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

Risk Assessment and Risk Management Plan

(consultation version) for

DIR 198

Clinical trial of a genetically modified alphavirus (Getah virus) for cancer treatment

Applicant: VRT Pharmaceutics Pty Ltd

22 August 2023

This RARMP is open for consultation until 05 October 2023.

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 genetically modified (GM) treatment **do not** fall within the scope of these evaluations as they are the responsibilities of other agencies and authorities.

PAGE INTENTIONALLY LEFT BLANK

Summary of the Risk Assessment and Risk Management Plan (consultation Version)

for

Licence Application No. DIR 198

Introduction

The Gene Technology Regulator (the Regulator) has received a licence application to conduct a clinical trial using a genetically modified organism (GMO). It qualifies as 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 (GM) Getah virus (GETV) as a treatment for cancer. The clinical trial is proposed to take place at Flinders Private Hospital in Bedford Park, South Australia (SA) and at other locations in SA as required.

The purpose of the clinical trial is 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 12 cancer patients would receive up to 15 doses of the GMO over a three-month period. 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, Fisheries and Forestry (DAFF) 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; an authorisation from the Department of Jobs, Skills, Industry and Regions - Agriculture Victoria in Victoria 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 **moderate** risks to 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 (Getah virus) for cancer treatment [1].
Parent organism	Getah virus (M1 strain, variant M1-c6), a member of the *Alphavirus* genus
Genetic modifications	Two single nucleotide changes have been introduced into the Getah virus 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 will be the first clinical trial to be undertaken in Australia. However, the applicant has applied for clinical trial approval in China, Japan, and United States of America (USA), which are under consideration. Furthermore, 27 participants have been administered with the GMO under an investigator-initiated trial for compassionate use in China, including 13 patients with advanced hepatocellular carcinoma (HCC) and 14 patients with different solid tumours.
Proposed limits and controls	
Proposed duration	5 years
Proposed release size	Up to 12 participants will be enrolled in the trial.
Proposed locations	Flinders Private Hospital, Bedford Park, South Australia (SA). Additional clinical trial sites in SA may be engaged. Other sites may be engaged for the storage of the GMO in New South Wales.
Proposed controls	• The GMO will be administered to trial participants in a hospital setting. • The GMO will be stored in an OGTR-certified physical containment level 2 (PC2) facility on arrival into Australia. Transport of the GMO and samples that may contain the GMO would be in accordance with IATA requirements UN 3373 or Regulator's *Guidelines for the Transport, Storage & Disposal of GMOs.* • Staff preparing and administering the GMO, or handling items contaminated with blood or body fluids from treated participants, will wear personal protective equipment (PPE). • Waste that may contain the GMO will be disposed of via the clinical waste stream, with destruction by autoclaving or high temperature incineration. • Participants will remain at the clinical trial site as an in-patient during the 5 days of administration and until 2 consecutive negative blood tests for viremia due to the GMO, at each treatment cycle. • Trial participants will be required to use barrier contraception during and for 90 days after treatment.

[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'.

	• Pregnant women will be excluded from the trial. • Trial participants may not donate blood or organs during the trial. • Staff will be informed that immunocompromised or pregnant individuals should not handle the GMO.

Risk assessment

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

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

The risk assessment concludes that the trial poses negligible risks to human health and safety and moderate risks to the environment. Specific risk treatment measures are included in the licence to manage these risks.

Risk management

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

As the level of risk is to the environment was assessed as **moderate**, licence conditions are proposed to manage this risk. In addition, since this is a clinical trial, the draft licence includes limits on the number of trial participants, types of facilities used and 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. There are also several general conditions relating to ongoing licence holder suitability, auditing and monitoring, and reporting requirements which include an obligation to report any unintended effects.

Table of contents

Abbreviations

AE	Adverse Event
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
AUSVETPLAN	Australian Veterinary Emergency Plan
BSC	Biosafety Cabinet
CCI	Confidential Commercial Information
CCID50	Cell culture infectious dose 50%
CDC	Centers for Disease Control and Prevention
CHIKV	Chikungunya virus
CRO	Contract Research Organisation
CTA	Clinical Trial Approval
CTN	Clinical Trial Notification
DAFF	Department of Agriculture, Fisheries and Forestry
DIR	Dealings Involving Intentional Release
DLT	Dose Limiting Toxicity
DNA	Deoxyribonucleic acid
EAD	Emergency Animal Disease
EADRA	Emergency Animal Disease Response Agreement
EEEV	Eastern equine encephalitis virus
EU	European Union
FSANZ	Food Standards Australia New Zealand
GETV	Getah virus
GM	Genetically modified
GMO	Genetically modified organism
HCC	Hepatocellular carcinoma
HREC	Human Research Ethics Committee
i.v.	Intravenous
IATA	International Air Transport Association
IBC	Institutional Biosafety Committee
ICF	Informed Consent Form
ICH-GCP	*Guidelines for Good Clinical Practice* of the International Council for Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use

IFN	Interferon
JEV	Japanese encephalitis virus
kb	kilobase
LOD	Limit of detection
LOQ	Limit of quantification
NATA	National Association of Testing Authorities
NHMRC	National Health and Medical Research Council
NPAAC	National Pathology Accreditation Advisory Council
nsP	Non-structural protein
NSQHS	National Safety and Quality Health Service
NT	Northern Territory
OGTR	Office of the Gene Technology Regulator
ORF	Open reading frame
PC2	Physical Containment level 2
PCR	Polymerase chain reaction
PPE	Personal protective equipment
QLD	Queensland
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
SAGV	Sugiyama virus
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
VEEV	Venezuelan equine encephalitis virus
VRT	VRT Pharmaceutics Pty Ltd
WEEV	Western equine encephalitis virus
WHO	World Health Organization
ZAP	Zinc-finger Antiviral Protein
ZIKV	Zika virus

Chapter 1 Risk assessment context

Section 1 Background

1. An application has been made under the *Gene Technology Act 2000* (the Act). 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.

2. 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.

3. 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.

4. 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.

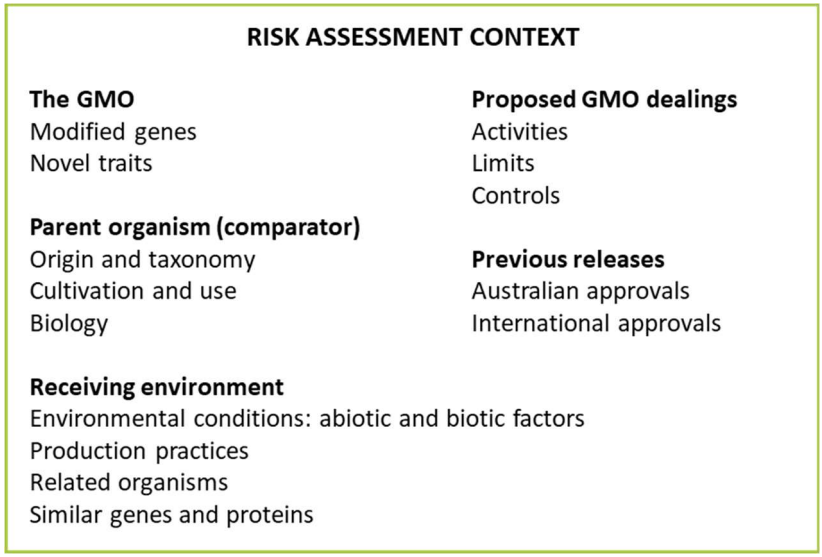

Figure 1. Summary of parameters used to establish the risk assessment context, within the legislative requirements, operational policies and guidelines of the OGTR and the RAF.

5. 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. However, the Regulator has sought advice from two experts on veterinary virology and alphavirus pathogenicity regarding the pathogenicity of the GMO. The advice provided by the experts was taken into consideration in the preparation of the consultation RARMP and is summarised in Appendix A.

6. 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

7. 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, Fisheries and Forestry (DAFF).

8. 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.

9. 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.

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

11. 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.

12. 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.

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

14. GETV is listed as a national notifiable animal disease (DAFF, 2019). It is also listed as a 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. The Victorian *Livestock Disease Control Act 1994* prohibits a person from possessing an exotic livestock disease agent without the authorisation from the Department of Jobs, Skills, Industry and Regions - Agriculture Victoria. Conditions of possession stipulated by the Livestock Disease Control Regulations 2017 include that the agent is maintained *in vitro* and is only used to perform tests, prepare reagents for tests or to undertake research for the diagnosis, monitoring or surveillance for the presence of the exotic disease in livestock in Australia[4]. Permits and approvals from the respective state governments would be required to conduct any dealings with the GM GETV in those states.

15. 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).

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

17. The National Pathology Accreditation Advisory Council (NPAAC) advises Commonwealth, State and Territory health ministers on matters relating to the accreditation of pathology laboratories. NPAAC plays a key role in ensuring the quality of Australian pathology services and is responsible for the development and maintenance of standards and guidelines for pathology practices. The standards include safety precautions to protect the safety of workers from exposure to infectious

[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*.

[4] These provisions are in accordance with section 39 of the *Livestock Disease Control Act 1994* and regulation 71 of the Livestock Disease Control Regulations 2017.

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 and by an Approved Pathology Practitioner (APP) employed by an Approved Pathology Authority (APA). Accreditation of pathology services is overseen by Services Australia (formerly Department of Human Services), and currently, the only endorsed assessing body for pathology accreditation is the National Association of Testing Authorities (NATA).

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

Section 2 The proposed dealings

19. 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.

20. The product sponsor is Guangzhou Virotech Pharmaceutical Technology Co. Ltd (Guangzhou Virotech) and the applicant VRT Pharmaceutics, will act as local sponsor and licence holder.

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 clinical trial is proposed to take place over a five-year period from the date of issue of the licence.

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

25. Up to 12 trial participants with locally advanced or metastatic cancer would be enrolled in the clinical trial.

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:

For dealings conducted with the GMO

- The GMO would be stored in an OGTR-certified physical containment level 2 (PC2) facility on arrival into Australia. Transport of the GMO and biological samples that may contain the GMO would be in accordance with International Air Transport Association (IATA) requirements UN 3373 or Regulator's Guidelines for Transport, Storage and Disposal of GMOs.
- The GMO will be prepared using a Biosafety Cabinet containment (BSC) level 2 or equivalent (i.e. negative pressure isolator).
- Staff would be informed that immunocompromised or pregnant individuals should not handle the GMO.
- Staff preparing or handling samples or items contaminated with the GMO will wear PPE, including disposable gowns and gloves. Staff administering the GMO will also wear safety glasses. Anyone with skin damage on their hands will be advised to wear double gloves.
- 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, urine or faeces.
- Waste that may contain the GMO will be disposed of via the clinical waste stream, with destruction by autoclaving or high temperature incineration.

For trial participants or close contacts

- Participants will remain at the clinical trial site as an in-patient during the 5 days of dosing and then until 2 consecutive negative test results for viremia is achieved after each treatment cycle with the GMO.
- Trial participants will be required to take the following precautions:
  - avoid exposure to mosquitoes for 7 days after each treatment, including by staying at home as much as possible, wearing long sleeves and long pants and by applying mosquito repellent every 12 hours to prevent mosquito bites when outdoors.
  - 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 sexually active, use barrier contraception during and for 90 days after treatment
  - avoid contact with newborns, immunocompromised and severely immunodeficient individuals.
  - cover any bleeding cuts or wounds to prevent their blood from contacting with people or animals.
- Pregnant women will be excluded from the trial.
- Trial participants may not donate blood or organs during the trial.

- 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. Trial participants and family members will both receive training sessions covering the trial instructions.

2.3 Details of the proposed dealings

2.3.1 Overview of the clinical trial

27. VRT Pharmaceutics (the Applicant) is seeking authorisation to conduct the proposed clinical trial in Australia. If the licence is issued, 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. Two successive patient cohorts of 3-6 participants will receive up to 15 doses of the GMO during 3 treatment cycles of 28 days each. 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. The applicant proposes that patients who respond well to the treatment 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.

29. The GMO will be administered via intravenous (i.v.) infusion once a day, on days 1 to 5 of each treatment cycle. As the clinical trial's secondary aim is to identify the Maximum Tolerated Dose (MTD) of the GMO, up to two dose levels (1.0×10^9 and 3.0×10^9 cell culture infectious dose 50% (CCID50[5])) will be tested. The first patient cohort will receive the lowest dose with the higher dose only administered if dose limiting toxicity (DLT), such as significant hematologic toxicity or severe immunotoxicity, is not observed. The presence of DLTs will be assessed by a Safety Review Committee (ESAC) prior to escalating or de-escalating the dose of GMO to be administered.

30. 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

31. The Applicant has provided details of how they will undertake dealings within facilities at Royal Adelaide Hospital (RAH), Flinders Private Hospital, Austech Medical Laboratories (Austech) 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.

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

Organisation or facility	Proposed dealing(s)	Notes
Austech - Bankstown NSW 2200	Storage and distribution of imported GMO vials	Storage and sample analysis in certified PC2 facility Cert-4594

[5] CCID50 correspond to the number of viable virus particles sufficient to cause a cytopathic effect in 50% of inoculated cultured cells. This value cannot be directly converted to genome copies of the virus. However, as a comparison, the applicant stated that, for at least one of their experiments, 1 CCID50 corresponded to approximately 750 viral genome copies.

	Storage and analysis of biological samples (GMO-related testing)	
Royal Adelaide Hospital Level 1 Clinical Trials Pharmacy - Bedford Park SA	Short term storage Preparation of treatment doses from lyophilised GMO	Procedure to be conducted within pharmaceutical isolator
Treatment room Flinders Private Hospital - Bedford Park SA	Administration of prepared GMO to trial participants Collection of biological samples from trial participants while patients are in hospital	Standard clinical facilities accredited to the NSQHS Standard
Flinders Private Hospital pathology laboratory	Collection of biological samples from trial participants Analysis of biological samples (clinical monitoring)	Standard pathology laboratory.
Flinders Centre for Innovation in Cancer - Bedford Park SA	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

32. Prospective participants will be screened against an extensive list of selection criteria. Inclusion criteria relevant to assessment of risk include that trial participant 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;
- if female and capable of child-bearing potential, have negative serum pregnancy test results within seven days before the first administration of the GMO;
- if capable of reproduction (male or female), agree to use effective contraceptive measures for at least 90 days after the final GMO treatment[6]; and
- agree 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).

33. Exclusion criteria include that trial participants must not:

- have evidence of an active infection, or immunosuppressive disorder;
- have received systemic immunosuppressive or immunomodulatory drugs within 14 days prior to first administration of GMO;
- 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.

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

2.3.4 Manufacturing of the GMO

34. The GMO will be manufactured by Guangzhou Virotech in China under Current Good Manufacturing Practice (cGMP)-like conditions 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 quality and product release tests, including verification that no sequence mutations have been introduced during production.

35. Vials will be clearly labelled with the product name, specifications, titre, batch number, expiration date, and name of the Sponsor. Approximately 1,170 vials of the GMO will be imported into Australia.

2.3.5 Import, transport and storage of the GMO

36. The GMO will be imported into Australia by specialist courier companies such as World Courier. GMO vials will be packaged and labelled for transport in accordance with the packaging and labelling requirements of the IATA code UN 3373 (Biological Substance, Category B). Briefly, the GMO vials will be packaged into unbreakable secondary packaging and then into a rigid unbreakable outer box. The outer packaging will be labelled "biohazard" and "contains GMO". The shipment will be maintained at 5 ± 3°C with cooler pads and contain a temperature data logger.

37. Upon arrival in Australia, the shipments will be delivered to Austech where the GMO will be stored in a PC2 facility (Cert-4594). Staff will unpack and inspect the vials before storage, then repackage the GMOs for shipment to the RAH Pharmacy (or other clinical trial site). The GMO will be double contained within two sealed, unbreakable containers, and clearly labelled as containing GMOs. Storage and transport will be in accordance with the OGTR's *Guidelines for the transport, storage and disposal of GMOs.*

38. At the RAH Pharmacy, the GMO will be diluted and prepared into i.v. infusion bags as needed for each trial participant and immediately transported to the Southern Oncology Clinical Research Unit (SOCRU), and then to Flinders Private Hospital (the trial site). Prepared infusion bags being transported from the RAH Pharmacy to the trial site will be sealed within a plastic bag and packaged in a non-breakable plastic container. 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.

39. The GMO and biological samples collected from trial participants will be treated as containing GMOs. Samples will be stored within sealed unbreakable primary and secondary containers, labelled as containing GMOs and stored in a locked freezer with access restricted to authorised persons until transported to analytical facilities within the clinical trial site or to third party analytical facilities located within Australia. Storage and transport will be in accordance with the Regulator's *Guidelines for the Transport, Storage and Disposal of GMOs.*

40. Waste containing GMOs 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 2.3.9).

41. 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.

2.3.6 Preparation of the GMO

42. Each dose of the GMO will be prepared for inoculation at the RAH Pharmacy by pharmacy staff who have been trained in their institution's Standard Operating Procedures (SOPs) for handling GMOs and associated waste. The procedure will be carried out in a Negative Pressure Isolator located in the RAH Pharmacy. Staff preparing or handling samples or items contaminated with the GMO will wear

PPE, including gowns and gloves. Anyone with skin damage on their hands will be advised to wear double gloves.

43. Each GMO vial will be reconstituted to yield a concentration of 2 x 10^8 CCID50/ml. The required volume will be transferred to an infusion bag containing 250 mL of saline, with proposed doses of the GMO requiring a total of one, four or ten vials each. Final concentrations will range from 1.2 x 10^6-1.2 x 10^7 CCID50/ml.

44. 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.

2.3.7 Administration of the GMO and post-administration activities

45. The GMO will be administered in a treatment room at Flinders Private Hospital. Staff administering the GMO will wear PPE including gowns, gloves and safety glasses. The procedure will be carried out in 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.

46. Trial participants will undergo 3 treatment cycle of 28 days each. For each cycle, trial participants will receive daily doses of the GMO for 5 consecutive days. A clinical trial nurse will administer the GMO by i.v. 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 the diluted GMO solution will be attached to the catheter via a secure Luer-Lok connector, creating a closed system. The GMO will be infused 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.

47. 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.

48. For each treatment cycle, trial participants will remain at the clinical trial site as an in-patient, in a private treatment room, until all 5 daily doses of the GMO are administered and then until 2 consecutive negative tests results for the presence of the GMO in blood samples is achieved. Viraemia will be assessed using quantitative polymerase chain reaction (qPCR) with a limit of quantification (LOQ) of 6.25 genome copies/µl serum, equivalent to 3.13 copies/µl whole blood.

49. 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.

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

51. After being discharged from the trial site, the participants will be instructed to avoid exposure to mosquitoes (see Section 2.2, this Chapter).

2.3.8 Sample collection and analysis

52. Biological samples (blood, saliva, nasal discharge, urine and faeces) will be collected at multiple time points 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 sampling schedule is detailed in Table 2.

Table 2. Schedule of sample collection

Biological samples	**Days of sample collection**												
	Treatment cycle 1							**Treatment cycles 2 and 3**					
	1	**5**	**6**	**7**	**14**	**21**	**28**	**1**	**5**	**7**	**14**	**21**	**28**
Blood	X	X*	X	X	X	X	X		X*	X	X		X
Saliva	X		X		X	X	X	X					X
Nasal swabs	X		X		X	X	X	X					X
Urine	X		X		X	X	X	X					X
Faeces	X			X	X	X	X	X			X		X

* Blood samples will be collected at 0.5h, 2h, 4h and 12h after the GMO administration

53. 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 (Cert-4594).

54. 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.

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

56. All biological samples will be treated as containing GMOs.

2.3.9 Decontamination and disposal of the GMO

57. 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.

58. 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%(v/v) ethanol, 1000 mg/L effective chlorine disinfectant or 1-2%(v/v) bleach.

59. 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 biohazard waste bin for collection and incineration by external waste contractors. Disposal or destruction will be documented.

2.3.10 Relevant training and experience of clinical trial personnel

60. 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 (NHMRC, 2019). 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. 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.

2.3.11 Contingency plans

61. In the event of accidental occupational exposure (via needlestick injury, splash to mucous membranes or skin) the exposed area will immediately be washed with soap or detergent and rinsed under running water. The person will be offered prompt medical attention. The medical practitioner will be given all relevant information about the GMO. The exposed person will need to take precautions to protect themselves from mosquito bites for 48 h.

62. All areas where the GMO will be handled will be provided with spill kits, including 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.

63. 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.

64. All serious adverse events (SAEs) will be reported to the Principal Investigator within 24 h and to the Regulator as soon as reasonably possible.

2.3.12 Informing persons covered by the licence about licence conditions

65. The applicant stated that, if issued, a copy of the licence will be provided to all participating trial sites. Staff involved in handling the GMO during receipt and storage, preparation, and administration to participants will be trained in the licence conditions. All training will be documented and kept within the clinical trial files.

Section 3 Parent organism

66. The characteristics of the parent organism provide a baseline for comparing the potential for harm from dealings with the GMO. The GM treatment is derived from the GETV strain M1.

3.1 Classification and genome characteristics

67. GETV is an enveloped and spherical virus containing a positive-sense single-stranded RNA (+ssRNA) genome. It belongs to 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. At least 32 alphavirus species have been identified and are globally distributed. Examples of alphavirus species include *Chikungunya virus* (CHIKV), *Barmah Forest virus* (BFV), *Eastern equine encephalitis virus* (EEEV), *Getah virus* (GETV), *Ross River virus* (RRV) and *Venezuelan equine encephalitis virus* (VEEV) (Chen et al., 2018).

68. The +ssRNA genome of the GETV is about 11-12 kb in length with a 5’ methylated cap and a poly-A tail at the 3’ untranslated region (UTR). It is organised in 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 multiple viral structural proteins, including the capsid (C) protein and 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 suggested to occur during translation of the 6K gene (Li et al., 2017b; Gotte et al., 2018; Ren et al., 2020; Nowee et al., 2021).The main functional protein is the E2, involved in infecting host cells, causing disease and triggering host immune responses (Li et al., 2022).

3.2 Lifecycle

69. Infection starts with binding of the viral E2 glycoprotein of the GETV virion to a cell receptor. 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 (Schulte et al., 2016; Gotte et al., 2018; Rangel and Stapleford, 2021).

3.3 Emergence, distribution and disease

70. GETV is viewed as an emerging mosquito-borne virus (Li et al., 2022). 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., 1962). 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). Since first identified, strains of GETV have gradually spread from tropical to temperate regions, causing disease outbreaks in animals in many countries in Asia (Li et al., 2022).

71. 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 (Kamada et al., 1980; Sentsui and Kono, 1980; Bannai et al., 2015; Bannai et al., 2016). 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; Zhai et al., 2008; Li et al., 2017b). From 2002 onwards, however, many GETV strains have been collected from wild-caught mosquitoes in geographically dispersed locations throughout China (Wang et al., 2006; Zhai et al., 2008; Li et al., 2017a; Li et al., 2017b). Multiple disease outbreaks in farmed pigs occurred between 2011 and 2018, again distributed across many provinces (Yang et al., 2018; Zhou et al., 2018; Lu et al., 2019; Xing et al., 2020). 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

72. According to recent analysis of available genome sequences and literature data, there are at least 169 strains of GETV (Li et al., 2022). Based on the phylogenetic analysis of region E2, GETV strains are classified 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 the 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 cold climates (Li et al., 2017a).

73. The majority of GETV isolates, including the parent organism M1 strain, 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 and animals such as pigs, horses, cattle and foxes, and has become the dominant group of circulating viruses (Ren et al., 2020; Xing et al., 2020). Over the past 20 years, all viruses causing animal diseases are group III isolates (Li et al., 2022).

3.5 Transmission

74. GETV is known to be transmitted by mosquitoes. However, transmission from a viraemic host to another is suggested to occur. 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).

75. Based on viraemia levels, both pigs and horses may act as amplifying hosts (Kumanomido et al., 1988b; 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.

76. GETV dissemination across large geographic distances suggests spread via long distance via transport or migration of hosts and/or vectors. 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). The high similarity of genome sequences of GIV strains isolated in China and Russia raised the possibility that migratory birds could disperse GETV. However, this hypothesis is yet to be proved (Sam et al., 2022).

3.5.1 Mosquito-vectored transmission

77. As an arbovirus, GETV is primarily transmitted by mosquitoes. The mosquito becomes infected by ingesting virus particles during a blood meal on a viraemic host. To be successfully transmitted to other hosts, a virus must survive mosquito ingestion, replicate and infect the mosquito's salivary glands. When an infected mosquito feeds on another animal or person, the virus present in its saliva is inoculated into the animal or person. Once infected, a mosquito remains infected for life and is able to transmit virus at each blood meal (Lim et al., 2018).

3.5.2 Direct inoculation

78. 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 – 2 x 10^6 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 3.7.1).

79. 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 in 2017, and 100% of the vaccinated sows proved seropositive for GETV (Zhou et al., 2020).

80. 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

81. 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 3.7.3 and 3.7.5).

3.5.4 Vertical transmission via milk

82. 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

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

84. 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).

85. 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 92 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 Invertebrate vectors

86. A wide variety of mosquito species are susceptible to GETV infection. GETV has been isolated from at least ten 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*[7] and two *Aedes* species[8], as well as from mixed populations within these genera (Matsuyama et al., 1960; Scherer et al., 1962; Scherer, 1984; Takashima and Hashimoto, 1985; Shirako and Yamaguchi, 2000; Wang et al., 2006; Zhai et al., 2008; Feng et al., 2012; Li et al., 2012; Zhou et al., 2012; Li et al., 2017b; Liu et al., 2019). Since 2005, additional species, including *Armigeres subalbatus, Armigeres obturbans*, *Anopheles sinensis and Cx. pipiens pallens*, have been found to carry GETV. These are widely distributed in China and have contributed to its recent spread (Zhai et al., 2008; Li et al., 2017b; Liu et al., 2019; Lu et al., 2020; Fang et al., 2021). 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).

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

Infective dose and transmission rate in mosquitoes

88. The infection of mosquitoes and transmission of GETV varies depending on the viral strain, number of viral particles ingested and the species of the mosquito. Laboratory studies showed that GETV strain MM2021 (isolated from *Cx. gelidus*) can infect *Ae. vexans* at 20-2,000 plaque-forming unit (PFU)/µl of blood with a transmission rate of 40-60%, while for *Cx. tritaeniorhynchus* comparable infection and transmission were only observed with an infection dose of 20,000 PFU/µl (Takashima and Hashimoto, 1985). Infection of mosquitoes with 1,000 PFU/µl of the GETV strain 12IH26 (isolated from *Cx. tritaeniorhynchus)* resulted in the infection of 74-93% of the mosquitoes and a transmission rate of ≥90% in *Cx. tritaeniorhynchus and An. stephensi,* and 36% in *Ae. albopictus* (Azerigyik et al., 2023). The infection rate indicates the ability of the virus to infect the midgut of a mosquito after the exposure to infected blood and replicate, while the transmission rate shows the virus ability to disseminate from the midgut to salivary glands allowing its transmission via saliva to a vertebrate host. The data discussed above and summarised in Table 3 suggests that although GETV can efficiently infect and replicate in a range of mosquitoes, the transmission of the virus depends on the GETV strain and mosquito species.

Table 3. Summary of GETV infection and transmission rates.

GETV strain	Mosquito species	Dose of infection (PFU[9]/µl)	Days post infection	Infection rate* (%)	Transmission rate** (%)
MM2021	*Ae. vexans*	~20-2,000	20	60-100	40-60
	Cx. tritaeniorhynchus	~125-20,000		11-94	0-53
12IH26	*Cx. tritaeniorhynchus*	1,000	15	74	90
	An. stephensi			74	96
	Ae. albopictus			93	36

Data from Azerigyik et al. (2023) and Takashima and Hashimoto (1985)
*n° of virus-positive mosquitoes/total n° of mosquitoes tested;
**n° of mosquitoes showing virus-positive saliva samples/total n° of mosquitoes tested

[7] *Cx. gelidus, Cx. tritaeniorhynchus, Cx. pseudovishnui, Cx. fuscocephala* and *Cx. annulus*

[8] *Ae. albopictus* and *Ae. vexans*

[9] To facilitate the comparison between data from different sources, further references to PFU in this RARMP will use genome copies/µL.

Capacity for geographic dispersal

89. As discussed in Section 3.5 of this Chapter, GEVT are manly transmitted via mosquito bites. GETV strains infect and replicate in many mosquito species with different dispersal capacity. Overall, mosquitoes have an average flight range between 25 m and 6 km; during migration this distance can vary between 50 m and 50 km (maximum flight range). Mosquitoes of the genus *Aedes* show the shortest flying distance a day (~55 m), followed by some species belonging to the genus *Cullex* (~920 m) and *Anopheles* (~1,000 m). *Cx. annulirostris* mosquitoes fly on average 2,200 m a day (Verdonschot, 2014). As mentioned in Section 3.5 of this Chapter, the isolation of GETV in distant locations suggests that spread via transport or migration of hosts and/or vectors may occur.

3.7 Vertebrate hosts and pathogenicity associated with GETV infection

90. Serological evidence suggests that GETV has a broad host range in nature and infects pigs and wild boar (Li et al., 1992; Sugiyama et al., 2009; Kuwata et al., 2018; Li et al., 2019), 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 higher (Li et al., 2019).GETV has also caused disease in foxes (Shi et al., 2019), and is used experimentally to infect mice, hamsters, guinea pigs and rabbits (Kumanomido et al., 1988a; Asai et al., 1991; Wang et al., 2021).

91. Humans are incidental or dead-end hosts to GETV and are unlikely to develop a level of viraemia sufficient to infect mosquitoes. Although GETV antibodies have been observed in individuals living in affected regions, GETV infections in humans are mostly subclinical (Li et al., 2022). Clinical disease associated with GETV infection in animals is discussed below.

3.7.1 Horses

92. 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). In a recent GETV outbreak in China, an infected horse developed a fever (body temperature ≥ 38.9 °C) that lasted for approximately 11 days. The highest temperature observed was 40.5 °C. No other clinical symptoms were observed (Lu et al., 2019).

93. An experimental dose-response study ranging from $10^{1.3}$ – $10^{6.3}$ CCID50 of the GETV strain MI-110 showed that specific symptoms were dose-related – the rash developed in horses receiving ≤ $10^{4.3}$; the submandibular lymph node was swollen only at ≥ 10^{5}. Affected horses appeared normal, without loss of appetite or respiratory signs. Most recovered within a week, and those with secondary complications recovered within 14 days (Kamada et al., 1991b). In documented outbreaks, all animals have made full recoveries without any ongoing complications (Fukunaga et al., 1981; Fukunaga et al., 2000).

94. While disease caused by GETV infection is not severe in horses, extensive outbreaks have caused significant disruption and economic loss. GETV poses a potential threat to the racehorse industry in affected countries (Timoney, 2017; Lu et al., 2019). As noted in Section 3.8 below, GETV infection is a notifiable equine disease in Australia.

3.7.2 Cattle

95. GETV infection of beef cattle in forest grazing areas and presenting with sudden onset of fever was documented in 2018 (Liu et al., 2019). 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 3.7.1, this Chapter 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.

3.7.3 Pigs

96. GETV has been isolated from healthy adult pigs (Matsuyama et al., 1967; Kumanomido et al., 1982) 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. Approximately 200 surviving piglets developed a rapidly fatal disease characterised by fever, tremors and uncoordinated movement, depression and diarrhoea and died five to ten days after birth. The GETV HuN1 strain was isolated from the cerebrum of an infected piglet. Histopathological changes were evident in the brain, lungs, kidneys, liver and spleen (Yang et al., 2018). GETV was broadly distributed, with virus and viral RNA isolated from multiple tissues of infected piglets (Yago et al., 1987; Yang et al., 2018). Foetal death was due to viral infection of the foetus, transmitted across the 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).

97. The infection of 5 (n=8) and 18-day (n=1) old piglets with $10^{5.5}$ genome copies of the Kanagawa strain of GETV resulted in the development of moderate to severe disease 20 h after inoculation. Symptoms included anorexia, depression, tremor, discoloration of the skin and incoordination of hindlimbs. Animals infected via the intramuscular route died within 5 days after infection, while 2 out of 3 piglets receiving the virus via oronasal route survived. The virus was recovered from all organs of dead animals, except for the heart, pancreas and small intestine of the 18 days old piglet (Kawamura et al., 1987). A study conducted with doses ranging from $10^{3.7}$-$10^{4.3}$ of the GETV strain 2078 resulted in viraemia but failed to induce clinical symptom in 9-days old piglets (Izumida et al., 1988)

98. The studies discussed above suggest that the ability to cause disease in young piglets may depend on the GETV strain and/or dose administered. In addition, reported GETV outbreaks have not been associated with disease in older pigs. Experimental infection of pigs which were five months or older and pregnant sows showed that all developed viraemia and antibodies to GETV, but no fever or other clinical signs occurred (Kawamura et al., 1987; Izumida et al., 1988).

3.7.4 Blue foxes

99. 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 displayed neurological symptoms and died on the third day of illness (Shi et al., 2019).

3.7.5 Small mammals

100. 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 1 x $10^{4\text{-}4.5}$ CCID50 of the V1 or Kanagawa strains died with paralysis 3-4 days post inoculation. Three- to four-day old mice infected intracranially or by oronasal exposure displayed impaired hind limb mobility at 4 days post inoculation, paralysis at 8 days post inoculation 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 (Yago et al., 1987; Wang et al., 2021).

101. 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 possibly with the viral strain (Kumanomido et al., 1988a; Wang et al., 2021).

102. Adult Syrian hamsters, guinea pigs and Japanese white rabbits have been experimentally infected with 1 x $10^{5.5\text{-}7.5}$ CCID50 of the GETV strain Sakura 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. The virus was observed sporadically in guinea pigs and rabbits and did not clearly associate with stage of gestation (Asai et al., 1991).

3.8 Duration of viraemia in susceptible animals

103. An experimental dose-response study in horses (Section 3.7.1) showed that the duration and magnitude of viraemia were both dependent on viral inoculum. Horses were infected by intramuscular injection and viraemia assessed by a virological assay. Viraemia was detected only in horses receiving 2 x $10^{3.3}$ CCID50 or higher, commencing on the first or second day after inoculation and lasting 4-5 days. Viraemia levels ranged from 1.6 to 158 CCID50/µL of serum (Kamada et al., 1991b).

104. Experimentally infected commercial pigs and piglets developed more transient viraemia. Virus was recovered from blood plasma samples at 1-2 days post inoculation (Kumanomido et al., 1988b), 1-2, 1-3 and 5-6 days post inoculation (Izumida et al., 1988). Although sample sizes were small, higher circulating virus levels were seen after i.v. infection with 1 x $10^{4.5}$ CCID50 than with intramuscular inoculation of 1 x 10^{6} CCID50 of GETV strain MIP-99 (Kumanomido et al., 1988b).

3.9 Mutation and recombination

105. Mutation is an important source of genetic variation in viruses. As with most RNA viruses, alphaviruses generate approximately 1 error per 10^{4} nucleotides copied (Drake and Holland, 1999). Viral evolution by mutation or self-recombination events may occur over time and usually involve repeated viral transmission to other hosts. However, the observed rate of divergence in nature is low. For example, Ross River virus (RRV) isolated at the beginning and end of an 11 month epidemic differed at only one nucleotide of 1600 base pair sequenced (reviewed in Strauss and Strauss, 1994). GETV has the capacity to adapt to different growth environments as *in vivo* pathogenicity is attenuated by serial passage in tissue culture. As these viruses cycle through both vertebrate and invertebrate hosts these adaptative mutations can increase or decrease the fitness of the virus in different hosts (Anishchenko et al., 2006; Rozen-Gagnon et al., 2014). 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, horses inoculated with virus after ten serial passages developed neither disease symptoms nor viraemia, although they still produced neutralising antibodies. Sequence variation was not investigated, but this suggests sufficient mutation capacity to enable phenotypic change over this period (Kamada, 1981).

106. Homologous recombination between viral strains requires both viruses to be present and replicating within the same infected host cell. Recombination of alphaviruses have been experimentally demonstrated. The recombination of Sindbis virus RNA in vitro has been documented in transfected cells (Weiss and Schlesinger, 1991). The co-administration of a plasmid encoding an attenuated salmonid alphavirus (SAV) deleted of the 6K-gene (a protein required for replication and production of infectious virus) and a plasmid encoding only the virus structural proteins and the 6K gene resulted in recombinant SAVs capable of causing disease in Atlantic salmon (Petterson et al., 2016). There are limited data regarding the recombination between alphaviruses in nature. The Western equine encephalitis virus (WEEV), is believed to have originated from the recombination between the Eastern equine encephalitis (EEEV) and a Sindbis -like virus (Hahn et al., 1988). However, RRRV and BFV have been found circulating in mosquitoes in the same geographic area (Kizu et al., 2019), with corresponding potential for vertebrate hosts to be coinfected. Analysis of thirty field isolated BFV genomes did not reveal any evidence of recent or historical recombination between these viruses (Michie et al., 2020b).

3.10 GETV in Australia

107. GETV was reportedly isolated from *A. amicus* and *Cx. 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., 2020b). No other GETV isolates have been reported, and GETV was described as exotic to Australia (Hodgson, 2002).

108. '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). There are no published data examining the consequences of GETV infection for Australian native animals.

109. The Australian Veterinary Emergency Plan (AUSVETPLAN) contains the nationally-agreed approach for responding to emergency animal disease incidents in Australia. Australia's policy for managing GETV disease notes that the virus could enter Australia through an introduced mosquito, a natural host or an infected animal. If the virus enters through the mosquito vector, there can be no effective response. If it enters via the import of an infected animal, the policy is to eradicate the disease using a combination of vector abatement, quarantine and movement controls, serological testing, tracing and surveillance to determine the source and extent of infection; and an awareness campaign to encourage cooperation of industry and the community (Animal Health Australia, 2018b).

110. GETV disease is currently included as a Category 4[11] disease in the Emergency Animal Disease (EAD) Response Agreement (EADRA), as an outbreak of the disease could cause production losses in the affected livestock industries. The EADRA is a legally binding agreement between government and livestock industries to cover management and funding of responses to EAD incidents. In this case, the costs of disease control would be shared 20% by governments and 80% by the relevant industries (Animal Health Australia, 2022).

3.11 GETV strain M1

111. 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 (IFN)-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; Zhang et al., 2020; Goncalves-Carneiro et al., 2021). M1 kills cancer cells by several mechanisms, including inducing endoplasmic reticulum stress-mediated apoptosis or necroptosis (Lin et al., 2014; Zhang et al., 2021).

112. When initially characterised, M1 was found to cause illness and death in newborn mice inoculated via 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 3.7.5).

[10] As of April 2019.

[11] The EADRA categorises listed diseases as 1-4, with category 1 including EADs that predominantly seriously affect human health and/or the environment but may only have minimal direct consequences to the livestock industries (e.g. rabies, Australian bat lyssavirus) and category 4 including diseases that cause mainly production losses in the affected livestock industries.

113. The strain was shown to replicate in *Ae. albopictus* and *Cx. quinquefasciatus.* 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.

114. 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.

115. A neuro adapted strain of the M1 virus, generated by serial passage of the M1 strain in the brains of suckling and weaning mice was sequenced in 2007 (Wen et al., 2007). This strain was later shown to induce apoptosis in cancer cells (Hu et al., 2009).

3.11.1 The virus used in this study

116. The applicant stated that the M1 strain was cultured for research purposes in three different institutes, resulting in three M1 strain variants. The parent organism used to construct the GMO was obtained following cell culture of one of the three M1 strain variants described above and is known as M1-c6 strain. The M1-c6 strain was selected due to its stable biological properties, virus replication and oncolytic effects. 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; Li et al., 2022). The applicant stated that the sequence of their virus strain and the M1 strain sequenced in 2007 have a homology of >99.8%. The pathogenicity of the wild type (WT) M1[12] and M1-c6 has been evaluated in animal models.

Pathogenicity studies in animals provided by the applicant

117. The applicant has provided data regarding the pathogenicity of the WT M1 strain in adult horses, adult cattle and in 18 days old piglets. Groups of 6 animals of each species were injected with 1-2 x 10^6 CCID50 of the viral strain via intramuscular (i.m) route and monitored for 28 days. Viraemia was assessed on days 1, 2, 5, 14, 21 and 28 in samples collected from horses and cattle, and on days 1, 2, 14 and 28 in serum samples from piglets. One horse, one cow and two piglets showed a transient increase in body temperature, returning to normal range within a day. One out of 6 horses had an increased body temperature[13] (38.9-39.3 °C) from day 2 to day 8, returning to normal on day 9, but showed no viraemia during this period. Another horse showed viraemia at days 14 and 21 after inoculation. No significant clinical symptoms, changes to body weight or haematological disorders were observed. Viraemia was also detected in 4/6 piglets at day 2 but was below the detectable level at days 14 and 28.

118. Although no clinical symptoms have been observed, the slight increase in the body temperature of 1/6 horses injected with the WT M1 strain is above the fever threshold (≥ 38.9 °C) previously observed during a GETV infection (Lu et al., 2019). In addition, the viraemia levels observed in one of the horses suggests that the WT M1 strain was capable of replicating. Given that Sanhe horses have a blood volume of approximately 28L (8% of body weight), the total virus level in the animal was far

[12] In this RARMP, the M1 strain variant cultured by the applicant's research Institute is called wild type (WT) M1 strain.

[13] Normal temperature in horses is 37-38.5 °C (NSW Department of Primary Industries, 2023), increased temperature is considered as ≥38.6 °C.

higher than the initial dose inoculated. However, as viraemia was not assessed between days 6-13 or 21-28, the duration of the viraemia and the maximum viral level observed cannot be determined.

119. In piglets, the administration of WT M1 failed to cause clinical symptoms. However, based on the viraemia levels detected at day 2, the total viral load in a piglet suggests viral replication. As virus level in serum was not assessed between days 2 and 14, the duration of the viraemia cannot be determined. As discussed in Section 3.7.3 of this Chapter, GETV infection can cause foetal/neonatal death and reproductive disorders in pregnant sows. The pathogenicity of the virus in piglets is highly dependent on their age. The data provided by the applicant shows the effects of WT M1 strain inoculation in 18-days old piglets, while the virus did not cause disease in 18-day old piglets, it is still unclear whether the WT M1 strain would cause disease in younger piglets or disorders in foetus of pregnant sows.

3.12 Environmental stability and inactivation

120. No specific information is available regarding disinfection of GETV but data available to other alphaviruses show sensitivity to chemicals such as 70% ethanol, sodium hypochlorite and quaternary ammonium compounds. They can also be inactivated by desiccation and temperatures above 58°C (Public Health Agency of Canada, 2010, 2011).

3.13 Prevention and treatment

121. 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).

122. 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 (Mair and Timoney, 2009; Bannai et al., 2015; Nemoto et al., 2015; Bannai et al., 2016). The vaccine is based on a GETV strain MI-110 isolated in Japan in 1978 and may not completely protect against currently circulating strains (Lu et al., 2020; Li et al., 2022).

3.14 Risk group classification of GETV

123. 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).

124. The criteria listed in the Australian Standard 2243.3:2022 Safety in Laboratories Part 3: Microbiological safety and containment (Standards Australia/New Zealand, 2022) are similarly consistent with classification as a Risk Group 2 microorganism, 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

125. The GMO is based on the GETV strain M1 that has been modified to enhance its ability to replicate in cancer cells. The final product proposed to be evaluated in this clinical trial is composed of two variants of the GMO: i) the original construct of the GMO as described in paragraph 127; and ii) a variant containing 2 adaptative single-point mutations that occurred during the manufacturing process. The applicant stated that this adaptative variant has similar levels of viral replication, and virulence to the GMO.

126. Some information about the genetic modifications and details of the two single adaptative point mutations have been declared as Confidential Commercial Information (CCI) under Section 185 of the Act. Under Section 187 of the Act, this information must not be disclosed except where it is made available to the Commonwealth or a Commonwealth Authority, a state agency or the Gene Technology Technical Advisory Committee in the course of carrying out their duties or functions under the Act or under a corresponding State law.

4.1 The genetic modifications and their potential effects

127. The GMO was produced by introducing two single-point mutations into the M1-c6 genome sequence by site-directed mutagenesis. The GM viral RNA was produced using *in vitro* transcription and used to transfect Vero cells. The primary virus seed was recovered from the supernatant of the cell culture and used to propagate the GMO in cell culture.

128. The two single nucleotide substitutions introduced in the GMO were identified during *in vitro* serial passage experiments with separate vials of the M1 strain, during which a genetic variant showed an enhanced cytopathic effect on cultured tumour cells. The GMO is replication competent and is not attenuated by the genetic modifications.

129. The applicant demonstrated that the GMO displays improved oncolytic activity compared with its M1-c6 strain. 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.

130. The applicant provided *in vitro* data showing a moderate reduction in the IFN-mediated antiviral response in cancer cells infected with M1-c6 genetically modified with mutation 1 alone ($M1^{mut\,1}$), compared with the response to M1-c6 strain. 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-c6.

4.2 Stability of the GMO during *in vitro* passage

131. 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 structural proteins. 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.

132. Following manufacturing of the GMO, two adaptative single-point mutations were identified in viral samples isolated from the final product (see paragraph 125). The applicant stated that the stability of the adaptative mutations was further evaluated during 5 consecutive serial passages in cancer and mosquito cell lines with no additional mutations observed. Details of these two adaptative single point mutations have been declared as CCI under Section 185 of the Act.

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

4.3 Pre-clinical and human toxicity studies

134. The GMO has been administered to 27 cancer patients, including 13 patients with advanced/metastatic hepatocellular carcinoma (HCC) and 14 patients with different solid tumours

under an ongoing investigator-initiated trial (IIT) or compassionate use access in China. The applicant stated that a total of 41 adverse events (AEs) have been reported in 5 subjects in the ongoing IIT study. Reported AEs included fever and transient, asymptomatic lymphopenia and were assessed as mild, self-limiting and consistent with those observed with other oncolytic viruses. No serious adverse events (SAEs) were reported and no subject has withdrawn from the study because of AEs.

4.4 Pathogenicity studies in animals

135. 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. The effects of the GMO in animals susceptible to GETV infection are discusses below.

Horses

136. The pathogenicity of the GMO was evaluated in horses following i.m. or i.v. administration. Intramuscular administration of 2 x 10^6 CCID50 of the GMO to male and female horses failed to induce clinical symptoms or changes in the body temperature. Viraemia was not detected at or below of the LOQ (<12.5 genome copies/ µL) in all time points evaluated (days 1, 2, 5, 14, 21 and 28) post-inoculation.

137. Horses receiving 2 x 10^8 CCID50 of the GMO via i.v showed a passive viraemia 30 min post-inoculation. The viraemia levels decreased over time to below the LOQ from day 3 to day 28. Viable viral particles ranged from 86.60 to 192.93 CCDI50/mL of serum collected at 30 min, 4, 12 or 24 h post-inoculation and were not detected after day 2. No clinical symptoms or changes in the animal's body temperature were observed at any time point.

Piglets

Intramuscular administration to 7-day old piglets

138. The i.m. administration of 1 x 10^6 CCID50 of the GMO to 7-day old piglets resulted in viraemia in 2/6 piglets at day 4 post-inoculation. Viraemia was below the LOQ at day 10. No diarrhea, fatigue, abnormal breathing, limb tremors, body reddening, or reaction at the administration sites were observed in any of the tested groups. No significant changes in body temperature[14] were observed from day 1 to day 10 post-inoculation. In this experiment, the HuN1 strain isolated during an outbreak of GETV infection in pregnant sows and neonatal piglets in China in 2017 (Yang et al., 2018) was included as a control. Five out of six piglets receiving 1 x 10^6 CCID50 of the HuN1 strain showed viraemia at day 4 post-inoculation, viraemia levels decreasing overtime. GETV HuN1 strain induced a slight increase in the body temperature (40.1-40.2 °C) in 2/6 piglets from day 1 to day 3 post-inoculation. No clinical symptoms were observed in HuN1-inoculated piglets throughout the 10 days of the study. It is unclear whether the HuN1 strain lost its pathogenicity or does not cause disease in 7-day old piglets. The applicant stated that since the isolation of the GETV HuN1 strain, researchers have been trying to reproduce the virus pathogenicity displayed in the original outbreak in experimentally infected animals without success.

Intramuscular administration to 18-day old piglets

139. Two out of 6, 18-day old piglets receiving 1 x 10^6 CCID50 of the GMO via i.m. injection showed slight increased body temperature (40.2-40.3 °C) at day 3 post inoculation. As a similar effect was

[14] Pigs have a normal temperature range of 38-40 °C (NSW Department of Primary Industries, 2006), increased temperature was considered as >40.1 °C.

observed in one animal receiving placebo, it is therefore unclear whether the changes in the body temperature was related to the GMO. Viraemia was not detected in any of the animals.

140. In a second experiment, when administered with a similar dose, viraemia was detected in 1/12 piglets at day 2. Body temperature ranged from 38.6-40.1 °C in most of the animals receiving the GMO and from 38.6-39.9 °C in animals from the control group. Two out of twelve animals showed a transient increase in body temperature ranging from 40.2-40.7 °C at weeks 3 and 4 after administration that returned to normal range withing one or two days; viraemia was not detected during this period.

141. No clinical symptoms were observed in any of the tested groups. Neutralizing antibodies varied depending on the experiment and were observed in 10/18 piglets receiving the GMO.

Intravenous administration to 18-day old piglets

142. The i.v. administration of 1.0×10^8 CCID50 to 18-day old piglets resulted in a viraemia peak at 12 h post-inoculation. The viraemia gradually decreased and remained below the LOQ from day 4 until the end of the experiment on day 28. The amount of viable viral particles varied depending on the animals and time-point evaluated, with the highest number of viable particles (2.4×10^4 genome copies/uL) observed at the 12 h post-inoculation time-point. No viable viral particles were observed from day 2 to day 4. No clinical symptoms were observed.

Cattle

143. The i.m. administration of 1×10^6 CCID50 the GMO to cattle induced increased body temperature[15] in 2/6 animals. Animal body temperature ranged from 39.3-40.1 °C on days 4 and 6 and returned to normal on day 7 after administration. Similar effects were observed in cattle receiving the WT M1 strain, with one animal showing a temperature increase to 39.2 °C on day 3, and one showing temperatures of 39.5-40.2 °C between days 3-4 after administration. Cattle receiving placebo showed a temperature range of 37.8-39.1 °C. Viraemia was not detected at or below of the LOQ (<12.5 genome copies/ µL) in all time points evaluated. Neutralizing antibodies were positive in all animals receiving the GMO or the WT M1 strain.

In summary, the data provided by the applicant suggest that the GMO do not cause disease in horses and/or cattle, which are animals susceptible to GETV infection. Although some infected animals experienced a relatively small and transient increase in body temperature, viraemia was not detected or was below the LOQ at all time point tested.

The pathogenicity of the GMO in piglets is unclear. Although the animals did not develop clinical symptoms, the viraemia data provided suggest that the GMO is capable of replicating but some uncertainty remains in the persistence of viremia in piglets between day 2 and 4. As discussed in Section 3.7.3 of this Chapter, GETV was shown to be pathogenic to foetal and neonatal piglets but asymptomatic in older piglets or adult pigs. The data provided by the applicant suggests that the GMO has lower ability to replicate in piglets than its parent organism. However, it is not possible to draw a conclusion as to whether the GMO and the WT M1 strain are capable of replicating but do not cause disease because of their attenuation, or if they cannot cause disease in 7 or 18-day old piglets.

[15] Body temperature in cattle ranges from 36.7 to 39.1 °C (Reviewed in Jacob R.H., 2004), increased body temperature was considered as > 39.1 °C.

4.5 Biodistribution and shedding

4.5.1 Biodistribution and shedding in animals without tumours

Single dose, i.m. administration

144. Blood distribution and shedding were evaluated in horses and piglets following a single i.m administration of the GMO. One out of six piglets showed 1.07 x 10^2 genome copies/uL of the GMO in serum collected on day 2. The GMO was not detected in serum of horses or cattle. Shedding studies failed to detect the GMO in anal and nasal swabs or saliva samples collected from horses at days 1, 2, 5, 14 and 28 and in nasal and anal swabs from piglets at days 2, 14 and 28.

Repeated doses, i.v. administration

145. Sprague Dawley rats and Cynomolgus macaques received two (rats) or three (macaques) different doses of the GMO daily for five consecutive days constituting a treatment cycle. This cycle was repeated three times, each cycle separated by 14 days. The biodistribution of the GMO, and its clearance from blood and shedding into urine, faeces and nasal secretions (macaques only) were assessed at different times.

- In both species, the serum concentration of the GMO 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 after the first dose than after the final dose.
- In rats, viral shedding into faeces was dose dependent, detectable throughout the treatment period and decreased over time.
- 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 2.43 x 10^2 -17 x 10^3 genome copies/µg of RNA sample and did not differ substantially between treatment groups and the animal species treated.

146. After the final treatment cycle, 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.

147. The administration of the GMO to animals without tumours suggests that shedding in faeces, urine and nasal secretion depends on the route of administration and is more likely to occur when the GMO is administered via i.v. infusion.

4.5.2 Biodistribution and shedding in mice with tumours

Single dose – i.v. administration to immunodeficient mice

148. Tumour-bearing Balb/c nude mice were treated with 6.75 x 10^9 CCID50 of the GMO. 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 (3-3.6 x 10^8 genome copies/µg of RNA) between 24-72 hours then declining steadily thereafter. Viral RNA decreased over time, but average virus concentration ranged from 1 x 10^3-1.5 x 10^4 genome copies/ug RNA on day 28, the last day of assessment.
- Viral RNA was maximal in serum at the initial 2 h time point (~1.8 x 10^4 genome copies/µL), declined logarithmically over the next seven days. Only 1/3 mice showed 9.8 x 10^3 genome copies/µL of serum at day 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.
- Shedding of viral RNA was observed in faeces, peaking from 12-48 hours after treatment and decreasing after day 7. The presence of viral RNA in urine was not assessed.

Repeated doses – i.v. administration to immunocompetent mice

149. Tumour-bearing C57 BL/6J mice received 1 x 10^7 CCID50 of the GMO via i.v. infusion once a day for 5 days. Blood samples were collected 2 h after each administration of the GMO (days 1-5), and then daily from day 6-28.

- Viral RNA in tumours was detected starting at 2 h after the first administration, peaked at day 4 (1.09 × 10^5 genome copies/µg of RNA) and gradually decreased. One out of 3 mice showed 1 x 10^2 genome copies/µg of RNA on day 15. Virus RNA were not detected or below the detection limit in tumours between days 16 and 28.
- In serum, viral RNA levels of 3.15 x 10^3 genome copies/µL were observed after 2 hours of the first administration of the GMO. Viraemia peaked at day 3 (2.92 × 10^4 genome copies/µL) and gradually decreased until it was not detected on day 10. Viraemia remained below the detection limit from day 11-28, suggesting that there is no influx of the GMO from the tumours into the bloodstream. Viable viral particles (viral titre) ranged from 1.2– 9.78 x 10^3 CCID50 (2 h after the first and third dose) and gradually decrease with 1/3 mice showing 3.31 x 10^2 CCDI50 of the GMO 2 hours after receiving the fifth dose. The presence of viable viral particles in serum was not detected on samples collected on days 6-9 and not tested afterwards.

In summary, *biodistribution and shedding studies in animals suggest the GMO is cleared from the blood stream within days following administration. The GMO reaches the tumour within hours following administration and can persist for longer in tumours of immunocompromised mice without resulting in active viraemia. Administration of the GMO via i.v. infusion may result in shedding in faeces, urine and nasal secretion.*

4.5.3 Biodistribution in human cancer patients treated with the GMO

150. Persistence in the bloodstream and shedding via body fluids (saliva, urine and nasal secretions) and faeces have been assessed in cancer patients receiving either their first treatment cycle or a second or later treatment cycle. Administration of the GMO resulted in neutralising antibodies in around half of patients within 30 days of initiating GMO treatment. It is expected these antibodies will reduce viraemia in trial participants and improve clearance of the GMO in subsequent treatment cycles.

Duration of passive viraemia following two individual doses of the GMO

151. Three patients with triple negative breast cancer received two i.v. doses of 3 × 10^9 CCID50 of the GMO with 14 days between each dose. Viraemia was assessed at 30 min, 4 h, and days 1-3, 6, 9 and 12 after administration. Viral RNA was detected in serum collected 30 min and 4 h after the first dose. Following administration of the second dose, viral RNA was only observed at the 30 min timepoint. Viraemia was below LOQ in samples collected at 24 h post the first or second dose or later.

Duration of passive viraemia following the first treatment cycle with multiple consecutive doses of the GMO

152. Data were obtained from eight hepatocellular carcinoma patients treated under a compassionate use access. Patients received i.v doses of 1 x 10^9 CCID50 of the GMO, once a day for 5 consecutive days. GMO levels were measured over 24 hours after the final (5^{th}) dose of the treatment cycle, then on days 14 and 28. Viral RNA was detectable in 5 out of 8 patients 30 min after the last dosing in treatment cycle 1. RNA levels were highly variable between individuals (range 3.04 x 10^1– 9.18 x 10^3 genome copies/µl); the reason for this, given patients were inoculated with the same quantity of GMO, is not known. A single patient showed detected levels of viraemia at 2 h after the last dosing. At 24 hours, viral RNA was below the detection limit in all eight patients. No viraemia was detected at days 14 and 28 in any patient, including the patient with previous viremia at 2 hours after administration.

Duration of passive viraemia following the second and subsequent treatment cycles with multiple consecutive doses of the GMO

153. Seven of the same group of hepatocellular carcinoma patients were monitored for viraemia after the second treatment cycle. Viral RNA was detected in five patients (range 2.77 x 10^1-1.59 x 10^3 genome copies/µl of serum) but only at 30 minutes after dosing. Viral levels were thus lower and persisted for a shorter duration than following cycle 1. As observed after the first cycle, variation between individuals was high. Three patients exhibited passive viraemia after both cycles, two patients returned low serum RNA levels after cycle 1 but below the detectable levels after cycle 2, while two patients experienced a brief viraemia after the second cycle but not the first.

154. Earlier compassionate-use data collected between 2018-2021 provides information about later treatment cycles. The GMO was administered to six patients with a range of solid tumours either as a monotherapy or in combination with other anti-cancer drugs. Doses ranged from 2.6 × 10^8 to 4.0 × 10^9 CCID50, covering the range of doses proposed for this trial (1.0 × 10^9 CCID50 and 3.0 × 10^9 CCID50). Patients received as many as 16 treatment cycles, each comprising 5-6 daily treatments with the GMO followed by an interval of 21-114 days before starting the next cycle. As the patients were not part of a formal clinical trial, samples were collected when convenient and data collated from a number of cycles. As treatments and cycles followed on from one another, samples collected before each administration would detect any 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 5.31 x 10^3 genome copies/µl.
- There was no clear relationship between the RNA level and time after administration. Where multiple samples were collected at a given time point, from the same or from different patients, 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) and came from only two patients. Given RNA detection was inconsistent at earlier time points and so few late time points were assessed, these data do not give certainty as to the maximum time the GMO remains in the circulation.

Measurement of active viraemia

155. Data relevant to active viraemia that may develop secondary to GMO replication in and release from tumours were collected from seven of the eight HCC patients discussed above. Study inclusion criteria required that these patients have primary tumours of at least 1 cm (longest diameter). The five

patients for whom the Applicant provided data had initial lesions measuring between 8.4-17.4 cm[16]. It is expected that the potential for viral replication and shedding back into the circulation would increase with the total quantity of viable cancerous tissue.

156. Patients were treated with the GMO once daily from day 1-5 of a 28-day treatment cycle. No viral RNA was detectable on D6 (24 hours after the 5th treatment), D14 or D28. There is a lack of data regarding viraemia levels during the first 4 days of GMO administration and whether active viraemia occurs in trial participants between days 6-14 and days 15-28.

157. Experiments conducted with tumour-bearing immunocompetent mice suggested that the GMO may undergo a few cycles of replication in the first 2 days after administration. However, GMO influx from the tumours into the bloodstream, characterised by a second peak of viraemia, was not observed (see 4.5.2, this chapter.)

GMO persistence in tumours

158. 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 presence of viable particles in tumour tissue was not assessed.

4.5.4 Shedding in human cancer patients treated with the GMO

159. In patients receiving two individual doses of the GMO, shedding was evaluated in saliva, nasal swab, urine and faeces at 30 min, 4 h, and days 2 and 12 after each dose. Viral RNA was consistently below LOQ at all tested timepoints.

160. Shedding was also assessed in nasal swabs, urine and faeces samples collected from 10 patients receiving multiple treatment cycles with doses of 1×10^9 CCID50 of the GMO, once a day for 5 consecutive days. Samples were collected 30 min, 4 h or 24 h after the 5th dose of treatment cycle 1 and 2, and on days 4, 14 and 28 of the following treatment cycles. the GMO was not detected in any of those samples.

161. In the earlier compassionate-use study, 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 (56 genome copies/µL) 5 days post-treatment and one positive urine sample (74 copies/µL) 2 days post-treatment. These data show that low levels of the GMO can be shed into urine and saliva for some days, but only at a very low level.

[16] Before treatment, one or two lesions were selected as target lesions and the sum of diameters was calculated. Measurements may therefore derive from either one or two lesions. Patients may have had more than two lesions but no information about total tumour burden was provided.

In summary, viraemia in trial participants can persist for at least 4 hours after a single i.v. administration of the GMO. Following the first treatment cycle consisting of 5 consecutive daily doses of the GMO, viraemia is anticipated to last for at least 12 h. In treatment cycle 2, viraemia is expected to last for at least 1 h after the 5th dose. The GMO is expected to be cleared within 24 h of the last administration at each treatment cycle. Very low level shedding into saliva and urine after the treatment is possible. The GMO is not expected to be shed in faeces or in nasal secretions. Shedding of the GMO in sperm was not assessed.

4.6 Mosquito transmission

162. The applicant provided data regarding the transmissibility of the GMO via *Cx. quinquefasciatus* fed on an artificial blood meal containing 5×10^7, 5×10^8 or 5×10^9 genome copies/mL of the GMO. The presence of the GMO RNA in mosquitoes' saliva was assessed 10 and 14 days post blood meal containing the GMO. One out of 14 saliva samples collected from mosquitoes infected with the mid-dose was positive at day 10. At day 14, the viral genome was detected in 3/15 saliva samples from animals receiving the high dose of the GMO. Samples collected from the low dose group were negative or below the LOQ in both time points. The study concluded that the minimum infective dose to allow the transmission of the GMO via mosquitoes is 5×10^8 copies/mL (5×10^5 copies/µl).

4.7 Stability in the environment and decontamination

163. As enveloped viruses, alphaviruses are relatively sensitive to desiccation. In addition, they are sensitive to high temperature, ethanol and chorine disinfectants.

164. The applicant has demonstrated that infectivity of the GMO decreases on hard surfaces after 24 hours at room temperature. The GMO was stable for 4 h in faeces, 1 day in saliva, urine, nasal swab and dressing, 3 days in skin swab, 7 days in serum and 14 days in blood samples at room temperature. The applicant reported that the GMO was unstable in faeces, stating that faeces is rich in complex organic matter, RNases and proteases that can affect the structural integrity of viruses and reduce their infectivity. However, the assay LOQ for faecal samples was 4×10^2 CCID50/ml, two orders of magnitude higher than for other sample types such as serum, saliva or urine. 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.

165. The applicant states that decontamination of the GMO via high temperature, ethanol and chorine disinfectants has been validated. This is consistent with methods for decontamination of other alphaviruses (see Section 3.12).

Section 5 The receiving environment

166. 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

167. 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, this chapter). There is a possibility that low levels of the GMO may be shed via body fluids such as urine and saliva (Sections 4.5.1 and 4.5.3, this chapter).

5.2 Clinical trial sites and associated locations

168. 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. The applicant has stated that if additional sites are engaged, they could be located anywhere in Australia.

169. 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 15) 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.

170. 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 17).

5.3 The wider environment

171. The principal routes by which the GMO could enter the wider environment are (a) by shedding or mosquito-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 vector that become infected.

5.3.1 Related viral species in the receiving environment

172. RRV is the closest relative of GETV amongst the alphaviruses (Forrester et al., 2012) and is endemic in Australia. First isolated from wild-caught mosquitoes in north Queensland (QLD) in 1959, it has since been isolated from over 40 mosquito species found across Australia (Doherty et al., 1963; Kizu et al., 2019). RRV causes disease in humans and horses, with human symptoms including polyarthritis, lethargy and rash (Dhama et al., 2014). Like GETV, RRV has multiple vectors and multiple hosts and its transmission dynamics are complex and not well understood (Michie et al., 2020) (Skinner et al., 2020). Between 1993 and 2020, Australia registered 130,271 cases of human RRV infections, with approximately 49% of the cases registered in Queensland (Reviewed in Qian et al., 2022). Humans are considered incidental hosts, and transmission cycles are maintained among mosquitoes and many nonhuman vertebrates, although the relative contributions of different animal species are unclear.

173. Species susceptible to RRV include marsupials (e.g. brushtail possums, kangaroos and wallabies, placental mammals (e.g. horses, pigs, sheep and rabbits) and birds (e.g. chickens, ducks and corellas). Potential reservoirs living in close proximity to humans include horses, which are common in peri-urban areas, brushtail possums which are abundant in urban environments, and flying foxes which are often found at high density close to human populations (Boyd et al., 2001; Wildlife Health Australia, 2015; Stephenson et al., 2018). Marsupials have long been considered important amplifying hosts for RRV, but the relevance of placental mammals in endemic transmission cycles has recently become evident in Fiji, which lacks marsupials (Togami et al., 2020).

174. Novel RRV lineages have emerged roughly every decade since the 1970s and expanded geographically within a relatively short time frame, displacing previously dominant lineages. For example, the G2 lineage was dominant between 1977 and 1995, G4 viruses were first detected in WA in 1994 and by 1996 had completely displaced G2. Biological and ecological factors driving these sweeps of genotype dominance are not yet understood (Michie et al., 2020).

175. BFV is another medically important alphavirus endemic to Australia. It was first isolated in the Barmah Forest in northern Victoria in 1974. Phylogenetic analysis places it as a more distant relative of both RRV and GETV than CHIKV (Forrester et al., 2012). Symptomatic disease in humans is similar to that caused by RRV, with fever, rash, and debilitating arthralgia, and cases have been reported in all states of Australia. As mentioned in Section 3.9 of Chapter 1, RRV and BFV have been found circulating in mosquitoes in the same geographic area with no evidence of recombination.

5.3.2 Relevant environmental factors

176. 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.

Availability of susceptible hosts

177. 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 3.7, this Chapter).

178. Livestock industries are of high economic importance to Australia, with an estimate of 34,000 livestock farms in the country (Department of Agriculture Water and the Environment, 2022). As of 2022, there were 4,300 pig production sites in Australia (Australian Pork, 2022). In South Australia, the livestock sector generated $4.1 billion in revenue in 2021-22 (Regions, 2023). The state engages extensively in intensive pig farming and horse racing, and houses thousands of pigs and horses (Dalgleish, 2021; Regional Development Australia (BGLAP), 2023).

179. Feral pigs are descendants of escaped domestic pigs and inhabit about 40% of Australia. Estimated numbers are 3.5 to 25.5 million, with the highest populations present in large drainage basins and swamps – areas also attractive to mosquitoes (Feral pig scan, 2023; Queensland Government, 2023). In south Australia they are found at Kangarro Island, Port Augusta and Riverland (National Feral Pig Action Plan, 2023).

180. Serosurveys conducted in Asia show that GETV infects a range of animal species– both domestic and wild (Li et al., 1992; Sugiyama et al., 2009; Li et al., 2019). 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 (Chapman, 2009). 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. Serology studies have observed anti-RRV antibodies in many Australian animals, including placental

mammals, marsupials and birds (Wildlife Health Australia, 2015). Anti-RRV antibodies have been shown to cross-react and neutralise GETV, reducing the risk of infection (Rawle et al., 2020a).

181. 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.

Availability of competent vectors

182. Of the 15 potential GETV vector species listed in Section 3.6, five have been observed in Australia: *Cx. quinquefasciatus* is widespread through the Australian mainland; *Cx. gelidus* has been observed in Northern Territory (NT) and QLD; *Cx tritaeniorhynchus*, also an established vector of *Japanese encephalitis virus* (JEV), was recently identified in the NT; *Ae. aegypti* is found in parts of QLD; and *Ae. albopictus is* present in some islands of the Australian Torres Strait (Whelan et al., 2000; Russell et al., 2005; Department of Health SA, 2006; Beebe et al., 2013; Knope et al., 2019; CSIRO, 2020; Lessard et al., 2021; Department of Health WA, 2023).

183. There are at least 300 mosquito species in Australia (Department of Health WA, 2023), it is possible that other mosquito species present in Australia could prove capable as vectors given the opportunity.

184. Ross RRV and BFV are also of particular concern in South Australia. In the 2021-22 financial year, there were 223 RRV and BFV human infections notified in South Australia. Both viruses were found in mosquitoes captured in different LGAs across South Australia between September 2022 and April 2023 (Department of Health SA, 2023).

185. RRV is the closest relative of GETV amongst the alphaviruses (Forrester et al., 2012) and its vectors include *Cx. annulirostris*, *Ae. vigilax* and *Ae. camptorhynchus*. 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 (Stephenson et al., 2018; Liu et al., 2020; Liu et al., 2021).

Physical conditions that may aid or restrict transmission

186. The risk of disease transmission from mosquitoes can vary depending on factors such as the prevalence of the disease in the area, the abundance of the mosquito species, and the effectiveness of mosquito control measures. As mosquitoes require water to breed, climatic influences such as unseasonably warm weather and heavy rainfall can also impact on mosquito prevalence. (Colón-González, 2021; Department of Health SA, 2021; CDC, 2022).

187. In South Australia, the peak period for mosquito breeding usually spans the months of September to April. Mosquito numbers in inland areas are influenced by temperature and rainfall (Department of Health SA, 2021). Higher than average rainfall due to La Nina weather patterns have increased mosquito breeding activity in South Australia in the last years, with the highest mean mosquito abundance registered in the 2022-2023 season (Department of Health SA, 2023). A mosquito and arbovirus transmission surveillance is conducted during the breeding season at twenty councils in high-risk areas for mosquito-borne diseases in SA (Department of Health SA, 2023).

188. As GETV is not currently known to be present in Australia, both humans and animals are not expected to have been previously exposed to the virus. However, sera from animals infected with GETV versus RRV have shown high levels of cross reactivity and cross protection (Rawle et al., 2020b). 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., 2020). Animal seroprevalence data specific to South Australia are not available.

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

189. GETV is not known to be present in the Australian environment (see Section 3.8). The genetic modifications include two single-point mutations. No heterologous gene has been introduced into the GMO genome.

Section 6 Relevant Australian and international approvals

6.1 Australian approvals

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

6.2 International approvals

191. The proposed clinical trial is a first-in-human study. The applicant stated that approval from Japan, USA and China for clinical trials with the GMO was under consultation stages at the time of this application. Previous administration of the GMO to patients in China was performed under Investigator-initiated trial or a compassionate use program.

Chapter 2 Risk assessment

Section 1 Introduction

192. 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.

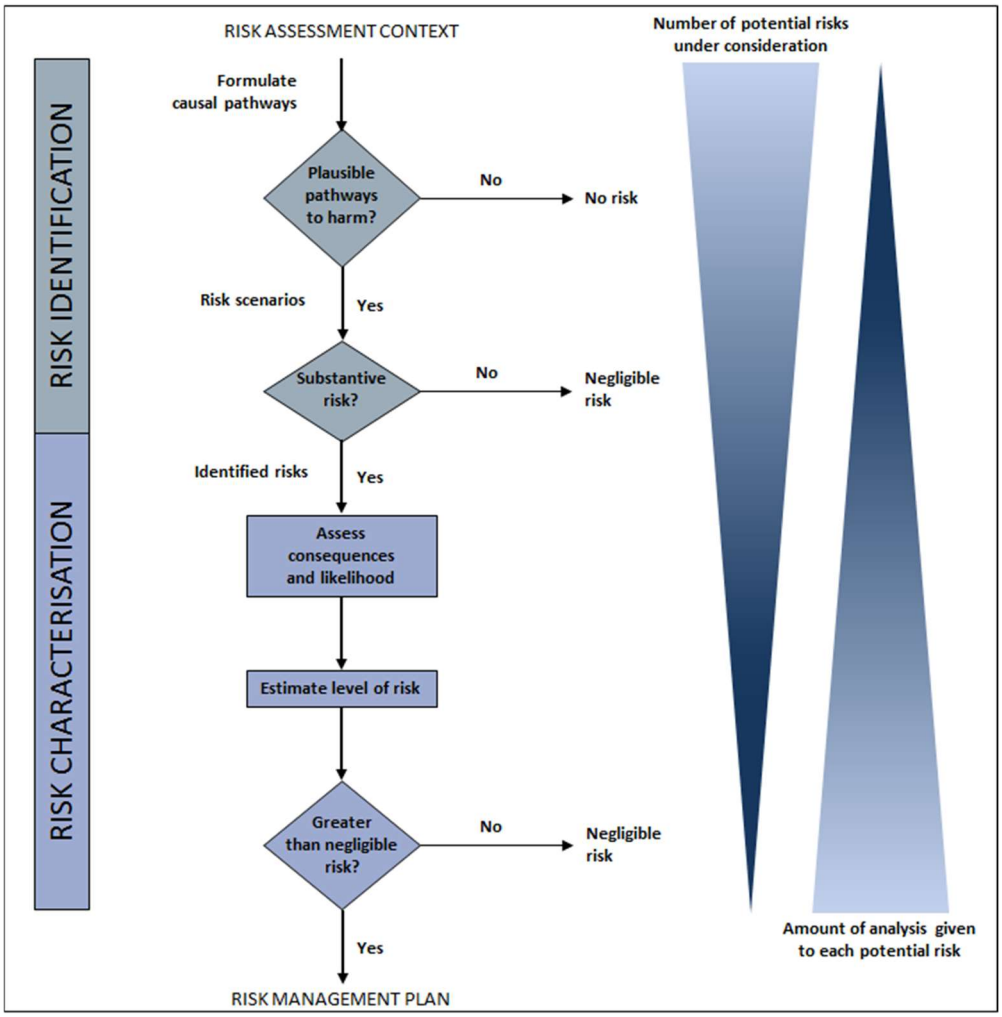

Figure 2. The risk assessment process

193. 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.

194. 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.

195. 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.

196. 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

197. 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.

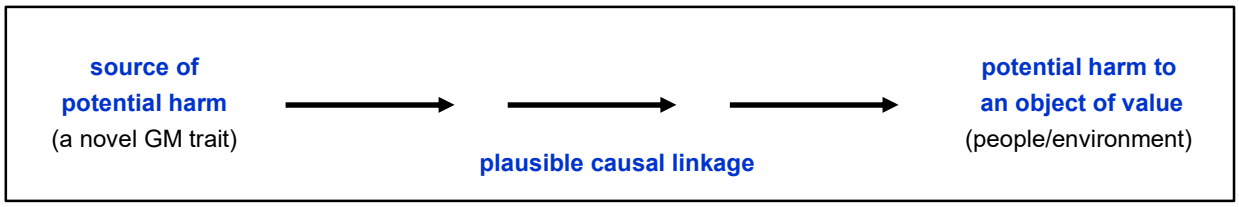

Figure 3. Components of a risk scenario

198. 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

199. The parent organism of the GMO is the variant M1-c6 of M1 strain of GETV. Details on the pathogenicity and transmissibility of GETV, and specific consideration of M1, are located in Chapter 1, Section 3.

200. 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.

201. 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

202. 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.

203. 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.

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

205. **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.

206. **Reversion to wild-type sequence**: The GMO contains two single point mutations that does not result in attenuation of the virus. Loss of either mutation would not increase the risk to human health and safety and the environment when compared to its parental organism, so this possibility will not be assessed further.

207. **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 1, Section 4.2). However, this behaviour is an attribute of the parent organism and not affected by the genetic modification so will not be considered further.

208. **Recombination with other alphaviruses in trial participants**: Recombination between two viruses requires that they co-infect the same cell at the same time, what is highly unlikely. In addition, given the low prevalence of alphaviral infection in humans in Australia (see Section 5.3, Chapter 1) and that data provided by the applicant suggesting the GMO is rapidly cleared by the human immune system, this causal pathway is not considered plausible and will not be further assessed.

209. **Recombination with other alphaviruses in animals.** In the event of mosquito-vectored transmission of the GMO to a susceptible or amplifying host, there is potential for recombination in animals co-infected with other alphaviruses endemic in Australia, including RRV and BFV. As mentioned previously, homologous recombination between viral strains requires both viruses to be present and replicating within the same infected host cell, and that is highly unlikely. In addition, as mentioned previously, animals exposed to RRV are likely to have cross-reactive immunity against the GMO. The GMO would likely be cleared by the host immune system prior to entering a cell and recombining Further, it should be noted that RRV and BFV have been found circulating in the same geographic area, and while relatively few full genomes of either species have been characterised, no phenotypically obvious recombinants have been reported to date (see Chapter 1, Section 5.4). It is

difficult to predict the consequences of such recombination, given that only one instance of recombination between different alphaviruses has been documented which resulted in a WEEV strain with similar host range but reduced pathogenicity and mortality than its parental EEEV (Government of Canada, 2012). As the GMO, RRV and BFV virus can cause a broad range of symptoms and infect humans and animals, it is unlikely that recombination would result in more pathogenic virus with an altered host range. The potential harm to animals exposed to a recombinant GETV strain would be comparable to the harm caused by a WT GETV strain or the GMO. This harm is discussed in the risk characterisation of Risk Scenario 2 (Section 3, this Chapter), therefore recombination will not be considered further.

210. **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 1, Section 3.7). 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 27 adult cancer patients receiving the GMO (Chapter 1, Section 4.3). 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.

211. **Exposure and infection of breast-feeding women:** The potential for transmission of arboviruses during breast-feeding was discussed in Chapter 1, Section 3.5. Transmission of GETV via breastfeeding 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), a member of the *Flaviviridae* family, 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 breast-feeding 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 1, Section 3.7), and women are unlikely to be conducting dealings or participating in the clinical trial so soon after giving birth. Therefore, exposure of breast-feeding 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

212. 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 animals or humans;
- the potential for the establishment of the GM GETV in the environment.

213. Potential harms to foetuses or infants infected *in utero* are discussed in the risk assessment. 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

214. Four risk scenarios were postulated and screened to identify substantive risks. These scenarios are summarised in Table 4.

215. In the context of the activities proposed by the applicant and considering both the short and long term, three of the four risk scenarios did not give rise to substantive risks that could be greater than negligible. One risk scenario was identified as posing substantive risk which warranted further assessment (characterised in Section 3; this chapter).

Table 4. Summary of risk scenarios from dealings with GM GETV

Risk scenario	Risk source	Possible causal pathway	Potential harm	Substantive risk	Reason
1	GM GETV	Treatment of trial participant with GMO ⬇ Passive viraemia or active viraemia following: ⬇ i. Infection of tumour cells followed by viral replication within tumour tissue ⬇ ii. Release of GMO into circulation (active viraemia) ⬇ Ingestion of GMO by mosquitoes during a blood meal ⬇ Mosquito-vectored transmission to other people ⬇ Infection with the GMO	• Miscarriage or foetal death. • Clinical symptoms of GETV disease (e.g. fever, rash, swelling of lymph nodes).	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 viraemia. • Viraemia in trial participants leaving the hospital is below the minimum infective dose to allow the transmission of the GMO via mosquitoes. • Subsequent active viraemia is not expected to occur. • No reports of disease in healthy, immunocompromised or pregnant individuals living in GETV affected areas. • Immunocompromised and pregnant individuals are excluded from participating in the trial.
2	GM GETV	Treatment of trial participant with GMO ⬇ Passive viraemia or active viraemia ⬇ Ingestion of GMO by mosquitoes during a blood meal ⬇ Mosquito-vectored transmission of the GMO to animals in the environment ⬇ Infection with the GMO ⬇ Infection of animal species who may be susceptible to	• Disease in susceptible adult animals, including Australian native animals. •Foetal/neonatal death in susceptible pregnant, nursing or neonatal animals, including native animals.	Yes	• GETV is known to infect a range of animals and cause disease in horses and pigs. • See Section 3 for risk characterisation.

Risk scenario	Risk source	Possible causal pathway	Potential harm	Substantive risk	Reason
		GETV-associated disease, and/or infection of amplifying hosts			
3	GM GETV	Treatment of trial participant with GMO ⬇ Passive or active viraemia ⬇ Exposure of other people in the event of: bleeding; or shedding of the GMO in body fluids or faeces ⬇ Infection with the GMO ⬇ Further transmission of the GMO to other people or animals	As per risk scenario 1	No	In addition to the reasons described in scenario 1: • Close contacts and carers will take precautions to minimise exposure to blood and body fluids from trial participants; • Trial participants would stay in the clinical trial site during the viraemic period; • Observed shedding into body fluids is very low. In addition, exposure via contact is not a typical route for the transmission of GETV.
4	GM GETV	Exposure of people undertaking dealings with the GMO via needle-stick injury, fomites or contact with broken skin during: (a) Preparation and administration of the GMO (b) Collection and analysis of biological samples ⬇ Viraemia ⬇ Ingestion of GMO by mosquitoes during a blood meal ⬇ Mosquito-vectored transmission to other people ⬇ Infection with the GMO	As per risk scenario 1	No	• Preparing the GMO will not require removal or recapping of a needle. • Staff handling the GMO or collecting biological samples would wear appropriate PPE. • 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 be trained to protect themselves from mosquito exposure for 48h. • Staff would be informed that immunocompromised or pregnant individuals should not handle the GMO.

2.4.1 Risk scenario 1

Risk source	GM GETV
Causal pathway	Treatment of trial participant with GMO ⬇ Passive viraemia or active viraemia following: ⬇ i. Infection of tumour cells followed by viral replication within tumour tissue ⬇ ii. Release of GMO into circulation (active viraemia) ⬇ Ingestion of GMO by mosquitoes during a blood meal ⬇ Mosquito-vectored transmission to other people, including immunosuppressed and pregnant people ⬇ Infection with the GMO
Potential harm	• Miscarriage or foetal death • Clinical symptoms of GETV disease (e.g. fever, rash, swelling of lymph nodes).

Risk source

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

Causal Pathway

217. 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 whom they subsequently bite.

218. As mentioned in Section 4.5 of Chapter 1, the administration of a single dose of the GMO to tumour bearing immunocompetent mice resulted in the detection of viraemia at the initial 2 h time point. The viraemia levels declined logarithmically over several weeks. However, in tumour bearing immunocompetent mice inoculated with multiple doses of the GMO, viraemia reached its highest level at day 3 and was no longer detected after day 10 (5 days after the last administration dose of the GMO).

219. Given the maximum GMO dose of 3×10^9 CCID50 and an average adult blood volume of 5L, the maximum concentration of circulating virus expected immediately after a single treatment dose with the GMO is 6×10^2 CCID50/µl of blood. This is expected to decline over time as the GMO enters cancerous tissue and is also cleared from the body. In the compassionate use study in China, the administration of a single dose of the GMO resulted in detection of viraemia 30 min after administration, viraemia levels decreased at the 4 h time point and was below the LOQ 24 h post-

administration. In another group of patients, the administration of 5 daily doses of 1 x 10^9 CCID50 of the GMO resulted in a viraemia level of 9.18 x 10^3 genome copies/µl of serum 30 min after the administration of the 5th dose of the GMO (the last dose of treatment cycle 1) (Chapter 1, Section 4.5.3). It decreased at the 2 h time point and was not detectable at the 12 h time point. Adjusting for the maximum dose to be used in this trial (3 x 10^9 CCID50), approximately 2.75 x 10^4 genome copies/µl might be expected to circulate soon after the administration of the 5th dose, representing a maximum of 2.75 x 10^4 viable virions/µl. At treatment cycle 2, viraemia was detected 30 min after the administration of the 5th dose of the GMO but not at later time points.

220. Preclinical studies indicate that active viraemia following infection of tumours does not occur. Murine models showed a steady logarithmic decline of the GMO in tumours from 48 hours onwards, without any GMO reappearing in the circulation. Based on this observation, the concentration of the GMO in the serum of trial participants is expected to range from 6 x 10^2 - 2.75 x 10^4 genome copies/µl of blood following the first treatment cycle. Viraemia is expected to be cleared over time with no increases due to viral replication in tumours. Viraemia levels are expected to be lower following multiple treatment cycles with the GMO.

221. The applicant has shown that the minimum transmission dose of the GMO to *Cx. quinquefasciatus* mosquitoes is 5 x 10^5 genome copies/µl of blood. It is unlikely that viraemia levels in trial participants could result in infection of *Cx. quinquefasciatus* mosquitoes. Advice given by an expert (Appendix A) in alphavirus pathogenesis suggests that natural transmission of alphaviruses via mosquitoes requires viraemia levels ≥10^4 viral particles/µL of blood. In laboratory settings, the establishment of a GETV infection in mosquitoes depend on the virus strain and mosquito species, with a minimum infection dose of 20 genome copies/µL of blood reported (see Section 3.5 of Chapter 1). Although unlikely, it is plausible that the number of viral particles in the blood of trial participants immediately after i.v. infusion or in the first days after completion of each treatment cycle could be sufficient to allow infection in a different competent mosquito.

222. It is not known whether any mosquito species present in the Australian environment, 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 1, Section 3.10) 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 climatic range (Chapter 1, Section 3.6). Australia has around 300 mosquito species, and South Australia at least three capable of transmitting the related RRV (Chapter 1, Section 5.3.1). It is plausible that at least one species of mosquitoes found in Australia can act as a competent vector for GETV. For example, *Cx. quinquefasciatus* mosquitoes are present around Australia and have been used in laboratory studies with GETV. If a trial participant gets bitten by a competent mosquito during the viraemic period, the GMO virus could be transmitted to other people.

223. The applicant proposed that trial participants will remain at the trial site as an in-patient for the 5 days of dosing and then until 2 consecutive negative test results for viremia is achieved after each treatment cycle with the GMO. Viraemia will be assessed using a qPCR assay with LOQ ≤ 6.25 genome copies/µl of serum or 3.13 genome copies/µl of whole blood. Participants leaving the hospital will have viremia levels below the minimum infection dose reported for infection of mosquitoes in laboratory settings. Therefore, the likelihood of mosquito-vectored transmission of the GMO to other people is highly unlikely.

Potential harm

224. If people are exposed to and infected with the GMO, a range of outcomes are possible. As discussed in Sections 2.2 and 2.3 of this chapter, immunocompetent individuals 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.

Potential harm to pregnant women and their foetuses

225. In pigs, mice and several other small mammals, GETV infects both the placenta and foetus and is associated with foetal and neonatal death (see Chapter 1, Section 3.7). However, 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 did not indicate any unusual reproductive difficulties (Li et al., 1992). In addition, several outbreaks of GETV have occurred in the past two decades (Chapter 1, Section 3.3) without reports of potential harm to pregnant women.

226. 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). Pregnant women are excluded from the clinical trial. In addition, trial participants must agree to take measures to prevent pregnancy for 90 days after receiving the final GMO treatment. It is unlikely that exposure of a pregnant woman to a small amount of the GMO via mosquito bites would result in miscarriage, or foetal death.

Potential harm to immunocompromised people

227. As discussed in paragraph 210, there are limited 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 alphaviruses, cells of the innate immune system (monocytes and macrophages) appear to promote the pathogenesis of acute RRV and CHIKV infection (Haist et al., 2017; Belarbi et al., 2019), 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. However, the applicant provided data showing that the i.v. administration of 14 consecutive doses of the GMO to immunocompetent mice resulted in in slight degeneration and necrosis of intestinal mucosa (≤ 20%), with no other administration-related adverse event observed. In addition, immunocompromised people are excluded from the clinical trial. It is unlikely that exposure to the GMO via mosquito bites would result in harm to immunocompromised people.

Conclusion

228. Risk scenario 1 is not identified as a substantive risk because potential exposure of trial participants to mosquito bites during viraemic period would be limited by the proposed controls. In addition, the potential for an adverse outcome as a result of mosquito-vectored transmission of the GMO to people is assessed as negligible. Therefore, this scenario does not warrant further assessment.

In summary, Risk Scenario 1 has taken into consideration that:

- *passive viraemia is limited to the number of viral particles administered and is expected to be cleared within 24 h following administration;*
- *active viraemia is not expected to occur;*
- *humans are dead-end host to GETV and the GMO is expected to be cleared over time;*
- *infection of mosquitoes requires high viraemia levels in the trial participants. This level of viraemia only occurs for a few hours following administration;*
- *trial participants will remain in the hospital during the administration of the GMO and until viraemia is below the limit of detection;*
- *there are no reports of disease in healthy, immunocompromised or pregnant individuals living in GETV affected areas;*
- *pregnant and immunocompromised people are excluded from the clinical trial.*

2.4.2 Risk Scenario 2

229. Risk Scenario 2 considers the potential harm to susceptible animals exposed to the GMO via mosquito bites. As Risk Scenario 2 is considered to be a substantive risk, a risk characterisation was conducted as detailed in Section 3.

2.4.3 Risk scenario 3

Risk source	GM GETV
Causal pathway	Treatment of trial participant with GMO ⬇ Passive or active viraemia ⬇ Exposure of other people in the event of: bleeding; or shedding of the GMO in body fluids or faeces ⬇ Transmission (e.g by direct inoculation) to other people or animals in the environment ⬇ Infection with the GMO
Potential harm	As for Scenario 1

Risk source

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

Causal Pathway

231. 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 shedding of the GMO does not occur in most of the patients, but low level shedding into saliva and urine is possible depending on the dose of the GMO and schedule of administration. Shedding in nasal secretions and faeces is unlikely (see section 4.5.3, Chapter 1). The presence of the GMO in sperm or vaginal secretions has not been evaluated. The duration of shedding is uncertain but will be investigated as part of the proposed clinical trial.

232. The risk of adverse effects from the exposure of people conducting dealings with the GMO is discussed in Risk Scenario 4. This risk scenario focuses on the likelihood and consequence of exposure of close contacts or animals outside the clinical trial site.

233. 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 for the 5 days of dosing and then until viraemia is not detected. 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. The dressing will be removed before the trial participant is discharged and disposed as GMO waste.

234. Once trial participants return home, carers and other close contacts or animals such as pets or livestock could be exposed to the GMO via direct contact with contaminated blood or body fluids. However, direct contact is not a typical route of GETV transmission and infection would require the direct inoculation of the GMO via a needle stick or sharps injury. Therefore, the likelihood of close contacts being exposed to the GMO via contact with blood or fomites is highly unlikley.

235. GETV is not known to be sexually transmitted. However, the shedding of the GMO in sperm or vaginal secretions has not been investigated. The applicant proposed that trial participants will be required to use barrier contraceptive if sexually active during the treatment and for at least 90 days after the final GMO treatment. Therefore, it is unlikely that a person will be exposed to the GMO during sexual activity.

236. Shedding of the GMO into secreted fluids such as saliva and urine is expected to be very low (Section 4.5.3, Chapter 1). As discussed in Section 3.5.5 of Chapter 1, studies with horses suggested that natural aerosol transmission of GETV to other animals is unlikely to occur. Therefore, it is unlikely that the GMO shed from trial participants would result in transmission to other people or animals via aerosols.

Potential harm

As discussed in Section 2.2 of this Chapter, the GMO is not expected to cause harm in immunocompetent individuals. As per Risk Scenario 1, exposure to small amounts of the GMO is unlikely to cause harm to pregnant or immunocompromised individuals. In addition, the trial participants will remain in the hospital during the potential viraemic period. If exposure occurs, it is unlikely that low levels of the GMO present in blood or shed into body fluids would cause harm to other people. The potential harm for animals exposed to the GMO is discussed in the Risk Characterisation in Section 3 of this Chapter.

Conclusion

237. Risk scenario 3 is not identified as a substantive risk because potential exposure routes to the GMO will be mitigated by the proposed controls. Therefore, this risk is assessed as negligible and does not warrant further detailed assessment.

In summary, in addition to risk scenario 1, this risk scenario has taken into consideration that:

- *only very low levels of the GMO, if any, is anticipated to be shed in saliva and urine;*
- *the presence of the GMO is not expected in nasal secretion and faeces;*
- *direct contact is not a typical route of GETV transmission.*

2.4.4 Risk Scenario 4

Risk source	GM GETV
Causal pathway	Exposure of people undertaking dealings with the GMO via needle-stick injury, fomites or contact with broken skin during: (a) Preparation and administration of the GMO (b) Collection and analysis of biological samples ⬇ Infection of people conducting the dealing and development of viraemia ⬇ Ingestion of GMO by mosquitoes during a blood meal ⬇ Mosquito-vectored transmission to other people ⬇ Infection with the GMO
Potential harms	• As per risk scenario 1.

Risk source

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

Causal Pathway

239. This scenario applies to people conducting dealings in the pharmacy or at the clinical trial site. There is potential for exposure of people to the GMO during the preparation and administration of the GMO, or during sample collection, via needle stick, sharps injury or contact with broken skin.

240. There is potential for exposure of people to the concentrated GMO during the preparation of the GMO via needle stick, sharps injury, aerosol formation, spills or eye splash. The GMO will be prepared in a negative pressure isolator. Personnel administering the GMO will wear face mask and safety glasses. These measures will minimise the potential for exposure via aerosol.

241. In the event of a needle stick injury, the exposed person would receive approximately 4 x 10^5 CCID50 (2 µl of the concentrated solution). As adults have an average blood volume of 5L, the exposure to the GMO via needle stick injury would result in a passive viraemia of 0.08 CCID50/µl of blood soon after the exposure. There is limited data regarding the replication of the GMO following a single administration to healthy individuals. However, based on the viraemia observed in a patient 30 minutes after receiving the 5th dose of the GMO (Chapter 1, Section 4.5.3), the exposure via needle stick injury could result in a viraemia peak of 3.67 CCID50/µl of blood and would gradually decrease.

242. Staff undertaking activities later in the clinical trial 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 during preparation of the GMO). As mentioned in Section 2.3.7, the GMO will be administered via a catheter inserted prior to the i.v. infusion. They will not be using sharps during the administration of the GMO, so exposure would only occur via contact of spilled material with broken skin.
- Staff collecting blood samples from trial participants, commencing 30 min after administration of the GMO, will use sharps to draw blood. As discussed in Risk scenario 1, approximately 2.75 x 10^4 genome copies/µl might be expected to circulate soon after the administration of the 5^{th} dose. In the event of a needle stick injury, the person conducting dealings could be exposed to a dose of ~5.5 x 10^4 genome copies resulting viraemia levels of ~0.011 genome copies/µL of blood, much lower levels of viraemia compared to exposure to the concentrated GMO.
- Staff collecting tumour samples may come into contact with virus that has concentrated within that tissue. However, preclinical data provided by the Sponsor suggests 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).

243. As discussed previously, the minimum dose for the transmission of GETV via mosquitoes in laboratory setting is 20 genome copies/µL (see Section 3.6, Chapter 1 and Risk Scenario 1). In the event of personnel exposure, it is unlikely that the viraemia levels expected in the exposed person could result in mosquito infection and subsequent transmission of the GMO to other people.

244. As discussed in Section 2.2 of Chapter 1, the preparation and administration of the GMO and sample collection will be carried out in the clinical trial site by authorised, experienced, and trained health professionals. All personnel working in settings where healthcare is provided are required to comply with the standard precautions for working with potentially infectious material, as described in the *Australian Guidelines for the Prevention and Control of Infection in Healthcare* (2019). Biological samples will be processed and analysed in PC2 laboratories (Chapter 1, Section 2.3.2), following standard PC2 laboratory practices. The applicant has proposed work practices that would minimise opportunities for exposure by direct inoculation. Pregnant and immunocompromised individuals will be instructed to not handle the GMO. The procedure for preparing i.v. infusion bags requires sharps but does not require high risk procedures such as removing or recapping needles. The needle/syringe unit will be disposed directly into a sharps container after use. Furthermore, appropriate training and use of PPE (e.g. gown, gloves, and when administering the GMO, face mask and safety glasses) would minimise the potential exposure of people to the GMOs via aerosol and contact with broken skin. Therefore, it is unlikely that personnel would be exposed to the GMO. In the event of exposure, it is highly unlikely that the viral levels of the GMO in the blood of the exposed person would result in mosquito infection.

Potential harm

245. As per risk scenario 1.

Conclusion

246. Risk scenario 1 is not identified as a substantive risk because inadvertent exposure by direct inoculation could transfer only a small quantity of the GMO that is highly unlikely to result in mosquito infection Therefore, this risk is assessed as negligible and does not warrant further detailed assessment.

In summary, Risk scenario 3 has taken into consideration that:

- *needles used in the preparation of the GMO will not be removed or recapped;*
- *personnel will wear appropriate PPE;*
- *personnel working in hospital setting are trained in standard precautions for working with potentially infectious material;*
- *in the event of exposure, the exposed person would receive a low dose of the GMO;*
- *viraemia would be limited to the number of viral particles inoculated and active viraemia is not expected to occur;*
- *immunocompromised or pregnant staff will be instructed to not handle the GMO.*

Section 3 Risk characterisation

247. Four risk scenarios were postulated and evaluated, as summarised in Table 4. The second risk scenario was identified as posing a substantive risk which warrants further assessment. This section provides more detail on the evaluation of this scenario.

Risk Scenario 2. Transmission of the GMO to susceptible animals

Risk source	GM GETV
Causal pathway	Treatment of trial participant with GMO ⬇ Passive viraemia or active viraemia ⬇ Ingestion of GMO by mosquitoes during a blood meal ⬇ Mosquito-vectored transmission of the GMO to animals in the environment ⬇ Infection of the animal with the GMO ⬇ Infection of animal species who may be susceptible to GETV-associated disease, and/or infection of amplifying hosts
Potential harm	• Disease in susceptible adult animals, including Australian native animals • Foetal/neonatal death in susceptible pregnant, nursing or neonatal animals, including native animals

Risk source

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

Causal Pathway and likelihood assessment

249. This scenario applies to cancer patients participating in the trial who will be inoculated with daily doses of the GMO for 5 consecutive days and for 3 treatment cycles. A female mosquito feeding on trial participants could ingest the GMO and transmit it to animals whom they subsequently bite.

250. As discussed in Risk scenario 1, the highest viraemia levels are expected immediately after each i.v. infusion. Viraemia is anticipated to decrease over time and be below detectable levels 24 hours after the administration of the 5th dose of treatment cycle 1. During the following treatment cycles, viraemia is expected to be detected for a shorter period due to an increased immune response to the GMO. As mentioned in Section 3.5 of Chapter 1, humans are considered dead-end hosts and viraemia levels are expected to remain below the minimum required for natural transmission of the virus via mosquitoes. In addition, trial participants will remain indoors, in a room at the clinical trial site, during the potential viraemic period. Participants will be discharged after two consecutive negative test results for viraemia and a second peak of viraemia (active viraemia) is not expected to occur. Therefore, it is unlikely that a trial participant could be bitten by a mosquito during the potential viraemic period.

251. In the unlikely event of a mosquito biting a trial participant during the viraemic period and becoming infected, the GMO will need to replicate in the mosquito midgut and disseminate to its

salivary glands. If the infected mosquito feeds on an animal, the GMO present in its saliva could then be inoculated into the animal. As mentioned, trial participants will remain indoors during the viraemic period. Exposure to mosquitoes would have to occur at the clinical trial site room. For transmission to occur, the infected mosquito would have to travel from the clinical trial site and feed on a GETV susceptible animal or an amplifying host to transmit the GMO. As discussed in Section 5.3.2 of Chapter 1, South Australia engages extensively in pig farming and horse racing. However, most mosquitoes have limited dispersal capacity (Section 3.6, Chapter 2). For an infected mosquito to travel significant distance, it would have to travel on a person or vehicle to regions where susceptible animals or amplifying hosts are present.

252. As discussed in Section 3.7 of Chapter 1, GETV has a broad host range. Although most infections are asymptomatic, clinical symptoms have been observed mainly in horses, pigs and cattle. Horses and pigs are also known as amplifying hosts for GETV. If an infected mosquito transmits the GMO to an amplifying host, the GMO could replicate in the animal and be further transmitted to other mosquitoes.

253. As mentioned previously, for a susceptible animal to acquire an infection from a mosquito bite, three events must occur: i) a viraemic trial participant would have to be bitten by a competent mosquito; ii) the GMO would have to replicate within the mosquito; and iii) the same mosquito would have to be in the presence of susceptible animals and bite it. This is a succession of unlikely events. Therefore, the likelihood of animals being infected as a result of exposure to the GMO via mosquito-vectored transmission is **highly unlikely**.

Consequence assessment

254. If animals in the environment are exposed to the GMO via mosquito bites, a range of outcomes are possible.

255. The applicant has shown that the GMO induced viraemia and an immune response but was not harmful to several non-human species including monkeys, dogs, Bama miniature pigs, chickens, adult rats, horses and piglets. However, GETV is not present in Australia and the susceptibility of Australian wildlife to GETV is unknown. It is plausible that the exposure to the GMO could result in outcomes equivalent to those associated with the wild-type virus in susceptible species.

Potential harm to animals susceptible to GETV associated disease

256. Characteristic GETV-associated pathologies affect many species including horses and cattle and cause foetal and neonatal death in pigs, as well as several small mammalian species (see Chapter 1, Section 3.7). Clinical signs of infection with a pathogenic WT GETV vary in severity and could negatively impact on the welfare of affected animals. In some cases, infection with pathogenic GETV can result in death. The inclusion of GETV disease as a category 4 disease, resulting in a production loss, in the EADRA (Chapter 1, Section 3.10) reflects the level of concern the livestock industries hold in relation to GETV.

257. Serosurveys conducted in Asia show that GETV infects a range of animal species– both domestic and wild (Li et al., 1992; Sugiyama et al., 2009; Li et al., 2019). Australia is home to a diverse and unique wildlife population, which have not been exposed to GETV. Any may be susceptible to infection with the GMO, the effects of which are unknown. Some species may experience disease, with any of the range of observed outcomes being possible, including severe disease comparable to an infection with a pathogenic WT GETV strain and adverse effects on reproduction.

258. 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 section 3.7, Chapter 1). 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 transmission via milk in mice is relevant to the prolonged lactation stage (Section 3.5.4, Chapter 1).The applicant has shown that the GMO does not cause disease in adult horses, cattle and 7- to 18-day old piglets, and only a transient fever was observed in some of the animals (see Section 4.4, Chapter 1). When administered intravenously, the GMO was rapidly cleared and not detected 3 or 4 days post-inoculation in adult horses or 18 day-old piglets. These data suggest that the GMO has shown reduced ability to replicate in horses and piglets compared to the parent organism. However, there is a lack of a positive control in these experiments and the effect of the GMO in pregnant sows has not been investigated. In addition, it is unclear whether the pathogenicity data provided by the applicant is relevant to GETV naïve livestock or wild animals in Australia.

259. However, as discussed in Section 5.3 of Chapter 1, many of the Australian wildlife, horses and pigs have been exposed to the closely related alphavirus RRV. Anti-RRV antibodies present in these animals could offer some level of cross-protection against the GMO. As this point, there is no data showing the extent of the cross-protection offered by anti-RRV antibodies against the GMO. Therefore, as a conservative approach, this risk scenario considers that the consequence of an eventual exposure of susceptible animals to the GMO via mosquito bites is **major** (major increase in damage to desirable components of the environment, with extensive biological or physical disruption to whole ecosystems, communities or an entire species, which persists over time).

Infection of an amplifying host and establishment of the GMO within the Australian environment

260. Like other arboviruses, GETV alternates between mosquito and vertebrate hosts. Many vertebrate species may support infection and viral replication, but not all develop sufficient viraemia to re-infect mosquitoes during a bloodmeal. Horses and pigs are thought to be natural amplifiers of GETV because of high viral titres produced after experimental infection and the high seroprevalence among pigs in GETV affected areas (Bannai et al., 2017). As mentioned previously, data provided by the applicant suggest that the GMO has low ability to replicate in horses and young piglets. However, many Australian native species serve as reservoir hosts for the closely related RRV and if infected with the GMO, may support sufficiently high viraemia to allow ongoing mosquito-vectored transmission. If animals are infected with the GMO and develop sufficient viremia to act as amplifiers, a natural transmission cycle could become established in the Australian environment.

261. In the unlikely event of GMO dispersal and persistence in the Australian environment, the adverse outcomes discussed above could continue in the long term, either at a low ongoing level or on an episodic basis. The related RRV offers an example of how a novel virus could establish in the environment, evolutionary analysis of RRV found that novel lineages have emerged repeatedly since the 1970s and expanded geographically within a relatively short time frame (Aaskov et al., 1981; Aubry et al., 2015; Lau et al., 2017; Michie et al., 2020).

262. Australia is currently free of GETV, which is listed as Prohibited Matter in several Australian states and causes a nationally notifiable equine disease (see Chapter 1, Section 3.11). If the GMO becomes established in the environment, it is unlikely that this outcome could be reversed. Current protocols for preventing the entry of GETV into Australia recognise that if the virus enters via infected mosquitoes, there can be no effective response (Animal Health Australia, 2018a). Therefore, the potential harm resulting from an infection of an amplifying host to the GMO is **major.**

Risk estimate

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

264. The potential consequence of mosquito-vectored transmission of the GMO to susceptible animals and amplifying hosts is considered **major**, with a probability of **highly unlikely**. The overall risk

is therefore estimated to be **moderate** (risk is of marked concern and will necessitate actions for mitigation that need to be demonstrated as effective).

In summary, this risk characterisation has taken into consideration that:

- *passive viraemia is limited to the number of viral particles administered and is expected to be cleared within 24 h following administration;*
- *active viraemia is not expected to occur;*
- *humans are dead-end host to GETV and the GMO is expected to be cleared over time;*
- *infection of mosquitoes requires high viraemia levels in the trial participants. This level of viraemia only occurs for a few hours following administration*
- *trial participants will remain in the hospital during the administration of the GMO and until viraemia is below the limit of detection; and*
- *the GMO does not cause disease in the animal models evaluated.*

However, there is lack of data regarding the effects of the GMO in pregnant sows and its potential harm to GETV naïve livestock or wild animals in Australia.

Section 4 Uncertainty

265. Uncertainty is an intrinsic part of risk analysis[17]. 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 (Clark and Brinkley, 2001; Hayes, 2004; Bammer and Smithson, 2008). These include:

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

266. Uncertainty is addressed by approaches such as using a weight-of-evidence, making 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.

[17] 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.

267. 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.

268. For DIR-198, uncertainty is noted in relation to a number of points, including the following.

- whether the data provided regarding the GMO pathogenicity towards GETV susceptible species such as horses, cattle and piglets is relevant to livestock species in Australia that are naïve to GETV.
- the outcomes of GMO infection for Australian native animals and other animal species in whom GETV infection has not been studied.
- the potential for active viraemia in trial participants after the administration of the GMO.
- the capacity of the GMO to cause placental and foetal infection in humans.
- whether mosquito species present on the Australian mainland can be infected by and transmit the GMO to animal hosts.

269. 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 5 Risk evaluation

270. 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.

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

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

272. Four risk scenarios were postulated whereby the proposed dealings might give rise to harm to people or the environment. This included consideration of whether people and animals can be exposed to the GMO via mosquito bites and whether there is a potential for recombination of the GMO with other alphaviruses. The potential for the GMO to persist in the environment and its effects was also considered.

273. A risk is only identified as substantive when a risk scenario is considered to have some chance of causing harm. Risk scenarios that do not lead to harm, or could not reasonably occur, do not represent an identified risk and do not advance any further in the risk assessment process. In the context of the control measures proposed by the applicant, one of the four risk scenarios was identified as a substantive risk requiring further assessment.

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

275. The risk due to mosquito-vectored transmission of the GMO to susceptible and amplifying hosts was estimated as posing a moderate risk to the environment. The applicant has proposed some

control measures related to these risks. Additional treatment measures to mitigate the identified risks should be applied, and are considered in Chapter 3

Chapter 3 Risk management plan

Section 1 Background

276. 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.

277. 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.

278. 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.

279. 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 risks 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

280. The risk identification process led to identification of one substantive risk, involving mosquito-vectored transmission of the GMO to susceptible animals in the environment, resulting in disease and establishment of the GMO in the environment. This risk was characterised in Chapter 2, Section 3, and risk evaluation proposed that this risk should be treated.

281. The applicant has proposed that trial participants will remain at the trial site during the 5 days of dosing and then until 2 consecutive negative test results for viremia are achieved. The qPCR assay proposed to assess the viraemia in trial participants has a LOQ of ≤ 5 viral genome copies/µL of whole blood or 10 viral genome copies/µL of serum, which is below the minimum infective dose observed in mosquitoes experimentally infected with GETV (see section 3.5, Chapter 1). These measures aim to minimise the exposure of trial participants to mosquito bites and the potential for mosquito infection and further transmission of the GMO to other people or animals. Therefore, these measures are proposed as draft licence conditions.

282. To further minimise the potential for exposure of trial participants to mosquito while in the hospital, draft licence conditions require:

- hospital room to be equipped with at least one electrical discharge insect control system (e.g. insect zapper)
- that trial participants leaving the room must wear long sleeves, long pants and apply mosquito repellent to prevent mosquito bites.

283. The applicant proposed that trial participants leaving the hospital will be instructed to also avoid exposure to mosquitoes for an additional 7 days (see Section 2.2, Chapter 1). In addition, they will be instructed to cover any bleeding cuts or wounds to prevent their blood from contacting other people or

animals. As mentioned previously, trial participants leaving the hospital will have very low levels of viral genome copies in their blood that are unlikely to result in mosquito infection. Therefore, these measures are not proposed as draft licence conditions.

284. The applicant also proposed that trial participants will be instructed to avoid contact with newborns and immunocompromised individuals. As discussed in Section 3.5 of Chapter 1, GETV is transmitted via mosquito bites, and to some extent, by direct contact. It is unlikely that a trial participant coming into contact with a newborn or immunocompromised individual would result in transmission of the GMO. Therefore, this measure is not proposed as a draft licence condition.

285. The applicant proposed that the clinical trial will be conducted at the Flinders Private Hospital in South Australia. However, other clinical trial sites could be engaged. There are uncertainties regarding the competence of mosquito species present in Australia to transmit GETV. While *Cx. quinquefasciatus* mosquitoes can be found throughout the Australian mainland, other potential GETV vectors are only found in the NT and QLD. Since *Cx. quinquefasciatus* is the only mosquito species in South Australia known to be competent for GETV infections, and the minimum infective dose for transmission of the GMO through these mosquitoes (Section 4.6, Chapter 1) is far higher than the LOQ of the qPCR assay proposed to assess the viraemia in trial participants draft licence conditions are proposed to limit the study to trial sites within South Australia.

Section 3 General risk management

286. 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

287. 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

288. The proposed clinical trial will involve a maximum of 12 participants within Australia, and most dealings with the GMOs will take place in medical facilities such as clinical trial units and hospitals or PC2 certified laboratories. The applicant has proposed that the trial will be completed within 5 years of commencement. Conditions maintaining the risk context and proposed limits of the trial, such as the maximum number of trial participants and duration of the study, have been included in the draft licence.

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

290. There are proposed inclusion and exclusion criteria for both trial participants (see Section 2.3.3, Chapter 1) and staff (see Section 2.2, Chapter 1). The inclusion and exclusion criteria for trial participants would be subject to approval by a HREC, who would consider the safety of the individuals involved in the

trial. The applicant proposed that immunocompromised and pregnant individuals will be excluded from the trial. Trial participants must agree to use effective contraceptives to prevent pregnancy while undergoing GMO treatment and for at least 90 days after the last treatment with the GMO. As there are no data regarding the shedding of the GMO in sperm or vaginal secretion, draft licence conditions require trial participants to use barrier contraceptive if sexually active for at least 90 days after the last treatment with the GMO. In addition, immunocompromised and pregnant staff will be instructed to not handle the GMO. These measures were taken into consideration in the assessment of Risk scenario 1 and Risk Scenario 4 and are proposed as draft licence conditions.

291. The applicant proposed that trial participants should refrain from donating blood or organs during the clinical trial. There is limited data regarding the persistence of the GMO in organs, eggs and its presence in sperm. Therefore, draft licence conditions require trial participants to refrain from donating blood, organs, eggs or sperm for at least 90 days after the last treatment with the GMO.

292. The applicant proposed to exclude individuals with active infection and those who have received a live attenuated vaccine within 4 weeks prior to the trial from the trial. As discussed in Section 3 of Chapter 2, recombination events between different alphaviruses are rare and GETV is not present in the Australian environment so people are highly unlikely to be infected. In addition, recombination between viral strains requires both viruses to be present and replicating within the same infected host cell, and that is highly unlikely. Therefore, these measures are not proposed as draft licence conditions.

293. The applicant advised that the GMO will be administered to trial participants via i.v. infusion by clinical staff at clinical trial sites. The GMO will be prepared in a negative pressure isolator, minimising the potential for exposure to the GMO via aerosol. The applicant has also proposed that clinical staff involved in the trial will wear PPE including gown, gloves and when administering the GMO, face mask and eye protection. Any broken skin not otherwise protected by PPE or clothing, will be covered by a waterproof dressing. In addition to the use of PPE, the applicant has proposed that procedures for preparing the GMO will preclude the recapping or removal of used needles from the syringe. These practices will minimise exposure of people handling and administering the GMOs (Risk Scenario 4) and are proposed as draft licence conditions.

294. Staff collecting biological samples 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, body fluids and faeces 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 collecting samples or caring for trial participants after their treatment wear PPE if likely to be exposed to blood and/or body fluids. PPE must include long-sleeved gown and gloves. Any other hospital staff required to perform medical procedures during their stay must be advised to do likewise.

295. In the event of exposure to the GMO, personnel will be offered prompt medical attention. The applicant proposed that exposed personnel will be instructed to take precautions to protect themselves from mosquito bites for 48 h following exposure. As discussed in

296. Risk Scenario 4, the level of the GMO in the blood of people accidentally exposed to the GMO is unlikely to result in levels that could be transmitted to mosquitoes. Therefore, this measure is not included as a draft licence condition.

297. The applicant proposed that carers and close contacts would be instructed to follow hygiene measures to prevent exposure to the GMO at home via contact with blood and body fluids. As trial participants will remain in the clinical trial site during the potential viraemic period these measures are considered unnecessary and are not included as draft licence conditions.

298. Conditions are included in the draft licence requiring the licence holder to ensure that all GMOs, including material or waste that has been in contact with the GMO, within the clinical trial site, are decontaminated by autoclaving, chemical treatment or by high-temperature incineration. Draft licence

conditions require that the licence holder must ensure that the GMO, or material or waste that has been in contact with the GMO, that is to be destroyed by external service providers, is through a clinical waste stream. This is considered satisfactory, provided that the licence holder is only permitted to engage persons who can adhere to appropriate standards to conduct the dealings.

299. 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.

300. 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

301. 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 12 adult participants, which are to be conducted at clinical trial sites
- require the trial participant to remain in the hospital during the 5 days of administration of the GMO, and then until two consecutive tests results for the presence of the GMO in blood.
- ensure that behavioural requirements are communicated to trial participants and their agreement obtained
- restrict access to the GMO
- ensure personnel involved in the trial are appropriately trained and follow appropriate behavioural requirements
- ensure appropriate PPE is used by staff exposed to blood and body fluids from trial participants after they receive treatment
- requiring decontamination of the GMO and materials and equipment that have been in contact with the GMO at clinical trial sites using effective disinfectants or disposal using a certified waste contractor in accordance with standard clinical waste disposal practices, as required by the relevant Australian and state legislation;
- transport and store the GMO and samples from GMO-treated participants in accordance with IATA shipping classification UN 3373 and/or the minimum requirements for packaging and labelling as detailed in the draft licence
- ensure the clinical waste stream to be used by external service providers is able to destroy untreated GMO and GMO-related waste.

3.2 Other risk management considerations

302. 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

303. 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.

304. 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.

305. 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

306. 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

307. 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 in trial participants.

308. 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

309. 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.

310. 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.

311. 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

312. 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 defining the active viraemia after each treatment cycle with the GMO; data assessing the effects of the

GMO in pregnant animals, including pregnant sows; and data assessing the cross-reactivity of anti-RVV antibodies against the GMO.

Section 5 Conclusions of the consultation RARMP

313. The risk assessment concludes that the proposed trial with the GM Getah virus poses a negligible risk to the health and safety of people and a moderate risk to the environment as a result of gene technology. The risk management plan concludes that the identified moderate risks can be managed to protect the health and safety of people and the environment by imposing risk treatment measures.

314. If the 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 in this licence, words and phrases used in this licence have the same meaning as they do in the Act and the Gene Technology Regulations 2001;

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

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

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

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

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

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

2. In this licence:

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

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

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

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

   (a) chemical treatment;

   (b) autoclaving;

   (c) high-temperature incineration; or

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

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

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

   ‘GM’ means genetically modified.

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

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

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

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

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

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

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

'Regulator' means the Gene Technology Regulator.

'Risk group 2 organism' means an organism that satisfies the criteria in AS/NZS 2243.3:2022 for classification as Risk Group 2.

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

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

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

'Storage facility' means a third-party facility offering logistical services and distribution of clinical supplies.

Section 2 General conditions and obligations

Holder of licence

3. The licence holder is 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 this licence

6. The persons covered by this licence are:

   (a) the licence holder, and any employees, agents and External service providers engaged by the licence holder; and

   (b) the project supervisor(s); and

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

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

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

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

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

Description of GMOs covered

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

Dealings authorised by this licence

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

(a) import the GMOs;

(b) make, develop, produce or manufacture the GMOs;

(c) conduct the following experiments with the GMOs:

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

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

iii) collect Samples from trial participants;

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

(d) transport the GMOs;

(e) dispose of the GMOs;

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

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

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

Conditions imposed by the Act

Note: The Act mandates the following 3 conditions.

Informing people of licence conditions (section 63)

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

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

(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 immediately 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.

Note 4: An example of informing immediately is contact made at the time of the incident via the OGTR free call phone number 1800 181 030.

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; and

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

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

Further conditions with respect to informing persons covered by the licence

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

Note: Information required under Condition 18 may be provided to External service providers who are engaged solely for storage and transport of the GMO through labelling of the outermost container of the GMOs in accordance with Condition 38(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;

        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 and controls on clinical trials conducted under this licence

22. The GMO may be administered to a maximum of 12 trial participants.

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

24. The administration of the GMO must only be conducted in Clinical trial sites within South Australia.

25. At each treatment cycle, trial participants must remain at the Clinical trial site, as an in-patient in an individual room, during the days of administration of the GMO, and then until two consecutive negative tests results for the presence of the GMO in blood is achieved, with a minimum of 24 hours between tests.

26. The licence holder must ensure that:

    (a) the Clinical trial site room described in licence condition 25 is equipped with at least one electrical discharge insect control system (e.g. insect zapper);

    (b) trial participants leaving their clinical trial site room wear long sleeves, long pants and apply mosquito repellent to prevent mosquito bites; and

    (c) the presence of the GMO in blood of trial participants is assessed using a quantitative polymerase chain reaction (q-PCR) with a limit of quantification ≤ 5 genome copies/µL of whole blood or ≤ 10 genome copies/µL of serum.

Preparation and administration of the GMOs

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

28. The following activities must only 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).

Conditions relating to trial participants

29. 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.

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

(a) pregnant and breastfeeding women;

(b) people with an immunosuppressive disorder; and

(c) those intending to become pregnant while participating in the trial or for the first 90 days following the last treatment with the GMO.

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

(a) use barrier contraception for 90 days after each treatment with the GMO; and

(b) not donate blood, sperm, ova, tissues or organs while participating in the trial and for 90 days after their last treatment with the GMO.

Conditions related to the conduct of the dealings

32. 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.

33. 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 dealings with 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.

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

Work practices at Clinical trial sites

35. For the purposes of Condition 33, 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) immunosuppressed and pregnant individuals must be advised to not undertake any roles in the clinical trial that involve handling of the GMO. A record that this advice has been given must be kept and made available to the Regulator on request;

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

(c) persons conducting dealings with the GMO must wear personal protective equipment (PPE, including gowns, gloves and, unless working in a Class II biosafety cabinet or negative pressure pharmaceutical isolator, eye protection and a surgical facemask;

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

(e) 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;

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

(g) equipment used for dealings with the GMO must be Decontaminated after use; and

(h) 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 or faeces from the trial participant.

Transport, storage and disposal of the GMO

36. 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 International Air Transport Association (IATA) shipping classification UN 3373.

37. Transport between a Storage facility and the Clinical trial site can also be done consistent with IATA shipping classification UN 3373 if the GMO is not repackaged at the Storage facility.

38. The licence holder must ensure that transport and storage of the GMO within the Clinical trial site, transport of Samples to an Analytical facility and, unless conducted according to Condition 37, any transport between a Storage facility and a Clinical trial site, follows these sub-conditions:

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

i) that it contains GMOs;

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

iii) the contact details for the licence holder; and

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

(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 the GMO can be accounted for and that a loss of GMOs 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 or Condition 19 has been met (i.e. the GMO are within a locked unit or an area which has restricted access). This includes situations where containers are left for collection in a holding area, or left unattended prior to Decontamination;

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;

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

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

(g) for the purposes of transport entirely within a building, where the GMO are accompanied by an authorised person for whom Condition 19 has been met, Conditions 38(a)iii), 38(a)iv) and 38(c) do not apply.

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

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

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

(c) by autoclaving, chemical treatment, high-temperature incineration or any other method approved in writing by the Regulator.

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

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

Contingency plans

41. The licence holder must ensure that any person (other than a trial participant) exposed to the GMO is offered prompt medical attention. The clinician must be provided with any relevant information about the GMO.

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

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

(b) persons cleaning up the GMO must wear appropriate PPE;

(c) the exposed area must be Decontaminated with an appropriate chemical disinfectant effective against the GMO,

(d) any material used to clean up the spill or PPE worn during clean-up of the spill must be Decontaminated; and

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

Section 4 Reporting and Documentation

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

43. 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.

44. 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/storage areas/Analytical facilities;

(b) the role and contact details for 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 all reportable events including but not limited Conditions 15, 16, 45 and 46;

(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 33 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.

45. For each Clinical trial site, the licence holder must notify the Regulator, in writing, of the end of the clinical trial, no later than 30 days after:

(a) the final dose being administered; or

(b) the decision that no further participants will be treated at that site.

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

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

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

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

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

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

Attachment A

DIR No: 198

Title: Clinical trial of a genetically modified alphavirus (Getah virus) for cancer treatment

Organisation Details

Postal address: VRT Pharmaceutics Pty Ltd
99 Cook Street
Forestville
New South Wales, 2087

GMO Description

GMOs covered by this licence:

Parent Organisms:

Common Name: Getah virus

Scientific Name: *Getah virus* (M1 strain)

Modified traits:

Categories: Human therapeutic

Description: The GMO, known as M1-c6v1, is a live Getah virus treatment derived from the M1 strain, modified to enhance its ability to replicate in cancer cells.

Table 1. Nucleic acid responsible for conferring the modified traits

Genetic modifications	
Source, identity, nature of modification	**Modified trait description**
• Introduction of a single point mutation to [redacted] gene • Introduction of a single point mutation to [redacted] gene	Enhance viral replication in cancer cells

Purpose of the dealings with the GMO:

To conduct clinical trials assessing the safety, tolerability and efficacy of a genetically modified Getah virus in patients with locally advanced or metastatic solid cancer.

Trial participants and route of administration of the GMO

Intravenous administration to adult humans with locally advanced or metastatic solid cancer.

Confidential commercial information (CCI)

Details of the two point mutations and the genes in which they are located were declared CCI under Section 185 of the *Gene Technology Act 2000*.

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 role and contact details for 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 all reportable events including but not limited to Condition 15, 16, 45 and 46. (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; (i) how compliance with Condition 33 will be achieved in relation to preparation of participant Samples for analysis subsequent to administering the GMO	44	At least 14 days prior to the first administration of the GMO at each Clinical trial site, or a timeframe agreed to in writing by the Regulator
Information to be provided at any time during the clinical trial	**Condition**	**Timeframe for reporting**
Any additional information related to the health and safety of people and the environment associated with the dealings covered by the licence, or any unintended effects of the dealings authorised by the licence	15(a), (c)	Immediately
Information related to any contravention of the licence by a person covered by the licence	15(b)	Immediately
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	45(a)	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	46(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	46(b), (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	46(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	38(f)	Within a timeframe stipulated by the Regulator
Any signed records or documentation collected under a condition of this licence	47	Within a timeframe stipulated by the Regulator

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

References

Aaskov, J.G., Mataika, J.U., Lawrence, G.W., Rabukawaqa, V., Tucker, M.M., Miles, J.A., and Dalglish, D.A. (1981). An epidemic of Ross River virus infection in Fiji, 1979. Am J Trop Med Hyg *30*, 1053-1059.

Aleck, K.A., Rosen, L., Pettitt, D.J., Boveington, C., and Bennett, P.H. (1983). Absence of intrauterine infection following Ross River virus infection during pregnancy. Am J Trop Med Hyg *32*, 618-620.

Animal Health Australia (2018a). AUSVETPLAN - Response Policy Briefs (Version 3.6) Australian Veterinary Emergency Plan (AUSVETPLAN) (Canberra, ACT: National Biosecurity Committee).

Animal Health Australia (2018b). Response policy briefs (Version 3.6). Australian Veterinary Emergency Plan (AUSVETPLAN). (Canberra, ACT).

Animal Health Australia (2021). Animal Health in Australia Anuual Report 2019-2020. (Canberra, Australia).

Animal Health Australia (2022). Government and live stock indutry cost sharing deed

Anishchenko, M., Bowen, R.A., Paessler, S., Austgen, L., Greene, I.P., and Weaver, S.C. (2006). Venezuelan encephalitis emergence mediated by a phylogenetically predicted viral mutation. Proc Natl Acad Sci U S A *103*, 4994-4999.

Asai, T., Shibata, I., and Uruno, K. (1991). Susceptibility of pregnant hamster, guinea pig, and rabbit to the transplacental infection of Getah virus. J Vet Med Sci *53*, 1109-1111.

Aubry, M., Finke, J., Teissier, A., Roche, C., Broult, J., Paulous, S., Despres, P.*, et al.* (2015). Silent Circulation of Ross River Virus in French Polynesia. Int J Infect Dis *37*, 19-24.

Australian Pork (2022). Industry facts.

Azerigyik, F.A., Faizah, A.N., Kobayashi, D., Amoa-Bosompem, M., Matsumura, R., Kai, I., Sasaki, T.*, et al.* (2023). Evaluating the mosquito host range of Getah virus and the vector competence of selected medically important mosquitoes in Getah virus transmission. Parasit Vectors *16*, 99.

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

Bannai, H., Nemoto, M., Niwa, H., Murakami, S., Tsujimura, K., Yamanaka, T., and Kondo, T. (2017). Geospatial and temporal associations of Getah virus circulation among pigs and horses around the perimeter of outbreaks in Japanese racehorses in 2014 and 2015. BMC Vet Res *13*, 1-8.

Bannai, H., Nemoto, M., Ochi, A., Kikuchi, T., Kobayashi, M., Tsujimura, K., Yamanaka, T.*, et al.* (2015). Epizootiological Investigation of Getah Virus Infection among Racehorses in Japan in 2014. J Clin Microbiol *53*, 2286-2291.

Bannai, H., Ochi, A., Nemoto, M., Tsujimura, K., Yamanaka, T., and Kondo, T. (2016). A 2015 outbreak of Getah virus infection occurring among Japanese racehorses sequentially to an outbreak in 2014 at the same site. BMC Vet Res *12*, 1-7.

Beebe, N.W., Ambrose, L., Hill, L.A., Davis, J.B., Hapgood, G., Cooper, R.D., Russell, R.C.*, et al.* (2013). Tracing the tiger: population genetics provides valuable insights into the Aedes (Stegomyia) albopictus invasion of the Australasian Region. PLoS Negl Trop Dis *7*, e2361.

Belarbi, E., Legros, V., Basset, J., Despres, P., Roques, P., and Choumet, V. (2019). Bioluminescent Ross River Virus Allows Live Monitoring of Acute and Long-Term Alphaviral Infection by In Vivo Imaging. Viruses *11*, 1-17.

Bick, M.J., Carroll, J.W., Gao, G., Goff, S.P., Rice, C.M., and MacDonald, M.R. (2003). Expression of the zinc-finger antiviral protein inhibits alphavirus replication. J Virol *77*, 11555-11562.

Boyd, A.M., Hall, R.A., Gemmell, R.T., and Kay, B.H. (2001). Experimental infection of Australian brushtail possums, Trichosurus vulpecula (Phalangeridae: Marsupialia), with Ross River and Barmah Forest viruses by use of a natural mosquito vector system. Am J Trop Med Hyg *65*, 777-782.

Brown, C.M., and Timoney, P.J. (1998). Getah virus infection of Indian horses. Trop Anim Health Prod *30*, 241-252.

Campos, G.S., Albuquerque Bandeira, A.C., Diniz Rocha, V.F., Dias, J.P., Carvalho, R.H., and Sardi, S.I. (2017). First Detection of Chikungunya Virus in Breast Milk. Pediatr Infect Dis J *36*, 1015-1017.

CDC (2022). Mosquitoes: Where Mosquitoes Live. Accessed: 16/05/2023.

Centers for Disease Control and Prevention (2020). Section VIII-F: Arboviruses and related zoonotic viruses. In Biosafety in Microbiological and Biomedical Laboratories (BMBL) (United States Department of Health and Human Services,).

Chapman, A.D. (2009). Numbers of Living Species in Australia and the World, 2 edn (Toowoomba, Australia).

Chen, R., Mukhopadhyay, S., Merits, A., Bolling, B., Nasar, F., Coffey, L.L., Powers, A.*, et al.* (2018). ICTV Virus Taxonomy Profile: Togaviridae. J Gen Virol *99*, 761-762.

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

Colón-González, F.J., Sewe, M.O., Tompkins,A.M., Sjödin,H., Casallas, A., Rocklöv, J., Caminade, C. Lowe, R. (2021). Projecting the risk of mosquito-borne diseases in a warmer and more populated world: a multi-model, multi-scenario intercomparison modelling study. Lancet Planet Health *5*, e404–414.

CSIRO (2020). Draft assessment report for Aedes albopictus (Asian Tiger Mosquito), CSIRO, ed.

DAFF (2019). National list of notifiable animal diseases. Accessed: 12/05/2023.

Dalgleish, M. (2021). Australian Pork - State of the Industry Report 2021

Department of Agriculture Water and the Environment (2022). Financial performance of livestock farms 2019–20 to 2021–22. Accessed: 16/05/2023.

Department of Health SA (2006). South Australian Integrated Mosquito Management Resource Package 2006, D.o.H. Environmental Health Service, ed. (Adelaide SA: Environmental Health Service, South Australian Department of Health), pp. 1-40.

Department of Health SA (2021). South Australian Arbovirus and Mosquito Monitoring and Control Annual Report 2020-2021.

Department of Health SA (2023). Arbovirus and mosquito monitoring reports. Accessed: 12/05/2023.

Department of Health WA (2023). Common Mosquitoes in Western Australia. Accessed: 13/07/2023.

Desgraupes, S., Hubert, M., Gessain, A., Ceccaldi, P.E., and Vidy, A. (2021). Mother-to-Child Transmission of Arboviruses during Breastfeeding: From Epidemiology to Cellular Mechanisms. Viruses *13*, 1-25.

Dhama, K., Kapoor, S., Pawaiya, R.V., Chakraborty, S., Tiwari, R., and Verma, A.K. (2014). Ross River Virus (RRV) infection in horses and humans: a review. Pak J Biol Sci *17*, 768-779.

Doherty, R.L., Carley, J.G., Mackerras, M.J., and Marks, E.N. (1963). Studies of arthropod-borne virus infections in Queensland. III. Isolation and characterization of virus strains from wild-caught mosquitoes in North Queensland. Aust J Exp Biol Med Sci *41*, 17-39.

Drake, J.W., and Holland, J.J. (1999). Mutation rates among RNA viruses. Proc Natl Acad Sci U S A *96*, 13910-13913.

Fang, Y., Zhang, W., Xue, J.B., and Zhang, Y. (2021). Monitoring Mosquito-Borne Arbovirus in Various Insect Regions in China in 2018. Front Cell Infect Microbiol *11*, 640993.

Feng, Y., Fu, S., Zhang, H., Li, M., Zhou, T., Wang, J., Zhang, Y*., et al.* (2012). Distribution of mosquitoes and mosquito-borne viruses along the China-Myanmar border in Yunnan Province. Jpn J Infect Dis *65*, 215-221.

Feral pig scan (2023).

Forrester, N.L., Palacios, G., Tesh, R.B., Savji, N., Guzman, H., Sherman, M., Weaver, S.C*., et al.* (2012). Genome-scale phylogeny of the alphavirus genus suggests a marine origin. J Virol *86*, 2729-2738.

Fukunaga, Y., Ando, Y., Kamada, M., Imagawa, H., Wada, R., Kumanomido, T., Akzyama, Y*., et al.* (1981). An Outbreak of Getah Virus Infection in Horses: Clinical and Epizootiological Aspects at the Miho Training Center in 1978. Bull Equine Res Inst *18*, 94-102.

Fukunaga, Y., Kumanomido, T., and Kamada, M. (2000). Getah Virus as an Equine Pathogen. Veterinary Clinics of North America: Equine Practice *16*, 605-617.

Garcia-Sastre, A. (2017). Ten Strategies of Interferon Evasion by Viruses. Cell Host Microbe *22*, 176-184.

Goncalves-Carneiro, D., Takata, M.A., Ong, H., Shilton, A., and Bieniasz, P.D. (2021). Origin and evolution of the zinc finger antiviral protein. PLoS Pathog *17*, e1009545.

Gotte, B., Liu, L., and McInerney, G.M. (2018). The Enigmatic Alphavirus Non-Structural Protein 3 (nsP3) Revealing Its Secrets at Last. Viruses *10*, 1-26.

Government of Canada (2012). Pathogen Safety Data Sheets: Infectious Substances – Eastern equine encephalitis (EEEV), Western equine encephalitis (WEEV).

Guernsey, M.W., Chuong, E.B., Cornelis, G., Renfree, M.B., and Baker, J.C. (2017). Molecular conservation of marsupial and eutherian placentation and lactation. Elife *6*, 1-19.

Hahn, C.S., Lustig, S., Strauss, E.G., and Strauss, J.H. (1988). Western equine encephalitis virus is a recombinant virus. Proc Natl Acad Sci U S A *85*, 5997-6001.

Haist, K.C., Burrack, K.S., Davenport, B.J., and Morrison, T.E. (2017). Inflammatory monocytes mediate control of acute alphavirus infection in mice. PLoS Pathog *13*, e1006748.

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

Hodgson, J. (2002). Equine exotic diseases. A manual for horse owners. (Canberra, Australia).

Hu, J., Cai, X.F., and Yan, G. (2009). Alphavirus M1 induces apoptosis of malignant glioma cells via downregulation and nucleolar translocation of p21WAF1/CIP1 protein. Cell Cycle *8*, 3328-3339.

ICH (2016). Integrated addendum to ICH E6(R1): Guideline for good clinical practice. Report No. E6(R2), available online https://database.ich.org/sites/default/files/E6_R2_Addendum.pdf.

Izumida, A., Takuma, H., Inagaki, S., Kubota, M., Hirahara, T., Kodama, K., and Sasaki, N. (1988). Experimental infection of Getah virus in swine. Japanese Journal of Veterinary Science *50*, 679-684.

Jacob R.H., S.V.S.M., Beatty D.T., Gardner G.E., Warner R.D. (2004). Grain feeding increases core body temperature of beef cattle. Animal Production Science *54*, 444-449.

Kamada, M., Ando, Y., Fukunaga, Y., Kumanomido, T., Imagawa, H., Wada, R., and Akiyama, Y. (1980). Equine Getah virus infection: isolation of the virus from racehorses during an enzootic in Japan. Am J Trop Med Hyg *29*, 984-988.

Kamada, M., Kumanomido, T., Wada, R., Fukunaga, Y., Imagawa, H., and Sugiura, T. (1991a). Intranasal infection of Getah virus in experimental horses. J Vet Med Sci *53*, 855-858.

Kamada, M., Wada, R., Kumanomido, T., Imagawa, H., Sugiura, T., and Fukunaga, Y. (1991b). Effect of viral inoculum size on appearance of clinical signs in equine Getah virus infection. J Vet Med Sci *53*, 803-806.

Kamada, M.A., Y.; Fukunaga, Y.; Imagawa, H.; Wada, R.; Kumanomido, T.; Tabuchi, E.; Hirasawa, K.; Akiyama, Y. (1981). Studies on Getah virus - Pathogenicity of the virus for horses. Bulletin of Equine Research Institute *18*, 84-93.

Kawamura, H., Yago, K., Narita, M., Imada, T., Nishimori, T., and Haritani, M. (1987). A fatal case in newborn piglets with Getah virus infection: pathogenicity of the isolate. Nihon Juigaku Zasshi *49*, 1003-1007.

Kizu, J., Neuman, C., Le Grand, L., and Liu, W. (2019). Discovery of Cocirculating Ross River Virus and Barmah Forest Virus At Wide Bay Military Training Area, Northeastern Australia. J Am Mosq Control Assoc *35*, 220-223.

Knope, K., Doggett, S.L., Jansen, C.C., Johansen, C.A., Kurucz, N., Feldman, R., Lynch, S.E*., et al.* (2019). Arboviral diseases and malaria in Australia, 2014-15: Annual report of the National Arbovirus and Malaria Advisory Committee. Report No. 2209-6051 (Electronic)

2209-6051 (Linking).

Kobayashi, D., Isawa, H., Ejiri, H., Sasaki, T., Sunahara, T., Futami, K., Tsuda, Y*., et al.* (2016). Complete Genome Sequencing and Phylogenetic Analysis of a Getah Virus Strain (Genus Alphavirus, Family Togaviridae) Isolated from Culex tritaeniorhynchus Mosquitoes in Nagasaki, Japan in 2012. Vector Borne Zoonotic Dis *16*, 769-776.

Kumanomido, T., Fukunaga, Y., Ando, Y., Kamada, M., Imagawa, H., Wada, R., Akiyama, Y*., et al.* (1982). Ecological Survey on Getah Virus among Swine in Japan. Bull Equine Res Inst *19*, 89-92.

Kumanomido, T., Wada, R., Kanemaru, T., Kamada, M., Akiyama, Y., and Matumoto, M. (1988a). Transplacental infection in mice inoculated with Getah virus. Vet Microbiol *16*, 129-136.

Kumanomido, T., Wada, R., Kanemaru, T., Kamada, M., Hirasawa, K., and Akiyama, Y. (1988b). Clinical and virological observations on swine experimentally infected with Getah virus. Vet Microbiol *16*, 295-301.

Kuwata, R., Shimoda, H., Phichitraslip, T., Prasertsincharoen, N., Noguchi, K., Yonemitsu, K., Minami, S.*, et al.* (2018). Getah virus epizootic among wild boars in Japan around 2012. Arch Virol *163*, 2817-2821.

L'vov, S.D., Gromashevski'i, V.L., Aristova, V.A., Morozova, T.N., Skvortsova, T.M., Gushchina, E.A., Petrova, E.S.*, et al.* (2000). [Isolation of Getah virus (Togaviridae, Alfavirus) strains in North- Eastern Asia]. Vopr Virusol *45*, 14-18.

Lau, C., Aubry, M., Musso, D., Teissier, A., Paulous, S., Despres, P., de-Lamballerie, X.*, et al.* (2017). New evidence for endemic circulation of Ross River virus in the Pacific Islands and the potential for emergence. Int J Infect Dis *57*, 73-76.

Lessard, B.D., Kurucz, N., Rodriguez, J., Carter, J., and Hardy, C.M. (2021). Detection of the Japanese encephalitis vector mosquito Culex tritaeniorhynchus in Australia using molecular diagnostics and morphology. Parasit Vectors *14*, 1-11.

Li, B., Wang, H., and Liang, G. (2022). Getah Virus (Alphavirus): An Emerging, Spreading Zoonotic Virus. Pathogens *11*.

Li, M.H., Fu, S.H., Feng, Y., Ye, X.F., Zhao, S.Y., Liu, C.T., and Liang, G.D. (2012). Investigation of arboviruses in different regions of Guizhou province, China in 2008. Chin J Vector Biol Control *23*, 417–420.

Li, X.D., Qiu, F.X., Yang, H., Rao, Y.N., and Calisher, C.H. (1992). Isolation of Getah virus from mosquitos collected on Hainan Island, China, and results of a serosurvey. The Southeast Asian Journal of Tropical Medicine and Public Health *23*, 730-734.

Li, Y., Fu, S., Guo, X., Li, X., Li, M., Wang, L., Gao, X.*, et al.* (2019). Serological Survey of Getah Virus in Domestic Animals in Yunnan Province, China. Vector Borne Zoonotic Dis *19*, 59-61.

Li, Y.Y., Fu, S.H., Guo, X.F., Lei, W.W., Li, X.L., Song, J.D., Cao, L.*, et al.* (2017a). Identification of a Newly Isolated Getah Virus in the China-Laos Border, China. Biomed Environ Sci *30*, 210-214.

Li, Y.Y., Liu, H., Fu, S.H., Li, X.L., Guo, X.F., Li, M.H., Feng, Y.*, et al.* (2017b). From discovery to spread: The evolution and phylogeny of Getah virus. Infect Genet Evol *55*, 48-55.

Lim, E.X.Y., Lee, W.S., Madzokere, E.T., and Herrero, L.J. (2018). Mosquitoes as Suitable Vectors for Alphaviruses. Viruses *10*, 2-17.

Lin, Y., Zhang, H., Liang, J., Li, K., Zhu, W., Fu, L., Wang, F.*, et al.* (2014). Identification and characterization of alphavirus M1 as a selective oncolytic virus targeting ZAP-defective human cancers. Proceedings of the National Academy of Sciences *111*, E4504-E4512.

Liu, H., Zhang, X., Li, L.X., Shi, N., Sun, X.T., Liu, Q., Jin, N.Y.*, et al.* (2019). First isolation and characterization of Getah virus from cattle in northeastern China. BMC Vet Res *15*, 1-7.

Liu, J., Hansen, A., Cameron, S., and Bi, P. (2020). The geography of Ross River virus infection in South Australia, 2000-2013. Commun Dis Intell (2018) *44*, 1-15.

Liu, J., Hansen, A., Cameron, S., Williams, C., Fricker, S., and Bi, P. (2021). Using ecological variables to predict Ross River virus disease incidence in South Australia. Trans R Soc Trop Med Hyg *115*, 1045-1053.

Lu, G., Chen, R., Shao, R., Dong, N., Liu, W., and Li, S. (2020). Getah virus: An increasing threat in China. J Infect *80*, 350-371.

Lu, G., Ou, J., Ji, J., Ren, Z., Hu, X., Wang, C., and Li, S. (2019). Emergence of Getah Virus Infection in Horse With Fever in China, 2018. Front Microbiol *10*, 1-9.

Mair, T.S., and Timoney, P.J. (2009). Getah Virus Infection. In Infectious Diseases of the Horse, T.S. Mair, and R.E. Hutchinson, eds. (Fordham, Cambridgeshire, UK: Equine Veterinary Journal Ltd.), pp. 155-158.

Matsuyama, T., Nakamura, T., Isahai, K., Oya, A., and Kobayashi, M. (1967). [Haruna virus, a group A arbovirus isolated from swine in Japan]. Gunma J Med Sci *16*, 131-134.

Matsuyama, T., Oya, A., Ogata, T., Kobayashi, I., Nakamura, T., Takahashi, M., and Kitaoka, M. (1960). Isolation of arbor viruses from mosquitoes collected at live-stock pens in Gumma Prefecture in 1959. Jpn J Med Sci Biol *13*, 191-198.

Michie, A., Dhanasekaran, V., Lindsay, M.D.A., Neville, P.J., Nicholson, J., Jardine, A., Mackenzie, J.S*., et al.* (2020). Genome-Scale Phylogeny and Evolutionary Analysis of Ross River Virus Reveals Periodic Sweeps of Lineage Dominance in Western Australia, 1977-2014. J Virol *94*.

Milner, A.R., and Marshall, I.D. (1984). Pathogenesis of in utero infections with abortogenic and non-abortogenic alphaviruses in mice. J Virol *50*, 66-72.

National Feral Pig Action Plan (2023). South Australia. Accessed: 16/05/2023.

National Health and Medical Research Council (2019). Australian Guidelines for the Prevention and Control of Infection in Healthcare. (Canberra: Commonwealth of Australia, available online https://www.nhmrc.gov.au/about-us/publications/australian-guidelines-prevention-and-control-infection-healthcare-2019#block-views-block-file-attachments-content-block-1).

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

Nemoto, M., Bannai, H., Tsujimura, K., Kobayashi, M., Kikuchi, T., Yamanaka, T., and Kondo, T. (2015). Getah Virus Infection among Racehorses, Japan, 2014. Emerg Infect Dis *21*, 883-885.

NHMRC (2019). Australian Guidelines for the Prevention and Control of Infection in Healthcare (2019).

Nowee, G., Bakker, J.W., Geertsema, C., Ros, V.I.D., Göertz, G.P., Fros, J.J., and Pijlman, G.P. (2021). A Tale of 20 Alphaviruses; Inter-species Diversity and Conserved Interactions Between Viral Non-structural Protein 3 and Stress Granule Proteins. Frontiers in Cell and Developmental Biology *9*, 1-13.

NSW Department of Primary Industries (2006). Basic pig husbandry – the litter. Accessed: 05/05/2023.

NSW Department of Primary Industries (2023). Taking a horse's temperature. Accessed: 04/05/2023.

OGTR (2013). Risk Analysis Framework 2013, 4th edn (Canberra: Office of the Gene Technology Regulator, available online: http://www.ogtr.gov.au/internet/ogtr/publishing.nsf/Content/raffinal5-toc).

Okada, Vaeteewoottacharn, and Kariya (2019). Application of Highly Immunocompromised Mice for the Establishment of Patient-Derived Xenograft (PDX) Models. Cells *8*, 1-18.

Petterson, E., Guo, T.C., Evensen, O., and Mikalsen, A.B. (2016). Experimental piscine alphavirus RNA recombination in vivo yields both viable virus and defective viral RNA. Sci Rep *6*, 36317.

Poh, C.M., Chan, Y.H., and Ng, L.F.P. (2020). Role of T Cells in Chikungunya Virus Infection and Utilizing Their Potential in Anti-Viral Immunity. Front Immunol *11*, 1-8.

Public Health Agency of Canada (2010). Pathogen Safety Data Sheets: Infectious Substances – Chikungunya virus. Accessed: 02/05/2023.

Public Health Agency of Canada (2011). Pathogen Safety Data Sheets: Infectious Substances – Ross river virus. Accessed: 02/05/2023.

Qian, W., Harley, D., Glass, K., Viennet, E., and Hurst, C. (2022). Prediction of Ross River virus incidence in Queensland, Australia: building and comparing models. PeerJ *10*, e14213.

Queensland Government (2023). Feral pig. Accessed: 12/05/2023.

Rangel, M.V., and Stapleford, K.A. (2021). Alphavirus Virulence Determinants. Pathogens *10*, 1-17.

Rawle, D.J., Nguyen, W., Dumenil, T., Parry, R., Warrilow, D., Tang, B., Le, T.T*., et al.* (2020a). Sequencing of Historical Isolates, K-mer Mining and High Serological Cross-Reactivity with Ross River Virus Argue against the Presence of Getah Virus in Australia. Pathogens *9*.

Rawle, D.J., Nguyen, W., Dumenil, T., Parry, R., Warrilow, D., Tang, B., Le, T.T*., et al.* (2020b). Sequencing of Historical Isolates, K-mer Mining and High Serological Cross-Reactivity with Ross River Virus Argue against the Presence of Getah Virus in Australia. Pathogens *9*, 1-17.

Regional Development Australia (BGLAP) (2023). Equine Industry Accessed: 23/05/2023.

Regions, S.D.o.P.I.a. (2023). Livestock, wool and dairy. Accessed: 16/05/2023.

Ren, T., Mo, Q., Wang, Y., Wang, H., Nong, Z., Wang, J., Niu, C*., et al.* (2020). Emergence and Phylogenetic Analysis of a Getah Virus Isolated in Southern China. Front Vet Sci *7*, 1-8.

Rozen-Gagnon, K., Stapleford, K.A., Mongelli, V., Blanc, H., Failloux, A.B., Saleh, M.C., and Vignuzzi, M. (2014). Alphavirus mutator variants present host-specific defects and attenuation in mammalian and insect models. PLoS Pathog *10*, e1003877.

Russell, R.C., Williams, C.R., Sutherst, R.W., and Ritchie, S.A. (2005). Aedes (Stegomyia) albopictus--a dengue threat for southern Australia? Commun Dis Intell Q Rep *29*, 296-298.

Sam, S.S., Mohamed-Romai-Noor, N.A., Teoh, B.T., Hamim, Z.R., Ng, H.Y., Abd-Jamil, J., Khor, C.S*., et al.* (2022). Group IV Getah Virus in Culex Mosquitoes, Malaysia. Emerg Infect Dis *28*, 475-477.

Scherer, W.F. (1984). Getah. In: The International Catalog of Arboviruses Including Certain Other Viruses of Vertebrates, The Centers for Disease Control and Prevention (CDC), ed. (Atlanta, GA: The Centers for Disease Control and Prevention (CDC),).

Scherer, W.F., Funkenbusch, M., Buescher, E.L., and Izumit (1962). Sagiyama virus, a new group A arthropod-borne virus from Japan. I. Isolation, immunologic classification, and ecologic observations. Am J Trop Med Hyg *11*, 255-268.

Schulte, T., Liu, L., Panas, M.D., Thaa, B., Dickson, N., Gotte, B., Achour, A*., et al.* (2016). Combined structural, biochemical and cellular evidence demonstrates that both FGDF motifs in alphavirus nsP3 are required for efficient replication. Open Biol *6*, 1-15.

Sentsui, H., and Kono, Y. (1980). An epidemic of Getah virus infection among racehorses: isolation of the virus. Res Vet Sci *29*, 157-161.

Sentsui, H., and Kono, Y. (1981). Pathogenicity of Getah virus for mice. Natl Inst Anim Health Q (Tokyo) *21*, 7-13.

Shi, N., Li, L.X., Lu, R.G., Yan, X.J., and Liu, H. (2019). Highly Pathogenic Swine Getah Virus in Blue Foxes, Eastern China, 2017. Emerg Infect Dis *25*, 1252-1254.

Shibata, I., Hatano, Y., Nishimura, M., Suzuki, G., and Inaba, Y. (1991). Isolation of Getah virus from dead fetuses extracted from a naturally infected sow in Japan. Vet Microbiol *27*, 385-391.

Shirako, Y., and Yamaguchi, Y. (2000). Genome structure of Sagiyama virus and its relatedness to other alphaviruses. J Gen Virol *81*, 1353-1360.

Skinner, E.B., Murphy, A., Jansen, C.C., Shivas, M.A., McCallum, H., Onn, M.B., Reid, S.A.*, et al.* (2020). Associations Between Ross River Virus Infection in Humans and Vector-Vertebrate Community Ecology in Brisbane, Australia. Vector Borne Zoonotic Dis *20*, 680-691.

Standards Australia/New Zealand (2022). Safety in laboratories, Part 3: Microbiological safety and containment AS/NZS 2243.3:2022, Seventh edition edn (Standards Australia/New Zealand).

Stephenson, E.B., Peel, A.J., Reid, S.A., Jansen, C.C., and McCallum, H. (2018). The non-human reservoirs of Ross River virus: a systematic review of the evidence. Parasit Vectors *11*, 1-13.

Strauss, J.H., and Strauss, E.G. (1994). The alphaviruses: gene expression, replication, and evolution. Microbiol Rev *58*, 491-562.

Sugiyama, I., Shimizu, E., Nogami, S., Suzuki, K., Miura, Y., and Sentsui, H. (2009). Serological survey of arthropod-borne viruses among wild boars in Japan. J Vet Med Sci *71*, 1059-1061.

Takashima, I., and Hashimoto, N. (1985). Getah virus in several species of mosquitoes. Trans R Soc Trop Med Hyg *79*, 546-550.

Timoney, P.J. (2017). Getah Virus: Significance as an Equine Pathogen. In Equine Disease Quarterly (Department of Veterinary Science, University of Kentucky College of Agriculture, Food and Environment).

Togami, E., Gyawali, N., Ong, O., Kama, M., Cao-Lormeau, V.M., Aubry, M., Ko, A.I.*, et al.* (2020). First evidence of concurrent enzootic and endemic transmission of Ross River virus in the absence of marsupial reservoirs in Fiji. Int J Infect Dis *96*, 94-96.

US Food and Drug Administration (2015). Design and analysis of shedding studies for virus or bacteria-based gene therapy and oncolytic products - Guidance for industry. Report No. FDA-2014-D-0852. (Silver Spring, MD: US Departmetn of Health and Human Services).

Verdonschot, P.F.M., Besse-Lototskaya, A.A. (2014). Flight distance of mosquitoes (Culicidae): A metadata analysis to support the management of barrier zones around rewetted and newly constructed wetlands. Limnologica *45*, 69-79.

Wang, A., Zhou, F., Liu, C., Gao, D., Qi, R., Yin, Y., Liu, S.*, et al.* (2021). Structure of infective Getah Virus at 2.8 A-resolution determined by cryo-EM. bioRxiv preprint.

Wang, H.Q., Liu, W.B., Yang, D.R., Liang, Y., Wang, J.W., Zhang, L.S., Liu, J.W.*, et al.* (2006). [Isolation and identification of arboviruses in Hebei Province]. Zhonghua Shi Yan He Lin Chuang Bing Du Xue Za Zhi *20*, 52-55.

Weiss, B.G., and Schlesinger, S. (1991). Recombination between Sindbis virus RNAs. J Virol *65*, 4017-4025.

Wen, J.-S., Zhao, W.-Z., Liu, J.-W., Zhou, H., Tao, J.-P., Yan, H.-J., Liang, Y.*, et al.* (2007). Genomic analysis of a Chinese isolate of Getah-like virus and its phylogenetic relationship with other Alphaviruses. Virus Genes *35*, 597-603.

Whelan, P., Hayes, G., Carter, J., Wilson, A., and Haigh, B. (2000). Detection of the exotic mosquito Culex gelidus in the Northern Territory. Commun Dis Intell *24 Suppl*, 74-75.

Wildlife Health Australia (2015). Ross River virus in Australian wildlife.

World Health Organisation (2021). Guideline: infant feeding in areas of Zika virus transmission, second edition. (Geneva: World Health Organisation,).

Xing, C., Jiang, J., Lu, Z., Mi, S., He, B., Tu, C., Liu, X*., et al.* (2020). Isolation and characterization of Getah virus from pigs in Guangdong province of China. Transbound Emerg Dis, 1-5.

Yago, K., Hagiwara, S., Kawamura, H., and Narita, M. (1987). A fatal case in newborn piglets with Getah virus infection: isolation of the virus. Nihon Juigaku Zasshi *49*, 989-994.

Yang, T., Li, R., Hu, Y., Yang, L., Zhao, D., Du, L., Li, J*., et al.* (2018). An outbreak of Getah virus infection among pigs in China, 2017. Transbound Emerg Dis *65*, 632-637.

Zhai, Y.-g., Wang, H.-Y., Sun, X.-h., Fu, S.-h., Wang, H.-q., Attoui, H., Tang, Q*., et al.* (2008). Complete sequence characterization of isolates of Getah virus (genus Alphavirus, family Togaviridae) from China. Journal of General Virology *89*, 1446-1456.

Zhang, B., Goraya, M.U., Chen, N., Xu, L., Hong, Y., Zhu, M., and Chen, J.L. (2020). Zinc Finger CCCH-Type Antiviral Protein 1 Restricts the Viral Replication by Positively Regulating Type I Interferon Response. Front Microbiol *11*, 1-14.

Zhang, J., Liu, Y., Tan, J., Zhang, Y., Wong, C.-W., Lin, Z., Liu, X*., et al.* (2021). Necroptotic virotherapy of oncolytic alphavirus M1 cooperated with Doxorubicin displays promising therapeutic efficacy in TNBC. Oncogene *40*, 4783-4795.

Zhou, F., Cui, D., Wang, A., Wang, X., Chang, H., Chen, L., Li, Y*., et al.* (2018). [Isolation and identification of the first getah virus (GETV) strain HNJZ-S1 from clinically suspected PRRS case of pig herd in Henan Province, China]. Chinese Journal of Virology *34*, 59-66.

Zhou, F., Wang, A., Chen, L., Wang, X., Cui, D., Chang, H., and Wang, C. (2020). Isolation and phylogenetic analysis of Getah virus from a commercial modified live vaccine against porcine reproductive and respiratory syndrome virus. Mol Cell Probes *53*, 1-5.

Zhou, X.F., Feng, Z.L., Hu, T.S., Qiu, W., Fan, Q.S., Li, L.J., and Zhang, F.Q. (2012). [Isolation and identification of Getah virus from Aedes albopictus in Yunnan]. Chin J Heal Lab Tech *22*, 103-106.

Appendix A. Summary of advice from experts

Expert	Summary of advice provided	Comment
1	Advised that:	
	• reversion of mutations only occurs if the virus undergoes multiple cycles of replication. However, humans are not known as amplifying hosts for GETV. It is unlikely that the GM virus will undergo several replication cycles in the trial participant.	Mutation and recombination of GETV and other alphaviruses are discussed in Chapter 1, Section 3.9 and Risk scenario 2 of the consultation RARMP.
	• transmission of alphaviruses by mosquitoes occurs when viremia is over 10^7 viral particles/mL of blood. As the virus is not expected to replicate in humans, it is unlikely that trial participants would develop such high levels of viraemia.	Transmission of GETV via mosquito bites in Chapter 1, Section 3.6. of the consultation RARMP. This information is also discussed in the Risk Scenarios 1, 2 and 3.
	• horses and cattle can act as amplifying hosts and develop high levels of viraemia when infected with WT alphaviruses. However, this is not expected to occur as the GM virus is less fit and unlikely to be transmitted from trial participants to animals via mosquito bites.	The effects of WT GETV and the GMO in horses and cattle is discusses in Section 3.7 and Section 4.4 of Chapter 1 of the consultation RARMP.
	• severe disease is caused when the virus reaches the brain of animals and that usually occur prior to maturation of the blood-brain barrier in foetal and neonatal piglets and experimentally infected mice. In the event of infection, virulence would be expected to be more severe in young animals.	The applicant has provided data regarding the effects of the GMO in 7- and 18-day old piglets. This information is discussed in Section 4.4 of Chapter 1 of the consultation RARMP.
	• Ross River virus (RRV) a closely related alphavirus is present in Australia. Most Australian animals, including marsupials, have been exposed to RRV and would have antibodies likely to cross-react with Getah virus.	The presence of RRV in Australia is included in Chapter 1, Section 5.3.1. The potential cross-reactivity of RRV antibodies with the GMO is further discussed in Chapter 2, Section 3 of the consultation RARMP.
	• recombination between the GM virus and RRV is unlikely to occur. Recombination only occurs if both viruses are present in the same cell at the same time. In addition,	As before, mutation and recombination of GETV and other alphaviruses are discussed in Chapter

Expert	Summary of advice provided	Comment
	recombination between alphaviruses is rare.	1, Section 3.9 and Risk scenario 2 of the consultation RARMP.
	• at least one mosquito species present in North Territory is competent for the transmission of Getah virus. Potentially to further reduce the risk of transmission of the virus by mosquito, the trial could be conducted in regions where the competent mosquitoes are not present.	Noted
2	• Raised concerns regarding:	
	• the potential harm to native wildlife as Getah virus is not endemic to Australia.	The potential harm to native animals posed by this proposed trial is considered is in Risk scenario 2 (Section 3, Chapter 2) of the consultation RARMP.
	• the potential for recombination between the GMO and other alphaviruses present in the environment (i.e. Ross River Virus (RVV)) resulting in a novel virus introduced in Australia.	Recombination between the GMO and other alphaviruses is considered in Risk scenario 2 (Section 3, Chapter 2) of the consultation RARMP.
	• the origin of the two mutations in the GMO and how does it compare to the original WT strain.	The origin of the two mutations inserted in the GMO is discussed in Section 4.1, Chapter 1. Some information about the genetic modifications have been declared as Confidential Commercial Information (CCI) under Section 185 of the Act.
	• the rationale of administering the GMO to susceptible animals via intramuscular injection while trial participants would receive it via intravenous infusion.	The applicant has provided data regarding the intramuscular and intravenous administration of the GMO to animals. This information is discussed in Sections 4.4, 4.5.1 and 4.5.2, Chapter 1 of the consultation RARMP.
	• the effects of the GMO and viraemia in immunocompromised (cancer) patients.	Viraemia and shedding of the GMO in cancer patients are discussed in Section 4.5.3 and 4.5.4, Chapter 1 of the consultation RARMP. It is important to note that patient safety and the quality and efficacy GM treatment do not fall within the scope

Expert	Summary of advice provided	Comment
		of this evaluation as they are the responsibilities of other agencies and authorities.
	• the limit of detection of the assay used to evaluate viraemia.	The applicant proposed that viraemia will be assessed using a qPCR assay with a limit of detection of 6.25 genome copies/µl serum (Section 2.3.7, Chapter 1 of the consultation RARMP). The sensitivity of this assay was considered in Risk scenario 1 and Risk scenario 2 (Section 3, Chapter 2) and is proposed as a draft licence condition.
	• the possibility of a second peak of viraemia in trial participants after leaving the hospital.	Experiments conducted in mice suggest that a second peak of viraemia is unlikely to occur. This information is included in Section 4.5.2, Chapter 1 and considered in Risk scenario 1 and Risk scenario 2 (Section 3, Chapter 2) of the consultation RARMP.
	• Suggested that additional data should be considered in the application, including:	
	• viraemia and shedding in individuals treated with the GMO overseas	As above.
	• the effects of intravenous administration of the GMO to healthy and immunocompromised animals	As above.
	• sequencing analysis of the GMO and WT Getah virus strains.	The homology among the WT M1 strain, the M1-c6 variant and other WT GETV strains is included in Section 3.11.1, Chapter 1 of the consultation RARMP.