Australian Government

Department of Health and Aged Care

Office of the Gene Technology Regulator

Risk Assessment and Risk Management Plan for

DIR 195

Trial of a genetically modified vaccine against devil facial tumour disease in Tasmanian devils

Applicant: University of Tasmania

14 June 2023

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Summary of the Risk Assessment and Risk Management Plan

for

Licence Application No. DIR 195

Decision

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

The applicant, University of Tasmania (UTAS), proposes to conduct a trial with a GM vaccine in Tasmanian devils. The GM vaccine consists of a replication defective human adenovirus serotype 5 (HAdV-5) vector that has been genetically modified to produce proteins capable of inducing an immune response against devil facial tumour cells.

The purpose of the study is to evaluate the immunogenicity, safety and efficacy of the GM vaccine for prevention and/or treatment of devil facial tumour disease. The GM vaccine would be administered to Tasmanian devils kept in enclosures within trial sites in Tasmania.

Veterinary medicines must be approved by the Australian Pesticides and Veterinary Medicines Authority (APVMA), which provides a national registration scheme for agricultural and veterinary chemical products under the *Agricultural and Veterinary Chemicals Code Act 1994* (AgVet Code), including vaccines. Therefore, in addition to approval by the Regulator, the University of Tasmania would require a permit from APVMA to use this GM vaccine.

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

The application

Project Title	Trial of a genetically modified vaccine against devil facial tumour disease in Tasmanian devils[1]
Parent organism	Human adenovirus serotype 5 (HAdV-5)
Genetic modifications	• Deletion of viral early-transcribed region 1 (E1) - to render virus unable to multiply • Deletion of viral early-transcribed region 3 (E3) - to increase host immune response to the virus • Insertion of antigen genes – to induce host immune response against tumour cells
Principal purpose	The proposed trial aims to evaluate the immunogenicity, safety and efficacy of a GM vaccine in Tasmanian devils for prevention and/or treatment of devil facial tumour disease
Previous trial	The proposed study would be the first trial to be conducted with the GM vaccine
Proposed limits and controls	
Proposed duration	5 years
Proposed trial size	22 Tasmanian devils
Proposed locations	Two contained trial sites in Tasmania
Proposed controls	• only registered veterinarians would administer the GMO • only trained and authorised personnel would access the trial sites • personnel would use personal protective equipment (PPE) • transport, storage and disposal of the GMO would be carried out according to the OGTR *Guidelines for the* T*ransport, Storage and Disposal of* GMOs.

Risk assessment

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

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

Credible pathways to potential harm that were considered include the potential exposure of people and animals other than the Tasmanian devils to the GMO and the potential for the GMO to transfer or

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[1] The title of the project as supplied by the applicant is "*Limited and controlled release of a genetically modified adenoviral vaccine for Tasmanian devils*"

acquire genetic material from other viruses. The potential for the GMO to be released into the environment and its effects were also considered.

Important factors in reaching the conclusions of the risk assessment included that the GMO is replication defective, and unintended exposure to the GMOs would be minimised by the proposed limits and controls measures.

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

Risk management

The risk management plan describes measures to protect the health and safety of people and to protect the environment by controlling or mitigating risk. The risk management plan is given effect through licence conditions.

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

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Abbreviations

AdV	Adenovirus
AICIS	Australian Industrial Chemicals Introduction Scheme
APVMA	Australian Pesticides and Veterinary Medicines Authority
BSC	Biosafety cabinet
CDC	Centers for Disease Control and Prevention
DFT	Devil facial tumour
DFTD	Devil facial tumour disease
DIOC	Direct instillation into the oral cavity
DIR	Dealings involving Intentional Release
DNA	Deoxyribonucleic acid
E1-2	Early regions 1-4
EU	European Union
FDA	United States Food and Drug Administration
FSANZ	Food Standards Australia New Zealand
GM	Genetically modified
GMO	Genetically modified organism
GTTAC	Gene Technology Technical Advisory Committee
HAdV	Human Adenovirus
i.m.	Intramuscular
i.t.	Intratumoural
IATA	International Air Transport Association
IBC	Institutional Biosafety Committee
ICH-GCP	*Guidelines for Good Clinical Practice* of the International Council for Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use
L1-5	Late regions 1-5
LD50	50% lethal dose
mGL	Monomeric green lantern
NHMRC	National Health and Medical Research Council
NRET	Department of Natural Resources and Environment Tasmania
OGTR	Office of the Gene Technology Regulator
PC2	Physical containment level 2
PCR	Polymerase chain reaction
PCR	Polymerase chain reaction
PPE	Personal protective equipment
PPNA	Polypeptide neoantigen
PVC	Polyvinyl chloride
RAF	*Risk Analysis Framework*

RARMP	Risk Assessment and Risk Management Plan
SOP	Standard Operating Procedure
TGA	Therapeutic Goods Administration
the Act	The *Gene Technology Act 2000*
the Regulations	The Gene Technology Regulations 2001
the Regulator	The Gene Technology Regulator
USA	United States of America
UTAS	University of Tasmania
WHO	World Health Organization
WIVA20	Wild Immunity Vector Adenovirus 20

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

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.

RISK ASSESSMENT CONTEXT

The GMO
Modified genes
Novel traits

Parent organism (comparator)
Origin and taxonomy
Cultivation and use
Biology

Receiving environment
Environmental conditions: abiotic and biotic factors
Production practices
Related organisms
Similar genes and proteins

Proposed GMO dealings
Activities
Limits
Controls

Previous releases
Australian approvals
International approvals

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.

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

local councils and the Minister for the Environment, and from the public. The advice from the prescribed experts, agencies and authorities and how it was taken into account is summarised in Appendix A. Two public submissions were received, and their consideration is summarised in Appendix B.

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 Chemicals Introduction Scheme (AICIS) and the Department of Agriculture, Fisheries and Forestry.

8.   The APVMA provides a national registration and permit scheme for agricultural and veterinary chemical products. It administers the provisions of the *Agricultural and Veterinary Chemicals Code Act 1994* (AgVet Code). For registration, the APVMA assesses whether a new veterinary vaccine meets the criteria set out in the AgVet Code before it is registered in the Register of Agricultural and Veterinary Chemical Products. A new veterinary vaccine that is not registered may be legally used, such as in animal trials, by obtaining a permit from the APVMA.

9.   As part of the permit process, the APVMA assesses the quality, safety and efficacy of the vaccine. Quality aspects could include batch-to-batch consistency in vaccine composition, purity and potency. The APVMA audits the Good Manufacturing Practice record of the applicant. Safety aspects include the toxicological profile of the vaccine and its residues, including metabolites and degradation products. Associated food safety risks and consumer dietary exposure are also considered by the APVMA. The APVMA approves the label, handling and directions for supply of veterinary vaccines to ensure safe use. The APVMA may also impose conditions on a permit for the supply of veterinary vaccines for research purposes.

10.   To conduct the trial, the applicant will also require approval by the relevant Tasmanian authority, the Department of Natural Resources and Environment Tasmania (NRET).

11.   Research involving animals has to be undertaken under the oversight of the relevant statutory framework within state and territory animal welfare acts. The statutory framework has been supplemented gradually by codes of practice and ethical principles developed by the National Health and Medical Research Council.

12.   The applicant stated they have obtained approval from the UTAS Animal Ethics Committee to conduct this trial. They are also applying for permits from the Tasmanian devil Captive Research Advisory Group and Biosecurity Tasmania.

Section 2   The proposed dealings

13.   Devil facial tumour disease (DFTD) is a transmissible cancer that affects Tasmanian devils[2]. Transmissible cancers are contagious and spread through populations by the physical transfer of living cancer cells. DFTD is transmitted from one devil to another by biting, especially during the mating season (Murchison, 2008; Metzger and Goff, 2016). Transmission via tumour cells shed into carcasses or via vectors seems unlikely (McCallum and Jones, 2006). The disease causes tumours on the face or inside the mouth of affected animals and can lead to death within 6-12 months. To date, two lineages of devil facial tumour (DFT) have been identified, DFT1 and DFT2. DFTD was first observed in 1996,

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

since then the disease has caused significant decline in the wild population of Tasmanian devils (Murchison, 2008; Metzger and Goff, 2016).

14.   The University of Tasmania (UTAS) is seeking authorisation to carry out a trial with a GM vaccine (the GMO) in Tasmanian devils. The purpose of the trial is to evaluate the immunogenicity, safety and efficacy of the GM vaccine for prevention and/or treatment of devil facial tumour disease. The GM vaccine would be administered via intramuscular (i.m.) or intratumoural (i.t.) injection or by direct instillation into the oral cavity (DIOC).

15.   The dealings involved in the proposed trial study are to:

(a)   conduct the following with the GMO:

i.   prepare the GMO for administration to Tasmanian devils;

ii.   administer the GMO to Tasmanian devils by i.m. or i.t. injection or by direct instillation into the oral cavity (DIOC);

iii.   collect samples from Tasmanian devils;

iv.   analyse the samples

(b)   transport the GMO;

(c)   dispose the GMO;

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

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

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

17.   Up to 22 Tasmanian devils would be included in the trial.

18.   Administration of the GMO to Tasmanian devils would be conducted by veterinarians.

19.   GMO-inoculated animals would be kept in enclosures within two trial sites in Tasmania.

20.   Only trained and authorised staff would be permited to access the animal enclosures.

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

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

- Staff preparing and administering the GMO would use personal protective equipment (PPE) including gowns, gloves, face masks and safety glasses;

- Staff entering the enclosures would use gloves and waterproof footwear. They would visit animals that have not been exposed to the GMO prior to entering the enclosures housing animals that have received the GMO. After exiting the enclosures, the footwear would be chemically decontaminated and kept on site in closed containers,

- Staff collecting biological samples would wear gloves;

- Devil drinking water would be replaced three times per week and waste water decontaminated in the first two weeks following administration of the GM vaccine;

- Devil's faeces would be removed from the enclosures daily in the first two weeks following administration of the GM vaccine. After this period, faeces would be collected at least twice a

week. Faecal waste containing GMO would be stored at the trial sites prior to being transported to UTAS for decontamination.

- Transport, storage and disposal of the GMO and any contaminated waste generated at the trial sites would be in accordance with the current version of the Regulator's *Guidelines for the Transport, Storage and Disposal of GMOs*.

2.3 Details of the proposed dealings

2.3.1 *Manufacturing of the GMO*

22.   The GMO would be manufactured at UTAS under a notifiable low risk dealing (NLRD) authorisation assessed by their Institutional Biosafety Committee (IBC). The GMO would be packaged into cryogenic vials and stored in -80 ℃ freezers within PC2 facilities at UTAS. Alternatively, the GM vaccine could be manufactured overseas and imported into Australia.

2.3.2 *Transport and storage of the GMO*

23.   If manufactured overseas, the GM vaccine would be imported into Australia by specialist courier companies and delivered to the UTAS or a storage facility. The GM vaccine would be packaged and labelled for transport in accordance with the packaging and labelling requirements of the International Air Transport Association (IATA) code UN N 3373/3425 (Biological Substance, Category B/GMO).

24.   The GMO would be transported from the storage facility or the PC2 laboratory at UTAS to the trial sites in a sealed primary container placed in an unbreakable secondary container labelled as containing GMOs. Unused vials of GMOs, biological samples, material and waste that may contain GMOs would be returned to the PC2 laboratories at UTAS for disposal and transported in sealed primary containers placed in unbreakable secondary containers labelled as containing GMOs.

25.   Faecal samples would be placed into a bag and a secondary unbreakable container and stored in freezers or on dry ice, in locked sheds within the study site. Samples would be transported weekly to PC2 facilities at UTAS for analysis.

26.   Transport and storage of the GMO, biological samples, material and any waste potentially containing GMO between the PC2 laboratory at UTAS and the trial sites would therefore be conducted in accordance with the Regulator's *Guidelines for the Transport, Storage and Disposal of GMOs*

27.   Access to the GMO, biological samples, material and any waste potentially containing GMO would be restricted to authorised personnel only.

2.3.3 *Trial sites*

28.   The trial would be carried out at two trial sites in Tasmania. Tasmanian devils inoculated with the GM vaccine would be kept individually in contained enclosures with restricted access.

29.   The animal enclosures proposed to be used in the trial were designed in consultation with experienced devil biologists and veterinarians. Both sites were built specifically to house the devils and are double fenced facilities. They contain multiple enclosures that are approximately 100 m^2 each, surrounded by a wire mesh security fence. The enclosures are built with corrugated iron or steel walls of at least 1.3 meters in height. Many of the enclosures are fitted with connecting doors which allow the devils to access adjacent enclosures if they are not occupied by another devil. To prevent digging, the enclosures and security fence in trial site 1 have a horizontal steel mesh. Trial site 2 has concrete walls buried to at least 0.4 m under the enclosure walls and a buried steel mesh under the security fence. A representative image of the enclosures is shown in Figure 2.

Figure 2. Representative image of the double contained enclosures

30.   The trial facilities would be equipped with remote monitoring sensors. If a person enters the facility outside of standard hours, an alert would be sent to the personnel involved in the trial, who would then check the site. The veterinary shed would be located next to (Trial site 1) or inside of (Trial site 2) the double contained enclosures.

2.3.4   *The trial design*

31.   The purpose of the trial is to evaluate the immunogenicity, safety and efficacy of a GM vaccine in Tasmanian devils for the prevention and/or treatment of devil facial tumours. The applicant proposed two trial designs:

Trial design 1 –Evaluation of the safety of the GM vaccine

32.   This trial would be conducted at trial site 1. It would evaluate the immunogenicity, safety and tolerability of the GM vaccine administered to healthy Tasmanian devils via i.m. injection into the masseter (jaw) muscle or direct instillation into the oral cavity (DIOC). Up to 10 animals would be included in the trial design 1.

- **Experimental Group 1A** – Dose escalation study. The first devil would receive an i.m. dose of 5 x 10^8 viral particles (vp) of the GM vaccine, followed by a second i.m. administration of 5 x 10^9 vp with a minimum of 4 weeks between doses. The animal would be returned to its enclosure after each dose and observed for adverse events. If no adverse events are observed, the next group would include 4 animals. Each of the animals would receive two i.m. doses of either 5 x 10^9 vp or 5 x 10^{10} vp with a minimum of 4 weeks between doses.

- **Experimental Group 1B** - The GMO would be administered via DIOC using micropipettes fitted with plastic pipette tips. Each of the animals would receive two doses of the GM vaccine with a minimum of 4 weeks between doses. The number of GMO viral particles to be administered via DIOC would be determined based on the results of the experimental group 1A.

Trial design 2 – Efficacy of the GM vaccine

33.   This trial would be conducted at trial site 2. It would assess the ability of GMO to prevent and/or treat DFTD in Tasmanian devils. Up to 12 animals would be included in the trial design 2.

- **Experimental Group 2A** – Devils would be allocated in 2 subgroups: Subgroup 2A.1 would receive a placebo; and Subgroup 2A.2 would receive two doses of the GM vaccine. The dose and administration route of the GM vaccine would be determined based on the results of the trial design 1. Following vaccination, the devils would be challenged with devil facial tumour 1 (DFT1) cells and monitored for the development of tumours.

- **Experimental Group 2B –** This experimental group would evaluate the efficacy of the GM vaccine in treating DFTD should devils in the placebo or GMO vaccinated subgroups develop tumours following challenge with DFT1 cells. Up to 5 doses of the GM vaccine would be administered via i.t. injection with a minimum of one week between doses. Devils would be monitored daily. Tumours would be measured on the dosing days or monthly once treatment is completed. If no reduction in tumour size is observed, devils would receive non-GM

immunotherapies. GM or non-GM treatment would be stopped and palliative care would be provided to devils showing tumours larger than 8 cm or if metastatic tumours were observed.

34.   In all cases, administration of the GM vaccine would be conducted while the animals are sedated in the on-site veterinarian shed.

35.   Biological samples would be collected at several time points (see section 2.3.6) and evaluated for the presence of the GM vaccine. The applicant proposes that animals showing negative test results for the shedding of the GM vaccine DNA in faeces, for at least a month, would be transferred to an alternative captive devil facility or free-range enclosure. Any of these animals with a negative test result for the GM vaccine which have active DFTD could also be transferred to an alternative facility. These animals would be returned to the double contained enclosures should they be selected to receive i.t. injections of the GMO. Animals sourced from the wild, if any, would be released back into their original home environment if they are negative for the presence of DFT1 and DFT2 DNA, tested in blood, oral swab samples or fine-needle aspirate from the administration site, and following six months of negative tests for the presence of GMO DNA in faecal samples.

2.3.5   *Preparation and administration of the GM vaccine*

36.   The GM vaccine would be transported on dry ice from the PC2 laboratories or the storage facility to the trial sites. It would be thawed and diluted in sterile saline on a bench at the administration facility using a micropipettor with a filter tip. Personnel preparing and administering the GM vaccine would use personal protective equipment (PPE) including gowns, gloves, face masks and safety glasses.

37.   Administration of the GM vaccine would be performed by veterinarians. Needles would be used to withdraw the prepared GM vaccine and for i.m. and i.t. injections. This procedure would not require removing or recapping needles. Needles and syringes would be discarded in an appropriated sharps container. Instillation of GM vaccine into the devil oral cavity would be performed using micropipettes fitted with plastic pipette tips.

38.   The applicant has proposed two ways of transporting the devils. Devils would be trapped using standard PVC pipe traps and placed individually in a burlap bag. The bag would be closed using a cable clamp and transported by hand from the enclosure to the veterinary shed. Alternatively, the devil would be transported inside the PVC trap and placed in a burlap bag at the veterinary shed. The applicant stated that in rare occasion, when a devil does not go into the trap on the day required, it would be captured by hand and placed into a burlap bag for transport. The devil would then be anaesthetised by delivery of isoflurane through a nose cone placed over the burlap bag. When the animal is sedated, it would be removed from the bag and kept under isoflurane anaesthesia until the administration of the GM vaccine is completed. In the case of the DIOC vaccination, the nose cone would be temporarily removed to access the oral cavity. After the procedure the devils would be placed in the burlap bag and transported back to the enclosure. The empty burlap bag would be transported to the PC2 laboratory facilities at UTAS for decontamination.

39.   The applicant stated that all devils involved in the study would be identified by a subcutaneously implanted microchip.

2.3.6   *Sample collection and analysis*

40.   Faecal samples would be collected daily in the first two weeks following administration of the GM vaccine. After this period, faeces would be collected at least twice per week. The collection of faecal samples would be performed using a pair of plastic tongs. Samples collected from the same animal over the course of the week would be pooled and tested for the presence of the GMO genome by polymerase chain reaction (PCR) test.

41.   Blood samples, oral and rectal swabs would be collected by veterinarians from anaesthetised animals at the on-site veterinarian shed. Samples would be collected on days 0, 7, 14 and 28, then

every 2-4 weeks for the duration of the trial. Additional samples would be taken when a subsequent dose of the GM vaccine is administered.

42.   Biological samples would be transported to PC2 facilities at the UTAS for analysis. Analysis of samples would be conducted using standard PC2 laboratory practices. Briefly, personnel analysing biological samples would wear PPE including gloves, lab coats and eye protection. Procedures that are likely to produce aerosols would be performed in a Class II Biosafety Cabinet. When working with the GMO outside a biosafety cabinet (BSC), the person would wear a face mask. Centrifugation of samples would be performed using closed primary containers and sealed rotors.

43.   As there is a limitation on how often the devils can be anaesthetised to collect samples, only a few tumour biopsies would be taken for analysis The persistence of the GMO in tumour cells over time would not be evaluated.

2.3.7   *Decontamination and disposal of the GMO*

44.   Equipment and surfaces exposed to the GMO would be decontaminated using 10% sodium hypochlorite solution (bleach), 0.9% solution Virkon S or 1% of F10.

45.   Used burlap bags would be decontaminated by autoclaving.

46.   Liquid waste likely to contain GMO would be decontaminated by adding a solution of sodium hypochlorite (bleach) to a final concentration of at least 0. 5% prior to disposal into the sewage system.

47.   Solid waste generated during preparation and/or administration of GM vaccine (e.g. pipette tips, needles and syringes) would be transported to PC2 facilities at UTAS for decontamination and disposal via the PC2 facility biohazard waste stream.

48.   In the event a devil dies from DFTD during the trial, the devil would be transported to UTAS and stored in a freezer within the PC2 facilities. Any devil remains and unused faecal samples would be autoclaved for 2 hours at 121 ℃. Internal temperature probes would be used to monitor the temperatures of the load. The applicant provided data showing the validation of this decontamination method. After decontamination, the waste would be disposed via the biohazard waste stream used by the PC2 facility.

2.3.8   *Training*

49.   The applicant's IBC declared that the training and experience of individuals involved in these dealings is satisfactory. Staff would be trained on the licence conditions. The GMO would be handled, prepared, and administered by qualified research and veterinary staff.

2.3.9   *Accountability and Monitoring*

50.   Records would be available in the UTAS electronic laboratory notebook and inventory management. This includes inventory of the GMO and experimental results.

2.3.10   *Contingency plans*

51.   In the event of inadvertent exposure of skin, eyes or mucosa to the GMO, persons who have been exposed would be instructed to wash the exposed area with excess of water and soap (skin) or 10% povidone solution (eyes and mucosa) and if required, seek medical attention.

52.   In the event of spill of the GMO on the floor, the area would be decontaminated with bleach, Virkon® or F10®.

53.   Unbaited artificial dens (e.g. boxes or barrels) would be placed outside the enclosures for the duration of the trial to trap devils in the unlikely event of an animal escapes from its enclosure. The animal would be returned to its enclosure.

54.   Any unplanned releases, including spills and/or adverse events would be reported to the UTAS Biosafety Officer and to the OGTR as soon as immediate safety and containment actions have been completed.

Section 3   Parent organism

55.   The characteristics of the parent organism provide a baseline for comparing the potential for harm from dealings with the GMO. The GM vaccine is derived from the human adenovirus (HAdV) serotype 5.

3.1   Classification and genome characteristics

56.   Adenoviruses (AdVs) are non-enveloped, icosahedral virions containing a linear double-stranded DNA genome. They infect a wide variety of vertebrate hosts, from humans to fish (Robinson et al., 2011; Yu et al., 2017). AdVs are classified into 6 genera within the family *Adenoviridae* (ICTV, 2022). Human adenoviruses (HAdVs) belong to the genus *Mastadenovirus*. Members of this genus infect mammals only, including bats, dogs, ruminants, horses, humans, swine and mice.

57.   HAdVs are grouped into seven species (A to G) containing at least 88 serotypes (Dhingra et al., 2019). A serotype is defined by the ability of infection in cell culture to be neutralised by specific antisera. The HAdV serotype 5 (HAdV-5) used as base to the proposed GM vaccine is classified as species C, along with HAdV-1, -2, and -6 (Wold and Toth, 2013a). All serotypes are similar in general structure and in functions of most proteins, but certain unique proteins contribute to the distinguishing properties of serotypes and species (Wold and Toth, 2013a).

58.   The adenoviral genome varies depending on its genus and serotype. In general, the genome is organised in into transcriptions units flanked by inverted terminal repeats (ITRs). HAdV-5 has a genome of about 36 kb and encodes approximately 40 proteins. Each transcription unit contains 1- 8 coding sequences. These units are transcribed in two phases – early (E) and late (L) stages of the viral reproduction cycle (Figure 3).
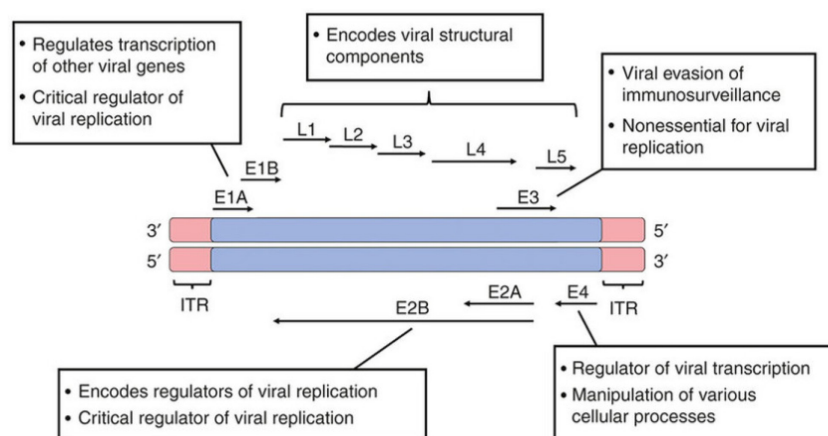
Figure 3. Schematic of general genomic organisation of human and mammalian AdVs, as presented in Afkhami et al. (2016).

59.   The E1 and E3 coding regions are exclusive to mastadenoviruses (human and mammalian AdVs) (ICTV, 2023). The E1 regions are essential for viral gene expression and replication (Roy et al., 2004; Saha and Parks, 2017). The E2 gene encodes E2 proteins which are mainly involved in viral DNA replication and transcription of late genes (Roy et al., 2004; Saha and Parks, 2017). The E3 gene encodes viral proteins that aid the virus in evading the host immune response. The E4 gene modulates cellular function and assists with viral DNA replication and RNA processing. The late transcription units

(L1-5) encode viral structural and non-structural proteins that are involved in the capsid formation and maturation of the new virions (Roy et al., 2004; Saha and Parks, 2017).

60.   The viral capsid is composed of 3 major (hexon, penton base and fibre) and four minor (IIIa, VI, VIII, and IX) proteins. In addition to the dsDNA genome, six other proteins (V, VII, μ, IVa2, terminal protein, and AdV protease) are encapsulated inside the capsid (Reddy and Nemerow, 2014).

3.2   Infection cycle

61.   The life cycle of AdV takes approximately 24–36 h and involves three major stages (infection, replication and assembling) (Giberson et al., 2012). During the infection, the AdV binds to receptors on the surface of the target cell triggering the internalisation of the virus particle via the process of endocytosis. Once internalised, the AdV is uncoated, and the viral genome is transported to the nucleus where it uses the host cell nuclear machinery to make copies of itself (Charman et al., 2019).

62.   Transcription of the AdV genes occurs in two phases – early (E) and late (L) – of the viral reproduction cycle. E1A protein is the first protein to be expressed and it is essential for the efficient expression of other adenoviral genes and progression of the viral life cycle. Replication of viral genome occurs after the E2 proteins are made and is concomitant with transcription of late genes (Charman et al., 2019). After DNA replication and the expression of late genes, the progeny viruses are assembled. At the last step the host cell is lysed releasing the virions (Wodrich et al., 2003).

63.   Alternatively, small amounts of AdV DNA can be maintained as episomal copies in the cytoplasm of infected cells (Garnett et al., 2009). In this case, the expression of E1A is repressed by the proteins of the host immune system. The reduced expression of EA1 supresses the expression of all subsequent viral genes and limits the production of infectious virions (Zheng et al., 2016). If the host becomes immunosuppressed, this latent form of the virus may be reactivated and undergo a new cycle of viral replication (Radke and Cook, 2018). Studies conducted in cells cultured in laboratory suggested that episomal copies of AdV DNA can persist in the cytoplasm of host cells for over 100 days (Zheng et al., 2016).

64.   AdVs do not have the machinery for efficient integration into the host genome. (Harui et al., 1999; Desfarges and Ciuffi, 2012; Hoppe et al., 2015; Dehghan et al., 2019). However, random integration of virus DNA into the host genome has been observed in very rare cases (Harui et al., 1999; Stephen et al., 2008).

3.3   Pathology

65.   HAdVs are common human pathogens and cause a wide range of illnesses such as common cold, sore throat, diarrhoea, conjunctivitis and others (Public Health Agency of Canada, 2014; CDC, 2022). Different HAdVs types have different tissue tropism, HAdV species -B, -C, and -E are the most common cause of respiratory diseases, while HAdV-A, -D, -F, and -G are mainly responsible for gastrointestinal infections and HAdV-D and E for ophthalmology diseases (Ismail et al., 2019).

66.   HAdV species C serotypes 1, 2, and 5 are a common cause of respiratory infection in young children. The infection is generally mild, and symptoms may include fever, nasal congestion, coryza, and pharyngitis. Immunocompromised individuals are a high-risk group for development of severe disease following HAdV infection. These include people who have received T-cell suppressive regimens, received allogeneic hematopoietic stem cell transplant (Dhingra et al., 2019), lymphoma patients receiving anti-CD52 antibody therapy, and solid-organ transplant recipients (Ljungman, 2004).

67.   Outbreaks of HAdVs-associated respiratory disease are more common in the late winter, spring and early summer, however infections can occur throughout the year. HAdVs are generally transmitted by aerosol droplets excreted from the respiratory tract of an infected individual. It is estimated that adults lacking specific anti-HAdV antibodies may be infected by inhaling as few as 5 viral particles. However, only 10 % of exposed individuals may become ill (Musher, 2003).

68.   HAdVs can also be transmitted by ocular secretions or by the oral-faecal route with food and water as possible vectors. They can be indirectly spread by towels, handkerchiefs, food, eating utensils and other items that were contaminated by an infected person (Pond, 2005).

69.   After natural HAdV infection, the incubation period of HAdVs ranges from 2 days to 2 weeks, depending on the viral species and serotype as well as the route of infection (Public Health Agency of Canada, 2014; Allard and Vantarakis, 2017). For respiratory infections, the incubation period is generally 4-8 days, whereas it is 3-10 days for intestinal infections (Allard and Vantarakis, 2017). The symptoms of mild infection usually last for a few days to a week but for the severe infections, symptoms may last longer.

3.4   Integration, mutation and recombination of AdV

70.   As described in Section 3.2 of this chapter, AdV DNA can be maintained as multiple episomal copies in the cytoplasm of infected cells (Harui et al., 1999). In addition, AdVs do not have the machinery for efficient integration into the host genome and exhibit extremely low levels of integration (Harui et al., 1999; Desfarges and Ciuffi, 2012; Hoppe et al., 2015; Dehghan et al., 2019). Random integration of virus DNA into the host genome has been observed in very rare cases (Harui et al., 1999; Stephen et al., 2008).

71.   Mutation and homologous recombination are important source of genetic variation in viruses. The HAdV-5 shows a mutation rate of 0.0046 substitutions per genome replication, this value is similar to other double-stranded DNA viruses (Risso-Ballester et al., 2016). Homologous recombination can occur when a host cell is infected by multiple AdVs at the same time. Recombination events among HAdV species C have resulted in at least three new AdVs strains in China (Yang et al., 2019; Zhang and Huang, 2019; Yu et al., 2020; Bastug et al., 2021). In addition, HAdV2/6 recombinant strains have been reported in clinical isolates in Turkey (Bastug et al., 2021).

72.   Homologous recombination appears to be restricted to members of the same species and occurs in the regions of high sequence homology (Lukashev et al., 2008). However, bioinformatic analysis suggested that HAdV-E4, a species E AdV was a result of a recombination event between species B and C (Gruber et al., 1993).

73.   Genomic analysis of 51 circulant strains showed that recombination among HAdV Species C is more likely to occur in the regions E4 and E1, while recombination events in the hexon and fibre gene and the E3 region were almost absent. As E1 and E4 regions encode genes that regulate the transcription and other cellular processes, recombination events in these regions do not result in serotype diversity (Dhingra et al., 2019). This is in contrast to that observed in HAdV Species D (HAdV-D), where recombination events between genes encoding the major capsid proteins (hexon, penton base, fibre) resulted in more than 50 HAdV-D serotypes (Robinson et al., 2013). Changes to structural proteins can result in increased ability to spread or cause disease, as it allows the virus to escape pre-existing immunity (Cook and Radke, 2017).

3.5   Host range

74.   Human and non-human AdVs have a range of vertebrate hosts including people, horses, cattle, pigs, sheep, goats and domestic fowl, wild birds, bats and reptiles (Allard and Vantarakis, 2017). Humans are the natural host for HAdVs (Custers, 2020). Although animal models have been used as tool to study HAdV infections, there is no report of natural HAdV infections of non-human hosts (Ismail et al., 2019; Bertzbach et al., 2021).

75.   HAdVs have been found in faeces of domestic animals living near humans, such as dogs, goats, pigs, and sheep, and in faeces from wild guinea pigs. HAdV and canine AdV were found concomitantly in faeces samples from pampas foxes. The presence of HAdV in the faeces of these animals was not considered a threat to the health of the animals, since it is more likely that the virus was ingested and eliminated in faeces but did not replicate in the gut of those animals (Monteiro et al., 2015; Pauly et al., 2015). However, these animals could act as reservoirs for humans and non-human adenoviruses.

76.　　The administration of HAdVs to mice, rabbits, rats, guinea pigs and non-human primates resulted in the development of specific antibodies. Limited to no clinical signs of a systemic disease have been observed in most of the infected animals, suggesting that HAdVs have restricted ability to replicate and cause disease in non-human hosts. However, experimental administration of high doses of HAdV can cause tumours in mice, rats and Syrian hamster. A LD50 of > 1.4 x 10^9 CCID50 has been reported for cotton rats and mice receiving the HAdV-5 via intranasal or intravenous route (Pereira and Kelly, 1957; Duncan et al., 1978; Prince et al., 1993; Chen et al., 2013).

3.6　　Shedding and Biodistribution

77.　　HAdV shedding is largely dependent on tissue and infection route. Respiratory infections are expected to generate the highest viral load soon after infection and virus persists for approximately 2 months post-infection as detected in respiratory samples (Huh et al., 2019). It estimated that sputum or oral secretions of infected adults contain 10^6 to 10^7 vp per milliliter (mL) (Musher, 2003).

78.　　HAdV shedding was also evaluated in faecal and oral swabs after the administration of a live, oral vaccine containing two serotypes of replication competent HAdV (HAdV-4 and HAdV-7). Over half of the vaccine recipients shed viable viral particles in faecal samples between 7-28 days following vaccination. No shedding was detected after 28 days of vaccination or at any time point in throat swabs (Allard and Vantarakis, 2017; FDA, 2019)

79.　　The presence of genomic DNA of HAdV species C was observed in human tonsil and adenoid tissues after surgical removal. Viable viruses were isolated following long-term culture of tissue samples with permissive cells, suggesting that low levels of infectious viruses may persist in these tissues in a latent form (Proenca-Modena et al., 2019).

80.　　In animal models, viable viral particles were recovered in cultures of spleen cells collected from rabbits and guinea pigs 8 weeks after intravenous administration of wild type (WT) HAdV-5. However, the number of viral particles was very low and only observed when cells where cultured for long periods (~70-100 days) (Pereira and Kelly, 1957; Faucon et al., 1974).

81.　　As discussed in Section 3.2, a small amount of WT AdV DNA can persist as episomal copies in the cytoplasm of infected cells. This latent form of the virus may be reactivated if the host becomes immunosuppressed, leading to a new cycle of infection and viral shedding (Radke and Cook, 2018).

3.7　　Prevalence

82.　　An estimation of the seroprevalence of HAdV-4, 5, -26 and -35 (serotypes commonly tested in the clinics or used in clinical/pre-clinical trials) is shown in Figure 4. This data is analysed based on approximately 30 studies published over the past 20 years (Mennechet et al., 2019). HAdV-5 is the most widely reported and has the highest seroprevalence globally. HAdV-26, appears to have high seroprevalence in Africa and Asia; and low in North America and Europe (Mennechet et al., 2019).

83.　　In Australia, the Laboratory Virology and Serology (LabVISE) reports from the Department of Health (1991-2000) showed an average of about 1400 reported cases of AdV infection per year over 10 years and only about 18 reported cases of HAdV-D26 infection (Spencer, 2002). From 1 January to 20 May 2023, New South Wales has registered 5,724 cases of HAdV infections, while Tasmania has recorded only 25 HAdV infections in the same period (NSW Health, 2023; Tasmania Health, 2023). It is important to note that the majority of AdV reported infections have not been serotyped and that testing for AdV infections may not be common in Australia. These numbers indicate that HAdVs are present in the Australian environment
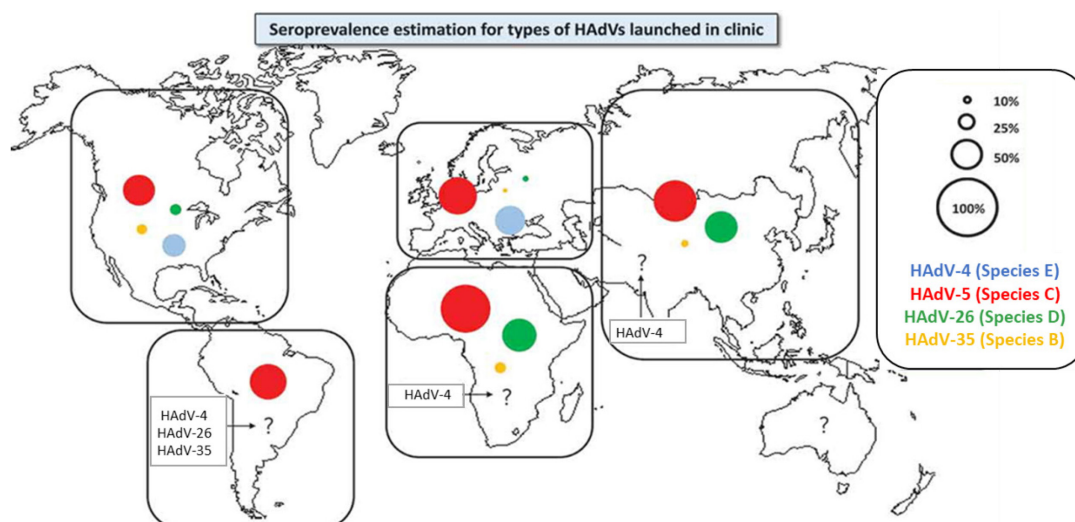
Figure 4. Seroprevalence for AdV types used in the clinic (Adapted from Mennechet et al., 2019)

3.8 Environmental stability and decontamination methods for human AdV

84.   AdVs are resistant to many common disinfectants and can remain infectious for long periods in the environment. Most serotypes are stable at 36 °C for a week, for several weeks at room temperature, and for several months at 4 °C. They are stable for weeks in tap water, sewage effluent and sea water and for 7 days to 3 months on dry surfaces (Public Health Agency of Canada, 2014; CDC, 2019).

85.   AdVs can be effectively inactivated using heat treatment (e.g. 56 °C for 30 min, 60 °C for 2 min or autoclaving) or chemical agents as such chlorine, formaldehyde or alcohol-based disinfectants (Rutala et al., 2006; Public Health Agency of Canada, 2014; Allard and Vantarakis, 2017; CDC, 2019). For example, surfaces can be decontaminated with 70% (v/v) ethanol, 0.2% (v/v) chlorine or 0.9% (v/v) Virkon S (>5 min contact time) (Rutala et al., 2006). Liquid waste may be treated with bleach with a final concentration of 10% (v/v) (~0.5% chlorine), for 15 minutes (Allard and Vantarakis, 2017).

3.9 Antiviral treatments for human AdV

86.   There is no specific treatment for AdVs. Most AdV infections are mild and do not require medical care (CDC, 2019). Antiviral drugs such as Cidofovir and Ribavirin may be used as treatment for severe AdVs disease in immunocompromised individuals (Yusuf et al., 2006; Waye and Sing, 2010; Lion, 2019).

3.10 Risk group of human AdVs

87.   The Australian Standard 2243.3:2022 Safety in Laboratories Part 3: Microbiological safety and containment (Standards Australia/New Zealand, 2022) classifies AdV as a Risk Group 2 organism.

Section 4   The GM Vaccine – nature and effect of genetic modifications

88.   The GM vaccine is known as Wild Immunity Vector Adenovirus 20 (WIVA20). It consists of a replication defective human AdV serotype 5 (HAdV-5) vector that has been genetically modified to produce proteins designed to trigger an immune response against devil facial tumour cells.

89.   Some information about the transgenes inserted into the GM vaccine has been declared 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

90.   The GM vaccine was produced using a commercial platform that allows customised DNA fragments containing overlapping sequences to be assembled into a single DNA molecule (Jang and Bunz, 2022). Fragments of the HAdV-5 DNA genome were combined to form a full-length adenoviral vaccine genome. The E1 region was replaced with the vaccine expression cassette and the E3 region was deleted (Figure 5).
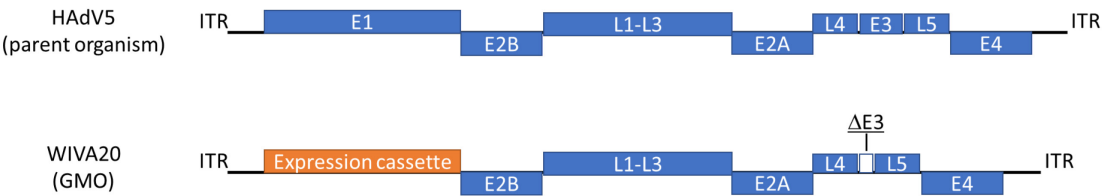

Figure 5. Schematic diagram of the parent organism and GMO genomes.

4.1.1   *Introduced genes and regulatory sequences*

91.   The introduced expression cassette contains a human cytomegalovirus (CMV) enhancer and promoter, coding sequences for two proteins (WIVA20 Antigens 1 and 2) linked by an internal ribosomal entry site (IRES) and a rabbit beta-globin polyadenylation signal (terminal signal). Details of the introduced genes are described below. Major elements included in Antigens 1 and 2 are summarised in Table 1.

92.   Particular details of element X, Y and Z have been declared as CCI under Section 185 of the Act.

Table 1. WIVA20 major elements

Genes of interest	Genetic modifications		
	Major elements	**Donor**	**Modified trait, description**
WIVA20 Antigen 1	Element X	*Sarcophilus harrisii* (Tasmanian devil)	Induces immune response
WIVA20 Antigen 2	Polypeptide neoantigen (PPNA)	*Sarcophilus harrisii*	Induces immune response
	Elements Y and Z	*Sarcophilus harrisii*	Facilitates the transport of polypeptide neoantigen to the desired location in the cell
	Monomeric Green Lantern (mGL)	*Aequorea victoria*	Facilitates the visualisation of polypeptide neoantigen expressed in GMO-transduced cells

93.   WIVA20 Antigen 1 consists of codon-optimised sequences of DNA for the expression of a specific antigen (here called Element X), a linker peptide and a polyhistidine-tag (His-tag). Element X is based on a protein of the vertebrate immune system. It was designed to induce an antibody-specific response against DFT1 cells. These antibodies are not expected to induce an immune response against healthy cells. Element X homologous proteins are expressed in most vertebrate cells (Frank, 2002).

94.   WIVA20 Antigen 2 is composed of codon-optimised sequences of DNA for the expression of a polypeptide neoantigen (PPNA), a hemagglutinin tag (HA-tag), two linker peptides, a myc-tag and a monomeric Green Lantern (mGL) protein. In addition, Antigen 2 contains sequences to direct the polypeptide neoantigen to the appropriate cellular location (Elements Y and Z).

95.   The PPNA consists of 18 individual peptides, ranging from 11 to 29 amino acids each, combined in a single polypeptide molecule. These peptides were identified by comparing genome sequences of

tumour cells to the genome of healthy cells of Tasmanian devils. Most of the selected peptides are derived from natural single point mutations or genomic rearrangements identified in the genome of DFT1 cells (Stammnitz et al., 2018; Stammnitz et al., 2022). Two peptides are derived from long-terminal repeat sequences of the DFT1 and DFT2 genomes that are aberrantly translated into proteins in the cancer cells.

96.   The PPNA was designed to induce antibody and T cell-mediated immunity targeting DFT cells. The applicant stated that transcriptomic and proteomic data suggest that these peptides are not expressed in healthy devil tissues. Additionally, individual peptides are not expected to maintain the function of the full-length proteins they are derived from, but interactions of the peptide motifs with cellular functions cannot be excluded. The presence of these peptides in cells of human and other animals has not been investigated.

97.   The purpose of Elements Y and Z included in Antigen 2 is to facilitate the transport of the polypeptide neoantigen to the desired location in the cell. This is expected to improve the immune response induced by the PPNA. Elements Y and Z are based on partial sequences of genes encoding proteins of the vertebrate immune system. Homologous sequences of elements Y and Z are found in humans and animals.

98.   The monomeric Green Lantern (mGL) is a fluorescent protein derived from *Aequorea victoria,* a bioluminescent jellyfish. It was designed for optimal expression and brightness in living cells (Campbell et al., 2020). The expression of mGL has been evaluate in mammalian cells cultured in a laboratory and in mouse models, with no cytotoxic effects reported (Campbell et al., 2020; Wang et al., 2022). The purpose of this protein is to facilitate the visualisation of Antigen 2 expressed in GMO-transduced cells.

99.   Linker peptides and tags are short sequences used to enhance protein structural stability, facilitate protein purification or as a cleavage site (Chen et al., 2013; Zhao et al., 2013). They can also facilitate the differentiation between GM and wild type (WT) genes and proteins. Linker peptides and tags have been included in vaccines and therapeutics evaluated in many animal models with no reports of toxicity or allergenicity (Rai et al., 2016; Jureczek et al., 2019; Zane et al., 2023).

100.   Regulatory sequences control the expression of the genes of interest. The expression of WIVA20 Antigens 1 and 2 is driven by the CMV promoter. The CMV promoter can drive the expression of recombinant proteins in a wide range of mammalian cells. It is commonly used in viral vector-based gene therapies and vaccines, including the commercially available COVID-19 vaccine Vaxzevria (MHRA, 2020). Other regulatory elements used include the IRES, to improve the translation of both protein of interest, and a terminal signal.

4.2   Effects of the genetic modifications

101.   As described in Section 3.1, this Chapter, the E1 region is essential for the viral replication, while the E3 region is involved in the viral immune evasion. The deletion of E1 and E3 regions removes the capacity of the GMO to replicate in cells and to evade the host immune response. As a result of these genetic modifications, the GMO can be used to deliver the genes of interest but cannot replicate itself or cause disease.

102.   The expression cassette allows the expression of WIVA20 Antigens 1 and 2 once the GMO transduces cells of a vaccinated Tasmanian devil. This should then induce an immune response to protect the devil against a future exposure to DFTD. As the GMO DNA does not integrate into the host genome, the expression of the transgenes would be transient. The GMO would be cleared by the host immune system within days or weeks, depending on the route of administration and biodistribution (see Section 4.4, this Chapter).

4.3   **Characterisation of the GM vaccine**

103.   In order to produce the GMO, the assembled WIVA20 vaccine genome was used to transfect HEK-293 packaging cells. The packaging cells supply the E1 region in *trans*, allowing the GMO to replicate. After replication, the GM vaccine was purified and stored at -80 C in freezers. The intended genetic modifications were confirmed by whole genomic sequencing. This analysis also showed the introduction of several single point substitutions in the GMO genome. These substitutions are in accordance with the expected HAdV-5 mutation rate per round of replication (Risso-Ballester et al., 2016). The applicant stated that these mutations are not expected to result in functional changes to the adenoviral vector. The expression of the WIVA20 Antigens 1 and 2 was confirmed in cells cultured in a laboratory.

104.   Prior to administration, the stability of the GM vaccine would be further tested. The deletion of the genomic E1 region would be confirmed by polymerase chain reaction (PCR). The presence of replication-competent AdV contaminants in the GM vaccine sample would be tested by infecting cells that do not supply the deleted E1 region in trans.

4.4   **Biodistribution and shedding of the GM vaccine**

105.   Biodistribution and shedding studies determine the location of a product after administration to a person or animal. For example, the GMO may travel from the administration site to other tissues or organs (biodistribution) or be excreted in body fluids or faeces (shed). As this is the first trial proposed with this GMO, its biodistribution and shedding has not been evaluated. It can be anticipated that the GM vaccine is likely to have a similar biodistribution and shedding as other HAdV vector-based vaccines and gene therapies. Studies conducted in animal models and humans suggest that biodistribution and shedding of replication defective adenoviral vectors depend on the dose and administration route. Biodistribution and shedding of AdV vectors are summarised in Table 2 and discussed below.

Intramuscular administration of adenoviral vectors

106.   Intramuscular administration of a single dose of a replication defective vaccine, based on the Chimpanzee Adenovirus (ChAd) serotype 68, resulted in the presence of the viral vector DNA in blood of rats from 4 hours to up to 4 days after injection. Blood samples tested negative for the presence of the viral vector at day 8. Similarly, blood samples of rhesus macaques that received 3 doses of the ChAd68-based vaccine tested negative for the viral vector DNA 7 days after the last vaccine administration (Dai et al., 2022). Analysis of biodistribution showed the presence of viral vector DNA at the administration site for up to 15 days, in spleen and stomach for up to 8 days, and in liver, prostate, colon, bone marrow, stomach, mesenteric lymph nodes, brain, kidney, lung, testis at days 1 and 2 following administration (Dai et al., 2022).

107.   Viral vector DNA was not detected in blood samples of mice following immunisation with a single i.m. dose of a vaccine based on the replication defective ChAd serotype Y25. Only 1 out of 160 faecal samples tested positive for the viral vector DNA on day 2. Biodistribution analyses showed the presence of the viral vector DNA at the administration sites, in axillary lymph node, bone marrow, heart, inguinal lymph node, liver, lung, mammary gland, mesenteric lymph node, sciatic nerve and spleen samples for up to 9 days (Stebbings et al., 2022).

108.   Biodistribution studies of HIV vaccines, based on HAdV-5 and HAdV-35 vectors, failed to detect viral vector DNA in blood of rabbits and mice that received a single i.m. dose of the vaccines containing $0.5 - 1 \times 10^{11}$ vp. The biodistribution of viral vectors varied depending on the animal model but viral vector DNA was primarily detected on day 9 at the administration sites (muscle), spleen, lymph nodes and liver (Sheets et al., 2008; Shimada et al., 2022). The number of viral particles decreased over time and low levels of viral DNA were detected at the administration site, spleen and lymph nodes samples collected from a small number of the rabbits (3-5 out of 10 animals) 3 months after administration.

109.   In humans, the i.m. administration of a gene therapy based on a replication-defective HAdV-5 failed to induce viraemia or viral shedding in faeces, throat swabs or urine samples. Samples were analysed on days 2, 7 and 14 as well as 4, 8 and 12 weeks after treatment (Matyas et al., 2005 reviewed in Brandon, 2008).

Intratumoural administration of adenoviral vectors

110.   Biodistribution and shedding of replication-defective HAdV-5 vectors following i.t. administration depends on the location of the injected tumour. Overall, viral vector DNA has been detected in blood from 30 min up to 7 days following administration. HAdV-5 vectors injected into human lung cancer led to shedding of viral vector DNA in urine for up to 14 days and sputum for up to 60 days. After two days, shedding was detected in faeces and throat swabs of patients injected with a high dose (>1 x 10^8 vp/dose) of HAdV vectors but not with lower doses. Viral vector DNA was present in tumour biopsies up to 90 days after administration (Tursz et al., 1996; Brandon, 2008)

111.   In patients with squamous cell carcinomas of the head and neck, the repeated administration of 3 x 10^{10} vp or higher doses of a replication defective HAdV-5 resulted in the presence of viable viral particles in blood 30 minutes up to 24 hours after injection of the adenoviral vector into the tumour. Viral DNA was detected in a dose-dependent manner. In addition, viable viral particles were detected in urine from patients who received at least 3 x 10^9 vp/dose and in sputum and saliva of patients who received doses of 1 x 10^{11} vp (Clayman et al., 1998). A study where HAdV-5 viral vector was injected into soft tissue sarcomas of the extremities failed to detect the viral vector in blood, sputum and urine samples collected 2 weeks after the last administration (Mundt et al., 2004).

Oral administration of adenoviral vectors

112.   Oral administration of replication competent AdV-based rabies vaccine (see Section 4.7, this Chapter) has been evaluated in target and non-target animals. Viral DNA was detected in oral and anal swabs samples collected from target animals such as raccoons, striped skunks and red foxes, from 6 h up to 34 days after administration of the vaccine. Oral and anal samples of most of Norway and cotton rats included in the study tested positive for the presence of the viral DNA for up to 3 days and samples of 8/9 Virginian Opossum were positive for up to 9 days. Low levels of viral DNA were detected in oral swab samples of 1/10 Virginia opossums on day 23, and in 1/9 cotton rats on day 17 and in anal swab sample of 1/10 Norway rats on day 34 (Sobey et al., 2019). Administration of a 10X dose of the same AdV-based rabies vaccine resulted in shedding of viral DNA in faeces of Eastern wild turkey (up to day 3), opossum (up to day 6), eastern cottontail (up to day 5), fox squirrel and wood rat (up to day 14). Viral DNA was detected in oral swab samples collected from wild turkeys, opossums and eastern cottontails on day 4 and in samples collected from fox squirrels on day 7 (Fry et al., 2013). Table 2 summarises the shedding and biodistribution data discussed above.

113.   It is important to note that most of the shedding studies reported the presence of viral vector DNA in biological samples but, with one exception, the presence of viable viral particles was not investigated (Clayman et al., 1998; Brandon, 2008). The detection of viral particles and the persistence of AdV vectors is likely to be overestimated in those studies as PCR is likely to detect fragments of viral DNA resulting from its clearance. In addition, shedding studies of the adenoviral rabies vaccine administered to non-target animals used 10 times the recommended dose of the vaccine. Further, the GMO is replication defective and unable to undergo reactivation. Therefore, viral shedding, if any, would be limited to the initial number of viral particles administered.

Table 2. Summary of biodistribution and shedding of AdV vectors in various animal species.

Administration route	Shedding of AdV vector DNA	AdV vector DNA in blood or other tissues	References
Intramuscular (i.m.)	*Positive:* - 1/160 faecal samples (day 2)	*Positive:* - blood (days 1-4) - administration site (up to 15 day)	Matyas et al. (2005); Brandon (2008); Sheets et al. (2008); Dai et al.

			(2022); Shimada et al. (2022); Stebbings et al. (2022)
		- spleen and stomach (up to day 9) - liver, prostate, colon, bone marrow, stomach, brain, kidney, lung, testis (days 1-2 days 1) - low levels in spleen and lymph nodes of 3-5/10 animals 3 months after administration	
	Negative: - throat swab (days 2-7; weeks 2-12) - urine (days 2-7; weeks 2-12) - faeces (days 3-7; weeks 2-12)		
Oral	*Positive:* - faeces (1-2 weeks) - oral swabs – target animals (up to day 34) - oral swabs – non-target animals (up to day 10**) - anal swabs – target animals - (up to day 23) - anal swabs – non-target animals (up to day 3**)	Not tested	Fry et al. (2013) Sobey et al. (2019)

[*]Presence and persistence of viral vector DNA vary depending on the tumour location
[**]Viral DNA was detected in oral swab samples of 1/10 Virginia opossum on day 23, and in 1/9 cotton rat on day 17. Anal swab sample of 1/10 Norway rat tested positive on day 34 (Sobey et al., 2019).

4.5 Host range of the GM vaccine

114. The GM vaccine is expected to infect the same range of hosts as the parent organism. As discussed in Section 3.5 of this chapter, humans are the natural host for HAdV-5, but animals have been experimentally infected with HAdVs and adenoviral vectors. There is no report of adenoviral infection in Tasmanian devils. However, experiments conducted in the laboratory indicate that HAdV-5 vectors can transduce DFT cells and deliver genes of interest (Kayigwe et al., 2022).

4.6 Stability in the environment and decontamination

115. The stability of GM vaccine in the environment has not been tested. However, AdVs can persist for long periods on surfaces and in water. It is expected that the survival of the GM vaccine in the environment would be similar to the parent organism. Methods of decontamination effective against the parent organism are expected to be equally effective against the GM vaccine (see Section 3.8, this chapter).

4.7 Adenoviral vector-based vaccines

116. Adenoviral vectors have been extensively investigated as vaccines, gene and cancer therapies. in non-clinical and clinical trials. They induce strong immune responses and have been shown to be effective and safe (Tolcher et al., 2006; Wold and Toth, 2013b; Stebbings et al., 2022). The TGA approved COVID-19 vaccines Vaxzevria (AstraZeneca) and Jcovden (Janssen) which are based on adenoviral vectors derived from the ChAdV-Y25 and HAdV-26, respectively (Mendonca et al., 2021; TGA, 2022). It is estimated that about 14 million doses of the Vaxzevria vaccine have been administered in Australia (TGA, 2023). Common side effects observed include injection site pain or

tenderness, tiredness, headache, muscle pain, fever and chills. Severe side effects were observed in rare cases and included severe allergic reaction, blood clots, myocarditis and pericarditis (Australian Government - Department of Health and Aged Care, 2023).

117. A rabies vaccine (ONRAB®) based on the replication-competent HAdV-5 encoding rabies glycoprotein has been used to protect wildlife in North America since 2006 (Rosatte et al., 2009). The vaccine consists of a suspension of the recombinant AdV encapsulated in a plastic blister and it is recommended for field vaccination of skunks, raccoons and foxes. It is distributed as baits in the environment by hand placement or dropped from a low flying aircraft. When an animal finds and chews the bait, it releases the vaccine into the animal's mouth, which will orally vaccinate the animal against rabies. Studies conducted in non-target animals (i.e. wood rats, eastern cottontail rabbits, Virginia opossums, eastern wild turkeys and fox squirrels) experimentally vaccinated with high doses of ONRAB®, showed that the vaccine does not induce behaviour changes, superficial or histological lesions. These results suggest that the vaccine offers a low risk of adverse effects to wild populations (Fry et al., 2013; USDA, 2019).

118. Adenoviral vectors have also been used to deliver neoantigen-based cancer therapies. Neoantigens are peptides or proteins expressed in tumour cells as result of random mutations in the cell genome. They are recognised by the host immune system as foreign *or non-self*, triggering an immune response to eliminate the cancer cell. As neoantigens are unique to each type of cancer, the risk of inducing autoimmunity is very low, qualifying them as safe immunogens with low risk of damage to healthy tissues (D'Alise et al., 2019). Non-clinical and clinical studies have shown that adenoviral vaccine delivering multiple neoantigens induce strong cellular immune against cancer cells capable of preventing or controlling tumour growth. (D'Alise et al., 2019; D'Alise et al., 2022; Palmer et al., 2022). Neoantigen-based therapies can be personalised to individual patients (Zhao et al., 2021) and have been considered safe and well tolerated (Palmer et al., 2022).

Section 5   The receiving environment

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

5.1   The trial sites

120. The intended primary receiving environment would be Tasmanian devils kept in contained enclosures at trial sites in Tasmania. The GM vaccine would be administered via an i.m. or i.t. injection or oral instillation.

121. The secondary receiving environment would be the trial sites including the enclosures and the veterinarian shed where the GM vaccine would be prepared and administered.

122. The principal route by which the GMO may enter the wider environment is by shedding. As discussed in Section 4 of this Chapter, the GMO is replication defective. In the event of shedding of the GMO at the administration sites, in faeces or body fluids, the number of viral particles excreted would be limited to the initial viral inoculum.

5.2   Related viral species in the environment

123. The presence of related viruses may offer an opportunity for introduced genetic material to transfer between the GMO and other organisms in the receiving environment.

124. AdVs belong to six genera: *Mastadenovirus* (infecting mammals), *Aviadenoviruses* (infecting birds), , *Atadenovirus* (infecting a broad range of hosts including reptiles, birds, ruminants, marsupials and tortoises), *Ichtadenovirus* (infecting fish), *Siadenovirus* (infecting one species of frog and tortoise and multiple species of domestic, wild and captive birds) and *Testadenovirus* (infecting turtles) (Tong et al., 2010; Lange et al., 2019; Vaz et al., 2020; Benko et al., 2022). As such, they are a common cause

of infection in animals and humans of all ages and can be found in all environments where humans or animals congregate in groups (Usman and Suarez, 2020).

125.   AdVs have been reported in Australian bearded dragons (*Pogona* spp) and native birds, including rainbow lorikeets (*Trichoglossus haematodus*), galahs (*Eolophus roseicapilla*), and sulphur-crested cockatoos (*Cacatua galerita*); and in brushtail possums (*Trichosurus vulpecula*) from New Zealand (Hyndman et al., 2019; Vaz et al., 2020). Although some of these animals may not be present in the Tasmanian environment (i.e. bearded dragons and rainbow lorikeets (Department of Natural Resources and Environment Tasmania, 2011; Latitude 42, 2011)), it can be anticipated that non-human AdVs are likely be present in the environment.

126.   Insects, ticks and leeches are present in the Tasmanian environment. These animals are not known to transmit AdVs but can be exposed to the virus while feeding on an infected animal. DNA of AdV Species C has been detected in ticks collected from wildlife in Kenya (Ergunay et al., 2022). Studies conducted in leeches showed that the viral DNA can persist for up to 50 days in animals experimentally fed with human blood containing 1.6 x 10^6 vp/mL of HAdV. The concentration of the viral DNA increased from day 1 to day 7 and subsequently decreased (Kampmann et al., 2017). It is important to note that both studies showed the presence of the adenoviral DNA but no viable viral particles. The increase of viral DNA concentration observed in leeches could result from viral replication in the remaining human blood or experimental variability. For example, individual leeches could have ingested different amounts of blood during the feeding experiment.

127.   The prevalence of HAdVs in Australia based on the reported cases and seroprevalence is low as mentioned in Section 3.7, this Chapter.

128.   In addition, the COVID-19 vaccine Vaxzevria commercially available in Australia, is based on a Chimpanzee adenoviral vector (MHRA, 2020). Other AdV vector-based gene therapies and vaccines have been evaluated in clinical trials. Therefore, AdV vectors could be present in people or the environment.

129.   Clonal transmissible cancers affect Tasmanian devils, dogs and bivalves such as clams and mussels (Ostrander et al., 2016). In dogs, the transmissible venereal tumour (CTVT) is transmitted by sexual contact. The disease is believed to have originated thousands of years ago and is common in free-roaming dogs around the word (Murchison et al., 2014). Bivalves are affected by a disseminated neoplasia, a lethal leukemia-like blood cancer. This disease was first observed in soft-shell clams (*Mya arenaria*) along the east coast of North America in the 1970s (Brown, 1977). To date, at least 5 lineages of disseminated neoplasia have been identified in bivalves living in the Atlantic Ocean and Mediterranean Sea (Metzger and Goff, 2016; Ostrander et al., 2016). Transmissible cancers usually spread among individuals of the same species. However, two cases of disseminated neoplasia transmission among bivalve species have been reported (Metzger and Goff, 2016). There is no evidence that DFTD can be transmitted to humans or other animals (Save the Tasmanian Devil Program, 2023).

5.3   Tasmanian devil habitat and behaviour

130.   Tasmanian devils are the largest surviving carnivorous marsupials in the world. They are found throughout Tasmania living in burrows, dens and caves. Devils are nocturnal and usually solitary animals. Both males and females build nests out of bark, grass and leaves in which they remain throughout the day. They typically stay within their home range, traveling an average of ~ 3 kilometres (km) during the night. Devils can climb trees, swim and run at speeds of up to ~12 km per hour.

131.   Devils are mainly scavengers feeding on whatever is available, but sometimes hunt for small animals. They eat native animals such as wallabies, possums and wombats. Reptiles, amphibians and insects have also been found in the stomachs of wild devils (Department of Natural Resources and Environment Tasmania, 2022). Devils are not considered a threat to humans. They do not display an aggressive behaviour unless they feel threatened, and in many situations, they would prefer to flee rather than fight (Bonorong Wildlife, 2022).

Section 6   Previous authorisations

132.   The Regulator has not previously approved any DIR or DNIR licences for dealings with the proposed GM vaccine.

133.   However, the Regulator has issued commercial and limited and controlled DIR licences (DIR-180, DIR-182 and DIR-184) utilising replication defective adenoviral vector-based vaccines for humans.

134.   In addition, the Regulator has issued several DNIR licences (DNIR-588, DNIR-599, DNIR-606, DNIR-609, DNIR-636, DNIR-637, authorising the clinical trial of replication defective adenoviral vector-based vaccines and gene therapies in humans.

Chapter 2   Risk assessment

Section 1   Introduction

135.   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 6). 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.
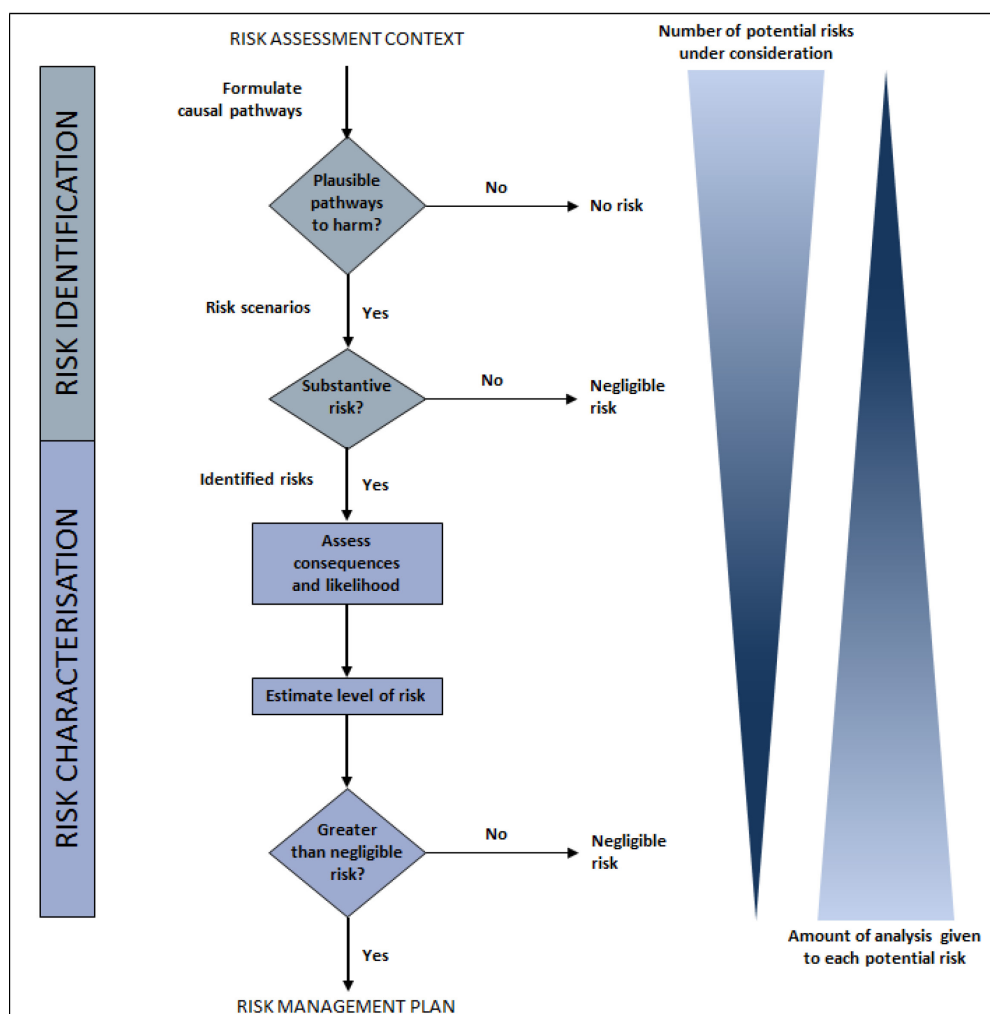

Figure 6. The risk assessment process

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

137.   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.

138.   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 6) i.e. the risk is considered to be no greater than negligible.

139.   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

140.   Postulated risk scenarios are comprised of three components (Figure 7):

     i.   the source of potential harm (risk source)

     ii.   a plausible causal linkage to potential harm (causal pathway)

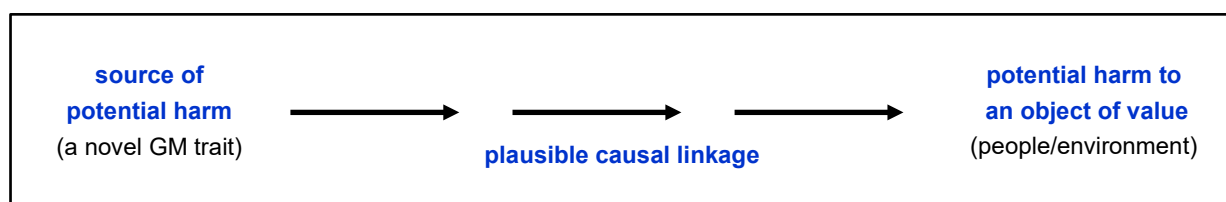   iii.   potential harm to people or the environment.

Figure 7. Risk scenario

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

- the proposed dealings

- the proposed limits including the extent and scale of the proposed dealings

- the proposed controls to limit the spread and persistence of the GMO and

- the characteristics of the parent organism(s).

2.1   Risk source

142.   The parent organism of the GMO is the human AdV serotypes 5 (HAdV-5). Details on the pathogenicity and transmissibility of HAdV-5 is discussed in Chapter 1, Section 3. Infection is generally the result of inhalation of aerosolised droplets excreted from respiratory, or ocular secretions containing the virus or, mucosal exposure to the virus or, via faecal-oral transmission. HAdV infects humans and causes common cold-like symptoms, eye infections or diarrhoea.

143.   Infection with AdV could result in latent infection in lymphoid tissues and increase the period of viral persistence in the body. However, the AdV remains episomal throughout the infection and does not integrate into the host DNA as discussed in Chapter 1, Section 3.4. Thus, the consequences of integration of viral DNA into a host cell genome will not be further discussed.

144.   As discussed in Chapter 1, Section 4, the GMO has been modified by deleting the E1 and E3 regions and inserting an expression cassette containing 3 major elements, element X, polypeptide neoantigen (PPNA) and monomeric Green Lantern (mGL). These introduced genes are considered further as a potential source of risk.

145.   The expression of the introduced genes is controlled by regulatory sequences. The regulatory sequences included in the GM vaccine are commonly used in viral vector gene therapies and vaccines. They are sequences of DNA that are not expressed as proteins and so exposure is to the DNA only and it has no toxicity (Society of Toxicology, 2003). Hence, potential harms from the regulatory sequences will not be further assessed for this application.

146.   Clonal transmissible cancers, such as DFTD, are rare and have been reported only in Tasmanian devils, dogs and bivalves such as clams and mussels (Ostrander et al., 2016). There is no evidence that DFTD can be transmitted to humans or other animals (Program, 2023) and the transmission of the disease will not be further discussed.

2.2   Causal pathway

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

- the proposed dealings, which are conducting experiments with the GMO, import, transport or disposal of the GMO, and possession (including storage) in the course of any of these dealings,

- proposed limits, including the extent and scale of the proposed dealings;

- characteristics of the parent organism;

- routes of exposure to the GMOs, the introduced gene(s) and gene product(s),

- potential effects of the introduced gene(s) and gene product(s) on the properties of the organism,

- potential exposure of other organisms to the introduced gene(s) and gene product(s) from other sources in the environment,

- potential exposure of other organisms to the GMOs in the environment,

- the release environment,

- spread and persistence of the GMOs (e.g. dispersal pathways and establishment potential),

- environmental stability of the organism (tolerance to temperature, UV irradiation and humidity),

- unauthorised activities, and

- practices before and after administration of the GMO.

148.   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.

149.   As discussed in Chapter 1, Section 1.1, the APVMA assesses the quality, safety and efficacy of the vaccine. The APVMA may also impose conditions on a permit for the supply of veterinary vaccines for research purposes.

150.   The Act provides for substantial penalties for unauthorised dealings with GMOs or non-compliance with licence conditions, and also requires the Regulator to have regard to the suitability of an applicant to hold a licence prior to the issuing of the licence. These legislative provisions are considered sufficient to minimise risks from unauthorised activities. Therefore, unauthorised activities will not be considered further.

2.3   Potential harm

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

- harm to the health of people or desirable organisms, including disease in humans or animals or adverse immune response to the GM vaccine

- the potential for establishment of the GMO that could cause harm to people or the environment harm to the health of people or desirable organisms, including toxicity/allergenicity

2.4   Postulated risk scenarios

152.   Three risk scenarios were postulated and screened to identify substantive risk. These hypothetical scenarios are summarised in Table 3 and discussed in depth in Sections 2.4.1-2.4.3 (this chapter).

Table 3. Summary of hypothetical risk scenarios from dealings with GM vaccine

Risk scenario	Risk source	Possible causal pathway	Potential harm	Substantive risk	Reason
1	GMO	Exposure of people undertaking dealings with the GMO via: needle-stick injury, aerosols, fomites, contact with broken skin or mucous membranes during (a) Preparation and administration of the GMO (b) Collection and analysis of biological samples (c) Animal bites or scratches (d) Transport, storage or disposal of the GMO ⬇ Transduction of cells by GMO ⬇ Expression of Antigens 1 and 2	Local inflammation, flu-like symptoms, or eye infection; or cross reactivity with the neoantigen resulting in autoimmune reaction	No	• Only veterinarians would administer the GMO. Procedures would not require removing or recapping the needles. • Only trained and experienced personnel would handle the GMO or access the enclosures. • Staff handling the GMO or collecting biological samples would wear appropriate PPE. • The GMO is replication defective. • The dose received through accidental exposure would be far smaller than that administered during vaccination/treatment based on adenoviral vectors (e.g. Covid vaccines). • WIVA20 antigens were designed to target DFT cancer cells. • Any reactions to Antigens 1 and 2 would be transient and the GMO would be rapidly cleared by the immune system. • Transport, storage and disposal between the PC2 laboratory and trial site would be performed in accordance with the Regulator's *Guidelines for the Transport, Storage and Disposal of GMOs*.
2	GMO	Administration of the GMO into Tasmanian devils ⬇ GMO is shed at the injection sites or via oral fluids or faeces	Local inflammation, flu-like symptoms, or eye infection, or cross reactivity	No	• The GMO is replication defective, and shedding would be limited to the number of viral particles administered.

Risk scenario	Risk source	Possible causal pathway	Potential harm	Substantive risk	Reason
		⬇ Exposure of non-target animals to the GMO through contact with inoculated devils or GMO-contaminated material ⬇ Transduction of cells by GMO ⬇ Expression of Antigen 1 and 2	with the neoantigen resulting in autoimmune reaction		• Following administration, the devils would be kept in contained enclosures. • Humans are the natural hosts for HAdVs. Exposure of other animals to the GMO is unlikely to cause disease. • As per risk scenario 1, if an animal is exposed to the GMO, the expression Antigens 1 and 2, and any adverse reaction would be transient.
3	GMO	Administration of the GMO into Tasmanian devils ⬇ a) The devil is coinfected with another AdV; or b) An animal or person is exposed to the GMO while infected with another AdV ⬇ GMO transduce a host cell co-infected with another AdV ⬇ (a) Complementation of E1 and E3 by AdV (b) Homologous recombination with AdV ⬇ Production of other recombinant AdVs	Local inflammation, flu-like symptoms or eye infection Disease in people or animals	No	• It is highly unlikely that both GMO and AdV would coinfect the same cell at the same time. • Recombination among AdVs is rare and usually restricted to the same species. • Homologous recombination in AdV-C is more likely to occur in E1 and E4 regions, which are not involved in virus tropism. • Multiple recombinations would be required to produce a replication competent AdV with altered tropism and immune evasion properties.

2.4.1   *Risk scenario 1*

Risk source	GMO		
Causal pathway	Exposure of people undertaking dealings with the GMO via needle-stick injury, aerosols, fomites, contact with broken skin or mucous membranes during: ⬇		
	(a)	Preparation and administration of the GMO	
	(b)	Collection and analysis of biological samples	
	(c)	Animal bites or scratches	
	(c)	Transport, storage or disposal of the GMO	
	⬇ Transduction of cells by GMO ⬇ Expression of Antigens 1 and 2		
Potential harm	Local inflammation, flu-like symptoms, or eye infection; or cross reactivity with the neoantigen resulting in autoimmune reaction		

Risk source

153.   The source of potential harm for this postulated risk scenario is the GM vaccine.

Causal Pathway

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

Exposure during preparation and administration of the GMO

155.   The GMO would be prepared at the trial sites and administered via i.m. or i.t. injection or via direct instillation into the devil's oral cavity. There is potential for exposure of people to the GMO during the preparation and administration via needle stick, sharps injury, aerosol formation, spills or eye splash.

156.   Needles would only be used during the administration of the GMO to the devils; this procedure would not require removing or recapping the needles. As discussed in Chapter 1, Section 2.3, only veterinarians would administer the GMO and needles and syringes would be disposed into appropriate containers. The devils would be kept under anaesthesia during the administration procedure. These measures would minimise the potential exposure of people to the GMOs via needle stick, sharps injury or animal bites. In the event of exposure the person would be instructed to wash the exposed area with excess of water and soap or antiseptic and seek medical attention if required. In addition, as AdVs usually cause respiratory, gastrointestinal or eye infections, it is unlikely that exposure via needle stick or sharps injury would result in infection.

157.   Dealings generating aerosols or accidental spills during preparation or administration could result in exposure via inhalation of aerosols or exposure through the oral route. However, personnel preparing and administering the GMO would wear PPE including gowns, gloves, face masks and safety glasses minimising the potential exposure of people to the GMOs via aerosol, spills or eye splash. Contact with broken skin is not a route of AdV infection, but it could result in exposure to the GMO. The applicant proposed that, in the unlikely event of exposure of skin, eye or mucosa to the GMO,

persons who have been exposed would be instructed to wash the exposed area with an excess of water and soap or an antiseptic solution. In addition, any spills on the floor or surfaces would be decontaminated using chemical disinfectants. (Chapter 1, Section 2.3.10).

Collection and analyses of biological samples

158.   Personnel entering the animal enclosures would wear waterproof footwear and gloves. Faecal samples would be collected using a pair of tongs and placed into a bag within a secondary unbreakable container. Both footwear and tongs would be decontaminated after use. Blood, oral and rectal swabs would be collected from anaesthetised devils at several timepoints. Needles would be used for the collection of blood samples by veterinarians or experienced staff using standard procedures.

159.   Samples would be analysed in PC2 laboratory facilities at UTAS. Analysis of samples would be conducted using standard PC2 laboratory practices including the use of a BSC when dealings with GMO are likely to generate aerosols (see Chapter 1, Section 2.3.6).

Animal bites

160.   Tasmanian devils would be handled during vaccination, sample collection and cage maintenance. They are wild animals and may be inclined to attack and bite, if they feel threatened. There is potential for exposure to the GMO via devil saliva following vaccination of devils via DIOC or i.t. injections. However, as described in section 2.3.5 devils would generally be trapped using standard PVC pipe traps and contained in a burlap bag. Devils would be anaesthetised before being removed from the bag for procedures.

161.   Tasmanian devils are nocturnal. Enclosure maintenance activities such as collecting faeces or changing drinking water would be conducted during daylight hours. In addition, Tasmanian devils are not considered a threat to humans, and they will not attack a person unless attacked or trapped (Section 5.3, Chapter 1). The animal staff entering the enclosure are experienced in handling these animals. It is highly unlikely that personnel entering the enclosures would be exposed to the GMO as these measures would minimise the potential exposure of people to the GMOs via animal bites

Transport, storage or disposal of the GMO

162.   If the GMO was unintentionally/accidentally spilled during import, transport or storage, this could result in exposure to people in the area via aerosol or liquid contact with eyes or mucous membranes/skin. Further, people could be inadvertently exposed to the GMO via contact with materials or surfaces contaminated with the GMO through subsequent hand to mouth transmission. However, the import of the GMO would be in accordance with the IATA packing instructions. This is considered satisfactory as it ensures that the GMOs are appropriately packaged and labelled. In Australia, the transport, storage and disposal of the GMO would be carried out in accordance with the current version of the Regulator's *Guidelines for the Transport, Storage and Disposal of GMOs*. This would mitigate exposure due to spills of the GMO during these dealings.

163.   The risk of exposure to the GMO in other people resulting in a sustainable infection is highly unlikely because the GMO is unable to form infectious viral particles.

Potential harm

164.   If people are exposed to the GMO, they could develop flu-like symptoms, eye infections or local inflammation for a short period of time before the virus is cleared by the immune system. They could also develop autoimmunity against the antigens included in the WIVA20 vaccine.

165.   As the GMO is replication incompetent, it is unable to produce further viral particles which are required to sustain an infection. In addition, any reactions to the expression of Antigens 1 and 2 would be transient and the GMO would be rapidly cleared by the immune system (See Section 4.2, Chapter 1).

166.   WIVA20 antigen 1 is specific to DFT cells and is unlikely to induce autoimmunity in humans. The mGL, included in WIVA antigen 2 is derived of GFP, a fluorescent protein extensively used in the study of cell biology. GFP overexpression can induce cytotoxicity and apoptosis of the transduced cell but has not been linked to autoimmunity (Ansari et al., 2016). The expression of mGL would be transient, and the GMO-transduced cell would be cleared by the immune system.

167.   The peptides included in the PPNA (WIVA20 antigen 2) are derived from random mutations present in the genome of DFT cells and are not found in healthy cells of Tasmanian devils. The potential cross-reactivity of the PPNA with peptides expressed by cells of humans or other animals has not been evaluated. However, as discussed in Section 4.7 of Chapter 1, neoantigen-based cancer therapies have been shown to be safe and well tolerated. As they target peptides exclusively expressed in cancer cells, the likelihood of cross-reactivity against healthy tissue is low. Further, if one peptide of the PPNA is identical to a gene sequence of a person exposed to the GMO, the peptide produce by the transduced cell would likely be recognised as a self-peptide and this would inhibit any immune response. Therefore, it is unlikely that an immune response generated against these antigens would target human cells or induce autoimmunity.

168.   Immunocompromised individuals are a high-risk group for development of severe disease following HAdV infection. However, the GMO cannot replicate or cause disease. In the event of exposure via needle stick/sharps injury, animal bites or mucosa/broken skin, the volume and hence the amount of GMO transferred would be far smaller than that administered during vaccination/treatment based on adenoviral vectors. For example, individuals immunised with Vaxzevria, an adenoviral Covid-19 vaccine received of 5×10^{10} viral particles per dose, corresponding to not less than 2.5×10^8 infectious units (AstraZeneca, 2022) and serious side effects were rarely observed (Chapter1, Section 4.7). WHO recommends that immunocompromised individuals should receive an additional dose of Vaxzevria, as these individuals are less likely to produce an efficient immune response against the COVID19 antigen following vaccination (WHO, 2022). It can be anticipated that the exposure of immunocompromised individuals to a small amount of virus would not induce a robust immune response against the WIVA20 antigens.

169.   Should severe adverse events occur, antiviral drugs such as Cidofovir and Ribavirin may be used as treatment to adenoviral infections. Therefore, the likelihood of serious side effect in in healthy or immunocompromised individual exposed to the GMO is unlikely.

170.   The exposure to small amount of GMOs and the transient nature of infection would be expected to result in very mild, or negligible symptoms, which would also minimise the potential for an adverse immune response to the GMO. Therefore, exposure to the GMO is not expected to result in an infection and is unlikely to result in an increased disease burden in humans.

Conclusion

171.   The potential for an unintentional exposure of people to the GMO resulting in a serious adverse immune reaction in humans is not identified as a risk that could be greater than negligible. Therefore, it does not warrant further detailed assessment.

2.4.2   *Risk scenario 2*

Risk source	GMO
Causal pathway	Administration of the GMO into Tasmanian devils ⬇ GMO is shed at the injection sites, or via oral fluids or faeces ⬇ Exposure of animals to the GMO through contact with inoculated devils or GMO-contaminated material ⬇ Transduction of cells by GMO ⬇ Expression of Antigens 1 and 2
Potential harm	Local inflammation, flu-like symptoms, or eye infection; or cross reactivity with the neoantigen resulting in autoimmune reaction

Risk source

172.   The source of potential harm for this postulated risk scenario is the GM vaccine.

Causal Pathway

173.   The principal route by which the GMO may enter the wider environment following vaccination is via shedding. People or animals could be exposed to the GMO by coming into contact with vaccinated devils or GMO-contaminated material. Risk scenario 1 explores the likelihood and consequences of people being exposed to the GMO. We will focus on the likelihood and consequence of exposure of other animals in this risk scenario.

Shedding of the GMO within the enclosures

174.   Shedding data available for vaccines and gene therapies based on AdV vectors suggests that the i.m. administration of the GMO is unlikely to result in shedding (see Chapter 1, section 4.4). There is limited data regarding oral administration of replication-defective adenoviral vectors. Shedding data for the replication competent AdV-based rabies vaccine suggests that the direct instillation of the GM vaccine into the oral cavity or injection into oral tumours of devils is likely to result in shedding of the GMO in oral fluids and faeces for the first two weeks. As the GMO cannot replicate, shedding would be limited to the initial number of viral particles administered. As mentioned, most shedding studies focused on the presence of viral DNA in biological samples but not viable viral particles. PCR based assays used to detect viral DNA are very sensitive and able to detect small fragments of DNA. Part of the GMOs detected in biological samples would consist of shredded viral DNA, the number of viable viral particles shed would be far smaller than the dose of the GMO initially administered and present for a shorter period than those reported.

175.   Following DIOC or i.t. administration, the GMO could be shed in faeces and saliva. The presence of GMO in urine is plausible but unlikely (Brandon, 2008). As discussed in Section 3.8 (Chapter 1), AdVs can persist in the environment for weeks or months and could be shed into the soil within the enclosure.

Exposure to the GMO in the wider environment

Escape of a devil from the enclosure

176.   If a vaccinated/treated devil escapes from the enclosure, it could shed the GMO into the wider environment via faeces or oral fluids or expose other animals during a fight/bite. However, the enclosures were designed and constructed to contain Tasmanian devils. In addition to the double

fencing, the enclosure was also designed to prevent digging. If a devil escapes from its enclosure, they would also have to escape through the security fence. In the unlikely event where an animal does escape from the double fenced site, they would likely remain in close proximity to the trial site areas as, as described in Section 5.3 of Chapter 1, devils usually stay within their home range. In addition, artificial dens would be placed outside each of the four perimeter fence walls for the duration of the trial in order to trap the devil and return it to its enclosure. Therefore, it is unlikely that a devil would be able to transfer the GMO to another animal via contact or shedding of the GMO into the environment outside of its enclosure.

Release into alternate facility or in the wild

177.   Based on the biodistribution data available for other adenoviral vector-based vaccines/therapies, it can be anticipated that viral DNA could be present in blood for up to 4 days and in spleen, liver and lymph nodes for the first two weeks following i.m. administration. It is important to note that although the presence of viral DNA has been detected by PCR, the presence of infectious viral particles in the animals is likely to be far smaller. In addition, adenoviral vectors are highly immunogenic and the administration of multiple doses of the GMO are likely to induce an immune response that would rapidly clear viable particles of the GMO. It is expected that an animal that received at least two doses of the GMO would have very low levels of the viral DNA by the time it would be transferred to an alternative facility or released into the wild. In contrast, i.t. administration of the GMO could result in the presence of the GMO at the administration site for at least 3 months. Animals displaying tumours would not be released into the wild.

178.   Furthermore, only animals no longer shedding the GMO in faeces would be relocated (Chapter 1, Section 2.3.4). For these animals to be transferred to an alternative facility, the GMO must not be detected in faeces for at least a month. At least 6 months of negative test results would be required prior to releasing an animal into the wild. Faecal samples would be tested by PCR, a very sensitive technique that can detect very low levels of DNA in biological samples. Tasmanian devils to be released into the wild would also need to show negative test results for the presence of DFT1 and DFT2 cells. Therefore, the transfer of animals that are no longer shedding the GMO to an alternative or free-range facility or their release into the wild is unlikely to result in exposure of other animals to the GMO.

179.   The proposed measures and controls would minimise the potential of exposure of other animals to the GMO outside the devil enclosures.

Incursion of other animals within the enclosure

180.   As the devils would be housed in open air enclosures, small animals such as possums, bats and birds could enter the enclosure. These animals could be exposed to the GMO via ingestion of food, soil or drinking water contaminated with small doses of the GMO shed by a vaccinated/treated devil. As mentioned in in Section 5.3 of Chapter 1, Tasmanian devils are carnivorous so it is likely that small animals may become prey, and would be unable to further disperse the GMO into the environment. However, given that devils are nocturnal animals, it is plausible that a bird or other animal could be exposed to the GMO shed within the enclosure during the day (e.g. via faeces, drinking water). It could then act as a vector and spread the GMO into the wider environment by the oral-faecal route. However, any animal entering the enclosure would only be exposed to a small dose of the GMO unable to trigger an infection. In addition, the applicant proposed that drinking water would be replaced frequently, and faeces would be collected from the enclosure (Section 2.2, Chapter 1), further minimising the likelihood of exposure of other animals to the GMO.

181.   High doses of the replication competent adenoviral rabies vaccine administered to non-target animals resulted in shedding of viral DNA in faecal samples of wild turkeys and opossum for up to 3 and 7 days, respectively. One out of 15 faecal samples of wood rats tested positive for the presence of viral DNA on day 14. It can be anticipated that the shedding of the GMO by exposed birds or other animals would be far smaller than the dose of the GMO initially administered to the devil. Further, adenoviral infections in birds are caused by AdV species belonging to the *Aviadenovirus* genus.

Vaccination of chickens with replication incompetent HAdV-5 vaccine encoding influenza antigens via an intranasal route failed to induce antibodies against the target antigens in 9 out of 10 chickens tested. Intramuscular administration of the vaccine induced an antibody response in all immunised chickens and protected them against a lethal challenge with Influenza virus. The poor immunogenicity of the vaccine when administered via the intranasal route was attributed to the limited ability of the HAdV-5 to infect respiratory tract cells of birds, a natural route for AdV infections (Gao et al., 2006).

182.   It is likely that oral exposure of birds to the GMO would not result in transduction of gastrointestinal cells in birds. If the GMO transduces cells of birds or other animals, it could lead to expression of WIVA20 antigens. As mentioned before, the vaccine antigens were designed to target DFTD cells in Tasmanian devils and are not expected to induce autoimmune or damage to healthy tissues. If an exposed animal shows gene sequences identical to one of the peptides included in the PPNA, the peptide would likely be recognised as a self-peptide and would inhibit any immune response. Although the administration of high doses of HAdV can lead to tumours or death in some animal models (Chapter 1, Section Chapter 13.53.5) HAdVs are not known to cause disease in birds or other animals in nature. Further, no adverse effects have been observed in animals exposed to a replication-competent HAdV-5 vaccines (see Section 4.7, Chapter 1). Therefore, it is unlikely that exposure to the GMO, a replication defective HAdV virus, would result in adverse effects or disease in birds or other animals.

183.   As discussed in Section 4.4 of Chapter 1, the GMO could be present in the blood of devils for up to 4 days following injection. Insects, ticks or leeches feeding on the devils could be exposed to the GMO. Although adenoviral DNA has been isolated from ticks and leeches, there is no evidence that they could transmit the virus while feeding on other animals. Studies conducted in mice showed the HAdV-5 vector has a half-life in blood of less than 2 min when administered intravenously (Alemany et al., 2000). In the event of transfer of the GMO to ticks and leeches via blood, it is unlikely that the GMO persists long enough to infect the next animal that the ticks or leeches feed on. The ingestion of GMO-exposed tick/leech by other animals (e.g. birds), could result in the presence of a small amount of GMO in the gastrointestinal tract. As discussed previously, it is unlikely that this could result in adverse effects or disease in birds or other animals.

Dilution of the GMO into the soil and contamination of waterways

184.   In the event of rain, the GMO shed within the animal enclosures could be diluted into the soil and eventually dispersed into waterways. Based on the parent organism's ability to survive in the environment, the GMO could persist in water for weeks (see Section 3.8, Chapter 1). However, due to its non-replicating nature, the GMO would be unable to maintain a stable presence in the environment for long periods and would eventually degrade. In addition, the GMO potentially contaminating waterways would be markedly diluted, minimising the potential for exposure of animals such as kangaroos or cattle via ingestion of contaminated water. As discussed in Chapter 1, Section 3.1., AdVs can infect a broad range of hosts including reptiles and fish, but humans are the natural hosts for HAdV. In addition, adenoviral infections in fish are rare and caused by a single species of AdV belonging to the *Ichtadenovirus* genus (Harrach et al., 2019). Further, as the amount of the GMO in waterways is expected to be very low, it is unlikely that the GMO would be transferred to animals. Therefore, the likelihood of potential infection of animals following exposure to an environmental source of the GMO is unlikely.

Potential harm

185.   As the GMO is replication defective, it is expected that shedding of viable particles of the GMO would be limited to a dose significantly lower than the initial dose administered. WT HAdV cannot replicate or cause disease in animals. In the event of exposure of an animal to the GMO, it is likely that the presence of the GMO would be transient, and it would be cleared by the host immune system. Similar effects would be expected in the event of further transmission to another animal. It is highly unlikely that the GMO would infect or cause disease via ingestion of contaminated water, in reptiles or fish.

186. HAdV-based vaccines have been shown to be safe in animals. However, the effects of WIVA20 antigens in animals have not been evaluated. As discussed before, WIVA20 antigen 1 and mGL are not expected to induce autoimmunity. Although, the cross reactivity of the PPNA with cells of other animals has not been investigated, neoantigen-based cancer therapies have been evaluated in non-clinical and clinical trials and are considered to be safe in animal models and in humans. Immune responses against the polypeptide neoantigens would be inhibited in animals expressing identical endogenous peptides. It is unlikely that exposure of animals to the GMO would result in autoimmunity.

187. Therefore, it is unlikely that the GMO shed by vaccinated devils would cause harm to other species in the Australian environment.

188. **Conclusion**

189. The potential of the GMO to be released into the environment and result in adverse immune reactions or disease in other animals is not identified as a risk that could be greater than negligible. Therefore, this scenario does not warrant further assessment.

2.4.3 *Risk scenario 3*

Risk source	GMO
Causal pathway	Administration of the GMO into Tasmanian devils ↓ a) The devil is coinfected with another AdV; or b) An animal or person is exposed to the GMO while infected with another AdV ↓ GMO transduce a host cell co-infected with another AdV ↙ ↘ Complementation of E1 and E3 by AdV / Homologous recombination with AdV in E1, E3 or other regions of high homology ↓ / ↓ Production of more replication incompetent GMOs with immune-evasion properties / (i) Formation of replication defective AdV expressing WIVA20 antigens 1 and 2 **AND** Replication competent GMO without WIVA20 expression cassette **OR** (ii) Replication competent AdV with defective immune evasion properties (E3) **AND** Replication incompetent GMO with immune evasion properties (E3) **OR** (iii) Replication competent AdV or replication incompetent GMO with altered tropism
Potential harm	Disease in people or animals

Risk source

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

Causal Pathway

191.   The transmission of the GMO can occur via the pathways mentioned in Risk Scenario 1 and 2 potentially resulting in transduction of host cells. If a devil, animal or a person exposed to the GMO has an existing AdV infection at the time of exposure or acquires an AdV infection while the GMO is present, this co-infection could potentially result in complementation and/or recombination of the GMO with WT AdVs and cause an adverse immune reaction and/or disease in people or animals. However, for the complementation or recombination to occur the GMO and a WT AdV must co-infect the same cell at the same time.

Complementation of E1 and E3 by AdV in devils and other mammals

192.   As discussed in Chapter 1, Section 3.5, AdVs can infect a broad range of hosts. However, as E1 and E3 regions are exclusive to mastadenoviruses (Chapter 1, Section 3.1), complementation is only possible if the co-infection involves the GMO and another mammalian AdV. As discussed in Chapter 1, Section 3.5, domestic and wild animals can act as a reservoir of mastadenoviruses and/or carry HAdVs in their faeces. As per Risk Scenario 2, GMO-inoculated devils would be housed in open air enclosures and small animals entering the enclosures could result in exposure of the devils to other adenoviruses. Although, there is no report of adenoviral infection in Tasmanian devils, it is plausible that devils could be susceptible to an adenoviral infection. Administration of the GMO via DIOC or injection into an oral tumour could result in the presence of the GMO in areas vulnerable to AdV infections (e.g. oral mucosa and gastrointestinal tissue). However, the likelihood of both GMO and another AdV co-infecting the same cell at the same time is low.

193.   As per Risk Scenario 2, it is unlikely that an animal other than the devils would be exposed to the GMO shed in the faeces or oral fluids of vaccinated/treated animals. If an animal ingests water, food or soil that has been contaminated with the GMO, it could transduce cells of the oral mucosa and/or gastrointestinal tissue. If the animal is infected with another mastadenovirus at the time of exposure or acquires an AdV infection while the GMO is present, it could result in co-infection. In the event of an exposure, the dose of GMO transferred to an animal other than the vaccinated devil is expected to be low. Therefore, it is highly unlikely that both the GMO and the AdV would co-infect the same cell at the same time to allow complementation.

194.   In the unlikely event of co-infection occurring in the same cell, the E1 and E3 regions provided in *trans* could lead to replication of the GMO, resulting in subsequent transduction of neighbouring cells. However, as HAdVs have restricted ability to replicate in animals (Section 4.5, Chapter 1), the GMO is not expected to replicate in animal cells even if the E1 region is provided in *trans*. Further, the vaccine was designed to trigger an immune response in the host. GMO-transduced cells expressing the vaccine antigens are expected to be cleared by the host immune system and the GMO destroyed. Therefore, it is highly unlikely that complementation of the GMO in animal cells would result in viral replication and increased harm to the devil or other animals.

Complementation of E1 and E3 by AdV in humans

195.   HAdV infects over 80% of the human population (Ismail et al., 2018). HAdVs are present in the Australian environment (Section 3.7, Chapter 1) and it is plausible that the E1 and E3 genes could be provided in *trans* from a pre-existing or acquired HAdV infection in persons accidentally exposed to the GMO if a co-infection in the same cell occurs. This could result in complementation by the HAdV leading to replication of the GMOs

196.   As discussed in Risk Scenario 1, the exposure of people undertaking dealing with the GMO is unlikely due to work practices that people conducting the dealings would follow. As AdVs are prevalent in respiratory, gastrointestinal or ocular tissue, it is unlikely that viral particles would be present in subcutaneous/skin cells in the case of a needle stick injury or contact with abraded skin. In the event of exposure of people to the GMO via aerosols or contact with mucous membranes, it is highly unlikely that the GMO and a WT HAdV would co-infect the same cell at the same. Further, HAdV infections are also self-limiting (Knight et al., 1962; Lichtenstein and Wold, 2004), if a cell co-infection is established, complementation would only result in the production of replication defective virions during the period of the co-infection. Once released, the GMO would be able to transduce only another round of host cells.

Homologous recombination with AdV

197.   Recombination is common among circulating WT AdVs in nature. It is seen as a key driver for adenoviral evolution and viruses in general. Similar to complementation, homologous recombination also requires the person or animals exposed to the GMO to be infected with a WT AdV at the same time.

198.   Recombination is more likely to occur between related viruses. The DNA homology between HAdV species is less than 20%. HAdVs belonging to Species C, as the parent organism, show up to 99% of DNA homology (Ghebremedhin, 2014). Thus, the GMO is more likely to recombine with a HAdV-C strain than with other species of human or non-human AdV. Recombination between the GMO and a human or non-human AdV strain could potentially result in the generation of different GM recombinants. These GM recombinants are described in Table 4.

Table 4. Plausible theoretical recombinants of GMO and wild-type AdVs

Recombinant region	Resultant recombinant	Outcome	Likelihood
E1 between • GMO • WT AdV	• Replication competent GMO without E3 gene • Replication incompetent AdV with WIVA20 Antigens	• Replication competent GMO that is still less immuno evasive than WT AdV • Replication incompetent AdV expressing WIVA20 Antigens	Unlikely
E3 between • GMO • WT AdV	• Replication incompetent GMO with E3 gene • Replication competent AdV without E3	• Replication incompetent GMO with modified immuno-evasive properties • Replication competent AdV without immuno-evasive properties (a WT AdV unable to evade the host immune system)	Unlikely
Capsid genes (hexon, penton and fibre) between • GMO • WT AdV	• Replication incompetent GMO with different hexon, penton or fibre. • Replication competent AdV without the WIVA20 Antigens but with different (hexon, penton or fibre)	• Altered tropism and host range of GMO • Altered tropism and host range of AdV	Highly unlikely

199.   As discussed in Risk Scenario 1, AdVs are prevalent in respiratory, gastrointestinal or ocular tissue and are unlikely to be commonly present in subcutaneous/skin cells in the case of a needle stick injury during administration. Exposure to the GMO by people via inhalation or contact with mucus membranes is plausible but unlikely due to the proposed work practices. As AdVs can infect a broad range of hosts, it is plausible that a devil could be infected with a non-human adenoviral strain. The likelihood of exposure of animals other than the devil to the GMO is unlikely (Risk Scenario 2).

200.   AdVs infecting birds are classified into the *Aviadenovirus* genus. In a recent study, an AdV variant showing 97.6 % similarity to murine adenovirus 2 was found in dropping samples and tissues of Australian cockatoos, tawny frogmouth and southern boobook. These birds are known to feed on the ground or eat small rodents and could have been exposed to the virus via ingestion of contaminated material. There is no evidence that any of these species can support the dissemination of mastadenoviruses (Wang and Zhao, 2019). In addition, the experimental inoculation of chickens with a HAdV-5-based vaccine showed that the virus has limited ability to infect bird cells, suggesting that the likelihood of co-infection between the GMO and other AdVs in birds exposed via ingestion of contaminated food or water is very low. In the event of co-infection, it is unlikely that the GMO would be present simultaneously with a WT-AdV in the cells of exposed people, birds or animals. Further, recombination is more likely to occur between viral strains of the same species. The parent organism is classified as a species C of human adenoviruses and therefore it is unlikely that recombination would occur between two different species or genera of AdVs.

201.   In the event of recombination, the WT-AdV could receive the expression cassette of the WIVA20 vaccine and express Antigens 1 and 2. The GMO could regain its E1 gene or corresponding region of another AdV, and become replication competent, but it would lose the expression cassette encoding WIVA20 antigens. This would result in a replication competent GMO without the WIVA20 antigens and E3; and a replication incompetent AdV expressing WIVA20 antigens. The resulting viruses are unlikely be more pathogenic than a WT-AdV strain.

202.   The GMO could regain its E3 gene and therefore its immuno-evasive properties but remain replication incompetent. The recombinant virus would not be able to replicate and would eventually be cleared by the immune system of the host. As an HAdV, the recombinant virus is not expected to cause disease in birds and animals.

203.   As discussed in Chapter 1, Section 3.4, recombination is an important source of genetic variation in viruses. Recombination of genes encoding structural proteins, such as hexon, penton and fibre regions of AdV can result in altered cell tropism. However, genomic analysis has shown that recombination in HAdV-C is more likely to occur in E1 and E4 regions. These regions are involved in the regulation of transcription and cellular process and are not expected to result in changes to the virus pathogenicity or host range. Therefore, the likelihood of homologous recombination at the hexon, penton and fibre regions of AdV, resulting in a GMO with an altered cell tropism is very low. In the event of recombination, the resulting AdV would remain replication incompetent.

204.   If a recombinant replication competent HAdV is produced, it could be shed from the original host and transmitted to other hosts (human, birds or animals) in the environment. These replication competent viruses would not include the WIVA20 antigens and would be similar to a WT-AdV. In addition, in order for a full reversion into a WT virus, multiple recombination events would need to occur, and this is highly unlikely.

205.   WIVA20 antigens were designed to induce a specific immune response against DFTD. Neoantigen-based cancer therapies have been considered safe in non-clinical and clinical studies. Further, as discussed in Risk scenarios 1 and 2, if cells of a host produce peptides that are identical to one of the antigens included in the GM vaccine, the antigen would likely be recognised as a self-peptide and would inhibit any immune response. Therefore, the increase expression of WIVA20 antigens in a host is unlikely to result in autoimmunity.

Potential harm

206.   If complementation were to occur, the number of replication incompetent GMO produced in the host cells would increase resulting in increased expression of WIVA20 antigens in the host. The expression of the vaccine antigens in the host could induce an antibody response against DFT cells. The potential for cross reactivity of these antigens with cells of human, birds or animals has not been investigated. However, due to their specificity to cancer, the risk of neoantigens inducing autoimmunity has been considered low (D'Alise et al., 2019). The temporary increase in the number of

adenoviral particles could result in mild symptoms such as local inflammation, flu-like symptoms or eye infection that are expected to resolve within one week (Chapter 1, Section 3.3).

207.   If homologous recombination were to occur, it could result in the formation of replication competent HAdV-5. The person exposed could potentially experience mild respiratory or eye infections depending on the route of exposure as described in Chapter 1, Section 3.3. These infections are self-limiting and rarely need medical intervention. If needed, antiviral therapies could be used (Chapter 1, Section 3.9). As discussed in Chapter 1, Section 3.5 and Risk Scenario 2, animals are not naturally infected with HAdVs. Although HAdV species C have been detected in the faeces of domestic and wild animals, including birds, there is no evidence of animal disease following a natural exposure to the human virus. Theoretically, if homologous recombination in the major capsid proteins (HAdV-C) or other AdV regions with high homology occurs, it could alter the tropism and host range of the virus. However, the risks of potential increased harm are negligible as AdVs do not typically cause severe disease and the resultant recombinants are unlikely to be more pathogenic than a WT-AdV strain.

Conclusion

208.   The exposure of people or animals to a GMO which has acquired the E1 gene, an AdV that has acquired WIVA20 transgenes or other recombinant viruses resulting in adverse immune response or disease in people or animals is not identified as a risk that could be greater than negligible. Therefore, it does not warrant further assessment.

Section 3   Uncertainty

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

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

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

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

212.   As trials are designed to gather data, there are generally data gaps when assessing the risks of a trial application involving GMOs. However, proposed 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.

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[3] A more detailed discussion is contained in the Regulator's *Risk Analysis Framework* available from the OGTR website or via Free call 1800 181 030.

213.   For DIR 195, uncertainty is noted in relation to:

- *Biodistribution and shedding of the GMO*. Data available for other adenoviral vector-based vaccines/therapies indicates that the GMO is likely to be shed in faeces and saliva following oral or i.t. administration. In addition, the GMO is likely to be present in the spleen, liver and lymph nodes for two weeks and in tumours for up to 3 months. However, there is uncertainty as to whether the data gathered in humans or other animals would be relevant to devils.

- *The potential for cross reactivity of the polypeptide neoantigens with healthy tissue of humans, birds or other animals*. Cancer therapies based on neoantigens peptides have been shown to pose low risk of autoimmunity in humans and animal models. However, there is uncertainty as to whether these data apply to the PPNA included in the WIVA20 vaccine.

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

Section 4   Risk evaluation

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

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

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

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

- the GMO is replication defective
- suitability of limits and controls proposed by the applicant

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

Chapter 3   Risk management plan

Section 1   Background

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

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

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

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

Section 2   Risk treatment measures for substantive risks

223.   The risk assessment of risk scenarios listed in Chapter 2 concluded that there are negligible risks to people and the environment from the proposed trial with the GMO. These risk scenarios were considered in the context of the scale of the proposed trial (Chapter 1, Section 2.1), the proposed controls (Chapter 1, Section 2.2), the proposed receiving environment (Chapter 1, Section 5), and considering both the short and long term effects of the GMO. Limits and controls proposed by the applicant and other general risk management measures are discussed below.

Section 3   General risk management

224.   The limits and controls proposed in the application were important in establishing the context for the risk assessment and in reaching the conclusion that the risks posed to people and the environment are negligible. Therefore, to maintain the risk context, licence conditions have been proposed to limit the number of animals, limit the locations to contained trial sites, limit the duration of the trial, as well as a range of controls to restrict the spread and persistence of the GM vaccine and its genetic material in the environment.

3.1   Limits and controls on the trial

225.   Sections 2.1 and 2.2 in Chapter 1 list the limits and controls proposed by University of Tasmania. Many of these are discussed in the risk scenarios considered in Chapter 2.. The appropriateness of the limits and controls is considered further in the following sections.

3.1.1 *Consideration of limits and controls*

226.   The proposed trial would involve a maximum of 22 Tasmanian devils. Animals would be kept in contained enclosures at two trial sites in Tasmania. The applicant proposed that the trial will be

completed within 5 years. Conditions maintaining the risk context and proposed limits of the trial, such as the maximum number of animals and duration of the study, have been included in the licence.

227.   Transport of the GMO, biological samples, material and waste reasonably expected to contain GMO would be conducted in accordance with the Regulator's *Guidelines for the Transport, Storage and Disposal of GMOs*. These are standard protocols for the handling of GMOs to minimise exposure to the GMOs, and are suitable for the GM vaccine. Therefore, the licence details the minimum requirements for packaging and labelling the GMO, samples, material and waste containing the GMO for transport and storage. These measures would limit the exposure of people and the environment to the GMOs.

228.   The applicant advised that most of the time the animals would be capture using a standard PVC trap but on rare occasions an animal may need to be captured by hand. As discussed in Chapter 1, Section 4.4, the administration of the GMO via DIOC or i.t. injection could result in shedding of the GMO in saliva for up to 2 weeks increasing the potential for exposure to the GMO via animal bites. Therefore, licence conditions have been included requiring the exclusive use of PVC traps to capture animals for at least 2 weeks after the administration of the GMO via DIOC or i.t. injection. An exemption is made when animals are sick or injured and in need of immediate veterinary care. To minimise the potential of exposure to the GMO via animal bites, personnel capturing animals in need of immediate care by hand must wear extra-long (elbow length) leather gloves.

229.   In addition, the applicant advised that animals would be kept under anaesthesia during the administration of the GMO and/or during collection of biological samples. These measures would further minimise the potential of exposure via animal bites or needle stick injury during administration of the GMO. Staff preparing and administering the GMO would wear PPE including, gowns, gloves, masks and eye protection minimising the risk of exposure to the concentrated GMO via spill or aerosol formation. The applicant proposed that staff collecting blood and swab samples would wear gloves. As discussed in Chapter 1, Section 4.4, blood samples collected at least 7 days after administration are not expected to contain GMO. However, blood and swab samples collected prior to 7 days are likely to contain GMO. Therefore, the use of gowns, gloves, masks and eye protection is recommended during preparation and administration of the GMO and during the collection of samples that are likely to contain GMOs. This practice would minimise exposure of people preparing or administering the GMO (Risk scenario 1) or collecting biological samples and have been included as licence conditions.

230.   The applicant proposed that preparation and administration of the GMO would be conducted in an on-site veterinarian shed. GMO-inoculated devils would be individually identified and kept in double barrier enclosures at the trial sites. Signs indicating the presence of the GMO would be placed at all entrances to the animal enclosures. In addition, the applicant has proposed that artificial dens would be placed outside each of the four perimeter fence walls for the duration of the trial to re-trap a devil in the event of an animal escaping. Effective identification and containment of the GMO-inoculated devils would minimise the potential for dispersal of the GMO in the environment and exposure of other animals outside the enclosures (Risk scenario 2). These measures have been included as licence conditions.

231.   The applicant proposed to replace the devils' drinking water three times per week and to decontaminate the waste drinking water prior to disposal for 2 weeks after injection. In addition, in the first two weeks following administration of the GMO, faeces would be collected from the enclosures daily, and subsequently at least twice a week. Faecal waste reasonably expected to contain the GMO would be stored at the trial sites prior to being transported to UTAS for decontamination. As discussed in Chapter 1, Section 4.4 i.m. administration of the GMO is unlikely to result in shedding. However, the administration of the GMO via DIOC or injection into oral tumours could result in shedding of the GMO in saliva or faeces for up to 2 weeks. Therefore, licence

conditions have been proposed requiring the replacement of devils drinking water at least three times per week and daily collection of faeces from the enclosures for at least two weeks after the administration of the GMO via DIOC or injection into oral tumours. Waste drinking water and faeces collected during this period must be decontaminated prior to disposal. These measures minimise the exposure of other animals to the GMOs present within the enclosures.

232.   The applicant proposes to collect samples (blood, faeces, oral and anal swabs) at various time points after administration of the GMO. As mentioned in Chapter 1, Section 4.4, the GMO could be present in blood samples in the first four days after i.m. administration of the GMO. Faecal, oral and anal swab samples are likely to contain GMO for the first two weeks after administration of the GMO via DIOC or injection into oral tumours. As discussed in Risk Scenario 2, part of the shed GMOs detected in biological samples are likely to consist of shredded viral DNA and not viable viral particles. However, as a precaution, the licence includes conditions that apply to blood samples collected from animals up to 7 days after i.m. immunisation, to faecal samples, oral and anal swabs collected animals up to 2 weeks after administration of the GMO via DIOC or i.t. injection and to any other sample, materials or waste, that are reasonably expected to contain the GMO.

233.   Biological samples collected post-administration would be stored in locked sheds at the trial sites prior to being transported to UTAS. Sample analysis would be performed in a certified PC2 laboratory at UTAS using standard PC2 work practices. The applicant proposed that personnel conducting dealings with the GMO outside a BSC would wear eye protection and face mask. These measures would minimise the potential of exposure of personnel to the GMO during sample analysis and have been included as licence conditions.

234.   The applicant proposed that faecal samples collected from GMO-inoculated devils would be tested for the presence of the GMO weekly. Animals showing at least 1 month of negative test results for the shedding of GMO DNA in faeces would be transferred to an alternative facility. Animals that have been vaccinated with the GMO and developed tumours but have not received i.t. injections of the GMO and are no longer shedding the GMO as a result of the vaccination, could also be transferred to these alternative facilities. Should these animals be selected to receive the GMO as a cancer treatment, they would be returned to the contained enclosures and treated as described above. The applicant proposed that animals sourced from the wild would be released back into the environment after 6 months of negative tests for GMO DNA in faeces; i.e. a total of 24 weekly tests would be undertaken. However, as the GMO is replication defective and unlikely to undergo reactivation, it is expected that samples from animals would be negative for the GMO after 2 weeks and weekly tests over a 6 month period were considered unnecessary. Therefore, as a precaution, licence conditions require at least 4 consecutive negative test results with a minimum of 1 week between each test, prior to transferring an animal to an alternative facility and at least 6 consecutive negative test results with a minimum of 4 weeks between each test, prior to releasing an animal into the environment. Animals to be released into the environment would also be tested for the presence of DFT1 and DFT2 cells; animals with active DFTD would not be released. These measures imposed as licence conditions, would ensure that the devils to be transferred to an alternative facility or released into the environment do not contain residual episomes from the GM vaccine.

235.   Conditions are included in the licence requiring the licence holder to ensure that all GMOs, including material or waste that has been in contact with the GMO be decontaminated by autoclaving, chemical treatment or by high-temperature incineration. Tasmanian devils may die from DFTD during the trial. The applicant proposed that devil remains reasonably expected to contain the GMO would be transported to UTAS and autoclaved or frozen and then autoclaved before disposal. The applicant has provided evidence that the autoclave cycle is adequate for decontamination.

236.   A standard condition is included in the licence requiring the licence holder to ensure that dealings are conducted to ensure containment of the GMO so as not compromise the health and safety of people and minimise unintentional exposure to the GMO.

237.   Other conditions included in the licence are standard conditions that state that only people authorised by the licence holder are covered by the licence, and that the licence holder must inform all people dealing with the GMOs, of applicable licence conditions.

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

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

- limit the trial to 22 Tasmanian devils, which are to be conducted in two contained trial sites in Tasmania;
- restrict access to the GMO;
- ensure personnel involved in the trial are appropriately trained and follow appropriate behavioural requirements;
- ensure appropriate PPE is used;
- restrict personnel permitted to administer the GMO;
- require decontamination of the GMO, biological samples, materials and equipment that have been in contact with the GMO at the trial site or UTAS using effective disinfectants or disposal methods.
- transport and store the GMO, samples, or other materials or waste, that are reasonably expected to contain the GMO according to the OGTR *Guidelines for the Transport, Storage and Disposal of GMOs*.

3.2   **Other risk management considerations**

239.   All DIR licences issued by the Regulator contain a number of conditions that relate to general risk management. These include conditions relating to:

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

3.2.1 *Applicant suitability*

240.   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.

241.   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.

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

3.2.2 *Reporting requirements*

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

- any additional information regarding risks to the health and safety of people or the environment associated with the dealings

- any contraventions of the licence by persons covered by the licence
- any unintended effects of the trial.

244.   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:

- expected date of administration with the GMO for each trial site
- cease of administration with the GMO for each trial site.

3.2.3 *Monitoring for compliance*

245.   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.

246.   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.

247.   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 or 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

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

- data regarding the shedding of the GMO by inoculated devils
- data regarding the safety of the GMO, including immune cross-reactivity studies of the polypeptides neoantigens, in the event of exposure of people or animals (other than a devil) that are likely to be present in the Australian environment.

Section 5　　Conclusions of the RARMP

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

250.   Conditions are 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.

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

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

Submission	Summary of issues raised	Comment
1	Has no concerns regarding the risk management proposed.	Submission has been noted.
2	Has no comments on the RARMP.	Submission has been noted.
3	Raised concerns regarding the vaccination of a wild population. Stated that if the trial is successful, it could lead to an uncontrollable dissemination of the GM vaccine, possibly across many mammalian species. The dissemination of the GM vaccine to the wild Tasmanian devil population could also have a positive effect.	The risks associated with exposure to the GM vaccine of other animals are discussed in Risk Scenario 2. As discussed in Section 3.5 of the RARMP, HAdVs have restricted ability to replicate and cause disease in animals. Furthermore, the GM vaccine is replication defective, it is highly unlikely that the exposure to the GM vaccine would result in harm to other animals. In addition, as this is a trial, DIR-195 licence includes limits and controls to minimise the potential for the GMO to spread in the environment.
	Recommended care and investigation during the trials to detect any unforeseen transmission to humans or other mammals in the enclosures, and whether this has adverse consequences.	Licence conditions require the licence holder to notify the Regulator of any unintentional release of the GMO and exposure of people. In addition, the licence holder must notify the Regulator in the event of an animal experiencing a serious adverse event that may be related to the GMO.
4	Agrees with the overall conclusions in Risk scenarios 1 and 2.	Submission has been noted.
	Recommended further discussion on Risk scenario 3 and amendments to include:	
	• that HAdV5 is the most common HAdV with the highest seroprevalence rates globally and it is unlikely that levels of HAdV are low in the Australian population.	Additional information has been included in Section 3.7 and Risk Scenario 3 to make it clear that HAdVs are present in the environment.

Submission	Summary of issues raised	Comment
	• that shedding duration can be different in different animals and sometimes AdV infections in animals can result in lifelong intermittent shedding and persistence in tissues for months. In addition, shedding is dependent on administration route.	The persistence of WT AdVs in tissues is discussed in Chapter 1, Section 3.2. This section has been amended to further clarify the potential for reactivation of the latent form of the WT virus. The potential of reactivation leading to shedding of WT AdVs is clarified in Section 3.6, same Chapter
		However, as the GM vaccine is replication defective, reactivation is not expected in the inoculated animals and shedding would be limited to the number of viral particles administered. Similar effects are expected in the event of exposure of other animals to the GMO. Section 3.6 and 4.4 of the RARMP have been amended to clarify the information included.
	• that AdVs are resistant to physical and chemical agents and can persist in the environment, including in fresh and marine water.	The environmental stability of WT AdV and the GM vaccine is discussed in Chapter 1, Section 3.8 and Section 4.6. The risk of exposure of other animals to the GMO in the environment is discussed in Risk Scenario 2.
	• that HAdVs and HAdV C species have been detected in domestic and wild animals and can accumulate in scavenger and carnivore species. The RARMP should discuss detection of HAdV C in species other than humans and the potential for animals, birds or carnivores to act as reservoirs or transmission vectors of wild type HAdVs to the vaccinated Tasmanian devils.	Section 3.5 of Chapter 1 has been amended to include the detection of HAdVs in faeces of domestic and wild animals. Risk Scenario 3 has been amended to include information relating to the potential for domestic and wild animal to act as a reservoir of mastadenoviruses.
	• relevant data on recombination of adenoviruses, especially for group C adenoviruses and HAdV5.	Information regarding the recent isolation of recombinant strains of HAdV species C has been added to Chapter 1, Section 3.4. Risk scenario 3 has been amended to further discuss recombination in the species.
	• further discussion on HAdV host range and the potential to cause disease and harm in animals.	Section 3.5 of Chapter 1 has been expanded to include additional information regarding experimental administration of HAdV to animals. Risk

Submission	Summary of issues raised	Comment
		scenario 2 and 3 were expanded to further discuss the HAdV host range and potential to cause harm in animals. However, it is to be noted that the data included in this section of the RARMP are in relation to replication competent human adenovirus which is not the case for the GM vaccine used in this application.
5	• Agrees that the risk assessment identifies all plausible risk scenarios by which the proposed dealings could potentially give rise to risks relating to the health and safety of people or the environment.	Submission has been noted.
	• Agrees that the limits and controls proposed in the draft licence to prevent the spread of the GM vaccine are appropriate for the trial.	Submission has been noted.
	• Agrees with the overall conclusion of the RARMP	Submission has been noted.
	• The Regulator should reconsider the necessity of 12 tests over 6 months before releasing the devils into the wild.	Licence condition 25(b) has been amended and requires at least 6 negative test results with a minimum of 4 weeks between each test.
	• The Regulator should further consider the risks from needle stick injuries, and devil bites and scratches	Chapter 1, Section 2.3.5 has been amended to clarify that needles would not be removed or recapped. The risk of exposure of people undertaking to GMO via needle stick injury, animal bites and others, was addressed in Risk scenario 1. This risk scenario has been expanded to included risk of exposure via animal scratches.
		Animal husbandry staff entering the enclosures to collect faeces samples are required to wear waterproof boots and gloves. This measure would minimise the risk of exposure to the GMO during sample collection
6	Agrees with the approach and considers that under strict adherence to the proposed control measures, the risk	Submission has been noted.

Submission	Summary of issues raised	Comment
	posed by the GMO to the health of humans and the environment is minimal.	
7	Supports the development of a vaccine against DFTD.	Submission has been noted.
	Noted that in addition to the APVMA permit, the applicant would require a separated application made under the provisions of the *Tasmanian Genetically Modified Organisms Control Act 2004* and the *Tasmanian Gene Technology Policy 2019-2029* and associated guidelines.	The interface with other regulatory schemes is described in Section 1.1, Chapter 1.
	Recommended: • In the event a person is exposed to the GMO, this should be reported to a local medical practitioner who is aware of the trial and specialises in infectious diseases.	The GM vaccine is replication defective and unlikely to cause infections and risks associated with accidental exposure to the GMO are considered negligible. Condition 47 of the licence requires that any person exposed the GMO is offered prompt medical advice. The clinician must be provided with relevant information about the GMO.
	• that immunocompromised individual should not handle the GMO.	The risks associated with exposure of immunocompromised individuals is discussed in Risk Scencario 1. As the GM vaccine is replication defective and acciddental exposure would involve a small number of viral particles, these risks were considered negligible.
	• amendments to the RARMP to clarify the prevalence of HAdV infections.	Additional information has been included in Section 3.7 and Risk Scenario 3 to make it clear that HAdVs are present in the environment.
	• the use of PPE all times during the trial, not only when samples are likely to contain GMOs	*The Gene Tecnology Act 2000* and *Gene Technology Regulations 2001* apply to dealings involving GMOs. When working with samples unlikely contain GMO, personnel should follow standard procedures for handling of biological samples.

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

The Regulator received 2 submissions from the public on the consultation RARMP. The issue raised in the submission is summarised in the table below. All issues that related to risks to the health and safety of people and the environment were considered in the context of currently available scientific evidence in finalising the RARMP that formed the basis of the Regulator's decision to issue the licence.

Submission	Summary of issues raised	Comment
1	Provided hypothesis for the emergence of the DFTD.	Submission has been noted.
2	Opposed to the application on ideological grounds.	Submission has been noted.