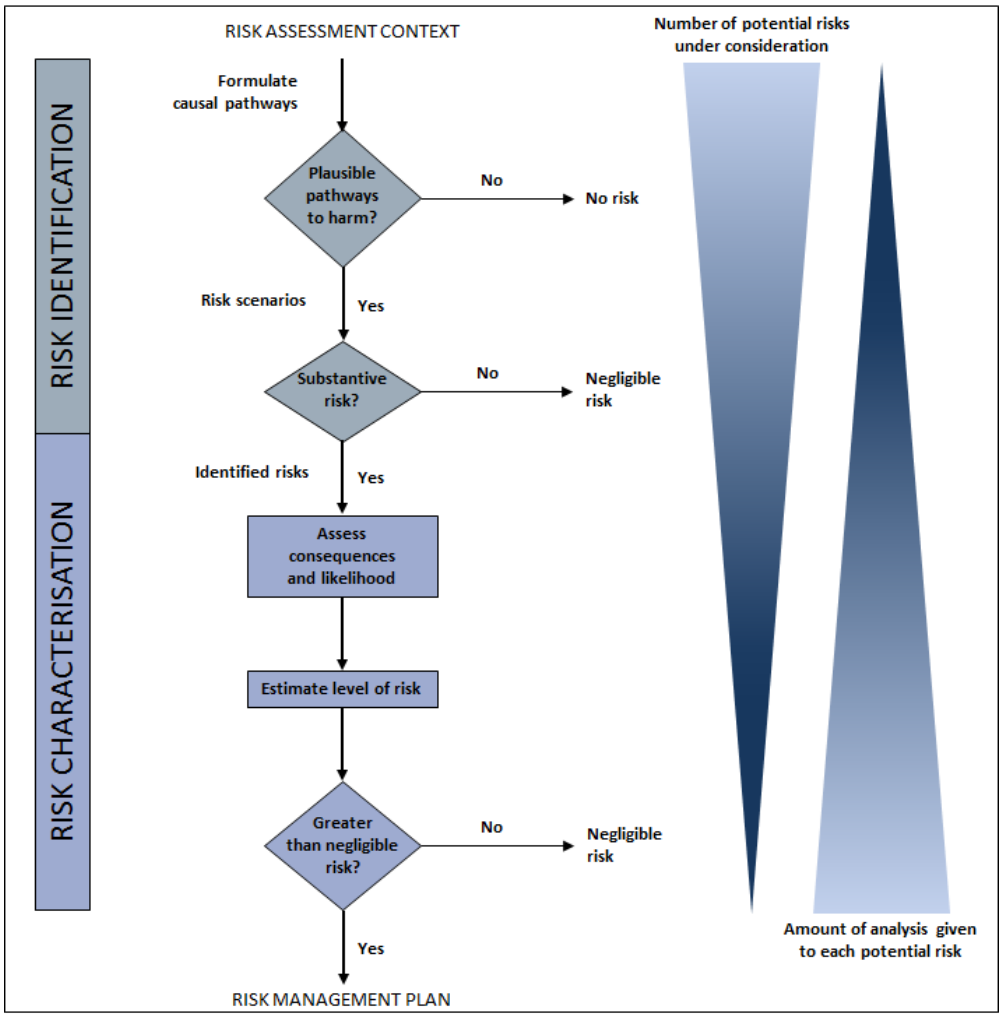

Figure 2. The risk assessment process

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

145. Risk scenarios examined in RARMPs prepared for licence applications for the same or similar GMOs, are also considered.

146. 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 plausible causal pathways that may give rise to harm for people or the environment from dealings with a GMO. These are risk scenarios.

147. Risk scenarios are screened to identify those that are considered to have a reasonable chance of causing harm in the short or long term. Pathways that do not lead to harm, or those that could not

plausibly occur, do not advance in the risk assessment process (Figure 3) i.e. the risk is considered to be no greater than negligible.

148. 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

149. Postulated risk scenarios are comprised of three components (Figure 3):

i. the source of potential harm (risk source)
ii. a plausible causal linkage to potential harm (causal pathway)
iii. potential harm to people or the environment.

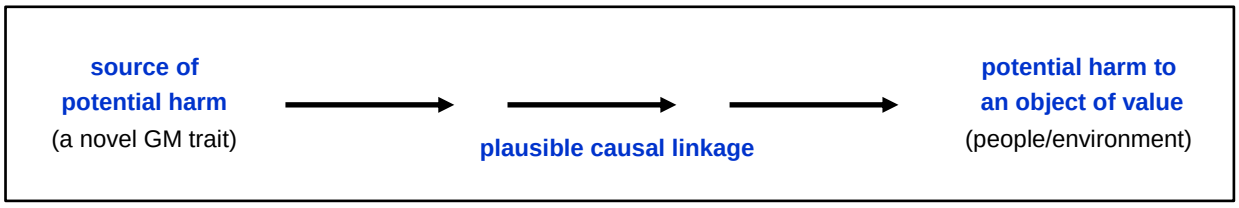

Figure 3. Components of a risk scenario

150. 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

151. The parent organism of the GMO is VACV. Details on the pathogenicity and transmissibility of VACV is provided in Chapter 1 (Section 3). Vaccination with VACV tends to produce a pustule at the inoculation site. Transmission of VACV from the vaccinee to other people and susceptible hosts, such as domestic pets, could occur from this site.

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

153. As discussed in Chapter 1 (Section 4), a 25 kb region of VACV genome was deleted. The introduction of human *anti-CTLA-4 antibody*, *FLT3L* and *IL-12p35* genes, intended to produce an oncolytic trait, are considered further as potential sources of risk.

154. The expression of the introduced genes are 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.

155. The genetic modifications involving introduction of genes have the potential to cause unintended changes to viral characteristics due to insertional effects such as interruptions, deletions, duplications or rearrangements of the genome. Pathways to any unintended effects in poxviruses

have already been considered in the RARMP for DIR-116, and found to be negligible. Their likelihood will be minimised by the imposed limits and controls. These include the requirement for any unintended effects to be reported to the Regulator immediately. Therefore, the potential for the processes of genetic modification to result in unintended effects will not be considered further.

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

2.2 Causal pathway

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

- proposed dealings
- proposed limits including extent and scale of the proposed dealings
- proposed controls to limit the spread and persistence of the GMOs
- 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 GMOs in the environment
- the environment at the site(s) of release
- spread and persistence of the GMOs (e.g. dispersal pathways and establishment potential)
- tolerance to abiotic conditions (e.g. temperature, UV irradiation and humidity)
- gene transfer by horizontal gene transfer (HGT)
- unauthorised activities, and
- practices during and after administration of the GMOs

158. Although all of these factors are taken into account, some are not included in the risk scenarios below as they may have been considered in previous RARMPs and a plausible pathway to harm could not be identified.

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

160. *Vaccinia virus* is transmitted through direct contact. Aerosol transmission is not considered as a viable route of infection for the GMO (see Paragraph 78). Therefore, aerosol transmission will not be considered further.

161. The GMOs and samples containing the GMO are proposed to be transported and stored in line with the Regulator's *Guidelines for the Transport, Storage and Disposal of GMOs*. These are standard protocols for the handling of GMOs to minimise exposure to the GMOs, so risks associated with such transport will not be further assessed.

162. The Act provides for substantial penalties for unauthorised dealings with GMOs or noncompliance 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 harm

163. In addition, 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
- the potential for establishment of a novel virus in the environment

2.4 Postulated risk scenarios

164. Three risk scenarios were postulated and screened to identify any substantive risks. These scenarios are summarised in Table 1 and examined in detail in Sections 2.4.1 - 2.4.3 (this Chapter).

165. 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.

Table 1. Summary of risk scenarios from the proposed dealings with the GMOs

Risk scenario	Risk source	Causal Pathway	Potential harm	Substantive risk?	Reasons
1	*GMO as a treatment*	i. Exposure of people undertaking dealings in clinical trial facilities to GMO via: ▪ needle stick/ sharps injury/ eye splash during GMO preparation, administration or sample analysis ▪ GMO contact with abraded skin ▪ contact with GMO contaminated materials ⬇ ii. Transduction of cells ⬇ iii. Expression of immunomodulatory transgenes	*Adverse immune response* *Vaccinia-like disease, including serious adverse reactions*	*No*	• Only trained and experienced personnel would prepare, administer and analyse the GMO. These personnel would also be experienced in the use and disposal of sharps. • Use of PPE (e.g. gown, gloves, mask and eye protection) minimises the potential for exposure to staff handling the GMO. • High-risk personnel are excluded from handing the GMO. • Sample testing would be conducted by qualified personnel in pathology or other testing laboratories. • The GMO is designed to selectively replicate in cancer cells. It is expected to be rapidly cleared by the immune response in healthy cells. • Accidental exposure would only involve a small dose of GMO. • Inadvertent exposures with wild-type VACV in healthy people documented to date did not lead to clinically significant symptoms or do not require treatment beyond first aid and observation.
2	*GMO as a treatment*	i. Trial participant injected/infused with the GMO ⬇ ii. The GMO or GMO products are dispersed/shed/ transmitted from the trial participant ⬇ iii. The GMO or GMO products are	*Adverse immune response* *Vaccinia-like disease, including serious adverse reactions*	*No*	In addition to the reasons described in Risk scenario 1: • High-risk trial participants, including immunocompromised persons and pregnant women, would be excluded. • High-risk staff would be excluded from providing care to patients post GMO administration. • Residual inoculum GMO is unlikely to be present at the site of administration as the line would be flushed and the injection/infusion site covered. The dressing would be

Risk scenario	Risk source	Causal Pathway	Potential harm	Substantive risk?	Reasons
		released to the environment, exposing other people or animals to the GMO or to the GMO products			changed daily by the trial participant until completely healed and dressing disposed in the provided biohazard bin, this would minimise contact transmission. • Trial participants would be educated on good hand hygiene and cough etiquette to prevent transmission. • All trial participants would be using barrier contraception to prevent pregnancy and transmission for at least 6 weeks after the last GMO treatment. • If the trial participant develops vaccinia-related lesions, they would be instructed to launder contaminated sheets and clothing in soapy water. • Bandages, dressings and other materials used to care for vaccinia-related lesions would be disposed in a biohazard bin. The biohazard bin would be returned to the clinical trial site for disposal. • The transmission rate from people who have received a replication-competent VACV vaccine to other people in recent vaccination programmes is low, as described in Paragraphs 82-83. • The limited number of clinical trial participants and education on transmission pathways is likely to reduce potential transmission. • If exposure occurred, it is likely to be at a low dose.
3	*GMO as a treatment*	i. Trial participant injected/infused with the GMO ⬇ ii. Trial participant becomes or is already infected with another compatible virus ⬇ iii. The GMO recombines with another virus in the host ⬇ iv. Produces a replication	*Novel disease in humans* *Establishment of novel virus with unknown pathogenicity in the environment*	*No*	• Prior treatment with an oncolytic virus is an exclusion criteria. This reduces the likelihood of similar genomic sequences that are available for recombination. • Vaccination with live vaccines 30 days prior to the first dose of GMO administration and while participating in the trial are also not permitted. • There is no reservoir of VACV in the Australian environment and limited opportunity for the GMO to come into contact with other related poxviruses. • For recombination to occur, the GMO and other poxviruses need to be present in the same cell at the same time.

Risk scenario	Risk source	Causal Pathway	Potential harm	Substantive risk?	Reasons
		competent recombinant virus ⬇ v. Recombinant virus shed ⬇ vi. Transduction of cells ⬇ vii. Recombinant virus infects other hosts			• As described in Paragraph 74, VACV is too large to persist in a transduced cell, which would limit interaction with a secondary compatible virus. • Viral factory compartmentalisation adds a further barrier for recombination. • The large 25 kb deleted region, housing multiple vaccinia genes would need to be acquired for the GMOs to regain its replicative ability in healthy cells.

2.4.1 Risk scenario 1

Risk source	GMO as a treatment
Causal pathway	i. Exposure of people undertaking dealings in clinical trial facilities to GMO via: ▪ needle stick/ sharps injury/ eye splash during GMO preparation, administration or sample analysis ▪ GMO contact with abraded skin ▪ contact with GMO contaminated materials ⬇ ii. Transduction of cells ⬇ iii. Expression of immunomodulatory transgenes
Potential harm	Adverse immune response Vaccinia-like disease, including serious adverse reactions

Risk source

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

Causal Pathway

167. 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

168. The GMO treatment would be prepared and administered into clinical trial patients with advanced solid cancerous tumours. Biological samples, including blood, would be collected throughout the trial. During these dealings, there is a potential risk of exposure to people involved in the trial via needle stick, sharps injury and or eye splash.

169. Controls proposed by the applicant, including appropriate training and the use of containment equipment, will minimise this risk. Use of PPE (e.g. gown, gloves, mask and eye protection) would minimise the potential for exposure of staff handling the GMO. Sample testing would be conducted by qualified personnel in pathology or other testing laboratories, which are required to adhere to national standards for handling of infectious substances. Additionally, appropriate decontamination and disposal practices would prevent persistence and spread of the GMO.

170. The above-mentioned limits and controls minimise the potential exposure of people to the GMOs via needle stick, sharps injury and/or eye splash.

Exposure via contact of abraded skin with injection site

171. As mentioned in Chapter 1, Section 3.6, VACV is transmitted through close physical 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, or directly with the GMO or GMO contaminated materials, they could be exposed to the GMOs.

172. Transmission of VACV from the treated trial participant to another person would require close contact with the skin lesions. The applicant has stated that the GMO would be flushed with sterile normal saline after administration and that the injection/infusion site would be covered by an occlusive dressing, this would limit the spread of the GMO from the injection/infusion site.

Exposure by contact with contaminated materials

173. As discussed in Chapter 1, Section 3.8, 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).

Potential harm

174. If people undertaking dealings in clinical trial facilities are exposed to the GMOs via needle stick, sharps injury, eye splash or via close contact to an unhealed vaccination site, they could suffer an adverse immune response or symptoms of VACV infection.

175. The standard Copenhagen vaccinia strain used as the parent organism to create the GMOs is capable of replicating in human cells, and thus can cause illness in humans (Chapter 1, Section 3.5).

176. However, as discussed in Chapter 1, Section 4, the GMO has been modified to be selectively replication competent in cancer cells. It is expected to be cleared by the immune system if it transduces healthy cells. The introduced genes all stimulate the immune response, therefore increasing its clearance ability. For example, when GM-VACV carrying IL-12 and IL-18 as transgenes were introduced in mice without cancers, clearance of the virus in the organs tested (spleen and ovaries) was enhanced. Clearance was associated with an increase in natural killer and T cells (Gherardi et al., 2003). Thus, any exposure of people undertaking dealings in clinical trial facilities is unlikely to result in viral infection/disease, and depending on the level of exposure, could only result in an acute reaction. Inadvertent exposures with wild-type VACV in healthy people documented to date did not lead to clinically significant symptoms or required treatment beyond first aid and observation (Cono et al., 2003; Fulginiti et al., 2003b; Maurer et al., 2003).

177. Persons undertaking dealings in clinical trial facilities who are likely to suffer a severe adverse reaction upon exposure due to conditions as stated in Paragraph 46 are to be excluded from handling or preparing the GMO.

178. The GMO encodes transgenes for three immunomodulatory genes, anti-CTLA-4 antibody, FLT3L, and membrane-bound IL-12. Production of IL-12 would be limited to the surface of cancerous cells as it is membrane bound and is therefore unlikely to be present in non-cancerous biological samples collected from the trial participant. Furthermore, a membrane-bound version of IL-12 has a better safety profile than systematically available IL-12 as described in Section 4.2.3. Moreover, Turnstone has provided transgene expression data and safety data from non-clinical studies. This data and its interpretation have been declared CCI under Section 185 of the Act. Relevant CCI was made available to the prescribed experts and agencies that were consulted on the RARMP for this application.

Conclusion

179. Risk scenario 1 is not identified as a substantive risk because potential exposure would be limited by the imposed limits and controls, and the GMO are designed to selectively replicate in cancer

cells. Therefore, this risk could not be greater than negligible and does not warrant further detailed assessment.

2.4.2 Risk scenario 2

Risk source	GMO as a treatment
Causal pathway	i. Trial participant injected/infused with the GMO ⬇ ii. The GMO or GMO products are dispersed/shed/transmitted from the trial participant ⬇ iii. The GMO or GMO products are released to the environment, exposing other people or animals to the GMO or to the GMO products
Potential harm	Adverse immune response Vaccinia-like disease, including serious adverse reactions

Risk source

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

Causal Pathway

181. Following GMO administration, there are a number of ways that the GMO could be dispersed, shed or transmitted from the trial participant.

182. As described in Paragraph 89, transmissible VACV of both high and low pathogenic strains can shed into the faeces and urine of experimentally infected mice. When VACV is administered as a vaccine, viremia (viral presence in the blood) and viruria (viral presence in urine) in humans is uncommon. It does occur in patients with progressive vaccinia and eczema vaccinatum (Lane and Fulginiti, 2003). However, these conditions are most likely to be manifested in persons who are immunocompromised and have a history of severe skin disease, respectively, see Paragraphs 102-103. Such persons are excluded from participating in the clinical trial. The GMO has been designed to selectively replicate in cancer cells. However, some level of GMO replication and immune evasion in healthy cells cannot be excluded. This could lead to GMO shedding into the environment.

183. The clinical trial would involve either i.t. injection or i.v. infusion as the mode of administration, this is different from the mode when used as vaccine against smallpox. Therefore, an increased amount of GMO is likely to be present in the blood when administered via i.v. The GMO could also be transmitted through the oral route. For example, 32% of trial participants (30 of 93) had low-levels levels of a similar, selectively replication-competent, oncolytic VACV treatment (JX-594) collected in throat swabs 4-8 days after i.v. administration. It is unclear if the low level of JX-594 detected was the result of swab collection of shed tumour cells and/or epithelial cells infected with JX-594, or free JX-594 itself. The number of infectious units detected was low (< 1% of the standard dose). There was no VACV detected in throat swab samples assayed at later time points, even in case of additional administrations of JX-594 after the first time point (Transgene, 2018). This data indicates that a persistent infection in the throat was not produced using JX-594. Furthermore, as stated in Paragraph 78, aerosol transmission from people vaccinated with VACV is considered unlikely. Moreover, as stated in Paragraph 176, the GMO is expected to be cleared by the immune system if it transduces healthy cells. The introduced genes all stimulate the immune response, therefore increasing its clearance ability.

184. The GMO could be transmitted from the trial participants should they develop vaccinia-related skin or oral pustules.

185. Trial participants who develop lesions would be instructed to follow the pustule management plan as described in Paragraph 26. The management plan would be explained to prospective participants during initial screening and anyone unwilling or unable to comply would not be enrolled in the trial. Trial participants would also be expected to seal contaminated disposable items in a provided primary container (e.g. press-sealed bag) and then place this into a provided secondary container (biohazard bin). At each visit, trial participants would return the biohazard bin to the clinical trial site for disposal as clinical waste. Participants would also be advised to launder contaminated fabrics in hot soapy water. The trial participant would also be instructed to change the dressing privately, unless they require assistance from a caregiver, and limit access to any pets, other animals and birds, or higher-risk individuals (see Paragraph 38). If an animal or other person develops a suspicious rash, this would be reported and may be examined by the clinical trial investigator. Together, these measures would minimise potential transmission of shed GMO and GMO products to other people and animals (including birds). Although birds are not known to be a host for VACV, replication from a GM VACV-based rabies vaccine was demonstrated in several Canadian bird species (Paragraph 69).

186. The transmission rate from people who have received a VACV vaccine to other people in recent vaccination programmes is low, as described in Paragraphs 82-83. The limited number of clinical trial participants and education on transmission pathways is likely to reduce potential transmission. Of relevance is the study where vaccination was carried out using replication-competent VACV strains. In this study, environmental samples were tested from 43 vaccinated individuals who suffered from major skin reactions. All of the environmental samples tested, that could have reasonably been in contact with a bandaged pustule, were found to be negative for live virus as determined by a plaque infectivity assay (Stark et al., 2006). The GMO has been designed to only replicate in cancer cells, reducing the potential for major skin reactions. Furthermore, high-risk staff would be excluded from providing care to patients post GMO administration. This would minimise the possibility of a serious adverse reaction should the trial participant develop a pustule.

187. Turnstone has provided shedding data from non-clinical studies. This data and its interpretation have been declared CCI under Section 185 of the Act. Relevant CCI was made available to the prescribed experts and agencies that were consulted on the RARMP for this application.

188. As described in Paragraph 127, 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 185, a pustule management plan would be employed in this scenario.

Potential harm

189. The potential harms from the GMO transmission are adverse immune responses and vaccinia-like diseases and reactions as described in Risk scenario 1.

Conclusion

190. Risk scenario 2 is not identified as a substantive risk because potential exposure would be limited by the imposed limits and controls (including bandaging of pustules), and the GMO are designed to selectively 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 as a treatment
Causal pathway	i. Trial participant injected/infused with the GMO ⬇ ii. Trial participant becomes or is already infected with another virus ⬇ iii. The GMO recombines with another virus in the host ⬇ iv. Produces a replication competent recombinant virus ⬇ v. Recombinant virus shed ⬇ vi. Transduction of cells ⬇ vii. Recombinant virus infects other hosts
Potential harm	Novel disease in humans Establishment of novel virus with unknown pathogenicity in the environment

Risk source

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

Causal Pathway

192. Should the trial participant be infected by other viruses, recombination can occur between viral types if they simultaneously infect the same cell. Similarly, another host could become infected with the GMOs through accidental exposure, and either have an existing viral infection or acquire one while the GMO is present. If recombination occurs, the introduced genes could potentially be transferred to other viruses, or genes that have been deleted from the GMO could be restored making it replication competent. The novel virus could then be shed from the recombination host.

193. Even though poxviruses can infect a wide range of organisms, specific poxviruses have a variable host range, and some are restricted to a single host (Oliveira et al., 2017). While recombination between different classes of virus can occur, the GM virus is more likely to recombine with another poxvirus than with an unrelated virus (see RARMP for DIR-116).

194. As described in the RARMPs for DIR-140 and DIR-170, *Molluscum contagiosum virus* (MCV) is likely to be present in the Australian population and MCV infection is more prevalent in school-aged children, adolescents, and young adults than in older adults (Konya and Thompson, 1999). 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.

195. A condition for participation is that the participants should not have had previous treatment with an oncolytic virus. Furthermore, trial participants would be selected on the basis that they have not received live vaccines within 30 days prior to the first dose of the GMO and agreement not to receive any live vaccines while participating in the trial (Paragraphs 36-37). These measures would reduce the likelihood of reassortment with similar genetic sequences between the viruses.

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

197. As discussed in Risk scenario 1, the GMO is designed to be selectively replication competent and is expected to localise to cancer cells and be cleared by the immune system in healthy cells. A barrier for potential recombination is in place, given that co-infection of the same cells with another poxvirus is needed for recombination.

198. As described in Paragraph 86, VAVC 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 from other viruses in the same cell (Paszkowski et al., 2016).

Potential harm

199. If the virus recombines with another virus, it could lead to a novel replication competent virus, it could then be shed from the recombination host and cause disease in humans or animals, or lead to the establishment of a novel virus in the environment.

200. Baseline information on the presence of the parental virus and introduced genes is provided in Chapter 1. The parental virus was one that was used in humans during the smallpox eradication programme. The introduced genetic elements are derived from human genes and they do not encode inherently toxic proteins, with the exception of overproduction of IL-12. However, the modification to limit the localisation of IL-12 to the cancer cell membrane should increase its safety as described in in Section 4.2.3.

201. If recombination between the GMO and another poxvirus occurred, it could result in viral progeny having any permutation of genomic segments of the two parent strains. Even in the unlikely scenario that recombination with a co-infecting poxvirus was able to generate a new replication competent poxvirus, it is not expected that recombination would lead to a virus that is more pathogenic or virulent than the wild type circulating poxvirus. This is because the deleted regions of the GMO are designed to reduce pathogenicity and the inserted genes are designed to enhance the immune response, which should result in enhanced viral clearance. Without functional characterisation, uncertainty, with respect to the potential recombinant virus, remains around this event.

Conclusion

202. Risk scenario 3 is not identified as a substantive risk because there is little potential for an adverse outcome as a result of recombination. Furthermore, there is a low likelihood of recombination occurring due to: the natural barriers to recombination, combined with the exclusion of participants who have previously received an oncolytic viral therapy or live virus vaccination 30 days prior to participating in the clinical trial. Therefore, this risk could not be greater than negligible and does not warrant further detailed assessment.

Section 3 Uncertainty

203. Uncertainty is an intrinsic part of risk analysis[12]. 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.

[12] 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.

204. Uncertainty is addressed by approaches such as balance of evidence, conservative assumptions, and there are several types of uncertainty in risk analysis (Clark and Brinkley, 2001; Hayes, 2004; Bammer and Smithson, 2008). These include:

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

205. 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.

206. 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, clinical trial applications are required to be limited and controlled. 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.

207. For DIR-179, uncertainty is noted in relation to the selective replication of cancer cells over healthy cells, biodistribution and shedding of the GMO in humans. As this is a first in human trial, Turnstone has conducted several non-clinical studies relating to these subject matters in a model species. These data have been declared CCI under Section 185 of the Act. Relevant CCI was made available to the prescribed experts and agencies that were consulted on the RARMP for this application. Due to the large deletions of vaccinia genes in the GMO (Paragraph 114), these modifications may have altered the GMO's ability to replicate in the cells of the model species. Therefore, there is uncertainty as to whether data gathered in the model species would be transferrable to humans. That being stated, the GMO is currently being evaluated in a Phase 1/2a clinical trial in the USA, with the intratumoural mode of administration (see Paragraph 142).

208. While some uncertainty remains, it is unlikely that the GMOs would behave very differently compared to the Copenhagen parental stain of VACV, which was used in humans during the smallpox eradication programme. That being stated, the deletion of vaccinia genes and introduction of genes, which enhance the immune response (Chapter 1, Section 4), are designed to increase the safety profile of the GMO should it be inadvertently exposed to people other than trial participants.

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

210. Additional data, including information to address these uncertainties, may be required to assess possible future applications with reduced limits and controls, such as a larger scale clinical trial or the commercial release of the GMO.

211. Chapter 3, Section 4, discusses information that may be required for future release.

Section 4 Risk evaluation

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

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

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

214. Three risk scenarios were postulated 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 these conclusions are summarised in Table 1 and include:

- the GMO has been designed to selectively replicate in cancer cells
- limited ability and opportunity for the GMOs to transfer the introduced genes through recombination
- suitability of limits and controls proposed by the applicant.

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

Chapter 3 Risk management plan

Section 1 Background

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

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

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

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

Section 2 Risk treatment measures for substantive risks

220. The risk assessment of risk scenarios listed in Chapter 2 concluded that there are negligible risks to people and the environment from the proposed clinical trial of GMO. These risk scenarios were considered in the context of the scale of the proposed clinical trial (Chapter 1, Section 2.1), the proposed controls (Chapter 1, Section 2.2), and the receiving environment (Chapter 1, Section 6), and considering both the short and the long term. The risk evaluation concluded that no specific risk treatment measures are required to treat these negligible risks. Limits and controls proposed by the applicant and other general risk management measures are discussed below.

Section 3 General risk management

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

3.1 Limits and controls on the clinical trial

222. Sections 2.1 and 2.2 in Chapter 1 list the limits and controls proposed by Novotech. Many of these are discussed in the three 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 proposed by Novotech

223. The proposed clinical trial would involve a maximum of 150 participants within Australia, and most dealings with the GMOs would take place in medical facilities such as clinical trial units, hospitals and

analytical laboratory facilities. Activities that would occur outside of medical facilities include transport, storage and disposal of the GMOs. The applicant has proposed to complete the study 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 and have been included in the licence.

224. The applicant advised that import and transport of the GMO and waste containing the GMO would be in accordance with IATA shipping classification UN 3373 [Category B] and/or the Regulator's *Guidelines for the Transport, Storage and Disposal of GMOs*. 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. These 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 GMO for export. These measures would limit the exposure of people and the environment to the GMOs.

225. There are proposed inclusion and exclusion criteria for both trial participants and staff as listed in Chapter 1, Section 2.3.4 and Paragraph 46. 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. 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. This also serves to minimise the potential for spread and persistence of the GMO as people in these groups are likely to experience a serious adverse reaction. When VACV is used to vaccinate against smallpox, potential pustule formation is likely to occur within seven days. Given this, licence conditions are imposed to exclude clinical trial staff providing direct care for whom exclusions under Paragraph 46 apply for at least seven days after each GMO administration or if pustules are present.

226. As stated in Paragraph 195, the likelihood of reassortment with the GMO and other similar genetic sequences between other viruses would be reduced by excluding trial participants who have received live vaccines within 30 days prior to the first dose of the GMO and agreed not to receive any live vaccines while participating in the trial (Paragraphs 36-37). However, reassortment can only occur between compatible viruses.. Therefore licence conditions are imposed where trial participants are excluded if they have received live vaccines, which are compatible for recombination with *Vaccinia virus*, 30 days prior to the first treatment with the GMO and also agree to forgo any vaccination with similar live vaccines, while participating in the trial. For example, compatible live vaccines, can include members of the *Poxviridae* family, or other live vaccines carrying any of the three immunomodulatory transgenes present in the GMO. This exclusion would not apply to the current COVID-19 vaccines.

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

228. 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 232.

229. 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 3.8) and compatible hosts such as rodents, marsupials and others as listed in Paragraph 68 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, other animals or birds to the GMOs.

230. 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 and cough etiquette practices. They would also provide trial participants a pustule kit and biohazard bin, as described in Paragraph 26. Together, these instructions, pustule kit and biohazard bin would limit the exposure of people, other animals or birds to the GMOs should pustules develop. The applicant has also stated that the trial participant would be instructed to launder sheets and clothing in hot (> 71°C) soapy water daily, should pustule(s) develop. Given that VACV can be inactivated with antimicrobial soap and water or bleach with 0.5% sodium hypochlorite (Paragraphs 90-91), the requirement for hot water (> 71°C), has not been imposed as a licence condition.

231. Part of the pustule management plan is for the trial participants to avoid high-risk individuals (Paragraph 26). Children under the age of 12 months are likely to develop serious adverse reactions should they become infected with VACV. As such, licence conditions include that trial participants would avoid direct physical contact with children under 12 months of age and excluded persons, from the time of each treatment with the GMO until after the respective follow-up visit to the clinical trial site that occurs on or after day 7 post-GMO administration.

232. A standard condition is included in the licence requiring the licence holder to ensure that dealings are conducted so as 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.

233. 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.

234. Further conditions imposed in the licence ensures 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 imposed to limit and control the clinical trial

235. 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 150 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 store the GMO and samples from GMO-treated participants in accordance with IATA shipping classification UN 3373 [Category B] and/or the minimum requirements for packaging, and labelling as detailed in the licence.
- clinical waste stream to be used by external service providers to destroy untreated GMO and GMO-related waste

3.2 Other risk management considerations

236. 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

237. 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.

238. The licence conditions include a requirement for the licence holder to inform the Regulator of any information that would affect their suitability.

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

3.2.2 Contingency plans

240. Novotech 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

241. 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, Novotech 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

242. The licence requires 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.

243. 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

244. 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.

245. 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.

246. 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

247. 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:

- information and data that would address the uncertainties noted in Chapter 2, Section 3. Specifically, information obtained from testing for selective replication competency in cancer cells, biodistribution and shedding of the GMOs in inoculated trial participants at the trial sites.

Section 5 Conclusions of the RARMP

248. 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.

249. Conditions are imposed to limit the number of trial participants, limit the location of the clinical trial to hospitals and clinical trial sites, limit the duration of the trial, and specify a range of controls to minimise the potential for the GMO and its genetic material to spread and persist 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 several submissions from prescribed experts, agencies and authorities[13] on the consultation RARMP. All issues raised in submissions relating 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 Committee agrees with the overall conclusions of the RARMP.	Noted.
	The Committee considered that all plausible risk scenarios have been identified.	Noted.
	The Committee did not identify any additional relevant information that should be considered.	Noted.
	The Regulator should consider further clarifying the uncertainties associated with transferability of non-clinical studies to the treatment of people.	Information was sought from the applicant with regards to the trial currently underway in the USA for i.t. administration NCT04301011. This information is being sought as CCI under Section 185 of the Act. The Regulator has reviewed this information in relation to the behaviour of the GMO in patients and the transferability of results from non-clinical studies.
	The Regulator should further consider limits and controls given uncertainties around data transferability and methods of administration.	The Regulator has taken a conservative approach with regards to the transferability of the findings in non-clinical studies to the administration of the GMO in humans. The Regulator has considered the potential for the GMO to be replication-competent in healthy cells and conditions are imposed in the licence to reflect this.
2	"The Shire has only ever formally considered the use of GM in crop situations, not in clinical or health settings. As such, we have no comment to make."	Noted.
3	"We have forwarded your correspondence to our Safer Communities Department for response."	Noted.
4	The Council has reviewed this application and offers the following feedback: While the Council is broadly supportive of DIR-179, it notes that there are some risks involved. • "The VACV used as the carrier in this treatment has the ability to cause pustules on the skin that carry the virus, which in this case will be the GM anti-tumour virus. These pustules carry the potential for	Noted.

[13] Prescribed expects, agencies and authorities include GTTAC, State and Territory Governments, Australian Government agencies and the Minister for the Environment.

Submission	Summary of issues raised	Comment
	secondary infection (Paragraph 26). The virus is also capable of infecting a wide range of animals, including common domestic and farm animals (Paragraph 68). • There is transmission of infection by contact with infected material and there is persistence of the virus in the environment (Paragraphs 78 and 87), so that the risk of spread of the virus is appreciable. • The proposed licence conditions recognise these risks and have a lengthy series of restrictions on the administration of the virus, handling, and care of the trial participants. • Monitoring of the patients and of the whole trial will be critical for personal and public safety. Contingency plans are required for risks emerging during the trials (Licence Condition 35)".	
5	The members "have reviewed the RARMP and in their opinion the draft licence conditions seems appropriate and commensurate with the level of risk of this clinical trial and in the context of the proposed risk management plan, including the proposed limits and controls." The members "supported the Regulator's conclusion of the RARMP 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 the gene technology."	Noted.
6	A report submitted concluded that Novotech's application has negligible risks to the health and safety of people and the environment. The submitters are satisfied of the measures taken to manage the short and long-term risks from the proposal.	Noted.
7	Comments are mainly in relation to the handling of GMO by trained staff as the submitter states that most clinical facilities do not have access to PC2 facilities.	
	"If issued, Novotech would have responsibility for ensuring training of personnel and compliance with licence conditions. • Is this direct responsibility or are they allowed to subcontract this to the clinical trial sites? • Will Novotech maintain a record of all staff involved in the trial and their training record?"	Licence conditions require the licence holder (Novotech (Australia) Pty Limited) to train people so that they can safely conduct the dealings in accordance with the conditions of the licence. Licence conditions require that these training records be made available within a timeframe stipulated by the Regulator.
	In case of exposure of people to the GMO, the applicant has proposed measures as described in Paragraph 58. • "Instead of soap, should it be disinfectant soap (like Microshield)?"	These contingencies were proposed by the applicant and were found to be satisfactory during the assessment of this application. Licence conditions require the licence holder to provider a contingency plan to

Submission	Summary of issues raised	Comment
		the Regulator at least 14 days prior to first administering the GMO at each Clinical trial site. This plan would include details of procedure for the decontamination of spills and disinfectants effective against the GMO at the Clinical trial site.
	"Paragraph 131. The principal route by which the GM VACV as a treatment could enter the wider environment is by shedding from the inoculated trial participants once they leave the hospital and return home. • If this occurs, isn't this a non-intentional release?"	• This is a first in human trial. By design, the GMO has been modified to reduce the likelihood of potential shedding. The potential shedding of the GMO was considered in the RARMP, its consequences assessed and found to be negligible. Conditions limiting the spread of the GMO are included in the licence. This application has been assessed as a 'Dealing involving Intentional Release' as it is a foreseeable consequence of the trial that GMOs may be released into the environment due to shedding from the patient.
	• "Will there be surveillance/testing of the wider environment to check for persistence?"	• The licence holder is required to contact the Regulator if any additional information involving risks to the environment, associated with the dealings authorised by the licence becomes available. This would include reporting significant shedding in the environment. The GMO has been designed to only replicate in human cancer cells and not healthy cells of humans and other hosts otherwise compatible with *Vaccinia virus*.
	• "Paragraph 230 states the applicant will provide patients with instructions on limiting exposure to GMO. How will compliance be ensured?"	• Licence conditions require the licence holder to obtain trial participant's written agreement to follow behaviours to limit exposure of others to the GMO.
	"Shedding is likely to be through contact with the pustules. What is the incidence of pustule formation likely to be? Based on the use of VACV in smallpox vaccination, this likelihood is high."	The GMO has been engineered to selectively replicate in human cancer cells. This should reduce the potential of pustule formation compared to VACV. Licence conditions require the licence holder to obtain trial participant's written agreement to follow behaviours to limit exposure of others to the GMO. This includes following processes to prevent people or animals coming into contact with pustules or virus shed from pustules. The trial participant would also be assessed for pustules during follow-up visits after GMO administration. The Regulator considers these requirements satisfactory to prevent transmission should pustules develop.
	"If the NYCBH strain was comparatively the most benign (Paragraph 93), why wasn't it used instead of the Copenhagen strain?"	The RARMP considers only the risks that may be associated with GMO proposed to be used by the applicant.
8	The GM virus is unlikely to pose substantial environmental risks due to the small and	Noted.

Submission	Summary of issues raised	Comment
	contained nature of the trial and barriers to recombination with wild type VACV in participants or in animals that could result in new variants of the virus emerging.	
	There is uncertainty around shedding routes into the environment and lack of biodistribution data and the potential for transmission of the GM virus by animals. This should be discussed further in risk scenario 2 (RS2). There is also uncertainty as to whether animal data is transferrable to the human situation.	Further discussion on shedding and transmission has been added to RS2 (Chapter 2, Section 2.4.2). Information was sought from the applicant with regards to the trial currently underway in the USA for intratumoural administration NCT04301011. This information is being sought as CCI under Section 185 of the Act. The Regulator has reviewed this information in relation to shedding and biodistribution of the GMO in human patients.
	The RARMP should provide data to support the statement that the GMO is expected to be cleared by the immune system.	Further information about IL-12 and its ability to enhance clearance of the virus has been added to Chapter 1, Section 4 and RS1 (Chapter 2, Section 2.4.1).
	The RARMP should discuss the risks regarding replication ability, shedding, and subsequent transmission to animals in more detail in RS2.	• Further information on transmission (oral route due to i.v. administration) has been added to RS2. • Further information on shedding and potential transmission by the model animal species has been added to the CCI attachment to the RARMP. • The selective replication of the GMO has been acknowledged in RS2 and included as a pathway for shedding into the environment.
	Information on VACV host range and reports of established VACV disease in native animals should be included in RS2.	Chapter 1, Section 3.2 has been expanded to include poxvirus infections in native Australian wildlife. Recent reports of established VACV disease in native animals in Brazil have already been described in the RARMP for DIR-170.
	The RARMP should include additional measures to reduce the risk of potential exposure of animals in the environment, given the possible replication ability, shedding, persistence, and broad host range of the GM virus.	Licence conditions are imposed to reduce the risk to all animals, including birds. The GMO has been engineered to selectively replicate in human cancer cells. This should reduce the potential of pustule formation. Licence conditions require the licence holder to obtain trial participant's written agreement to follow behaviours to limit exposure of others to the GMO. This includes following processes to prevent people or animals coming into contact with pustules or virus shed from pustules. The trial participant would also be assessed for pustules during follow-up visits after GMO administration. The Regulator considers these requirements satisfactory to prevent transmission should pustules develop.

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

The Regulator received one submission from a member of the public on the notification of the application. 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	Quote from submission: "God help the patients."	Noted.