Australian Government
Department of Health
Office of the Gene Technology Regulator

Risk Assessment and Risk Management Plan

(consultation version) for

DIR 187

Clinical trial of a genetically modified alphavirus for treatment of cancer

Applicant: VRT Pharmaceutics Pty Ltd

22 November 2021

This RARMP is open for consultation until Friday 7 January 2022.

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

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

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

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

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Summary of the Risk Assessment and Risk Management Plan (consultation Version)
for
Licence Application No. DIR 187

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, VRT Pharmaceutics Ptd Ltd (VRT Pharmaceutics) proposes to conduct a clinical trial of a genetically modified *Getah virus* as a treatment for cancer. The clinical trial is proposed to take place at Flinders Private Hospital in Bedford Park, South Australia and at other locations in Australia as required. The trial will run for a period of up to five years. Its objectives are to evaluate the safety and tolerability of the GMO in adult participants with locally advanced or metastatic cancer. Trial participants' immune response to the GMO, as well as its biodistribution and shedding, will also be assessed. A maximum of 18 cancer patients would receive up to seven treatments with intravenously delivered GMO. Four different dose levels would be tested. Patients who respond well to the treatment would have the opportunity to continue to receive the GMO for another two years after the study protocol is complete.

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, VRT Pharmaceutics would require authorisation from the 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. VRT Pharmaceutics would also require approval from the Department of Agriculture, Water and the Environment for import of the GMO into Australia. In addition, they may require approval from the Chief Inspector of Stock before bringing the GMO into South Australia, and a Prohibited Matter Permit from New South Wales, Queensland and Western Australia if they wish to conduct dealings in those states.

The Regulator has prepared a Risk Assessment and Risk Management Plan (RARMP) for this application, which concludes that the proposed clinical trial poses negligible risks to human health and safety and the environment. Licence conditions have been drafted for the proposed clinical trial. The Regulator invites submissions on the RARMP, including draft licence conditions, to inform the decision on whether or not to issue a licence.

The application

Project Title	Clinical trial of a genetically modified alphavirus for treatment of cancer [1]
Parent organism	*Getah virus* (M1 strain), a member of the *alphavirus* genus
Genetic modifications	Two single nucleotide changes have been introduced into the *Getah virus* (M1) genome, each altering one amino acid in separate viral proteins.
Principal purpose	The trial will evaluate the safety and tolerability of the GMO in adult participants with locally advanced or metastatic cancer. Trial participants' immune response to the GMO, as well as its biodistribution and shedding, will also be assessed.
Previous clinical trials	The proposed study is the first formal clinical trial to be undertaken. However, 14 patients with different solid tumours have been treated with the GMO under compassionate use access in China.
Proposed limits and controls	
Proposed duration	5 years
Proposed release size	Up to 18 participants will be enrolled in the trial
Proposed locations	Flinders Private Hospital, Bedford Park SA; Royal Adelaide Hospital, Adelaide SA; and Austech Medical Laboratories, Bankstown NSW. Additional clinical trial sites in Australia may be engaged if sufficient participants cannot be recruited in Adelaide.
Proposed controls	• The GMO will be administered to trial participants in a hospital setting • Staff preparing and administering the GMO will use personal protective equipment • Waste that may contain the GMO will be disposed of by high temperature incineration • Participants will remain in hospital for at least 24 hours after the first treatment and 2 hours after subsequent treatments • Trial participants will take the following precautions: – Take measures to avoid exposure to mosquitoes for 7 days after each treatment – If sexually active, use barrier contraception for 30 days after each treatment – Avoid contact with newborns, immunocompromised and severely immunodeficient individuals • Pregnant women will be excluded from the trial • Trial participants may not donate blood or organs during the trial • Immunocompromised or pregnant clinical trial staff should avoid direct contact with the GMO and with participant injection sites, excreta and secretions

[1] The title of the application submitted by VRT Pharmaceutics Pty Ltd was 'Clinical trials with alphavirus M1 GMO (M1-c6v1) in patients with solid tumours'.

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 short- and long-term impacts are considered.

Credible pathways to potential harm that were considered include transmission of the GMO from trial participants to other people and animals via mosquitoes or direct contact with blood or body fluids, transmission of the GMO from exposed clinical trial staff to other people and animals, and transplacental transmission from a pregnant clinical trial staff member to their unborn child. Potential harms that were considered in relation to these pathways included more severe forms of *Getah virus* (GETV)-associated disease.

Important factors in reaching the conclusions of the risk assessment that unintended exposure to the GMO would be minimised by proposed limits and controls.

As risks to the health and safety of people, or the environment, from the proposed trial of the GM GETV as a cancer 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. Draft licence conditions are detailed in Chapter 4 of the RARMP.

As the level of risk is considered negligible, specific risk treatment is not required. However, since this is a clinical trial, the draft licence includes limits on the number of trial participants, locations limited to facilities similar to the hospitals and associated storage and distribution site described in the application, limits on the duration of the trial, as well as a range of controls to minimise the potential for exposure of people other than trial participants, and exposure of animals, to the GMO. 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

AHSSQA	Australian Health Service Safety and Quality Accreditation
AICIS	Australian Industrial Chemical Introduction Scheme
APA	Approved Pathology Authority
APL	Accredited Pathology Laboratory
APP	Approved Pathology Practitioner
APVMA	Australian Pesticides and Veterinary Medicines Authority
CCI	Confidential Commercial Information
CDC	Centers for Disease Control and Prevention
CHIKV	Chikungunya virus
CRO	Contract Research Organisation
CTA	Clinical Trial Approval
CTN	Clinical Trial Notification
DAWE	Department of Agriculture, Water and the Environment
DIR	Dealings involving Intentional Release
DNA	Deoxyribonucleic acid
FSANZ	Food Standards Australia New Zealand
GETV	Getah virus
GM	Genetically modified
GMO	Genetically modified organism
kb	kilobase
HREC	Human Research Ethics Committee
IATA	International Air Transport Association
iv	Intravenous
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
NHMRC	National Health and Medical Research Council
NPAAC	National Pathology Accreditation Advisory Council
nsP	Non-structural protein
NSQHS	National Safety and Quality Health Service
OGTR	Office of the Gene Technology Regulator
ORF	Open reading frame
PCR	Polymerase chain reaction
PPE	Personal protective equipment
qPCR	Quantitative polymerase chain reaction
RAH	Royal Adelaide Hospital
RARMP	Risk Assessment and Risk Management Plan
Regulations	Gene Technology Regulations 2001
Regulator	Gene Technology Regulator

RRV	Ross River virus
SAE	Serious adverse event
SOCRU	Southern Oncology Clinical Research Unit
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
VRT Pharmaceutics	VRT Pharmaceutics Pty Ltd
WHO	World Health Organization

Chapter 1 Risk assessment context

Section 1 Background

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

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

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

4. The *Risk Analysis Framework* (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.

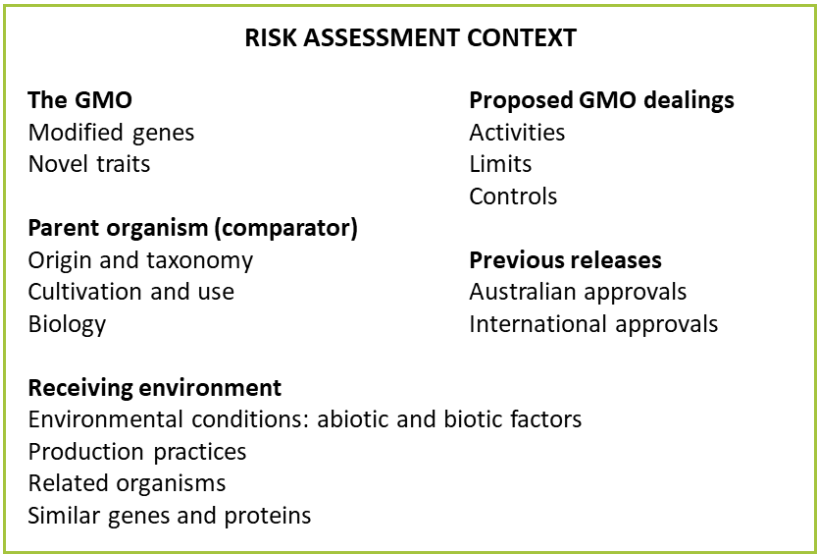

Figure 1. Summary of parameters used to establish the risk assessment context, within the legislative requirements, operational policies and guidelines of the OGTR and the RAF.

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 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, State and Territory Governments, Australian Government authorities or agencies prescribed in the Regulations, Australian local councils and the Minister for the Environment - and from the public.

1.1 Interface with other regulatory schemes

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

14. 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. GETV is listed as prohibited matter under the New South Wales *Biosecurity Act 2015* and Queensland *Biosecurity Act 2014* and is a declared prohibited organism under Western Australia's Biosecurity and Agriculture Management Regulations 2013[2]. GETV is a declared notifiable disease in South Australia, pursuant to section 4 of the *Livestock Act 1977*, and a person must not, without the approval of the Chief Inspector of Stock[3], bring or cause a notifiable disease to be brought into the state. Permits and approvals from the respective state governments may be required to conduct dealings with GM GETV in those states.

16. The clinical trial activities described in the application would take place in hospitals and associated pharmacies. Analysis of biological samples collected from trial participants treated with the GMO would occur at clinical trial sites or pathology laboratories. These facilities are regulated by State and Territory governments and adhere to professional standards for safety (NSQHS), disease control (Australian Guidelines for the Prevention and Control of Infection in Healthcare (2019)) and handling of pathology samples (NPAAC).

17. 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.

18. 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).

19. 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

[2] GETV is assigned to the Category 1-Exclusion 'control category', which applies to prohibited organisms whose introduction into the state should be prevented, and to the Prohibited 'keeping category' whereby keeping the organism in the state is prohibited except under the authority of a permit, including for scientific purposes.
[3] Appointed under Part 8 of the *Livestock Act 1977*.

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

20. VRT Pharmaceutics Pty Ltd (VRT Pharmaceutics) has proposed a Phase 1 clinical trial of a GM *Getah virus* (GETV). The M1 strain of GETV has been shown to preferentially target cancer cells and is under investigation as an oncolytic virus. The genetic modifications enhance this selectivity, increasing viral replication in cancer cells but not in non-cancerous cells. The primary purpose of the trial is to assess the safety and tolerability of the GMO. Trial participants' immune responses to the GMO, as well as its biodistribution and shedding, will also be assessed.

21. The GMO will be manufactured in China and imported into Australia. It will be administered intravenously to adult cancer patients who meet specific disease-related criteria. Biological samples that may contain the GMO will be collected from trial participants for analysis in laboratories within Australia.

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

(a) import the GMO;

(b) conduct the following experiments with the GMO:

i. prepare the GMO for administration to trial participants;

ii. administer the GMO to trial participants by intravenous infusion;

iii. collect biological samples from trial participants;

iv. analyse the samples described in 22(b)iii;

(c) transport the GMO;

(d) dispose of the GMO;

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

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

23. The proposed clinical trial would run for five years.

24. The clinical trial would take place at Flinders Private Hospital, Bedford Park SA. Additional clinical trial sites in Australia may be added if required.

25. Enrolment would be limited to 18 trial participants with locally advanced or metastatic cancer.

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

26. The Applicant has proposed controls to minimise exposure to the GMO, and to restrict the spread and persistence of the GMOs in the environment. These include that:

- The GMO would be administered to trial participants in a hospital setting;
- Only trained and authorised staff would conduct dealings with the GMO;
- Staff preparing and administering the GMO would use personal protective equipment;

- Transport, storage and disposal of the GMO and any contaminated waste generated at a clinical trial site would be in accordance with the current version of the Regulator's *Guidelines for the Transport, Storage and Disposal of GMOs;*
- Trial participants would be required to remain at the clinical trial site for a specified time after GMO treatments and instructed in behavioural measures to prevent GMO transmission;
- Pregnant women would be excluded from the trial; and
- Immunocompromised or pregnant staff would be advised to avoid direct contact with the GMO and with participant injection sites, excreta and secretions.

2.3 Details of the proposed dealings

2.3.1 Overview of the clinical trial

27. VRT Pharmaceutics (the Applicant) is applying for authorisation to conduct the proposed clinical trial in Australia on behalf of an international sponsor (the Sponsor). If the licence is approved, a Contract Research Organisation (CRO) with responsibility for managing the trial will be engaged.

28. The trial is a Phase 1 open-label dose-escalation study that will evaluate the safety and efficacy of the GMO (M1-c6v1) as a treatment for locally advanced or metastatic cancer, as well as evaluating its immunogenicity, tissue distribution and shedding characteristics. Four successive patient cohorts will receive up to seven treatments with the GMO at different dose levels. Patients who respond well to the treatment will have the opportunity to continue to receive the GMO for another two years after the study protocol is complete, during which time safety and tolerability will continue to be assessed. Participants will preferentially be recruited from the area local to the trial site but could live elsewhere in Australia and return home in between treatments.

29. Up to three dose levels (3.0×10^8 CCID50[4], 1.0×10^9 CCID50 and 3.0×10^9 CCID50) will be tested. As the Applicant is seeking to identify the Maximum Tolerated Dose, the first patient cohort will receive the lowest dose, with higher doses only administered once this is shown to be safe. If the first cohort cannot tolerate the 3.0×10^8 CCID50 dose, then the next cohort would receive 1.0×10^8 CCID50.

30. The GMO will be administered on the first day of each of seven treatment cycles. The interval between the first and second dose will be 21 days, followed by a 14 day interval before each subsequent dose. The treatment period will run for a total of 106 days. Follow up visits to the clinical trial site will take place monthly for the first year, and bi-monthly for the second year.

31. Participants will remain in the trial until they complete the study protocol unless they experience confirmed disease progression, cannot tolerate the treatment or withdraw their consent.

2.3.2 Organisations involved in the trial

32. The Applicant has provided details of how they would undertake dealings within facilities at Royal Adelaide Hospital (RAH), Flinders Private Hospital, Austech Medical Laboratories and the Flinders Centre for Innovation in Cancer (see Table 1). However, they may wish to engage additional sites over the course of the trial if sufficient participants cannot be recruited in Adelaide.

[4] CCID50 is a measure of infectious virus titre and equals the amount of virus required to produce a cytopathic effect in 50% of inoculated tissue culture cells.

Table 1 Clinical and other facilities in which proposed dealings will take place

Organisation or facility	Proposed dealing(s)	Notes
Austech Medical Laboratories Bankstown NSW 2200	Storage and distribution of imported GMO vials Storage and analysis of biological samples (GMO-related testing)	Storage and sample analysis in certified PC2 facility Cert-4594
Royal Adelaide Hospital Level 1 Clinical Trials Pharmacy	Preparation of treatment doses from lyophilised GMO	Procedure to be conducted within pharmaceutical isolator located in Isolator Room 1F132
Southern Oncology Clinical Research Unit, Flinders Private Hospital, Bedford Park SA	Administration of prepared GMO to trial participants Collection of biological samples from trial participants	Standard clinical facilities accredited to the National Safety and Quality Health Service Standards (NSQHS Standards)
Flinders Private Hospital pathology laboratory	Analysis of biological samples (clinical monitoring)	Standard pathology laboratory.
Flinders Centre for Innovation in Cancer	Analysis of biological samples (GMO-related testing)	Sample analysis in certified PC2 facility Cert-3824

* Certified under Regulator's *Guidelines for the Certification of a Physical Level 2 Laboratory*, V3.2 (issued 1 March 2013)

2.3.3 Selection and management of trial participants

33. Prospective participants will be screened against an extensive list of selection criteria. Inclusion criteria relevant to assessment of risk include that participants must:

- be over 18 years;
- fully understand and be able to sign the informed consent form (ICF);
- be willing to follow and have the ability to complete all trial procedures;
- have locally advanced or metastatic cancer with at least one measurable tumour[5];
- have a performance status on the Eastern Cooperative Oncology Group (ECOG) scale one week before first use of the study drug of either 0 (fully active, able to carry on all pre-disease activities without restriction) or 1 (restricted in physically strenuous activity but ambulatory and able to carry out work of a light or sedentary nature, e.g. light house work or office work);
- if female and of child-bearing potential, have negative serum pregnancy test results within seven days before the first use of the study drug;
- if capable of reproduction (male or female), agree to use effective birth control measures with their partners for at least 90 days after the final GMO treatment[6];
- consent to stay in the treatment facility for at least 24 hours after receiving the first GMO dose and for 2 hours after receiving each subsequent dose;
- consent to apply Ultrathon™ Insect Repellent Lotion to uncovered skin every twelve hours for seven days following each treatment with the GMO, whenever they intend to leave a physically mosquito-protected area (such as a building fitted with flyscreens).

[5] As defined in RECIST Guidelines version 1.1 Eisenhauer, E.A., Therasse, P., Bogaerts, J., Schwartz, L.H., Sargent, D., Ford, R., Dancey, J., Arbuck, S., Gwyther, S., Mooney, M.*, et al.* (2009). New response evaluation criteria in solid tumours: revised RECIST guideline (version 1.1). Eur J Cancer *45*, 228-247. i.e. with a minimum diameter of 10 mm by CT scan or caliper measurement by clinical exam, or 20mm by chest X-ray.

[6] Note that the use of barrier contraception will be recommended but not required.

34. Exclusion criteria include that trial participants must not:

- have received a live attenuated vaccine within 4 weeks before the first use of the study drug; and
- have previously received an oncolytic virus or other gene therapy treatment.

2.3.4 Manufacture and import of the GMO

35. The GMO will be manufactured in China and packaged into borosilicate glass vials sealed with a flexible stopper. Each vial will contain the GMO (3.0×10^8 CCID50) supplied as a lyophilised powder. The product will only be released after passing all quality and product release tests, including verification that no sequence mutations have been introduced during production.

36. Vials will be clearly labelled with the product name, specifications, titre, batch number, expiration date, and name of the Sponsor.

2.3.5 Transport of the GMO

37. Imported shipments will be delivered to Austech Medical Laboratories (Austech) in Sydney, NSW where GMO vials will be stored then transported to the RAH Pharmacy (or other clinical trial site) as required. At the RAH Pharmacy, the GMO will be diluted into intravenous (iv) infusion bags as needed for each trial participant and immediately transported to the Southern Oncology Clinical Research Unit (SOCRU) at Flinders Private Hospital.

38. Transport from the border to Austech, and then to the clinical trial site, will be contracted to a specialised courier company, such as World Courier, with experience in handling GMOs. The GMO will be packed to meet the requirements of International Air Transport Association (IATA) shipping classification UN3373 (Biological Substance, Category B). This classification applies to infectious substances which are known or reasonably expected to contain non-Category A pathogens and which are shipped for diagnostic or investigational purposes. For international transport, packaging and labelling must be in accordance with IATA packing instruction 650[7]. The Applicant stated that the required sift-proof primary receptacles and secondary packaging will also be unbreakable, and the outer packaging will carry 'biohazard' and 'contains GMO' labels. In particular, prepared iv infusion bags being transported from the RAH Pharmacy to SOCRU will be sealed within a plastic bag and packaged in a non-breakable plastic container. The IATA packaging requirements and additional labelling together meet the requirements stipulated in the Regulator's *Guidelines for the Transport, Storage and Disposal of GMOs* for microorganisms requiring PC2 containment.

39. Waste containing the GMO will be sealed in designated waste bins and transported from the clinical trial site to the site of destruction by an external service provider (see Section 2.3.12).

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

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[7] For solid substances, this specifies:

- a primary receptacle and secondary packaging that are both sift-proof, and a rigid outer packaging;
- that primary receptacles will be packed such that, under normal conditions of transport, they will not break, be punctured or leak their contents into the secondary packaging;
- that multiple fragile primary receptacles placed within a single secondary package must be individually wrapped or separated so as to prevent contact between them;
- that dry ice must be placed outside the secondary packaging;
- a rigid outer packaging that is strong enough for its capacity, weight and intended use; and
- labelling must include the name and address of the shipper and consignee, together with the name and telephone number of the person responsible

2.3.6 Storage of the GMO

41. On arrival in Australia, the GMO will be received and stored in the Austech Medical Laboratories PC2 facility (Cert-4594), in accordance with the Regulator's *Guidelines for the Transport, Storage and Disposal of GMOs*. Staff will unpack and inspect the vials before storage, then repackage for shipment to the RAH Pharmacy.

42. Short term storage at the RAH Pharmacy may also be required before the GMO is prepared for administration to trial participants. Again, vials will be unpacked and inspected before storage.

43. The GMO and biological samples collected from trial participants will be stored within sealed unbreakable primary and secondary containers and placed in a locked freezer with access restricted to authorised persons. The outer container and freezer will each be labelled to indicate that it may contain a GMO. Storage will be in accordance with the Regulator's *Guidelines for the Transport, Storage and Disposal of GMOs.*

44. The CRO contracted to manage the trial will maintain a consolidated electronic record of all GMOs being stored across the separate locations. VRT Pharmaceutics, as licence holder and local sponsor of the trial, will be updated on a regular basis.

2.3.7 Preparation of the GMO

45. Each dose of GMO will be prepared for inoculation by pharmacy staff who have been trained in their institution's SOPs for handling GMOs and associated waste disposal. The procedure will be carried out in a Negative Pressure Isolator located in the RAH Pharmacy. The Regulator has approved use of this isolator for preparing GMOs associated with at least three other clinical trials (DIR-140, DNIR-571 and DNIR-598).

46. Each GMO vial will be reconstituted to yield a concentration of $1.1x10^8$ CCID50/ml. The required volume will be transferred to an infusion bag containing 250 mL of saline, with the different GMO doses requiring a total of one, four or ten vials each. Final concentrations will range from $4x10^5$-$1.2x10^7$ CCID50/ml.

47. Dose preparation will require handling of sharps in association with the GMO. A needle attached to a syringe will be used to add water to the sealed GMO vials, withdraw the reconstituted GMO solution, and inject it into the injection port of the infusion bag The syringe with needle still attached will be discarded directly into a sharps container. The needle will not be removed or recapped.

2.3.8 Administration of the GMO and post-administration activities

48. The GMO will be administered in a clinical area at SOCRU. This will be a standard single treatment room, not shared with other patients and thoroughly cleaned as soon as each trial participant departs. When in use for the trial, the room will be supplied with a spill kit, GMO sampling kit, spare unbreakable plastic containers and GMO labels.

49. A designated clinical trial nurse will administer the GMO by in infusion using standard hospital equipment. Before starting the procedure, a catheter or other suitable intravenous device will be inserted into a peripheral vein. No further use of sharps will be required. The infusion line attached to the prepared infusion bag containing diluted GMO solution will be attached to the catheter via a secure Luer-Lok connector, creating a closed system. GMO will be infused slowly over one hour (approximately 4.2 ml/minute). No other substances will be administered through the infusion line while GMO administration is in progress, ensuring that sharps associated with other treatments do not come into contact with the GMO.

50. After infusion is complete, the infusion line will be detached from the catheter. The exposed end of the infusion line will be enclosed with an ethanol wipe to prevent drips and the infusion bag

and wipe discarded as GMO waste. The catheter will remain in place - its exposed end will be disinfected with 70% ethanol, allowed to dry for 30 seconds, flushed with 10 ml saline to remove any residual GMO, disinfected again, then sealed with a sterile cap.

51. The catheter will be removed two hours before the trial participant is due to leave the hospital. The insertion site will be disinfected and covered with an occlusive dressing. This will be removed by a staff member just before the participant departs and disposed of as GMO waste.

52. All equipment and surfaces potentially in contact with the GMO will be decontaminated by wiping with a chlorine-containing disinfectant solution.

53. After the first GMO treatment, trial participants will remain at the clinical trial site as an in-patient for at least 24 hours, in the treatment room where the GMO was administered. After each subsequent treatment, they will remain in the treatment room for a minimum of two hours.

2.3.9 Sample collection and analysis

54. Biological samples (blood, saliva, nasal discharge, urine and stool) will be collected for clinical monitoring of participants and for tests related to the GMO (assessment of GMO content, both viral genomes and infectious particles), immunogenicity, cytokine production and expression of molecular markers). The Applicant plans a detailed time course after the first two GMO treatments, with multiple samples collected on day 1, followed by single time points on days 2, 3, 8, and 15 for all sample types except stool. Sampling after later treatments will be reduced to days 8 and 15 during cycle 3, and day 15 only during cycles 4-7. If early results indicate that shedding has ceased, or it extends beyond 106 days, this schedule may be amended.

55. Stool samples will be collected on day 1 for all treatment cycles, on days 8 and/or 15 during cycles 1-3, then on the final day of the treatment period (day 106).

56. All biological samples for GMO-related tests will be collected at the clinical trial site. These samples will be processed and analysed in the certified PC2 laboratory at either the Flinders Centre for Innovation in Cancer (Cert-3824) or at Austech Medical Laboratories in Sydney (Cert-4594).

57. Blood and urine samples for clinical monitoring will be collected either at the clinical trial site or at local pathology laboratories and analysed at the Flinders Private Hospital pathology laboratory.

58. Tumour tissue samples may be collected between Days 1-49 following the first GMO treatment. Procedural details would depend on participants' specific tumours, however collection would be done by a specialist medical practitioner and involve the use of sharps. Samples would be processed for detection of the GMO *in situ* and for immunohistochemical analyses.

59. All biological samples will be treated as though they contain the GMO.

2.3.10 Personal protective equipment and other precautions

<u>For clinical trial staff:</u>

60. The Applicant will recommend that pregnant or immunocompromised individuals not undertake any roles that involve handling the GMO and will include this in training materials.

61. Staff preparing the GMO will wear PPE that includes a disposable gown and gloves, and must wash or sanitise their hands after removing the gloves. Staff administering the GMO will add a mask and safety glasses. Anyone with skin damage on their hands must wear double gloves.

62. Any clinical trial staff caring for trial participants, or other hospital staff required to perform procedures as part of their medical care, will be advised to wear disposable protective suits and gloves if they may be exposed to blood, secreta, urine or faeces. As above, double gloves should be worn if a

person has broken skin on their hands, or if gloves are likely to tear during the procedure. Staff will be reminded to pay particular attention to avoiding sharps injuries when performing invasive procedures.

For close contacts of trial participants:

63. Close contacts of trial participants will be advised to pay attention to personal protection when handling items contaminated by blood and body fluids from trial participants. Gloves should be worn and hands washed or sanitized immediately after removing them.

2.3.11 Behavioural requirements for trial participants

64. During the entire treatment period, trial participants will be instructed to avoid close contact with newborns, and known immunocompromised or severely immunodeficient people. They must also not donate blood or organs during the trial.

65. Along with the requirement to use effective contraception during the treatment period and for 90 days afterwards (Section 2.3.3), barrier contraception will be recommended for sexually active trial participants and their partners to prevent transfer of body fluids during sexual contact.

66. Trial participants will be instructed in a range of behavioural measures intended to minimise opportunities for being bitten by mosquitoes:

- They must remain inside the treatment facility for at least 24h after their first GMO treatment and for a minimum of 2h after each subsequent treatment, when passive viraemia following infusion of the GMO is expected to be highest.
- For seven days after each treatment, they must apply Insect Repellent Lotion (to uncovered skin every twelve hours whenever they intend to leave a physically mosquito-protected area (e.g. a building fitted with flyscreens). The clinical trial site will provide the repellent. They should reapply the lotion as required, particularly if swimming or travelling to areas with higher mosquito prevalence.
- They will be instructed to take steps to control mosquitoes around their homes – e.g., by emptying standing water, ensuring windows and external doors are fitted with effective flyscreens, and using mosquito netting around beds or sitting areas if needed.
- If they live in or travel to an area where mosquitoes are present, trial participants will be instructed to wear long-sleeves and long pants, stay indoors where possible, and choose accommodation that excludes mosquitoes (e.g. air-conditioned or fitted with insect screens).
- Prospective participants will be informed of the risks associated with the GMO treatment and these behavioural requirements before they consent to take part in the study. Trial participants and family members will receive training sessions covering these instructions.

2.3.12 Decontamination and disposal of the GMO

67. During preparation and administration of the GMO, all disposable materials that have come into contact with the GMO, such as pharmaceutical vials, syringes, needles, cotton balls, gauze blocks and gloves will be disposed as GMO waste in accordance with the Regulator's *Guidelines for the Transport, Storage and Disposal of GMOs*. They will be discarded into study-specific biohazard waste bins provided and collected by an external waste contractor experienced in disposal of GMO waste, and destroyed by high temperature incineration.

68. Work surfaces and equipment exposed to the GMO will be decontaminated as soon as practicable after use and before use for any other purpose. Surfaces will be wiped down with either 70% ethanol, 1000 mg/L effective chlorine disinfectant or 1-2% bleach.

69. Any unused vials of GMO remaining at the end of the trial will be disposed of either by returning to the Sponsor or placing in a study-specific waste bin for collection and incineration by external waste contractors. Disposal or destruction will be documented.

2.3.13 Relevant training and experience of clinical trial personnel

70. Medical staff responsible for preparing and administering the GMO must comply with clinical standards published by the Department of Health in their state and have completed all competency assessments required by relevant healthcare authorities e.g. The Australian Health Practitioner Regulation Agency (AHPRA) and National Boards (Medical Board of Australia, Nursing and Midwifery Board of Australia, Pharmacy Board of Australia etc). Relevant competencies include *Aseptic technique*, *Hand hygiene* and *Infection Prevention and Control Practices*. The Applicant stated that competency assessments are completed during tertiary education and via professional continuing education programs. Staff at the RAH Pharmacy will be trained in handling and disposal of the GMO according to the site SOP.

71. The CRO managing the trial will check the qualifications and training records of each staff member either while qualifying the clinical trial site or prior to the Site Initiation Visit.

2.3.14 Contingency plans

72. In the event of a needlestick injury or the GMO solution contacts damaged skin, the exposed area will immediately be washed with soap or detergent and rinsed under running water for at least 5 minutes. If liquid splashes into the eyes or contacts other mucosal tissues, the area will be rinsed with running water. Blood samples will be collected daily (from the day after exposure) and tested for GMO viral genomes until two consecutive negative results are obtained. The exposed person would need to take precautions to protect from mosquito bites while awaiting the negative results.

73. If a person other than a trial participant is exposed to the GMO, they will be offered prompt medical attention. The medical practitioner will be given all relevant information about the GMO.

74. All staff handling the GMO will be provided with spill kits, including those in the pharmacy, the clinical area, pathology laboratories and couriers. Staff will be trained in the use of the spill kit and appropriate spill clean-up procedures.

75. Any spill or loss of the GMO at a clinical trial location or during transport, or exposure of a person other than a trial participant to the GMO, will be reported to the Regulator as soon as possible.

76. All serious adverse events (SAEs) will be reported to the Regulator within 24 hours.

2.3.15 Informing persons covered by the licence about licence conditions

77. A copy of the licence will be provided to all participating locations. Staff involved in handling the GMO during receipt and storage, preparation, and administration to participants will be trained in licence conditions. Up-to-date instructions for handling the GMO will be included in the Study Protocol and Pharmacy Manual. All training will be documented in training logs.

78. External service providers will be informed that they are handling a GMO via labelling on the outermost container. In addition, a copy of the licence will be included in shipping documentation and provided to the waste disposal provider when entering into the service agreement.

Section 3 Parent organism – *Getah virus*

79. *Getah Virus* (GETV) is an enveloped, single-stranded, positive sense RNA virus of the genus *Alphavirus* within the family *Togaviridae*. Alphaviruses generally exhibit broad host tropism and are transmitted to various vertebrate hosts, including humans, via invertebrate vectors, usually mosquitoes, in which they establish persistent and asymptomatic infections. In contrast, infection in the vertebrate host is acute and can cause severe disease. To date, at least 32 alphavirus species have

been identified and are globally distributed across all continents except Antarctica. They are categorised as either 'Old World' or 'New World' alphaviruses based on their E1 protein genetic diversity and geographic origin. Old World alphaviruses tend to be associated with arthritic symptoms and New World alphaviruses with encephalitic symptoms and a more frequently lethal outcome (Abdullah et al., 2021; Gotte et al., 2018; Nowee et al., 2021).

3.1 Classification and genome characteristics

80. As an alphavirus, the GETV genome is a single positive-stranded RNA molecule of about 11.5 kb. It mimics cellular RNA as it has both a 5' cap and a poly-A tail at the 3' untranslated region (UTR). The genome contains two large open reading frames (ORF1 and ORF2), both encoding polyproteins. The polyprotein translated from ORF1 is processed in several stages into four non-structural proteins (nsP1 to nsP4). These are responsible for viral RNA transcription, replication, polyprotein cleavage and RNA capping. ORF2 is located downstream of ORF1 and encodes the structural proteins capsid (C), three envelope proteins (E1, E2 and E3), a 6 kDa protein (6K) and the transframe protein (TF), comprised of a C-terminal extension of the 6 K protein after a frameshift event (Gotte et al., 2018; Li et al., 2017b; Nowee et al., 2021; Ren et al., 2020).

3.2 Lifecycle

81. Infection of a cell starts with binding of the GETV virion to its receptor, mediated by the viral E2 glycoprotein. This initiates receptor-mediated endocytosis followed by fusion of viral and endosomal membranes. The nucleocapsid is released into the cytoplasm and disassembled, releasing the viral genome. ORF 1 of the positive strand RNA genome is immediately translated, producing the viral non-structural proteins (nsP) 1–4 which together form the viral replicase complex. These are expressed as a polyprotein and processed in a highly regulated manner into individual proteins by viral protease nsP2. NsP2 and nsP3 provide replicase activity and recruit host cell factors, while nsP4 contributes polymerase activity. Replicase complexes, together with a number of cellular proteins, associate with the plasma membrane and replicate the viral genome via a negative strand intermediate. Structural proteins, which form the virus particle, are expressed from a subgenomic mRNA which is synthesized during viral replication (Gotte et al., 2018; Rangel and Stapleford, 2021; Schulte et al., 2016).

3.3 Emergence, distribution and disease

82. GETV is viewed as an emerging mosquito-borne virus. The prototype strain, MM2021, was first isolated from *Culex gelidus* mosquitoes collected in Malaysia in 1955 (Scherer, 1984). Sugiyama virus (SAGV), later classified as a GETV strain, was isolated in Japan a year later (Scherer et al., 1962a). In China, GETV was first identified in Hainan Province in 1964, when the strain designated M1 was isolated from a pool of *Culex* mosquitoes (Li et al., 1992).

83. The first GETV-associated disease outbreaks occurred among Japanese racehorses in 1978 and 1979. Subsequent major outbreaks re-emerged in 1983 and again in 2014 and 2015 (Bannai et al., 2015; Bannai et al., 2016; Kamada et al., 1980; Sentsui and Kono, 1980). A clinically similar equine outbreak in India in 1990 was serologically identified as GETV-related without genomic confirmation (Brown and Timoney, 1998). In China, GETV has expanded in prevalence only in the past two decades. After isolation of M1 in 1964, no further GETV strains were identified during multiple arbovirus surveys up to the 1990s (Li et al., 1992; Li et al., 2017b; Zhai et al., 2008). From 2002 onwards, however, many GETV strains have been collected from wild-caught mosquitoes in geographically dispersed locations throughout China (Li et al., 2017a; Li et al., 2017b; Wang et al., 2006; Zhai et al., 2008). Multiple disease outbreaks in farmed pigs occurred between 2011 and 2018, again distributed across many provinces (Lu et al., 2019; Xing et al., 2020; Yang et al., 2018; Zhou et al., 2018). In recent years, GETV disease occurred for the first time in foxes (Shi et al., 2019) and cattle (Liu et al., 2019).

3.4 GETV strains and phylogenetic relationships

84. Phylogenetic analyses of the many GETV isolates place them into four distinct evolutionary groups (I-IV), with the most recent common ancestor estimated to have existed about 150 years ago (Li et al., 2017b). The oldest, Group I, is represented only by strain MM2021, isolated in Malaysia in 1955. Group II diverged next and contains two SAGV strains isolated in Japan in 1956. Group IV emerged most recently and again has only two representatives, isolated in Russia in 2000 and Yunnan in southern China in in 2012. This pair is particularly striking for being collected thousands of kilometres apart from one another and in very different environments, indicating an ability to survive and adapt to both warm and frigid climates (Li et al., 2017a).

85. The majority of GETV isolates fall into group III. While the few strains belonging to groups I, II and IV were isolated exclusively from mosquitoes (Xing et al., 2020), Group III includes strains isolated from mosquitoes, pigs, horses, cattle and foxes, and has become the dominant group of circulating viruses (Ren et al., 2020; Xing et al., 2020).

3.5 Transmission

3.5.1 Mosquito-vectored transmission

86. As an arbovirus, GETV is primarily transmitted by mosquitoes. During a blood meal on a viraemic host, a mosquito ingests virus particles which reach the midgut and replicate in epithelial cells. Progeny virions are released and disseminate to various internal organs, including the salivary glands, where they replicate further. When an infected mosquito feeds on another animal, virus present in its saliva is inoculated into the animal. Once infected, a mosquito remains infected for life and is able to transmit virus at each blood meal (Lim et al., 2018).

87. Viraemia refers to the presence of circulating virus particles in the bloodstream, with access to all organs and tissues. Direct inoculation of virus into the blood, such as by mosquitoes, physical breaches, or blood transfusions, is known as *passive viraemia*. *Active viraemia* follows viral replication within infected tissues, with progeny virions released back into the bloodstream. Not all host species achieve a level of active viraemia sufficient to reinfect mosquitoes. Species that do achieve sufficient viral concentrations in the blood for transmission to mosquitoes are known as *amplifying hosts* and play a crucial role in *mosquito-host-mosquito* transmission cycles. Species that do not reach a sufficient viral titre in the blood are considered *dead-end hosts* as they cannot perpetuate the transmission cycle (Lu et al., 2020).

88. Based on viraemia levels, both pigs and horses may act as amplifying hosts (Kumanomido et al., 1988c; Ren et al., 2020). It has also been suggested that wild boar may be involved in the natural transmission cycle in Japan (Sugiyama et al., 2009). GETV seropositivity in wild animals other than boars has not been surveyed.

89. GETV dissemination across large geographic distances suggests spread via long distance migration of infected mosquitoes or birds. For example, GETV has reached northern Russia from the over-wintering places of migratory birds (Lvov et al., 2015). GETV strains isolated during 2012 and 2014 equine outbreaks in Japan were more closely related to Chinese and South Korean strains than to earlier isolates collected in Japan (Kobayashi et al., 2016). Investigation of three closely located infections in Guangdong and Hunan provinces, China in 2017 and 2018 also revealed distinct origins. Rather than reflecting local transmission, the closest relatives of a GETV strain isolated from pigs in Guangdong in 2018 were a porcine strain collected from distant Henan province, followed by strains isolated in Japan between 2012 and 2016 (Lu et al., 2019; Xing et al., 2020).

3.5.2 Direct inoculation

90. GETV can be transmitted by direct inoculation into blood or tissues, as evidenced by the many experimental animal models of infection described in the literature. An experimental dose-response

study in horses showed that clinical signs observed after intramuscular injection of GETV were dose dependent. The dose range was 20 – $2x10^6$ CCID50, increasing by factors of ten. There was no minimum dose as all horses developed a neutralising antibody response, but at 20-200 CCID50, there was no viraemia and horses developed only a rash without any of the other characteristic clinical signs of GETV-associated illness (see Section 3.8.1).

91. Contamination of an attenuated commercial vaccine for *porcine reproductive and respiratory syndrome virus* with live GETV demonstrated an inadvertent direct inoculation. This came to light while investigating unexplained abortions in pregnant sows at a pig farm in China, and 100% of the vaccinated sows proved seropositive for GETV (Zhou et al., 2020).

92. In an alternative setting, experimentally infected mice transmitted GETV to uninfected cage mates, apparently by biting and scratching. When the experiment was carried out using two-day old neonates, there was no transmission. However, all mice became GETV-positive when two-month old males were housed together. As the animals bore multiple wounds, GETV transmission was concluded to occur through physical injuries caused by fighting (Wang et al., 2021).

3.5.3 Transplacental transmission

93. GETV infection is associated with foetal and neonatal death in pigs, mice and several other small mammals. In adults of these species, GETV can cause viraemia and a humoral immune response but does not lead to symptomatic disease. However, GETV can cross the placental barrier, establishing infection in both placenta and foetus and causing foetal death. In pigs and mice, the efficiency of transplacental transmission varies with the stage of gestation (see Sections 3.8.3 and 3.8.5).

3.5.4 Vertical transmission via milk

94. GETV transmission from dam to offspring via milk has been observed in experimentally infected mice (Sentsui and Kono, 1981). Considering other alphaviruses, the possibility of Chikungunya virus (CHIKV) transmission by this route was raised after viral genomes were detected in human breast milk, persisting for over three weeks after onset of disease symptoms in the mother. In the reported case, however, there was no transmission to the baby (Campos et al., 2017).

3.5.5 Aerosol transmission

95. The possibility of aerosols or direct contact as mechanisms for GETV spread between horses has been considered due to (a) lack of mosquito activity when some horses became ill, (b) viral shedding into nasal secretions of experimentally infected horses, and (c) because suckling mice can be infected by the intranasal route (Kamada et al., 1991a; Sentsui and Kono, 1980; Wang et al., 2021).

96. Wang et al used mice to demonstrate that GETV was not airborne over a distance. There was no transmission between mice housed in separate cages connected by a screened pipe that allowed air, but not mice, to access both. Infected neonates also did not transfer GETV to uninfected cage mates (Wang et al., 2021).

97. Intranasal infection via aerosol spray has been demonstrated in horses and is often used experimentally. Development of neutralising antibody responses confirmed that GETV infection was established across the full dose range tested. However, the observed pattern of symptoms (rash and slight fever, without hind leg or lymph node swelling) suggests that the higher end of the dose range tested equated to the middle of the dose range tested by intramuscular inoculation ((Kamada et al., 1991b) and see paragraph 103 below). The authors concluded that aerosol transmission between horses was unlikely to occur in nature as a high viral titre was needed to establish symptomatic infection by the intranasal route, and viral shedding into nasal secretions was too low to achieve this (Kamada et al., 1991a).

3.6 Competent vector species

98. GETV has been isolated from at least ten mosquito species spanning four different genera and found under diverse climactic conditions, ranging from tropical southeast Asia to the frozen tundra of northern Russia. It was initially thought to be carried and spread primarily by mosquitoes of the *Culex* and *Aedes* genera as it has been isolated from at least five *Culex*[8] and two *Aedes* species[9], as well as mixed populations within these genera (Feng et al., 2012; Li et al., 2012; Li et al., 2017b; Liu et al., 2019; Matsuyama et al., 1960; Scherer, 1984; Scherer et al., 1962a; Shirako and Yamaguchi, 2000; Takashima and Hashimoto, 1985; Wang et al., 2006; Zhai et al., 2008; Zhou et al., 2012). Since 2005, additional species, including *Armigeres subalbatus, Armigeres obturbans* and *Anopheles sinensis*, have been found to carry GETV. These are widely distributed in China and have contributed to its recent spread (Li et al., 2017b; Liu et al., 2019; Lu et al., 2020; Zhai et al., 2008). GETV is notable for its presence in Mongolia and Russia and is the only alphavirus found under such severe climatic conditions (L'vov et al., 2000).

99. Laboratory studies have also shown that *Ae. japonicus*, *Ae. aegypti*, *Cx pipens pallens*, *Tripteroides bambusa* and *Cx. fatigans* are susceptible to GETV infection (Li et al., 1992; Takashima and Hashimoto, 1985).

3.7 Vertebrate hosts

100. Serological evidence suggests that GETV has a broad host range in nature and infects pigs and wild boar (Kuwata et al., 2018; Li et al., 1992; Li et al., 2019; Sugiyama et al., 2009), horses, mules, goats and humans (Li et al., 1992), and beef cattle (Li et al., 2019). Low seropositivity rates have been observed in chickens (2%) and ducks (6%); for comparison, seropositivity for pigs (46%) and cattle (79%) in the same area were relatively high (Li et al., 2019).

101. GETV has also caused disease in foxes (Shi et al., 2019), and used experimentally to infect mice, hamsters, guinea pigs and rabbits (Asai et al., 1991; Kumanomido et al., 1988b; Wang et al., 2021).

3.8 Pathogenicity associated with GETV infection

102. Serological evidence suggests widespread exposure to GETV among vertebrates, including humans, in affected regions but many infections are subclinical. Clinical disease associated with GETV infection has been described in horses, pigs, blue foxes and cattle. In addition, GETV causes disease in several small mammalian species when infected experimentally.

3.8.1 Horses

103. Clinical signs of GETV infection in horses include fever lasting from one to four days, a rash on the neck, shoulders and hind quarters, swelling of the hind limbs and swelling of the submandibular lymph nodes. Specific combinations of symptoms, and their timing relative to one another vary (Fukunaga et al., 1981; Fukunaga et al., 2000). An experimental dose-response study showed that specific symptoms were dose-related – the rash developed in horses receiving the lower 2/3 of the dose range, the submandibular lymph node was swollen only at the upper 1/3 of the dose range, and the remaining clinical signs appeared in the upper 2/3 of the dose range (Kamada et al., 1991b). Affected horses appear normal, without loss of appetite or respiratory signs. Most recover within a week, and those with secondary complications recover within 14 days. In documented outbreaks, all animals have made full recoveries without any ongoing complications (Fukunaga et al., 1981; Fukunaga et al., 2000).

104. While disease caused by GETV infection is not severe, extensive outbreaks have caused significant disruption and economic loss. GETV poses a potential threat to the racehorse industry in

[8] *Cx. gelidus, Cx. tritaeniorhynchus, Cx. pseudovishnui, Cx. fuscocephala* and *Cx. annulus*
[9] *Ae. albopictus* and *Ae. vexans*

affected countries (Lu et al., 2019; Timoney, 2017). As noted in Section 3.9 below, GETV infection is a notifiable equine disease in Australia.

3.8.2 Cattle

105. GETV infection of beef cattle in forest grazing areas and presenting with sudden onset of fever was documented in 2018. The authors noted some uncertainty as to whether GETV was responsible for the observed symptoms as few seropositive cattle were symptomatic. However, the reported data concerning symptoms, viraemia and neutralising antibody titre are consistent with observations in experimentally infected horses (Section 3.8.1 and Kamada et al., 1991b). GETV infection of cattle may thus be associated with a self-limiting febrile illness as is well-documented in horses.

3.8.3 Pigs

106. GETV has been isolated from healthy adult pigs (Kumanomido et al., 1982; Matsuyama et al., 1967) but can cause foetal/neonatal death and reproductive disorders in pregnant sows (Liu et al., 2019). In the largest reported outbreak, over 150 pregnant sows had stillbirths or foetal mummies and approximately 200 surviving piglets died five to ten days after birth (Yang et al., 2018).

107. Infected neonates experienced a rapidly fatal disease characterised by fever, tremors and uncoordinated movement, depression and diarrhoea, dying within ten days after birth. Histopathological changes were evident in the brain, lungs, kidneys, liver and spleen. GETV was broadly distributed, with virus and viral RNA isolated from multiple tissues (Yago et al., 1987; Yang et al., 2018). Foetal death was due to viral infection of the foetus, transmitted across its placenta (Shibata et al., 1991; Yang et al., 2018). Poor reproductive outcomes were linked to maternal infection before the middle stage of pregnancy (Izumida et al., 1988).

108. Reported GETV outbreaks have not been associated with disease in older pigs. Experimental infection of nine-day old piglets, five month old pigs and pregnant sows showed that all developed viraemia and antibodies to GETV, but no fever or other clinical signs (Izumida et al., 1988).

3.8.4 Blue foxes

109. In 2017, GETV caused a rapidly fatal illness in 5 month old farmed blue foxes in Shandong province in eastern China. Animals showed symptoms of sudden fever, anorexia and depression, and six of the twenty-five affected foxes had onset of neurological symptoms and died on the third day of illness (Shi et al., 2019).

3.8.5 Small mammals

110. Newborn mice are routinely used as an animal model to test for and amplify GETV, and experience hind limb paralysis and death. However, their susceptibility to infection lasts only a few days. Day old mice infected intracranially with GETV died with paralysis 3-4 days post inoculation (DPI). Three- to four-day old mice infected intracranially or by oronasal exposure displayed impaired hind limb mobility at 4 DPI, paralysis at 8 DPI and died 2-4 days later. In contrast, seven-day old mice infected intranasally, and adult mice infected by either route, displayed no clinical signs (Wang et al., 2021; Yago et al., 1987)

111. GETV crosses the placental barrier in mice, establishes infection in both placentas and foetuses and causes foetal death. The efficiency of this transmission route varies with the stage of pregnancy, and the timing possibly with the viral strain (Kumanomido et al., 1988b); (Wang et al., 2021).

112. Adult Syrian hamsters, guinea pigs and Japanese white rabbits have been experimentally infected with GETV by the subcutaneous route. Transplacental infection occurred in all three species. It occurred most readily in hamsters and was independent of the stage of pregnancy – after inoculating females at different stages of the gestation period, GETV was recovered from all placentas and all but one foetus. It was observed sporadically in guinea pigs and rabbits and did not clearly associate with stage of gestation (Asai et al., 1991).

3.9 GETV in Australia

113. GETV was reportedly isolated from *Anopheles amictus amicus* and *Culex bitaeniorhynchus* mosquitoes in north eastern Queensland in 1961, however a recent re-analysis found that this was likely due to sample contamination within the testing laboratory (Rawle et al., 2020). No other GETV isolates have been reported, and GETV was later described as exotic to Australia (Hodgson, 2002).

114. 'Infection with GETV' is currently included in the National list of notifiable animal diseases[10] under *Equine diseases and infections*. A notifiable disease is one that represents a major threat to Australian livestock industries and access to overseas export markets and must be reported to agricultural authorities. GETV is further listed as a notifiable animal disease in all Australian states and territories. Australia is considered free of GETV disease, with no occurrences ever reported (Animal Health Australia, 2021).

3.10 GETV strain M1

115. The M1 strain of GETV was isolated from mosquitoes collected in Hainan Island in southern China in 1964 (Li et al., 1992). It was later shown to have oncolytic activity, selectively killing a range of cancer cells *in vitro* and in mouse tumour models *in vivo*. Selectivity for cancer cells was based on a deficiency in zinc-finger antiviral protein (ZAP), a known antiviral gene and tumour suppressor that is commonly down regulated in human cancers (Lin et al., 2014). ZAP is an interferon-stimulated gene that inhibits the replication of certain viruses, including members of the alphavirus genus, by degrading and/or blocking the translation of incoming RNA (Bick et al., 2003; Goncalves-Carneiro et al., 2021; Zhang et al., 2020). M1 kills cancer cells by several mechanisms, including inducing endoplasmic reticulum stress-mediated apoptosis or necroptosis (Lin et al., 2014; Zhang et al., 2021).

116. When initially characterised, M1 was found to cause illness and death in newborn mice inoculated by any of the intracranial, subcutaneous and intraperitoneal routes. Degeneration, atrophy, necrosis and inflammatory changes were evident in skeletal muscle fibres of diseased newborn mice, and pathological changes were also seen in the brain. Older mice (2-3 weeks) were only exposed to M1 by intracranial inoculation, and at this age, illness and death occurred in only about 20% of animals (Li et al., 1992). These findings are similar to those observed with other GETV strains that also caused disease in neonates but not in older mice (see Section 3.8.5).

117. When *Ae. albopictus* mosquitoes infected with M1 were allowed to feed on newborn mice, the mice became ill within 3-6 days and viral antigens were isolated from brain tissue (Li et al., 1992), indicating that the M1 strain was also capable of causing disease when introduced by its natural transmission route.

118. Serology in humans and livestock on Hainan Island was not assessed until 15-18 years after the isolation of M1. During serosurveys conducted in 1980 and 1982, 26% of human patients with fever of unknown cause had antibodies to M1, compared with 10.9% of serum specimens from healthy people in the rural Baoting County where M1 was originally collected and 3.4% from healthy people in Haikou City. The authors suggested a possible etiologic role for the virus in febrile illnesses on Hainan Island (Li et al., 1992). There are, however, no subsequent reports associating GETV with disease in humans.

119. M1 was not studied further until assessed for its ability to induce apoptosis in cancer cells (Hu et al., 2009). Its ability to reproduce the disease outcomes associated with other GETV isolates has not been investigated. However, phylogenetic analysis consistently places M1 in evolutionary Group III, reflecting its similarity to more recent GETV isolates obtained from livestock species during outbreaks of epidemic disease. (Li et al., 2017b; Ren et al., 2020).

120. Other than the newborn mouse studies described above, M1 has not been assessed in any animal models that would reveal the characteristic pathogenicity associated with GETV infection (see

[10] As of April 2019.

Section 3.8). However, as the oldest isolate within phylogenetic Group III, M1 has similarity to members of the older Groups I and II as well as to more recent Group III isolates. Group II is represented by two SAGV strains isolated in 1956, which caused similar pathogenicity in horses to that associated with recent epidemic strains of GETV ((Kumanomido et al., 1988a) and Section 3.8.1). Likewise, SAGV induced antibody production but did not cause disease in weanling mice, rabbits, guinea pigs and 2-3-month old pigs (Scherer et al., 1962b). Given that this selectively pathogenic phenotype both predates the isolation of M1 and is common in more recent Group III isolates, it is reasonable to assume that M1 shares this attribute with the entire GETV group.

3.11 Genetic stability

121. Alphaviruses replicate their genome with relatively low fidelity, but the observed rate of divergence in nature is low. For example, RRV isolated at the beginning and end of an 11 month epidemic differed at only one nucleotide of 1600 sequenced (reviewed in Strauss and Strauss, 1994). Nonetheless, GETV has the capacity to adapt to different growth environments as *in vivo* pathogenicity is attenuated by serial passage in tissue culture. A study in horses found that GETV isolates passaged zero to three times *in vitro* retained pathogenicity, with animals developing clinical signs similar to those arising from natural infection. In contrast, horse inoculated with virus after ten serial passages developed neither disease symptoms nor viraemia, although they still produced neutralising antibodies (Kamada, 1981). Sequence variation was not investigated, but this suggests sufficient mutation capacity to enable phenotypic change over this period.

3.12 Environmental stability and inactivation

122. No specific information is available regarding disinfection of GETV, but most lipid enveloped viruses are sensitive to chemicals such as 70% ethanol, sodium hypochlorite and quaternary ammonium compounds (Public Health Agency of Canada, 2010).

3.13 Prevention and treatment

123. There is no specific treatment for disease caused by GETV. Control of GETV infection in endemic areas relies on controlling the mosquito vector such as by eliminating or reducing mosquito breeding sites, and use of larvicides and adulticides (Mair and Timoney, 2009).

124. An inactivated whole-virus vaccine to prevent equine GETV infection has been available in Japan since 1979 and is mainly administered to thoroughbred racehorses. Recommended administration is twice in the first year and then annually as a booster before onset of the mosquito season (Bannai et al., 2015; Bannai et al., 2016; Mair and Timoney, 2009; Nemoto et al., 2015). The vaccine is based on a GETV strain (MI-110) isolated in Japan in 1978 and may not adequately protect against currently circulating strains (Lu et al., 2020).

3.14 Risk group classification of GETV

125. The American Committee on Arthropod-Borne Viruses (ACAV) Subcommittee on Arbovirus Laboratory Safety (SALS) provides biosafety recommendations for each virus registered in the *International Catalogue of Arboviruses, including Certain Other Viruses of Vertebrates*. Biosafety level 2 (BSL2) containment is recommended for GETV on the basis that it causes disease in sheep, cattle or horses (Centers for Disease Control and Prevention, 2020).

126. The criteria listed in the Australian Standard 2243.3:2010 Safety in Laboratories Part 3: Microbiological safety and containment (Standards Australia/New Zealand 2010) are similarly consistent with classification as a Risk Group 2 micro-organism, based on pathogenicity in susceptible animal species. PC2 containment and work practices are therefore appropriate when working with unmodified GETV.

Section 4 The GMO – nature and effect of the genetic modifications

4.1 The genetic modifications and their potential effects

127. GETV strain M1 was modified by introducing two point mutations that localise to different viral proteins and have distinct effects on viral phenotype. These single nucleotide substitutions were identified during *in vitro* serial passage experiments with separate vials of the M1 strain, during which a genetic variant showing enhanced cytopathic effect towards cultured tumour cells was observed. It is not known whether these mutations arose during serial passage or reflect pre-existing clonal variation within the M1 isolate. Both mutations were then introduced into the working M1 sequence by site-directed mutagenesis. The GMO remains replication competent and is not attenuated by the genetic modifications.

128. Details of the two point mutations and the genes in which they are located are under consideration to be declared as Confidential Commercial Information (CCI) under Section 185 of the Act. This information will be made available to the prescribed experts and agencies consulted on this application. CCI is not available to the public.

129. No derivation history was provided to document how the working M1 virus stock relates to the original M1 isolate collected in 1964 and discussed in Section 3.10. Any impact on viral phenotype is therefore unknown (see Section 3.11).

130. The Sponsor demonstrated that the GMO displays improved oncolytic activity compared with its M1 parent. The GMO showed greater selectivity for human cancer cell lines over non-tumorigenic cell lines derived from the same tissues. It also more effectively suppressed *in vivo* growth of solid tumours in a murine model.

131. The Sponsor provided *in vitro* data showing a moderate reduction in the interferon (IFN)-mediated antiviral response in cancer cells infected with M1 genetically modified with mutation 1 alone ($M1^{mut\ 1}$), compared with the response to M1. This creates a more permissive cellular environment for viral replication (Garcia-Sastre, 2017). They propose that non-cancerous cells are protected from replication of $M1^{mut\ 1}$ or the GMO by constitutively expressed antiviral genes such as ZAP, which degrades and/or blocks translation of viral RNA (Zhang et al., 2020). In certain cancer cells where ZAP is deficient but IFN signalling is still active, the GMO has a replicative advantage over M1.

132. The cellular IFN response is the first line of defence against viral pathogens. Most cells respond to viral infection by secreting IFNs which bind to neighbouring cells and trigger expression of an array of antiviral proteins that make the cell refractory to viral replication. Viruses in turn have mechanisms to evade or subvert the host IFN response. A cell's susceptibility to infection depends on the balance between the level and activity of cellular antiviral effectors, and the ability of a particular virus to counteract them (Garcia-Sastre, 2017).

133. Disease caused by alphaviruses is known to be modulated by their ability to stimulate an IFN response, as well as their sensitivity to it. For example, a RRV field isolate that stimulated very high levels of IFN-β production, and was highly sensitive to IFN, was compared with a prototype RRV strain that provoked a lower IFN response. The field isolate induced milder disease in mice, which displayed mild hind limb weakness compared with severe hindlimb weakness and loss of gripping strength in mice infected with the prototype strain (Liu et al., 2020b). More severe CHIKV-associated symptoms were also associated with inefficient type I IFN signalling in mice (Couderc et al., 2008).

134. The reduced IFN response stimulated by $M1^{mut\ 1}$ is expected to shift the balance towards increased viral replication. As the reduction is moderate rather than extreme, the GMO is anticipated to have a moderate replication advantage over M1. Preclinical studies showed no disease in several animal species (Section 4.3), but there are no data on the GMO's ability to infect animal cells that are normally permissive for GETV replication and involved in its characteristic pathologies (see Section 4.4

below). The genetic modifications could exacerbate the clinical symptoms that follow GETV infection in animals susceptible to disease - thus the GMO could display enhanced pathogenicity towards these species.

4.2 Stability of the GMO during *in vitro* passage

135. Genetic and phenotypic stability of the GMO were assessed during serial passage for twenty generations in cultured Vero cells. Three new point mutations appeared, two of which caused single amino acid changes in the E2 and capsid proteins, respectively. One mutation occurred only at generation 7 while the other was present at generations 8-12, 14-16 and 18. Neither mutation was associated with any change in viral titre or replication kinetics, cytotoxicity towards a non-cancerous cell line, or relative replication capacity in cancerous vs normal cell lines.

136. The Applicant stated that reversion of the introduced mutations to the original nucleotide sequence has not been observed during amplification of viral stock during construction of multiple seed banks and manufacturing bulk lots of the GMO for clinical use. In the above serial passage experiment, the point mutations specific to the GMO were unchanged.

137. These data indicate a low-level capacity for random mutation during viral replication. Phenotypic outcome is dependent on the specific mutation. This behaviour is an underlying characteristic of the parent organism and not affected by the genetic modifications.

4.3 Pre-clinical and human toxicity studies

138. The Sponsor undertook toxicity studies in healthy adult animals from several non-human species including monkeys, dogs, Bama miniature pigs, chickens and rats. No obvious abnormalities or toxicity attributable to the GMO were observed.

139. The GMO was administered to nine human patients with a range of solid tumours either as a monotherapy or in combination with other drugs. Doses ranged from 2.6×10^8 to 4.0×10^9 CCID50. Each patient underwent multiple treatment cycles (up to 16), each comprising 5-6 daily GMO doses followed by an interval of 16-110 days before starting the next treatment cycle. The Sponsor reported that adverse events for the first two treatment cycles were mild, self-limiting and consistent with those observed with other oncolytic viruses.

140. The Sponsor also reported that 55% of patients demonstrated an antibody response to the GMO within 30 days of initiating treatment.

4.4 Effect of the genetic modifications on characteristic GETV infection outcomes

141. As noted above, the GMO was assessed in healthy adult monkeys, dogs, chickens, rats and Bama miniature pigs. The studies in monkeys, dogs and chickens demonstrate that the GMO has not acquired pathogenicity in circumstances where wild-type GETV is not known to be pathogenic.

142. There are no *in vivo* data that allow direct assessment of the GMO in situations where GETV is known to cause disease – such as in horses, foxes, neonatal mice and commercial breed piglets, or during murine or porcine pregnancy.

143. Adult rats were exposed to the GMO by intracranial inoculation. No clinical abnormalities were noted over a 22-day observation period, and no histopathological abnormalities were seen in brain and spinal cord tissues. Intracranial inoculation has not been reported in rats, but has been widely used in mice and neurological disease is limited to neonates within a few days of birth (paragraph 110) As a consequence, no conclusions can be drawn as to the effect of the genetic modifications on characteristic GETV pathogenicity in rodents.

144. Commercial pig breeds are established vertebrate hosts for wild-type GETV. Adult pigs develop a brief viraemia without clinical signs, followed by a neutralising antibody response (Izumida et al., 1988). Virus has been recovered from spleen and several lymph nodes at three DPI (Kumanomido et al., 1988c). The major disease impact is seen in neonatal piglets and through trans-placental infection of foetuses (Section 3.8.3). As an alternative host, the relevance of Bama miniature pigs is unclear. These are a highly inbred strain based on the primitive Bama xiang pig subspecies, which is indigenous to an isolated area of China, has a distinct genetic background and is phenotypically very different to commercial pig breeds (Chen et al., 2021; Liu et al., 2008; Xie et al., 2016; Zhang et al., 2019). Different breeds or subspecies have been observed to respond very differently to GETV infection. For example, a serosurvey in Yunnan, China found that beef and dairy cattle in the same locality differed greatly in rates of seropositivity (71.9% vs 13.3%) and antibody titre (1/640 and over vs 1/10–1/20) (Li et al., 2019).

145. The Bama pig toxicity study involved animals that were 4-5 months old, not pregnant, and thus could not exhibit neonatal or foetal disease. No clinical signs, gross pathological or histopathological abnormalities were observed, consistent with GETV infection outcomes in adults of commercial pig breeds. The neutralising antibody response was low and slow to develop, which does support low infectivity of the GMO in this model. However, observed lack of viraemia and low viral recovery from tissues are not comparable with published GETV studies in commercial pig breeds as samples were not collected at time points when viraemia and viral isolation from tissues have been reported (Izumida et al., 1988; Kumanomido et al., 1988c). Again, no conclusions can be drawn as to the effect of the genetic modifications on characteristic GETV pathogenicity in commercial pig breeds.

4.5 Biodistribution and shedding

146. The Sponsor examined biodistribution and shedding of the GMO in monkeys and rats without tumours, and in mice with tumours. Serum concentration and shedding have also been monitored in human cancer patients treated with the GMO.

4.5.1 Biodistribution and shedding in animals without tumours

147. Sprague Dawley rats and Cynomolgus macaques were inoculated with two (rats) or three (macaques) different doses of the GMO daily for five consecutive days. This treatment was delivered in three cycles each separated by 14 days (43 days total). GMO biodistribution, clearance from blood and shedding into urine, faeces and nasal secretions (macaques only) were assessed at varying times.

- In both species, the serum concentration was highest immediately after inoculation, declined rapidly over the first 30-60 min and then more slowly over the next 5-23 hours. GMO levels were higher immediately after the first dose than after the final dose.
- Viral shedding into faeces was dose dependent and more reliable in rats than in macaques. In rats, the GMO was detectable throughout the treatment period and decreased over time. In monkeys, GMO was detected only in the highest dose group on the final day of treatment.
- Shedding into urine in both species was dose dependent, decreased over time, and persisted for the full treatment period in rats and for the duration of the first cycle in macaques.
- The GMO was detectable in nasal secretions throughout the treatment period, ranged from 243-17,000 copies/µg RNA and did not differ substantially between treatment groups.

148. After the final GMO treatment, the GMO localised mainly to the spleen and inguinal lymph nodes (located in the groin). Low levels were found in the heart, lung, liver, kidney, brain, ovary, testes and epididymis. Four weeks later, residual virus was detectable only in the spleen, inguinal lymph nodes and at the injection site.

4.5.2 Biodistribution and shedding in mice with tumours

149. Tumour-bearing Balb/c nude mice were treated with the GMO at 150 times the highest dose proposed for this study, amplifying detection. Adaptive immune responses, including T cell-mediated responses and antibody formation, are defective in these mice (Okada et al., 2019) which allowed the GMO to be followed over time in the absence of immune clearance.

- Viral RNA was detected mainly in tumours, rising sharply to peak between 24-72 hours then declining steadily thereafter. Viral RNA was comparatively low by day 7 but remained above the assay detection limit for the full 28 days assessed.
- Viral RNA was maximal in serum at the initial 2 hour time point, declined logarithmically over the next seven days, and was at or below the detection limit on days 14 and 28. While viral RNA was peaking and declining in the tumour, there was no influx into serum suggestive of an active viraemia due to release of viral progeny from the tumour.
- Viral RNA was initially high in tissues with extensive blood flow, including the liver, heart, spleen, kidney and lung. As with serum, viral RNA declined logarithmically in these tissues, with no accumulation suggestive of infection and viral replication.
- Some viral RNA was eliminated by the faecal route, peaking from 12-48 hours after treatment. Shedding into urine was not assessed.

150. Tumour-bearing immune competent mice were treated with the GMO at the equivalent of the highest dose proposed for this study. GMO levels were very low, even in tumour tissue. As observed in nude mice, GMO levels in the tumour were highest at 48 hours, dropped 10-fold over the next two days and were below the limit of detection by day 7. Viral RNA was undetectable in faeces or urine.

4.5.3 Biodistribution and shedding in human cancer patients treated with the GMO

151. Persistence in the bloodstream and shedding via body fluids (saliva, urine and nasal secretions) and faeces were assessed in two groups of cancer patients, one receiving their first treatment cycle and the second receiving a second or later treatment. The Sponsor has observed a neutralising antibody response in around half of patients within 30 days of initiating GMO treatment, and expects this to contribute to reduced viraemia and more rapid clearance of the GMO in cycles after the first.

152. **Biodistribution and shedding after the first treatment cycle:** Five patients with hepatocellular carcinoma were treated with the GMO for five consecutive days. GMO levels in serum were measured over 24 hours from the final (5th) dose. RNA levels were undetectable in two patients. In the remaining three, the GMO concentration was highest at 0.5 h then declined rapidly over 2-18 hours. The presence of infectious virus at 0.5 and 2h was demonstrated using a tissue culture assay. No GMO was found in urine, nasal swabs and stool samples of these patients up to 24 h after the 5th treatment, or 9 days later.

153. **Biodistribution and shedding after the second and subsequent treatment cycles:** The GMO was administered to six patients with a range of solid tumours either as a monotherapy or in combination with other drugs. Doses ranged from 2.6×10^8 to 4.0×10^9 CCID50. Patients received multiple treatment cycles, each comprising 5-6 daily treatments followed by an interval of 21-114 days before starting the next cycle. Persistence of the GMO in the bloodstream and shedding via body fluids (saliva and urine) and faeces were assessed at various time points after administering the GMO. As the patients were not part of a formal study, samples were collected when convenient and data collated from a number of treatment cycles.

154. Persistence in the bloodstream was assessed by collecting serum before treatment, and at several time points within the first few hours after administering the GMO. As treatments and cycles followed on from one another, each 'before' measurement would detect any residual GMO remaining from the previous dose (24 hours earlier) or cycle (21-109 days earlier).

- Viral RNA was detected in serum as early as 5 min and for up to 1 hour after administering the GMO. RNA copy numbers ranged from below the detection limit to 5310 copies/µl.
- There was no clear relationship between RNA level and time after administration. Where multiple samples were collected at a given time point, readings were highly variable.
- The test report concluded that RNA was detectable in serum for up to one hour after administering the GMO. However, only four of the 33 serum samples were collected at time points later than one hour post-administration (1.5, 2, 3 and 4.5 hours). Given RNA detection was inconsistent at earlier time points and so few late time points were assessed, the maximum time the GMO remains in the circulation is considered uncertain.
- Viral RNA was consistently undetectable in serum 24 hours post-administration.

155. A small quantity of viral RNA was detected in lung tumour tissue by *in situ* hybridisation 35 days after GMO administration. This was assessed in a single patient after one treatment cycle comprising five daily doses of 1×10^9 CCID50. The sectioned tumour tissue appears viable.

156. Viral shedding was assessed in saliva, urine and faecal samples from five patients. For each patient, samples were collected at several time points ranging from 1-8 days after the final dose of a given treatment cycle, and this time course was repeated across a number of cycles. A total of 100-102 samples of each type were collected across the five patients. In most samples, viral RNA was below the detection limit. Patient #5 returned one positive saliva sample (day 5 post-treatment) and one positive urine sample (day 2 post-treatment). RNA levels in both were very low. These data show that the GMO can be shed into urine and saliva for some days, but only at a very low level.

157. These data show that viraemia following the first GMO dose lasted at least 2 h, and at least 1 h after the second dose, and less than 24 h in both cases, but the exact durations are uncertain. Low level shedding into saliva and urine for some days after treatment is possible. In view of the nasal shedding demonstrated by macaques (paragraph 147) and limited sampling in humans (paragraph 152), the capacity of humans to shed via nasal secretions is unclear.

4.6 Stability in the environment

158. As enveloped viruses, alphaviruses are relatively sensitive to desiccation. They typically transfer directly from host to host and have limited ability to survive in the environment. The Applicant provided stability testing data at different temperatures (Table 2).

Table 2 Observed GMO stability[1] over time in serum, saliva, nasal secretions and urine at the indicated temperatures

Biological material (human origin)	Room temperature	2 to 8°C	-20° to -70°C
Serum	7 days	>70 days	>70 days
Saliva	1 day	21 days	>70 days
Nasal swabs	1 day	21 days	>48 days
Urine	3 days	28 days	>70 days

[1] Data provided by Applicant

159. The Applicant reported that the GMO was unstable in faeces, stating that faeces matrix is rich in complex organic matter, RNases and proteases that can affect the structural integrity of viruses and reduce their infectivity. However, the assay limit of detection for faecal samples was $4x10^2$ CCID50/ml, two orders of magnitude higher than for other sample types ($5x10^0$ CCID50/ml for serum, saliva and urine and $3.33x10^0$ CCID50/ml for nasal swabs). Industry guidance from the US Food and Drug

Administration advises that the complex faeces matrix can adversely affect assay performance. In PCR testing, for example, proteases, nucleases, ions and salts can degrade template DNA and affect DNA polymerase activity. Microbial shedding can thus be underestimated (US Food and Drug Administration, 2015), and GMO levels in faeces could be higher than they appear.

Section 5 The receiving environment

160. 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.

5.1 Clinical trial participants

161. The primary receiving environment will be the clinical trial participants, who will be inoculated intravenously. The GMO will circulate through the blood supply, organs and tissues, and is intended to be taken up by tumour cells anywhere in the body. Pre-clinical and human compassionate use data suggest that virus levels in tumour tissue will rapidly decline, but without re-entering the circulation to cause active viraemia (see Sections 4.5.2 and 4.5.3). There is a possibility that the GMO may be shed via body fluids such as urine, saliva and nasal secretions (Sections 4.5.1 and 4.5.3).

5.2 Clinical trial sites and associated locations

162. The secondary receiving environment will be the laboratory, pharmacy and clinical trial unit where the GMO will be stored, prepared, administered and waste disposed of, and the pathology and certified laboratories where biological samples will be analysed. Most facilities described in the application will be located in Adelaide, SA, while initial storage and distribution and some sample analysis will take place in Sydney, NSW. If additional sites are engaged, they could be located anywhere in Australia.

163. As part of a hospital, the pharmacy and clinical trial unit will be accredited to the National Safety and Quality Health Service (NSQHS) Standards (see paragraph 16) and equipped to handle infectious agents and conduct procedures in accordance with the *Australian Guidelines for the Prevention and Control of Infection in Healthcare* (National Health and Medical Research Council, 2019). Procedures will comply with relevant Australian healthcare guidelines and clinical standards, including but not limited to NSQHS Standard 3 (Preventing and Controlling Healthcare Associated Infections) and the National Hand Hygiene Initiative.

164. Certified PC2 laboratories undertaking sample analysis will follow work practices described in the Regulator's *Guidelines for the Certification of a PC2 Laboratory*. It is expected that uncertified pathology laboratories will comply with NPAAC standards and guidelines which include safety precautions to protect workers from exposure to infectious microorganisms (see paragraph 18).

5.3 The wider environment

165. The principal routes by which the GMO could enter the wider environment are (a) by shedding or vector-mediated transmission of the GMO from treated trial participants once they leave the hospital; and (b) by exposure and infection of clinical site staff, particularly those involved in preparing and administering the GMO. The tertiary receiving environment includes trial participants' homes, any places they visit while capable of shedding the GMO or infecting an insect vector, and the wider environment accessible to any insect vectors that become infected.

5.4 Relevant environmental factors

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

5.4.1 Susceptible hosts

167. Pigs, horses, humans, goats, cattle, rodents, and rabbits are susceptible to GETV infection and are widely found in Australia. Of these, GETV is known to cause disease in horses, was associated with similar disease aetiology in cattle, and causes death in foetal/neonatal pigs and mice (see Section 3.8).

168. The clinical trial site described in the application is in metropolitan Adelaide, and any additional sites recruited at a later time are also likely to be in urban locations. Farms and livestock are thus not expected to be nearby. However, patients seeking experimental cancer treatments often come from further afield and could live in rural areas close to livestock.

5.4.2 Availability of competent vectors

169. There are at least 300 mosquito species in Australia, with a relatively small number involved in transmitting arboviral diseases such as Dengue fever, Australian encephalitis, RRV disease and Barmah Forest virus disease (Department of Medical Entomology NSW, 2020). Of the 15 potential GETV vector species listed in Section 3.5, *Ae. albopictus* is currently present only in some islands of the Australian Torres Strait (Beebe et al., 2013; CSIRO, 2020; Russell et al., 2005), while *Ae. aegypti,* is found in North, Central and parts of Southern Queensland (Knope et al., 2019).

170. Given the number and climactic range of mosquito species infected by GETV in Asia, it is possible that mosquito species present in Australia could prove capable as vectors given the opportunity. The southernmost parts of Australia are at lower latitudes (less than 45°S) than the northernmost regions where GETV has been found in the northern hemisphere (70°N) (Lvov et al., 2015). RRV is the closest relative of GETV amongst the alphaviruses (Forrester et al., 2012). RRV vectors include *Cx. annulirostris*, *Ae. vigilax* and *Ae. camptorhynchus*, which are found in South Australia. RRV disease occurs periodically and is most prevalent in the Riverland region of the state along the Murray River, with vector populations and rainfall being important factors associated with transmission. Transmission rates in metropolitan Adelaide are among the lowest in populated areas of South Australia (Liu et al., 2020a; Liu et al., 2021; Stephenson et al., 2018).

5.4.3 Physical conditions that may aid or restrict transmission

171. Higher than average rainfall due to La Nina weather patterns have increased mosquito breeding activity in South Australia in the last year, and travel restrictions due to the global COVID-19 pandemic have seen more regional tourism within riverine areas of the state. Surveillance data to January 2021 show increased mosquito numbers along the Murray River although no isolation of arboviruses. Nonetheless, notifications of confirmed and probable RRV infections in January 2021 were 25-fold higher than in the two years prior (SA Health, 2021).

172. As GETV is not currently known to be present in Australia, both humans and animals would be immunologically naïve and have no protection due to prior infection. However, sera from animals infected with GETV versus RRV have shown high levels of cross reactivity and cross protection (Rawle et al., 2020). Humans and animals with prior exposure to RRV may therefore have cross-protective antibodies and a measure of resistance to GETV infection. Notification data suggest this would apply to only a small percentage of the human population – state-wide mean annual rates in South Australia from 2000-2013 were 16.8 cases per 100,000 people (0.0168%) (Liu et al., 2020a). Animal seroprevalence data specific to South Australia are not available.

5.5 Related viral species in the receiving environment

173. As noted above, RRV is the closest relative of GETV amongst the alphaviruses and periodic outbreaks have occurred in South Australia. RRV infects humans, and animal hosts present in the Australian environment include a range of marsupials and placental mammals (Stephenson et al., 2018; Wildlife Health Australia, 2015).

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

174. GETV is not known to be present in the Australian environment (see Section 3.9).

Section 6 Relevant Australian and international approvals

6.1 Australian approvals

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

6.2 International approvals

176. The proposed clinical trial is a first-in-human study and there are no relevant international approvals. Patients in China were treated under a compassionate use programme.

Chapter 2 Risk assessment

Section 1 Introduction

177. 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.

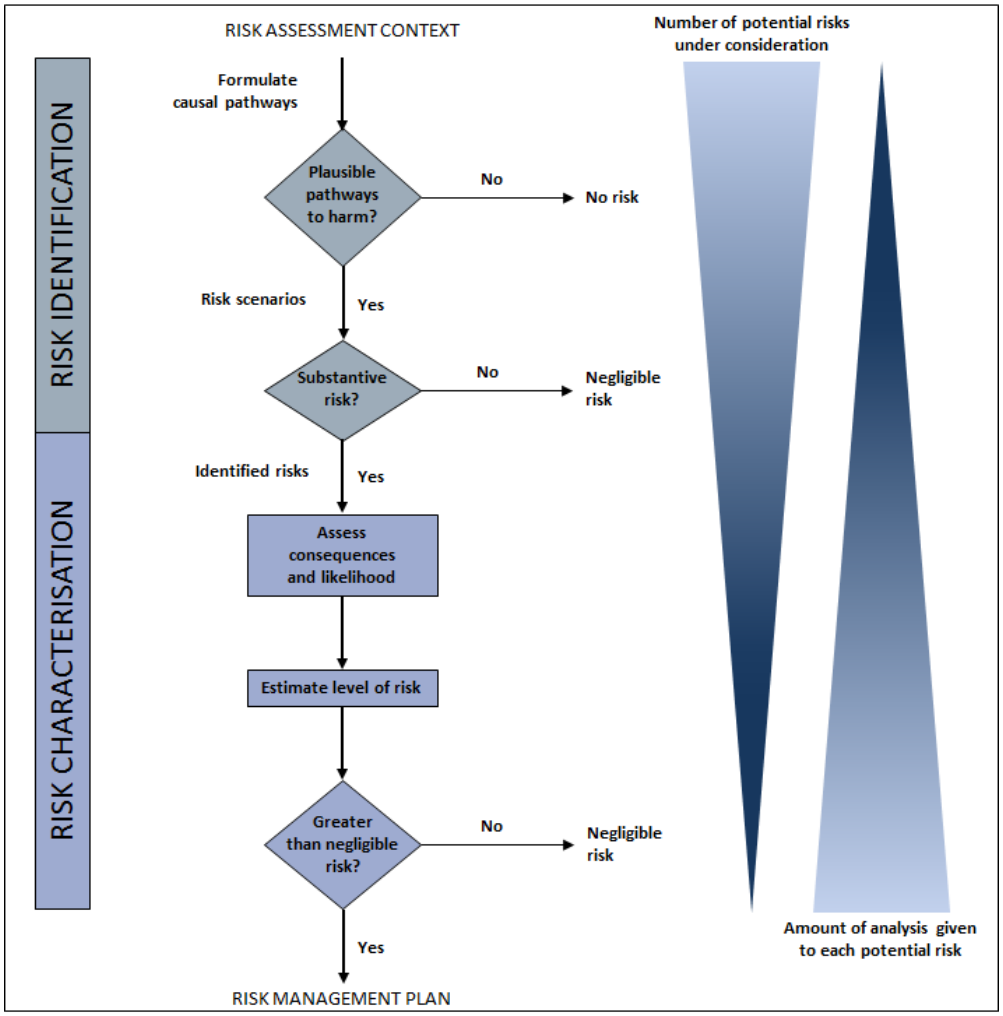

Figure 2. The risk assessment process

178. The Regulator uses a number of techniques to identify risks, including checklists, brainstorming, reported international experience and consultation (OGTR, 2013). Risk scenarios examined in RARMPs prepared for licence applications for the same or similar GMOs, are also considered.

179. 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.

180. 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.

181. 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

182. 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.

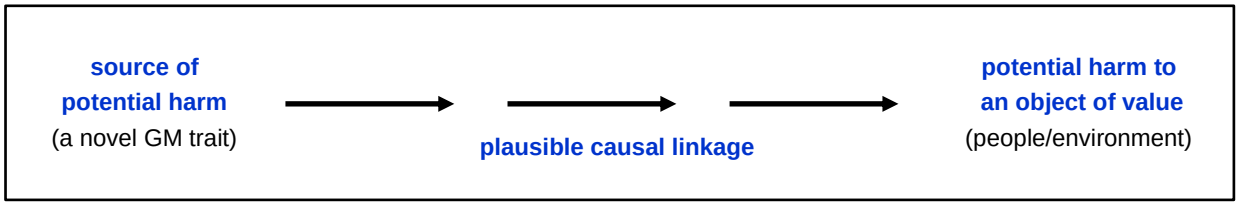

Figure 3. Components of a risk scenario

183. 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.

2.1 Risk source

184. The parent organism of the GMO is the M1 strain of GETV. Details on the pathogenicity and transmissibility of GETV, and specific consideration of M1, are located in Chapter 1, Section 3.

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

186. The introduction of point mutations that increase viral replication in cancer cells, and potentially in other cells that are naturally permissive for GETV replication, is considered as a potential source of harm.

2.2 Causal pathway

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

- potential effects of the point mutations and modified proteins on the properties of the parent organism;
- the proposed dealings;
- proposed limits, including the extent and scale of the proposed dealings;
- proposed controls to limit the spread and persistence of the GMO;

- practices during and after administration of the GMO;
- unauthorised activities;
- routes of exposure to the GMO;
- the release environment;
- potential exposure of other people and animals to the GMO in the wider environment;
- spread and persistence of the GMO (e.g. dispersal pathways and establishment potential);
- environmental stability of the GMO (ability to survive outside of a host cell, and influence of temperature, humidity and UV irradiation); and
- gene transfer by horizontal gene transfer.

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

189. As discussed in Chapter 11.1, 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 participating in the trial, and to the environment.

190. **Unauthorised activities:** 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.

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

192. **Reversion to wild-type sequence**: the GMO contains two point mutations which have potential to increase pathogenicity in species already susceptible to GETV-associated disease. Loss of either mutation would not increase the risk to human health and safety and the environment, so this possibility will not be assessed further.

193. **Acquisition of additional mutations during viral replication in tumours**: as RNA viruses, alphaviruses have an underlying error rate during genome replication and random mutations occurred at low frequency during a serial passage experiment (Chapter 14.2). However, this behaviour is an attribute of the parent organism and not affected by the genetic modification so will not be considered further.

194. **Recombination with other alphaviruses such as RRV**: recombination between two viruses requires that they co-infect the same cell. Given the low prevalence of alphaviral infection in Australia (see Section Chapter 15.4.3), this causal pathway is not considered plausible and will not be further assessed.

195. **Exposure and infection of immune competent people:** Serosurveys conducted on Hainan Island in the early 1980s identified a possible association between febrile illness of unknown origin and exposure to the M1 strain of GETV (see Chapter 13.10). Since that time, however, there have been many well-documented GETV outbreaks amongst livestock, including at major racehorse training centres in Japan. For example, the Miho training centre of the Japanese Racing Association and surrounding farms were the site of sequential equine disease outbreaks in 2014 and 2015 (Bannai et al., 2015; Bannai et al., 2016). Closely related GETV strains were circulating amongst farmed pigs in the area at the same time, suggesting mosquito-vectored transmission from pigs to horses (Bannai et al.,

2017). There were no coinciding reports of unusual human febrile illness in the area. Furthermore, the GMO was not associated with unexpected pathogenicity in the 14 adult cancer patients granted compassionate use access to the GMO (Chapter 14.3). Therefore, exposure leading to infection of immune competent people with the GMO is not considered a plausible pathway to harm and will not be considered further.

196. **Exposure and infection of breast feeding women:** the potential for MTCT of arboviruses during breastfeeding was discussed in Chapter 13.5.4). MTCT of GETV was observed in a single murine study in the early 1980s, and of the more prevalent alphaviruses, there has been a single report of CHIKV in human breast milk, which did not infect the baby. There is more evidence for transmission of *Zika virus* (ZIKV) by this route (Desgraupes et al., 2021), but the World Health Organisation recommends that infants born to ZIKV-infected mothers continue to be fed according to normal infant feeding guidelines as the benefits of breastfeeding outweigh any potential risk of ZIKV transmission (World Health Organisation, 2021). In addition, in species that experience severe disease in neonates (e.g. pigs and mice), this is limited to those infected in the first few days after birth (Chapter 13.8.3 and Chapter 13.8.5), and women are unlikely to be conducting dealings or participating in the clinical trial so soon after giving birth. Therefore, exposure of breastfeeding women to the GMO, with transmission to infants via ingestion of contaminated milk, is not considered a plausible pathway to harm and will not be considered further.

2.3 Potential harm

197. The following factors were taken into account when postulating hypothetical risk scenarios for this licence application:

- harm to the health of people or desirable organisms, including disease in humans or animals
- the potential for a GETV variant that has greater virulence in susceptible species establishing in the environment

198. Potential harms to foetuses or infants infected *in utero* are discussed in the risk assessment (risk scenario 4). However, the clinical trial selection criteria exclude pregnant women from enrolling in the trial. Participants must also agree to use effective contraception for 90 days after receiving the final GMO treatment. As no pregnant women will be exposed to the GMO via participation in the proposed clinical trial, associated risks have not been considered further. This aspect of the risk context will be maintained through imposition of licence conditions.

2.4 Postulated risk scenarios

199. Four risk scenarios were postulated and screened to identify substantive risks. These scenarios are summarised in Table 3 and examined in detail in Sections 2.4.1 to 2.4.4 (this Chapter).

200. In the context of the activities proposed by the applicant and considering both the short and long term, neither risk scenario gave rise to substantive risks that could be greater than negligible.

Table 3 Summary of risk scenarios from dealings with GM GETV

Risk Scenario				Substantive risk?	Reasons
#	Risk source	Causal Pathway	Potential harm		
Section 2.1: Risks to people and animals from exposure to GMO					
1	GM GETV	i. Treatment of trial participant with GMO ⬇ ii. Passive viraemia ⬇ iii. Infection of tumour cells followed by viral replication within tumour tissue ⬇ iv. Release of GMO into circulation (active viraemia) ⬇ v. Ingestion of GMO by mosquitoes during a blood meal ⬇ vi. Mosquito-vectored transmission to other people or animals in the environment including individuals and species who may be susceptible to viral infection ⬇ ii. Infection with the GMO	Miscarriage/Developmental abnormalities or foetal death Disease in susceptible adult animals, or foetal/ neonatal death in susceptible pregnant or nursing animals Viraemia sufficient to infect additional mosquitoes, leading to spread of GMO within the environment	No	• Opportunities for trial participants to be bitten by mosquitoes will be minimised by requiring them to remain indoors at the clinical trial site during the period of highest viraemia. • Subsequent active viraemia is not expected to occur • Participants will take precautions to avoid exposure to mosquitoes for a further seven days after leaving the clinical trial site

2	GM GETV	i. Treatment of trial participant with GMO ⬇ ii. Passive viraemia and shedding of GMO, or bleeding due to injury or menstruation ⬇ iii. Infection of tumour cells followed by viral replication within tumour tissue ⬇ iv. Release of GMO into circulation (active viraemia) and shedding of GMO, or bleeding due to injury or menstruation ⬇ v. Transmission (e.g by direct inoculation) to other people or animals in the environment, including individuals and species who may be susceptible to viral infection ⬇ vi. Infection with the GMO	As for Scenario 1	No	• Trial participants will be at the hospital and not in the home environment while passive viraemia is highest • Active viraemia is not expected to occur • Medical procedures will take place in a hospital setting, following clinical procedures and guidelines • Close contacts and carers will take precautions to minimise exposure to blood and body fluids from trial participants • Observed shedding into body fluids to date is very low
3	GM GETV	i. Exposure of people undertaking dealings in the pharmacy or clinical facilities to GMO by direct inoculation (i.e. sharps injury or contact with broken skin) while preparing the GMO for administration, administering it to trial participants or collecting blood or tumour samples ⬇ ii. Passive viraemia ⬇	As for scenario 1	No	• Preparing the GMO will not require removal or recapping of a needle • Use of PPE (gown, gloves) will protect staff from direct contact with the GMO, and afford some protection from sharps injury • Accidental exposure while collecting blood samples or carrying out medical procedures would involve a very low GMO dose which is not expected to produce a level of viraemia infective towards mosquitoes • Staff exposed while preparing or administering the GMO will

		ii. Ingestion of GMO by mosquitoes during a blood meal ⬇ v. Mosquito-vectored transmission to other people or animals in the environment including individuals and species who may be susceptible to viral infection ⬇ v. Infection with the GMO			take measures to protect themselves from mosquito exposure until they receive two consecutive negative serum test results for viral genomes
4	GM GETV	i. Exposure of pregnant woman undertaking dealings in the pharmacy or clinical facilities to GMO by direct inoculation (i.e. sharps injury or contact with broken skin) while preparing the GMO for administration, administering it to trial participants or collecting blood or tumour samples ⬇ ii. Passive viraemia ⬇ ii. Infection of susceptible tissues, including placenta	Developmental abnormalities or foetal death	No	• The Applicant will advise prospective clinical trial staff that pregnant women should not undertake roles in the trial that involve handling the GMO • Measures will be in place to minimise exposure to the GMO • There is uncertainty as to the effect of the GMO on human pregnancy

2.4.1 Risk scenario 1

Risk source	GM GETV
Causal pathway	i. Treatment of trial participant with GMO ⬇ ii. Passive viraemia ⬇ iii. Infection of tumour cells followed by viral replication within tumour tissue ⬇ iv. Release of GMO into circulation (active viraemia) ⬇ v. Mosquito-vectored transmission to other people or animals in the environment, including individuals and species who may be susceptible to viral infection ⬇ vi. Infection with the GMO
Potential harms	• Miscarriage/Developmental abnormalities or foetal death • Disease in susceptible adult animals, or foetal/neonatal death in susceptible pregnant or nursing animals • Viraemia sufficient to infect additional mosquitoes, leading to spread of GMO within the environment

Risk source

201. The source of harm for this postulated risk scenario is the GMO, which may be more pathogenic towards species susceptible to GETV-associated disease.

Causal Pathway

202. This scenario applies to cancer patients participating in the trial who will be inoculated with the GMO. The GMO will circulate in the blood (passive viraemia) before being taken up by tumour tissue, and if replication ensues, viral progeny could be released back into the blood stream (active viraemia). During either viraemic period, female mosquitoes could ingest the GMO during a blood meal and transmit it to other people or animals whom they subsequently bite.

203. As discussed in Chapter 14.5.2 and Chapter 14.5.3, in immune deficient mice given an extremely high dose of the GMO, GMO inoculated into the blood declined logarithmically over several weeks. However, in immune competent mice, only a low level of GMO could be detected in serum at 48 hours post inoculation. In human cancer patients, viral RNA was detectable in serum beyond two hours after the first GMO treatment and for at least one hour after subsequent treatments. The Applicant has acknowledged uncertainty regarding the duration of passive viraemia, given that the few human treatments to date were not part of a clinical trial and data collection was not systematic.

204. Given the maximum GMO dose of $3x10^9$ CCID50 and an average adult blood volume of 5L, the maximum concentration of circulating virus immediately after GMO treatment is $6x10^5$ CCID50/ml. This is expected to decline over time as the GMO enters cancerous tissue and is also cleared from the body. Preclinical studies indicate that active viraemia following infection of tumours does not occur. Whether or not the GMO replicated in tumour tissue, murine models showed a steady logarithmic decline in tumours from 48 hours onwards, without any GMO reappearing in the circulation. Based on

this observation, the GMO concentration in serum immediately after iv infusion is expected to be the highest that will be attained; there will be no increase due to viral replication.

205. The Sponsor used qPCR to measure viral RNA in the serum of cancer patients within the first hour after treatment with the GMO (Chapter 14.5.3). The highest value of 5310 RNA copies/µl was detected in a patient treated with $2x10^9$ CCID50. Adjusting for the maximum dose to be used in this trial ($3x10^9$ CCID50), approximately 8000 RNA copies/µl might be expected to circulate soon after the GMO is administered, representing a maximum of 8000 virions/µl. Mosquitoes ingest around 3-5 µl during a bloodmeal (Jove et al., 2020) which could contain up to $2.4x10^4$-$4 x10^4$ GMO particles if the patient were bitten immediately. There are no studies on the minimum 'mosquito infective dose' for GETV and none were found for other alphaviruses. Considering other arboviruses, Zika virus readily infected and was transmissible from mosquitoes at 10^2-10^3 PFU/µl (Chouin-Carneiro et al., 2020), so it is possible that the passive viraemia following GMO treatment offers transmission potential.

206. It is not known whether any mosquito species found in Australia, and near Adelaide in particular, are competent vectors for transmission of GETV or of the GMO. GETV is not known to be present in Australia (Chapter 13.9) and potential vectors have not been investigated. However, at least 15 competent vector species have been identified in Asia, encompassing four different genera and covering a wide climactic range (Chapter 13.6). Australia has around 300 mosquito species, and South Australia at least three capable of transmitting the related RRV (Chapter 15.4.2). RRV disease occurs periodically in South Australia and both mosquito numbers and RRV incidence have been higher than usual this year (Chapter 15.4.3). Mosquitoes are less prevalent in metropolitan Adelaide, but trial participants could be recruited from anywhere in the state or further afield and return home between treatments. They will also be ambulatory and potentially capable of travelling for work or leisure (Chapter 12.3.3). With intervals of two to three weeks between treatments, they could visit other parts of Australia where vector species may be present.

207. Given the uncertainty regarding the duration of viraemia and the potential for encountering mosquitoes capable of becoming infected with and transmitting the GMO, the Applicant has proposed measures to limit this possibility. After each treatment with the GMO, trial participants:

- will be required to remain indoors at the clinical trial site for a time exceeding the passive viraemic period observed in prior human studies (24 hours after the first treatment and 2 hours after subsequent treatments); and
- must undertake a range of measures intended to prevent exposure to mosquitoes for the first seven days after leaving the clinical trial site. These are detailed in Chapter 12.3.11.

These proposed controls will minimise the likelihood of GMO transmission to other people or animals in the environment.

Potential harm

208. If people or animals in the environment are exposed to and infected with the GMO via mosquito bites, a range of outcomes are possible. As discussed in Sections 2.2 and 2.3 (this chapter), some groups are not expected to suffer an adverse outcome and are not considered in this scenario. However, for other groups, infection with the GMO may cause a more severe manifestation of disease associated with GETV infection, appropriate to the species and developmental stage of the affected individual. Affected groups and potential disease manifestations are described below.

2.4.1.1 Potential harm to pregnant women and their foetuses

209. In pigs, mice and several other small mammals, GETV infects both placenta and foetus and is associated with foetal and neonatal death. As with humans, GETV infection does not cause clinical disease in adults. Horses, in whom placental transmission has not been reported, respond very differently to GETV infection. It is possible then that humans share with pigs and small mammals the capacity for transplacental transmission to the foetus, with potential for developmental abnormalities or foetal death (Auriti et al., 2021).

210. There is uncertainty regarding this outcome as there are no data regarding the effects of the GMO or other GETV strains on human pregnancy. Serosurveys documenting human exposure to the M1 strain of GETV in China found an overall seropositive rate of 14.4% and made no report of unusual reproductive difficulties (Li et al., 1992). However, the surveys were undertaken in a small rural province, the sample size was small and access to healthcare is unknown.

211. For comparison, GETV's closest relative RRV infects and kills mouse foetuses *in utero* (Milner and Marshall, 1984), but an investigation of first trimester pregnancies during an RRV epidemic in the Cook Islands in 1980 found 39 women with serological evidence of infection and no evidence that any of their infants had been infected (Aleck et al., 1983).

2.4.1.2 Potential harm to immunocompromised people

212. As discussed in paragraph 195, there is little data on human infection with GETV and none concerning outcomes of infection in immunocompromised people. In susceptible animal species, the humoral (B cell mediated) immune response develops after active viraemia has waned and so may protect against subsequent exposures but not against a first infection. Considering disease caused by other Old World alphaviruses, cells of the innate immune system (monocytes and macrophages) appear to promote the pathogenesis of acute RRV and CHIKV infection (Belarbi et al., 2019; Haist et al., 2017), and certain T cell subsets are also involved in disease pathology attributed to CHIKV (Poh et al., 2020). In contrast, adaptive immunity contributes to suppressing replication of RRV that persists in tissues at low levels and may underly the chronic phase of the disease (Belarbi et al., 2019). As the immunologic mechanisms controlling alphavirus infection are not well defined, and given the limited data regarding GETV infection outcomes in humans, a conservative viewpoint allows for the possibility that the GMO could adversely affect an immunocompromised person.

2.4.1.3 Potential harm to animals susceptible to GETV associated disease

213. Characteristic GETV-associated pathologies affect horses, foxes and cattle and cause foetal and neonatal death in pigs and several small mammalian species (see Chapter 13.8). The GMO could induce more severe forms of disease in these species.

214. Serosurveys conducted in Asia show that GETV infects a range of animal species– both domestic and wild (Li et al., 1992; Li et al., 2019; Sugiyama et al., 2009). Australia is home to a diverse and unique wildlife population, comprising some 386 native mammalian species and 828 native birds which are not currently exposed to GETV and represent an immunologically naïve population. Any may be susceptible to infection with the GMO. Some may experience disease, with any of the range of observed outcomes being possible, including adverse effects on reproduction.

215. Foetal infection and death during pregnancy may be particularly relevant to native rodents as this has been demonstrated in three rodent species to date (see Chapter 13.8.5). Many Australian natives are marsupials; however, these still undergo a short pregnancy supported by a placenta. Foetal development then continues during an extended lactation period (Guernsey et al., 2017). Transplacental transmission as described in pigs, rodents and rabbits could lead to infection of young during early development, and the report of MTCT via milk in mice is relevant to the prolonged lactation stage (Chapter 13.5.4).

2.4.1.3 Infection of an amplifying host

216. Like other arboviruses, GETV alternates between mosquito and vertebrate hosts. Many vertebrate species may support infection, but not all develop sufficient viraemia to reinfect mosquitoes during a bloodmeal. Pigs are thought to be natural amplifiers of GETV because of high viral titres produced after experimental infection and the high seroprevalence amongst pigs in the field (Bannai et al., 2017).

217. If pigs, or an Australian animal capable of sufficient viraemia to act as an amplifier is infected with the GMO, a natural transmission cycle could become established in the Australian environment. The GMO could spread and persist in the Australian environment and the adverse outcomes discussed in Sections 2.4.1.1–2.4.1.3 could continue in the long term, either at a low ongoing level or on an episodic basis.

Conclusion

218. Risk scenario 1 is not identified as a substantive risk because although passive viraemia induced in trial participants may be sufficient for mosquitoes to become infected, should competent vector species be encountered, the Applicant has proposed measures to minimise opportunities for trial participants to come into contact with mosquitoes for the first seven days after each treatment with the GMO. Therefore, this risk could not be greater than negligible and does not warrant further detailed assessment.

2.4.2 Risk scenario 2

Risk source	GM GETV
Causal pathway	i. Treatment of trial participant with GMO ⬇ ii. Passive viraemia and shedding of GMO, or bleeding due to injury or menstruation ⬇ iii. Infection of tumour cells followed by viral replication within tumour tissue ⬇ iv. Release of GMO into circulation (active viraemia) and shedding of GMO, or bleeding due to injury or menstruation ⬇ v. Transmission (e.g by direct inoculation) to other people or animals in the environment, including individuals and species who may be susceptible to viral infection, as listed in scenario #1 ⬇ vi. Infection with the GMO
Potential harm	As for Scenario 1

Risk source

219. The source of harm for this postulated risk scenario is the GMO, which may be more pathogenic towards species susceptible to GETV-associated disease.

220.

Causal Pathway

221. As described in Risk Scenario 1, trial participants will be inoculated with the GMO and experience passive viraemia as the GMO circulates through the body and concentrates in cancerous tissue. If the GMO replicates in cancer cells, there is potential for viral progeny to re-enter the circulation, causing a period of active viraemia. However, as previously noted, preclinical data suggest that active viraemia does not occur and the duration of the initial passive viraemia in human cancer patients is uncertain. Human data indicate that low level shedding into saliva and urine are possible, while data regarding shedding via nasal secretions is limited. The duration of such shedding is uncertain but will be investigated as part of the proposed clinical trial.

222. Susceptible people and animals could be exposed to viraemic blood or to body fluids containing the GMO in a number of ways.

Exposure of people conducting dealings

223. Staff caring for trial participants while they remain at the hospital, performing medical procedures unrelated to the trial, and collecting blood or tumour samples could be exposed to blood or body fluids. Post-treatment care will be for up to 24 hours after the GMO is administered, and therefore when shedding is likely to be at its highest. Collection of blood samples will commence 30 minutes after the first treatment, when the circulating GMO concentration is also expected to be high, and will involve the use of sharps with concomitant risk of direct inoculation. Tumour samples will be collected within 49 days of the first treatment and would likely involve a surgical procedure, also presenting an opportunity for sharps injury and direct blood contact.

224. The Applicant has considered these exposure pathways and proposed controls to minimise their impact. The GMO will be administered in a hospital setting and clinical staff involved in the trial will be required to comply with clinical standards published by the Department of Health in their state and have completed all required competency assessments (see Chapter 12.3.13). Any medical procedures unrelated to the trial would also involve qualified medical staff, and given the participants have cancer, are also likely to take place in a hospital. The Applicant will advise any clinical trial staff caring for trial participants, or other staff required to perform procedures as part of their medical care, to wear disposable protective suits and gloves if they may be exposed to blood, secretions, urine or faeces from the trial participant. If the staff member has broken skin on their hands or is performing a procedure where their gloves are likely to tear, they will be advised to wear two pairs of gloves.

225. Sharps injuries are most prevalent in the clinical setting, given that most sharps usage takes place in this environment (Guest et al., 2010, 2014). However, clinical trial staff are expected to be competent in sharps handling via their experience and ongoing training. The Applicant will also remind them to be aware of correct sharps handling technique when performing invasive procedures.

226. Required procedures for sample analysis are not known, and analytical facility staff could undergo sharps injury or otherwise contact the GMO. However, testing will be conducted by qualified personnel in pathology or other testing laboratories, which are required to adhere to national standards for handling of infectious substances, or in certified PC2 laboratories. The standards and work practices adhered to in these environments will minimise the likelihood of exposure to the GMO.

Exposure of other people or animals

227. Once trial participants return home, carers and other close contacts or animals such as pets or livestock could be exposed to the GMO. Direct inoculation with contaminated blood or body fluids carries the highest risk. In the home setting, this is most likely to involve contact with damaged skin.

228. The Applicant has proposed measures to minimise exposure of other people and animals to potentially contaminated blood and body fluids. Firstly, participants will remain at the clinical trial site while viraemia is highest, keeping them out of the home environment. The catheter used to inoculate

the GMO will be removed and the insertion site covered with an occlusive dressing for at least 2 hours before the participant leaves the hospital, which will ensure any bleeding has stopped.

229. The Applicant acknowledges uncertainty regarding clearance time for the GMO inoculum. Accordingly, they will advise close contacts to wear gloves when handling items contaminated by blood and body fluids from trial participants, and to wash or sanitise their hands after removing them. Trial participants and family members will both receive training sessions covering the trial instructions.

230. Blood may be transferred during sexual activity, either due to abrasion or menstruation by a female trial participant. Trial participants will be required to use effective contraception to prevent pregnancy, and the Applicant will recommend that barrier protection be used.

231. No measures were recommended to minimise exposure of animals. Bleeding due to a small cut or injury could expose another human, pet or livestock animal to a participant's blood, but the quantity and GMO concentration involved are expected to be low. A more serious wound would be attended to by a carer or family member and addressed by the advice given above.

232. The small quantity of shedding seen thus far into secreted fluids such as saliva and urine is unlikely to facilitate transmission via the types of contact likely between participants and household contacts, both human and animal.

Potential harm

233. See Scenario 1.

Conclusion

234. Risk scenario 2 is not identified as a substantive risk because potential exposure routes to the GMO will be mitigated by the proposed controls. Therefore, this risk could not be greater than negligible and does not warrant further detailed assessment.

2.4.3 Risk scenario 3

Risk source	GM GETV
Causal pathway	i. Exposure of people undertaking dealings in the pharmacy or clinical facilities to GMO by direct inoculation (i.e. sharps injury or contact with broken skin) while preparing the GMO for administration, administering it to trial participants or collecting blood or tumour samples ⬇ ii. Primary viraemia ⬇ iii. Ingestion of GMO by mosquitoes during a blood meal ⬇ iv. Mosquito-vectored transmission to other people or animals in the environment including individuals and species who may be susceptible to viral infection, as listed in scenario #1 ⬇ v. Infection with the GMO
Potential harm	As for Scenario 1

Risk source

235. The source of harm for this postulated risk scenario is the GMO, which may be more pathogenic towards species susceptible to GETV-associated disease.

Causal Pathway

236. This scenario applies to people conducting dealings in the pharmacy or at the clinical trial site. GETV can be transmitted via direct inoculation (see Chapter 13.5.2), such as via needlestick injury or contact with physically damaged skin. Sharps will be used while preparing the GMO for administration, during invasive medical procedures such as collecting tumour samples, and to collect blood samples commencing 30 minutes after the first GMO treatment.

237. Reconstituting lyophilised GMO carries the highest risk in this scenario as the GMO concentration will be at a maximum. The GMO will be reconstituted to $1.1x10^8$ CCID50/ml. If 2 µl of this solution (containing $2.2x10^5$ CCID50) were transferred via a needlestick puncture and dispersed in the bloodstream of the exposed person, an average blood volume of 5L would yield a final concentration of 44 CCID50/ml. Mosquitoes ingest about 3-5 µl during a bloodmeal (Jove et al., 2020), which would contain up to 0.22 CCID50 of GMO. This does not readily translate to the number of virus particles, however the Sponsor used qPCR to measure viral RNA in the serum of cancer patients within the first hour after treatment with the GMO. The highest value of 5310 RNA copies/µl was detected in a patient treated with $2x10^9$ CCID50. On a proportionate basis, a $2.2x10^5$ CCID50 inoculum would equate to 0.58 RNA copies/µl in the blood and a feeding mosquito could ingest 2-3 virus particles.

238. As noted in Risk Scenario 1, the minimum mosquito infective dose for GETV is not known. The observed minimum blood concentration of dengue virus needed for successful transmission from infected humans to mosquitoes ranges from 10^3-10^4 infectious units/ml, corresponding to ingestion of 1-20 infectious units (Duong et al., 2015; Nguyet et al., 2013). Zika virus was transmitted to mosquitoes when orally challenged with a viral concentration equating to 0.5-5 PFU per 5 µl inoculum, although they were unable to transmit it onwards (Chouin-Carneiro et al., 2020). Given these data and

the lack of information regarding the minimum MID for the GMO, there is a small possibility that viraemia persisting until the GMO is cleared from the body could be sufficient to infect mosquitoes.

239. Staff undertaking activities later in the clinical trial workflow would be exposed to progressively lower concentrations of the GMO. For example:

- Staff administering the GMO would be exposed to a maximum GMO concentration of $1.2x10^7$ CCID50/ml (about 10-fold less than the maximum concentration handled in the pharmacy). They will not be handling the GMO in association with sharps, so direct inoculation would only be via spilled material contacting broken skin.
- Staff collecting blood samples from trial participants, commencing 30 min after administration of the GMO, will use sharps to draw blood. The maximum GMO concentration they could be exposed to, based on an average blood volume of 5L, is $6x10^5$ CCID50 (about 1/180 of the maximum concentration handled in the pharmacy); in reality the concentration would be lower due to viral uptake by the tumour.
- Staff collecting tumour samples may come into contact with virus that has concentrated within that tissue. However, preclinical data provided by the Sponsor suggest that the GMO does not persist beyond a few days in tumours, and only a small amount of viral RNA was detected in a human tumour sample 35 days after treatment (see Chapter 14.5.3).

These low concentrations of circulating GMO, relative to the maximum estimated after exposure to reconstituted GMO stock solution, are unlikely to be sufficient to infect mosquitoes.

240. The Applicant has proposed work practices that would minimise opportunities for exposure by direct inoculation. The procedure for preparing iv infusion bags requires sharps but does not require high risk procedures such as removing or recapping needles. The needle/syringe unit will be deposited directly into a sharps container after use. Staff will also wear gloves. A single glove layer has been shown to reduce the volume of liquid transferred during accidental puncture by a hollow bore needle by 52% compared with no glove (Krikorian et al., 2007).

241. Staff preparing or administering the GMO could be exposed via contact with broken skin. Both groups will wear gloves and a protective gown, and staff with damaged skin on their hands will wear double gloves. Spill kits and training in their use provided to all staff will enable prompt removal of spilled GMO. These measures will minimise opportunities for direct inoculation with the GMO.

242. In the event that a person preparing or administering the GMO is exposed to it, the Applicant has proposed to carry out daily serum testing for viral RNA, from the day following exposure until two consecutive negative results are obtained. The exposed staff member must protect themselves from mosquito bites while awaiting the results. This measure, combined with the small GMO dose expected, would effectively minimise the chance of mosquitoes feeding on the exposed person and becoming infected by the GMO.

Potential harm

243. See Scenario 1 (Section 2.4.1, this chapter).

Conclusion

244. Risk scenario 3 is not identified as a substantive risk because inadvertent exposure by direct inoculation could transfer only a small quantity of the GMO, and the Applicant has proposed measures to both minimise opportunities for exposure and then to prevent contact with mosquitoes during any resulting period of viraemia if this occurs. Therefore, this risk could not be greater than negligible and does not warrant further detailed assessment.

2.4.4 Risk scenario 4

Risk source	GM GETV
Causal pathway	i. Exposure of pregnant woman undertaking dealings in the pharmacy or clinical facilities to GMO by direct inoculation (i.e. sharps injury or contact with broken skin) while preparing the GMO for administration, administering it to trial participants or collecting blood or tumour samples ⬇ ii. Passive viraemia ⬇ iii. Infection of susceptible tissues, including placenta ⬇ iv. Infection of foetus
Potential harms	Developmental abnormalities or foetal death

Risk source

245. The source of harm for this postulated risk scenario is the GMO, which may be more pathogenic towards species susceptible to GETV-associated disease.

Causal Pathway

246. This scenario applies to pregnant women conducting dealings in the pharmacy or at the clinical trial site. There is potential for GETV to be transmitted via direct inoculation during these activities, as discussed in Risk Scenario 3.

247. If the GMO enters the bloodstream, biodistribution data provided by the Sponsor suggests that the GMO would initially circulate through the body, be cleared rapidly from non-permissive tissues and organs but concentrate in any tissues that are permissive for GETV infection. If the human placenta is permissive for GETV infection, as it is in several other species, this could lead to placental and then foetal infection, potentially causing developmental abnormalities or foetal death.

248. The Applicant proposes to advise prospective clinical trial staff that pregnant women should not undertake roles in the trial that involve handling the GMO, noting that they cannot require personal information to be disclosed and also that women may be unaware they are in an early stage of pregnancy. Acknowledging these caveats, this action would reduce the number of pregnant women with the opportunity for parenteral exposure to the GMO.

249. Other limiting factors include that pregnant women make up only a small percentage of the population: Australian birth and population data suggest that approximately 4.3% of the female population of child-bearing age gave birth in the last year. The clinical trial will be small, enrolling up to18 participants. Statistically then, pregnant women have a low chance of being involved.

250. As discussed in Scenario 3, the Applicant has proposed measures to minimise exposure of staff preparing and administering the GMO by direct inoculation. In addition, the quantity of GMO to which staff collecting blood or tumour samples would be exposed to by this route is very small.

251. The efficiency of transplacental transmission varies with the stage of gestation at which viral infection occurs. There is no information about when this would be for GETV or the GMO, but it further limits the likelihood of foetal infection.

Potential harm

252. Potential harms during human pregnancy are discussed in Risk Scenario 1 (Section 2.4.1.1).

Conclusion

253. Risk scenario 4 is not identified as a substantive risk because it requires that a pregnant woman be involved in the trial despite being advised against it, the small trial size and low percentage of pregnant women in the population, and be at an appropriate stage of gestation for foetal injury to occur. The Applicant has also proposed measures to minimise exposure of all staff. Therefore, this risk could not be greater than negligible and does not warrant further detailed assessment.

Section 3 Uncertainty

254. Uncertainty is an intrinsic part of risk analysis[11]. 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, and there are several types of uncertainty in risk analysis (Bammer and Smithson, 2008; Clark and Brinkley, 2001; Hayes, 2004). These include:

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

255. 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.

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

257. For DIR-187, uncertainty is noted in relation to a number of points, including the following.

- whether the GMO is pathogenic toward GETV susceptible species such as horses, cattle and commercial pig breeds. There are no data on the ability of the GMO to cause disease in animal species and/or breeds known to be susceptible to GETV-associated disease (see paragraph 134 and Chapter 14.4). In addition, the derivation history of the M1 working stock and any impact on natural pathogenicity are unknown (see paragraph 129).
- the duration of passive and active viraemia after the GMO is administered (see Chapter 14.5.3).
- the capacity of the GMO to cause placental and foetal infection in humans (see Chapter 2, Section 2.4.1.1).

[11] 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.

- outcomes of GMO infection for Australian native animals and other animal species in whom GETV infection has not been studied (see Chapter 2, Section 2.4.1.3).
- whether mosquito species present on the Australian mainland can be infected by and transmit the GMO to animal hosts (see Chapter 15.4.2 and paragraph 206).

258. The uncertainties outlined above have been accommodated by taking a conservative approach to the risk analysis. 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 trial or the commercial release of this GMO. Chapter 3, Section 4, discusses information that may be required for future release.

Section 4 Risk evaluation

259. 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 the Applicant should be required to collect more information.

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

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

261. Four 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 3 and include the suitability of limits and controls proposed by the applicant.

262. 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 proposed release do not pose a significant risk to either people or the environment[12].

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

Chapter 3 Risk management plan

Section 1 Background

263. 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.

264. 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.

265. 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.

266. 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

267. 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 clinical trial and the proposed controls (Chapter 1Section 2) and the receiving environment (Chapter 1, Section 4.6), 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

268. The limits and controls proposed in the application were important in establishing the context for the risk assessment and in reaching the conclusion that the risks posed to people and the environment are negligible. Therefore, to maintain the risk context, draft licence conditions have been imposed to limit the number of trial participants and duration of the trial, limit the locations to those proposed in the application, as well as a require a range of controls to restrict the spread and persistence of the GMO in the environment. The conditions are discussed and summarised in this Chapter and listed in detail in the draft licence.

3.1 Limits and controls on the clinical trial

269. Sections 2.1 and 2.1 in Chapter 1 list the limits and controls proposed by VRT Pharmaceutics. Many of these are discussed in the four 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 VRT Pharmaceutics

270. The clinical trial was proposed to involve a maximum of 18 participants within Australia. The draft licence increases this limit to 20 to accommodate replacement of any participants who withdraw or are withdrawn after enrolling in the trial (Chapter 12.3.1). Most dealings with the GMO would take place in hospital clinical areas, hospital pharmacies, certified PC2 laboratories and other analytical facilities. Activities that would occur elsewhere include transport and disposal of the GMO. The Applicant has proposed to complete the study within five years of commencement. Conditions maintaining the risk context and proposed limits of the trial such as the maximum number of trial participants, duration of the study and types of facility in which it will be conducted have been included in the draft licence.

271. The Applicant advised that import and transport of the GMO would be in accordance with IATA shipping classification UN 3373 (Biological Substance, Category B) and/or the Regulator's *Guidelines for the Transport, Storage and Disposal of GMOs*, as applicable to risk group 2 microorganisms These provide standard protocols for handling and minimising exposure to the GMOs Once at the storage facility or clinical trial site, access to the GMO would be restricted to appropriately trained personnel. These proposed arrangements are suitable for the GMO. Therefore, the draft licence details the minimum requirements for packaging and labelling the GMO for transport and storage between and within the storage facility, pharmacy and clinical trial site, as well as transport of the GMO during import. These measures limit the exposure of people and the environment to the GMOs.

272. Conditions are included in the draft licence requiring the licence holder to ensure that all GMOs, including material or waste that has been in contact with the GMO, within the clinical trial site, are decontaminated by autoclaving, chemical treatment or by high-temperature incineration. Draft licence conditions require that the licence holder ensure that any destruction of the GMO, or material or waste that has been in contact with the GMO, by external service providers is destroyed by autoclaving or high temperature incineration. Given that the GMO is modified from a parent organism classified as risk group 2, is potentially more pathogenic in susceptible hosts, and waste may include vials of unused GMO, disposal methods in accordance with the Regulator's *Guidelines for the Transport, Storage and Disposal of GMOs* for risk group 2 are appropriate.

273. Proposed criteria for selecting trial participants are listed in Chapter 12.3.3. These are subject to approval by a HREC, who will consider safety of the individuals involved in the trial. The draft licence requires that trial participants be adults, not be pregnant at commencement of the GMO treatment, agree to use effective contraception to ensure they don't become pregnant while undergoing GMO treatment, and be willing to follow and able to complete all procedures required by the clinical trial protocol. These procedures include remaining at the clinical trial site during their anticipated viraemic period and undertaking behavioural measures to minimise their exposure to mosquitoes for an additional seven days after each treatment. Both measures will minimise the potential for participants to transmit the GMO to mosquitoes in the environment, and for subsequent transmission from mosquitoes to other susceptible people or animals.

274. Staff involved in the trial or providing other medical care, and other people and animals with whom trial participants interact, could be exposed to the GMO via contact with blood and body fluids containing the GMO. The Applicant has proposed a range of measures to limit this, including conducting the trial in a hospital setting and engaging staff qualified for their roles. Draft licence conditions require that clinical trial staff caring for trial participants after their treatment wear PPE if likely to be exposed to blood and/or body fluids, and that any other hospital staff required to perform medical procedures during their stay be advised to do likewise. To minimise exposure of carers and close contacts at home to blood and body fluids, draft licence conditions require that these people be instructed in similar measures. The inoculation site will also be covered with an occlusive dressing for at least two hours before participants leave the clinical trial site. As 30 minutes should be sufficient to ensure that bleeding has ceased, draft licence conditions require a dressing to be in place for this time frame and removed before the participant's departure.

275. As blood could be transferred during sexual activity, draft licence conditions amend the requirement for participants to use effective contraception to include that such contraception must also provide barrier

protection. Although not proposed by the Applicant, the draft licence requires trial participants to cover any bleeding cuts or wounds, to prevent contact with people or animals.

276. Direct inoculation with the reconstituted GMO concentrate could lead to passive viraemia and infection of mosquitoes in the environment. This applies to staff preparing the GMO and the Applicant has proposed measures to minimise their exposure. Draft licence conditions require that staff preparing the GMO wear PPE that includes a gown and gloves. To protect any broken skin not otherwise protected by PPE or clothing, this must be covered by a waterproof dressing. Procedures for preparing the GMO must preclude the recapping or removal of used needles from the syringe.

277. In the event of exposure by direct inoculation, the Applicant has proposed to carry out daily serum testing for viral RNA, from the day after exposure until two consecutive negative results are obtained. The exposed staff member must also protect themselves from mosquito bites while awaiting the results; this would be for a minimum of two days. Comparable serum testing within an hour of treating cancer patients with high doses of the GMO gave inconsistent results, fluctuating between high RNA readings and readings below the detection limit (see Chapter 14.5.3). A starting viraemia of about 6 µg RNA/ml blood (see Risk Scenario 3) is therefore unlikely to be detectable at any time after exposure. Given the lack of information regarding a minimum MID for the GMO or for alphaviruses in general, and therefore the capacity for an inadvertently exposed person to infect a mosquito taking a blood meal, draft licence conditions require that staff exposed by direct inoculation while preparing the GMO (i.e. sharps injury or contact of the GMO concentrate with broken skin) take specified precautions to protect themselves from mosquito bites for a period of 48 hours after exposure. Serum testing for viral RNA is not required.

278. There is uncertainty as to whether GETV and/or the GMO could cause foetal infection in humans. To manage any risks posed to pregnant women and their unborn children, the Applicant has proposed to advise prospective clinical trial staff that pregnant women should not undertake roles that involve handling the GMO. Together with measures to protect all staff from direct inoculation with the GMO, and the statistically low chance of pregnant women becoming involved with a small trial, this will minimise the opportunity for women to be exposed to the GMO during pregnancy. Draft licence conditions require that this advice be given to staff considering involvement in the trial.

279. Staff caring for trial participants while they remain at the hospital, collecting blood or tumour samples, and performing medical procedures unrelated to the trial could be exposed to blood or body fluids. A standard condition is included in the draft licence requiring the licence holder to ensure that dealings are conducted so as not compromise the health and safety of people and minimise unintended 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.

280. Other conditions included in the draft licence are standard conditions stating that the licence covers only those people authorised by the licence holder, and that the licence holder must inform all people dealing with the GMO, other than external service providers, of applicable licence conditions.

281. Further conditions included in the draft licence ensure that a compliance management plan is in place for each clinical trial site before administration of the GMO commences at that site. The compliance management plan must detail how the licence holder intends to comply with the licence conditions, including listing persons responsible for site management, proposed reporting structures, staff training procedures and transport and disposal processes.

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

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

- limit the trial to 20 adult participants, who are not pregnant
- require the trial 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
- restrict personnel permitted to administer the GMO
- ensure appropriate PPE is used by staff, and by carers exposed to blood and body fluids from trial participants after they receive treatment
- ensure that behavioural requirements are communicated to trial participants and their agreement obtained
- transport and store the GMO and samples from GMO-treated participants in accordance with IATA shipping classification UN 3373 and/or the minimum requirements for packaging and labelling as detailed in the draft licence
- destroy untreated GMO and GMO-related waste, with disposal by autoclaving or high temperature incineration to be used by external service providers

3.2 Other risk management considerations

283. All DIR licences issued by the Regulator contain 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

284. 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.

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

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

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

287. If issued, the persons covered by the licence would be the licence holder and employees, agents or contractors of the licence holder and other persons who are, or have been, engaged or otherwise authorised by the licence holder to undertake any activity in connection with the dealings authorised by the licence. Prior to dealings with the GMOs, VRT Pharmaceutics 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.

3.2.3 Reporting requirements

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

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

289. 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 clinical trial sites where the GMO will be administered to trial participants
- expected date of first administration of the GMO for each clinical trial site
- date of final administration of the GMO for each clinical trial site

3.2.4 Monitoring for compliance

290. 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.

291. 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.

292. 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

293. Additional information has been identified that may be required to assess an application for a larger clinical trial, a commercial release of the GMO, or to justify a reduction in limits and controls. This includes data assessing behaviour of the GMO in animal models that would reveal whether it shares the pathogenicity characteristic of other GETV strains. e.g. horses, pregnant and neonatal mice and pigs from commercial breeds; data defining the duration of passive and active viraemia after the GMO is administered; and data addressing whether mosquito species present on the Australian mainland can be infected by and transmit the GMO to vertebrate hosts.

Section 5 Conclusions of the consultation RARMP

294. The risk assessment concludes that the proposed clinical trial of the GMO 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.

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

Chapter 4 Draft licence conditions

Section 1 Interpretations and Definitions

1. In this licence:

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

    (b) words denoting a gender include any other gender;

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

    (d) words denoting persons include a partnership and a body whether corporate or otherwise;

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

    (f) where any word or phrase is given a defined meaning, any other part of speech or other grammatical form in respect of that word has a corresponding meaning;

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

2. In this licence:

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

'Analytical facility' a laboratory in Australia accredited to undertake testing of human diagnostic Samples, such as a medical testing laboratory accredited by the National Pathology Accreditation Advisory Council (NPAAC), or a PC2 Laboratory certified by the Regulator.

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

Note: The clinical trial facility/hospital and Pharmacy need not be co-located or part of the same organisation.

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

a) chemical treatment;

b) autoclaving;

c) high-temperature incineration; or

d) a method approved in writing by the Regulator.

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

'External service provider' means a person engaged by the licence holder solely in relation to transport, storage and/or disposal of the GMO other than at a Storage facility or Clinical trial site, or Sample analysis other than at a Clinical trial site, and who is not undertaking any dealings with the GMO that are not for those purposes.

'GM' means genetically modified.

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

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

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

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

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

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

'Pharmacy' means a location where authorised staff store, prepare, and dispense medications in a medical environment.

'Regulator' means the Gene Technology Regulator.

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

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

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

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

Section 2 General conditions and obligations

Holder of licence

3. The licence holder VRT Pharmaceutics Pty Ltd.

Remaining an accredited organisation

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

Validity of licence

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

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

Persons covered by the licence

6. The persons covered by this licence are the licence holder, and any employees, agents of, or External service providers contracted by the licence holder, or the project supervisor(s), or other persons who are, or have been, engaged or otherwise authorised by the licence holder or the project supervisor to conduct any of the dealings authorised by this licence.

7. To the extent that any activity by a trial participant may be considered to be a dealing for purposes of the Act, that dealing is authorised by this licence.

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

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

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

Description of GMOs covered

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

Note: Attachment A is not included in the draft licence as the GMO is described in this Risk Assessment and Risk Management Plan.

Dealings authorised by this licence

11. The dealings authorised by this licence are to:

a) import the GMO;

b) conduct the following experiments with the GMO:

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

ii) administer the GMO to adult trial participants by intravenous infusion;

iii) collect Samples from trial participants;

iv) prepare and/or analyse the Samples described in 11b)iii);

c) transport the GMO; and

d) dispose of the GMO

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

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

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

Conditions imposed by the Act

Note: The Act mandates the following 3 conditions.

Informing people of licence conditions (section 63)

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

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

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

(c) the surrender of the licence.

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

Monitoring and audits (section 64)

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

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

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

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

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

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

Note 1: For the purposes of this condition:

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

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

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

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

Informing the Regulator of any material changes of circumstance

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

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

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

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

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

Further conditions with respect to informing persons covered by the licence

18. If a particular condition, including any variation of it, applies to a person with respect to any dealing, the licence holder must not permit a person covered by this licence to conduct that dealing unless the person has been informed of the condition, including any variation of it.

Note: Information required under Condition 18 may be provided to External service providers who are engaged solely for storage and transport of the GMO through labelling of the outermost container of the GMOs in accordance with Condition 39(a).

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

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

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

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

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

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

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

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

Note: The licence may be made available electronically.

Section 3 Limits and control measures

Limits on clinical trials conducted under this licence

22. The GMO may be administered to a maximum of 20 trial participants.

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

Preparation and administration of the GMO

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

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

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

(b) administration of the GMO to trial participants.

Note: Before any of these activities take place, the details of each Clinical trial site must have been notified to the Regulator in accordance with Condition 44(a).

26. The licence holder must ensure that clinical trial staff are advised that pregnant individuals should not undertake any roles in the clinical trial that involve handling the GMO. A record that this advice has been given must be kept and made available to the Regulator on request.

Conditions relating to trial participants

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

28. The licence holder must ensure that criteria used in selecting trial participants include (though are not limited to) the following:

(a) Trial participants must be aged at least 18 years;

(b) Trial participants must be willing to follow and have the ability to complete all clinical trial procedures; and

(c) Trial participants must not be pregnant or attempting to become pregnant when they commence treatment with the GMO.

29. The licence holder must ensure that each trial participant remains indoors at the clinical trial site for at least 24 hours after administering the initial GMO treatment and at least 2 hours after each subsequent GMO treatment, in an area that is physically protected from exposure to mosquitoes.

30. The licence holder must ensure that before each trial participant leaves the Clinical trial site following each administration of the GMO, the participant is (or has already been) provided with a supply of an effective topically-applied mosquito repellent sufficient for at least seven days use, and is educated about its correct use.

Conditions relating to post-administration preventive practices

31. Before inoculating any trial participant with the GMO, the licence holder must obtain written agreement from the trial participant that they will:

(a) Stay indoors at the clinical trial site for at least 24 hours after receiving the first GMO treatment and for at least 2 hours after receiving each subsequent treatment;

(b) Implement measures intended to prevent exposure to mosquitoes, including but not limited to:

i) apply and reapply the topical mosquito repellent provided by the Clinical trial site to uncovered skin as directed by the Clinical trial site for seven days following each treatment with the GMO, whenever they intend to leave a physically mosquito-protected area (such as a building fitted with effective flyscreens);

ii) for the duration of the treatment period, take steps to control mosquitoes indoors and outdoors around their homes, e.g. by emptying standing water where mosquitoes may breed, ensuring windows and external doors are fitted with effective flyscreens, and using mosquito netting around beds or sitting areas if required;

iii) if living in or travelling to an area where mosquitoes may be present, wear long-sleeves and long pants, stay indoors within mosquito-protected buildings as much as possible.

(c) For seven days after each treatment with the GMO, implement hygiene measures intended to prevent transmission of the GMO to other people and animals, including but not limited to:

i) in the event of bleeding, e.g. from a cut or injury, cover the affected area with an occlusive dressing until bleeding has stopped and seal the dressing in a container (e.g. a press sealed bag) before disposing of it.

(d) If capable of reproduction, use effective birth control measures with their partners from the first GMO treatment to at least 90 days after the final GMO treatment;

(e) If sexually active, also use barrier protection capable of protecting all partners from blood and body fluids from the first GMO treatment to at least seven days after the final GMO treatment; and

(f) For the duration of the treatment period, avoid close contact with newborn infants and people known to be immunocompromised.

32. The licence holder must ensure that carers and close contacts of trial participants are instructed in hygiene measures to minimise exposure to blood or body fluids produced by the trial participant, e.g. by wearing gloves and washing or sanitising their hands immediately after removing them. A record of this advice must be kept and provided to the Regulator on request.

Conditions related to the conduct of the dealings

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

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

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

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

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

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

Work practices at Clinical trial sites

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

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

(b) persons conducting dealings with the GMOs must wear personal protective equipment (PPE) including (but not limited to) a long-sleeved gown and gloves;

(c) any broken skin (e.g. cuts, scratches, dermatitis) of persons conducting dealings, or caring for trial participants after GMO treatment, and not covered by PPE or clothing must be covered with a waterproof dressing;

(d) needles used during preparation of the GMO for administration must not be recapped or removed from the syringe before being discarded into a sharps container;

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

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

(g) persons caring for trial participants while they remain at the clinical trial site must wear PPE including (but not limited to) a long-sleeved gown and gloves if they may be exposed to blood, body fluids, urine or faeces from the trial participant;

(h) after removing the catheter or other intravenous device used to administer the GMO from the trial participant, the inoculation site must be covered with an occlusive dressing for at least 30 minutes. The dressing must be removed before the participant leaves the Clinical trial site and disposed of as clinical or GMO waste.

Transport, storage and disposal of the GMOs

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

38. Transport between a storage facility and the clinical trial site may also be consistent with IATA shipping classification UN 3373 if the GMO is not repackaged at the storage facility.

39. The licence holder must ensure that transport and storage of the GMO within the Pharmacy, transport of the GMO from the Pharmacy to the Clinical trial site, transport of Samples to Analytical facilities and, unless conducted according to condition 38, any transport between a Storage facility and a Pharmacy or Clinical trial site, follows these sub-conditions:

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

i) that it contains a GMO; and

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

iii) the contact details for the licence holder; and

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

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

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

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

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

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

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

(g) For the purposes of transport entirely within a building, where the GMOs are accompanied by authorised persons for whom Condition 18 has been met, Conditions 39(a)iii), and 39(c) do not apply.

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

40. The licence holder must ensure that all GMO and waste reasonably expected to contain the GMO are decontaminated:

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

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

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

41. Where Decontamination is carried out by an External service provider, the licence holder must ensure that the GMO, or waste reasonably expected to contain the GMO, is decontaminated by autoclaving or high-temperature incineration.

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

Contingency plans

42. In the event that clinical trial staff are exposed to the reconstituted GMO concentrate by needlestick injury or contact with broken skin, the exposed person(s) must, for a period of 48 hours following the exposure, implement measures intended to prevent exposure to mosquitoes, including but not limited to:

(a) apply and reapply an effective topically-applied mosquito repellent provided by the Clinical trial site to uncovered skin as directed by the Clinical trial site whenever they intend to leave a physically mosquito-protected area (such as a building fitted with effective flyscreens).

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

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

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

(c) the exposed area must be decontaminated with an appropriate chemical disinfectant effective against the GMOs; and

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

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

Section 4 Reporting and Documentation

The following licence conditions are imposed to demonstrate compliance with other conditions and facilitate monitoring of compliance by staff of the OGTR.

Notifications to the Regulator

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

44. The licence holder must notify the Regulator, in writing, of the name and address of each storage facility before commencement of dealings at that location.

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

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

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

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

(d) the proposed reporting structure for the trial at the site and how the reporting structure enables the licence holder to become aware of any self-reported incidents for the purposes of Condition 47;

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

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

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

(h) the expected date of first administration; and

(i) how compliance with Condition 34 will be achieved in relation to preparation of participant Samples for analysis subsequent to administering the GMO.

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

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

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

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

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

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

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

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

Attachment B – Summary of reporting requirements

Prior to the commencement of the trial	Condition	Timeframe for reporting
The name and address of each Storage facility	44	Before commencement of dealings at that location
A written Compliance Management Plan for each Clinical trial site: (a) the name, address and description of the Clinical trial site, including any associated Pharmacies/storage areas/Analytical facilities; (b) the key persons responsible for the management of the trial at the site; (c) that the Institutional Biosafety Committee (IBC) associated with the site (if any) has been notified of the trial; (d) the proposed reporting structure for the trial at the site and how the reporting structure enables the licence holder to become aware of any self-reported incidents for the purposes of Condition 47; (e) details of how the persons covered by the licence (for that type of dealing) will be informed of licence conditions applicable to them and how they will be trained to safely conduct the dealings; (f) the person(s) or class of persons administering the GMO; (g) where, within the site, the GMO is expected to be administered; (h) expected date of first administration;	45	At least 14 days prior to the first administration of the GMO at each Clinical trial site, or within a timeframe agreed to in writing by the Regulator
Information to be provided at any time during the clinical trial	**Condition**	**Timeframe for reporting**
Any additional information related to the health and safety of people and the environment associated with the dealings covered by the licence, or any unintended effects of the dealings authorised by the licence	15(a), (c)	As soon as the licence holder becomes aware
Information related to any contravention of the licence by a person covered by the licence	15(b)	As soon as the licence holder becomes aware
Any relevant conviction of the licence holder	16(a)	Immediately
Any revocation or suspension of a licence or permit held by the licence holder under a law of the Commonwealth, a State or a foreign country	16(b)	Immediately
Any event or circumstances that would impact the licence holder capacity to meet the licence conditions	16(c)	Immediately
Provide notification to the Regulator, in writing, of the final GMO administration of the last trial participant at each Clinical trial site	46	Within 30 days of the decision to cease GMO administration at that particular Clinical trial site.

Any Serious adverse event which may be related to the GMO	47(a)	As soon as reasonably possible
Any loss or spill of the GMO, or exposure of a person other than the trial participant to the GMO	47(a), (c)	As soon as reasonably possible after becoming aware of the event
Any event where a trial participant has not followed the procedures described in the instruction provided by the licence holder	47(d)	As soon as reasonably possible after becoming aware of the event
Information to be provided on request by the Regulator		
Information related to the persons covered by the licence	9	Within a timeframe stipulated by the Regulator
Information related to the licence holder's ongoing suitability to hold a licence	17	Within a timeframe stipulated by the Regulator
Copies of signed and dated statements and training records	19	Within a timeframe stipulated by the Regulator
A consolidated record of all GMOs being stored	39(f)	Within a timeframe stipulated by the Regulator
Any signed records or documentation collected under a condition of this licence	48	Within a timeframe stipulated by the Regulator

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

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