Australian Government
Department of Health
Office of the Gene Technology Regulator

February 2021

# Risk Assessment and Risk Management Plan

for

# DIR 177

## Clinical trial of genetically modified human adenovirus for bladder cancer treatment

**Applicant** – Novotech (Australia) Pty Limited

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# Summary of the Risk Assessment and Risk Management Plan

**for**

# Licence Application No. DIR 177

## *Decision*

The Gene Technology Regulator (the Regulator) has received a licence application to conduct a clinical trial using a genetically modified organism (GMO). It qualifies as a DIR licence application under the *Gene Technology Act 2000* (the Act). The applicant, Novotech (Australia) Pty Limited, proposes to conduct a clinical trial to assess the efficacy of the genetically modified (GM) human adenovirus for bladder cancer treatment in participants whose tumours are unresponsive to standard treatment.

Transitional cell carcinoma (TCC) is the most common type of bladder cancer. Each year almost 2,700 new cases and approximately 1100 deaths of TCC are recorded in Australia. Current treatment includes surgery to remove the bladder tumour, chemotherapy or immunotherapy. The combination of treatments give the best results but cancer reoccurrence rates are still high.

The proposed GM adenovirus treatment is predicted to significantly increase survival rates and limit the reoccurrence in participants that have been unresponsive to other treatments. The GM human adenovirus would be manufactured overseas and imported into Australia. It would be administered into the bladder to a maximum of 60 participants at hospitals located in New South Wales (NSW) and Victoria (VIC).

Clinical trials in Australia are conducted in accordance with requirements of the *Therapeutic Goods Act 1989*, which is administered by the Therapeutic Goods Administration (TGA). Therefore, in addition to approval by the Regulator, Novotech (Australia) Pty Limited would require authorisation from TGA before the trial commences. Clinical trials conducted in Australia must also be conducted in accordance with the *National Statement on Ethical Conduct in Human Research* and with the *Guidelines for Good Clinical Practice* of the International Council for Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use.

The international sponsor for the trial is a privately held, clinical-stage biopharmaceutical company CG Oncology, which is based in the United States. Novotech, a clinical research organisation, is applying for authorisation to conduct the proposed clinical trial in Australia and is responsible for ensuring that the licence conditions are met.

Novotech (Australia) Pty Limited would also require approval from the Department of Agriculture, Water and the Environment for import of the GMO.

The Regulator has prepared a Risk Assessment and Risk Management Plan (RARMP) for this application, which concludes that the proposed clinical trial poses negligible risks to human health and safety and the environment, and that any risks posed by the dealings can be managed by imposing conditions on the trial.

## *The application*

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| ***Project Title*** | Clinical trial of a genetically modified human adenovirus for treatment of bladder cancer [1]. |
| ***Parent organism*** | Human adenovirus type 5 (Ad5) |
| ***Principal purpose*** | The proposed trial is a phase 3 study designed to evaluate the efficacy of the genetically modified human adenovirus for bladder cancer treatment in participants whose tumours are unresponsive to standard treatment. |
| ***Genetic modifications*** | Modified human adenovirus:<br>Partial deletion of viral *gene E3 and E1a promoter* and insertion of*:*<br>- A promoter providing tumour specificity – *human hE2F-1 promoter*<br>- A gene stimulating anti-tumour response – *human hGM-CSF gene*<br>- pA signal protecting from transcriptional read-through |
| ***Previous clinical trials*** | Three clinical trials have been conducted:<br>Phase 1 clinical trials in the United States and Canada<br>Phase 2 clinical trial in the United States (study 1)<br>Phase 2 clinical trial in the United States (study 2) |
| ***Proposed limits and controls*** | |
| *Proposed duration* | 5 years |
| *Proposed trial size* | Up to 60 clinical trial participants in Australia |
| *Proposed locations* | Hospitals in NSW and VIC |

## *Risk assessment*

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

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

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

Important factors in reaching the conclusions of the risk assessment include: that the GMO replicates preferentially in cancer cells; the GMO has limited ability to stimulate the immune response in healthy

[1] The title of the project as submitted by the applicant was: 'Clinical Trials with an Oncolytic Treatment Vaccine (CG0070)'

hosts and other animals; and the exposure to the GMO would be minimised by the imposed limits and controls.

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

## *Risk management plan*

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

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

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# Abbreviations

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|---|---|
| Act | *Gene Technology Act 2000* |
| AICIS | Australian Industrial Chemicals Introduction Scheme |
| ARTG | Australian Register of Therapeutic Goods |
| °C | Degrees Celsius |
| Ad5 | Adenovirus serotype (type) 5 |
| AdV-C | Adenovirus species C |
| AE | Adverse Event |
| BCG | Bacillus Calmette-Guérin |
| bp | Base Pair |
| BSC | Biosafety Cabinet |
| cDNA | Complementary Deoxyribonucleic Acid |
| CFDA | China Food and Drug Administration |
| CIS | Carcinoma *in situ* |
| Cth | Commonwealth of Australia |
| DAWE | Department of Agriculture, Water and the Environment |
| DDM | n-dodecyl-β-D-maltoside |
| DIR | Dealings Involving Intentional Release |
| DNA | Deoxyribonucleic Acid |
| DNIR | Dealing Not Involving Intentional Release |
| DP | Dimerization Protein |
| FSANZ | Food Standards Australia New Zealand |
| GCP | Good Clinical Practice |
| GI | Gastrointestinal |
| GM | Genetically Modified |
| GM-CSF | Granulocyte-Macrophage Colony Stimulating Factor |
| GMO | Genetically Modified Organism |
| GMP | Good Manufacturing Practice |
| gp | Glycoprotein |
| h | Hour |
| HAdV | Human Adenovirus |
| HAdV-C | Human Adenovirus species C |
| hGM-CSF | Human Granulocyte-Macrophage Colony-Stimulating Factor |
| HGT | Horizontal Gene Transfer |
| HREC | Human Research Ethics Committee |
| HSCT | Hematopoietic Stem Cell Transplant |
| HSV | Herpes simplex Virus |
| IATA | International Air Transport Association |
| IBC | Institutional Biosafety Committee |

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|---|---|
| ICH-GCP | *Guidelines for Good Clinical Practice* of the International Council for Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use |
| ITR | Inverted terminal repeat |
| IVE | Intravesical |
| L | Litre |
| mL | Millilitre |
| NHMRC | National Health and Medical Research Council |
| NLRD | Notifiable Low Risk Dealings |
| NMIBC | High-grade Non-Muscular Invasive Bladder Cancer |
| NPAAC | National Pathology Accreditation Advisory Council |
| NSQHS | The National Safety and Quality Health Service |
| NSW | New South Wales |
| OGTR | Office of the Gene Technology Regulator |
| pA | Polyadenylation |
| PC | Physical Containment |
| PCR | Polymerase Chain Reaction |
| PPE | Personal Protective Equipment |
| pRb | Retinoblastoma tumour-suppressor Protein |
| PVC | Polyvinyl Chloride |
| QC | Quality Control |
| RARMP | Risk Assessment and Risk Management Plan |
| Rb | Retinoblastoma |
| Regulations | Gene Technology Regulations 2001 |
| Regulator | Gene Technology Regulator |
| RNA | Ribonucleic Acid |
| TGA | Therapeutic Goods Administration |
| Th1 | Type 1 T helper cells |
| TSD | Transport, Storage and Disposal of GMOs |
| TCC | Transitional Cell Carcinoma |
| TURBT | Transurethral Resection of Bladder Tumour |
| T-VEC | Talimogene laherparepvec |
| US | United States |
| USFDA | United States Food and Drug Administration |
| VIC | Victoria |
| vp | Viral Particles |
| vp/mL | Viral Particles per Millilitre |
| WHO | World Health Organisation |
| WT | Wild-type |

# Chapter 1 Risk assessment context

## Section 1 Background

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

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

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

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

5. Figure 1 shows the information that is considered, within the regulatory framework above, 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 clinical trial are assessed within this context. Chapter 1 provides the specific information for establishing the risk assessment context for this application.

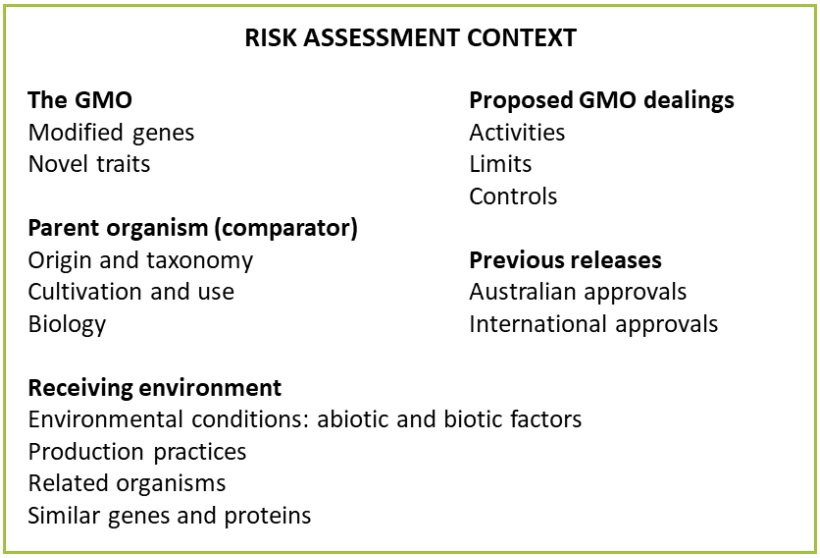


**Figure 1. Summary of parameters used to establish the risk assessment context, within the legislative requirements, operational policies and guidelines of the OGTR and the RAF.**

6. In accordance with Section 50A of the Act, this application is considered to be a limited and controlled release application, as the Regulator was satisfied that it meets the criteria prescribed by

the Act. Therefore, the Regulator was not required to consult with prescribed experts, agencies and authorities before preparation of the RARMP.

7. Section 52 of the Act requires the Regulator to seek comment on the 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.

**Interface with other regulatory schemes**

8. Gene technology legislation operates in conjunction with other regulatory schemes in Australia. The GMOs and any proposed dealings conducted under a licence issued by the Regulator may also be subject to regulation by other Australian government agencies that regulate GMOs or GM products, including Food Standards Australia New Zealand (FSANZ), the Australian Pesticides and Veterinary Medicines Authority (APVMA), the Therapeutic Goods Administration (TGA), the Australian Industrial Chemicals Introduction Scheme (AICIS) and the Department of Agriculture, Water and the Environment (DAWE). Proposed dealings may also be subject to the operation of State legislation declaring areas to be GM, GM free, or both, for marketing purposes.

9. Medicines and other therapeutic goods for use in Australia are required to be assessed for quality, safety and efficacy under the *Therapeutic Goods Act 1989* and must be included in the Australian Register of Therapeutic Goods. The TGA is responsible for administering the provisions of this legislation. Clinical trials of therapeutic products that are experimental and under development, prior to a full evaluation and assessment, are also regulated by the TGA through the Clinical Trial Approval (CTA) scheme or the Clinical Trial Notification (CTN) scheme.

10. For clinical trials, the TGA has regulatory responsibility for the supply of unapproved therapeutic products. In terms of risk to individuals participating in a clinical trial, the TGA (as the primary regulatory agency), the trial sponsor, the investigators and the Human Research Ethics Committee (HREC) at each trial site all have roles in ensuring participants' safety under the *Therapeutic Goods Act 1989*. However, where the trial involves a GMO, authorisation is also required under gene technology legislation. To avoid duplication of regulatory oversight, and as risks to trial participants are addressed through the above mechanisms, the Regulator's focus is on assessing risks posed to people other than those participating in the clinical trial, and to the environment. This includes risks to people preparing and administering the GM virus, and risks associated with import, transport and disposal of the GMO.

11. The International Council for Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use – Guidelines for Good Clinical Practice (ICH-GCP) is an international ethical and scientific quality standard for designing, conducting, recording and reporting trials that involve the participation of human subjects (ICH 1996). The guideline was developed with consideration of the current good clinical practices of the European Union (EU), Japan, and the United States of America (USA), as well as those of Australia, Canada, the Nordic countries and the World Health Organization (WHO). The TGA has adopted the ICH-GCP in principle as Note for Guidance on Good Clinical Practice (designated CPMP/ICH/135/95) (Therapeutic Goods Administration 2000), which provides overarching guidance for conducting clinical trials in Australia which fall under TGA regulation.

12. The National Health and Medical Research Council (NHMRC) has issued the *National Statement on Ethical Conduct in Human Research* (National Health and Medical Research Council et al., 2018). This document sets the Australian standard against which all research involving humans is reviewed.

The *Therapeutic Goods Act 1989* requires that the use of a therapeutic good in a clinical trial must be in accordance with the ethical standards set out in this document.

13. Approval by a Human Research Ethics Committee (HREC) is also a fundamental requirement of a clinical trial. HRECs conduct both ethical and scientific assessment of the proposal and in addition often consider issues of research governance. Other elements of governance of clinical trials that are considered by HRECs include appropriate informed consent, specific inclusion and exclusion criteria, data monitoring and vaccine accounting and reconciliation.

14. The Department of Agriculture, Water and the Environment administers Australian biosecurity conditions for the importation of biological products under the *Quarantine Act 1908*. Biological products include animal or microbial derived products such as foods, therapeutics, laboratory materials and vaccines (including GM vaccines). Import of GM virus is subject to regulation by the Department of Agriculture, Water and the Environment and the Regulator.

15. All clinical trial sites would be located at medical facilities including out-participant settings, hospitals and associated pharmacies. Analysis of biological samples collected from trial participants administered with the GMO would occur at clinical trial sites, or at pathology laboratories. These facilities are regulated by State and Territory governments and adhere to professional standards for safety (NSQHS), disease control (Australian Guidelines for the Prevention and Control of Infection in Healthcare (2019) and handling of pathology samples (NPAAC).

# Section 2 The proposed dealings

16. Novotech (Australia) Pty Ltd has proposed clinical trials of a GM human adenovirus that preferentially replicates in bladder cancer cells and stimulates the immune system in the tumour.

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

- importing the GMO;
- preparing the GMO for administration;
- instilling the GMO in the bladder;
- collecting samples that may contain the GMO;
- analysis of the samples mentioned above;
- transporting and storing the GMO;
- disposing of the GMO, and

the possession, supply or use of the GMO for the purposes of, or in the course of, any of the above.

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

18. The trial is proposed to take place for up to 5 years from the date of issue of the licence. The applicant intends to treat up to 60 participants with the GMO. Participants may receive up to 24 doses over a period of 21 months, first weekly for 6 weeks then less frequently depending on the participant immune reaction to the treatment.

19. The trial would take place at five clinical sites in Australia. While the selection of clinical sites has not been finalised, participating hospitals will be located in NSW and VIC. Proposed clinical trial sites include Royal Melbourne Hospital, Wollongong Private Hospital, and Barwon Health, Geelong. Only trained and authorised staff would be permitted to conduct dealings with the GMO.

## 2.2. The proposed controls to restrict the spread and persistence of the GMOs

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

- ensuring that the GMO is administered by authorised, appropriately trained medical staff in clinical facilities in accordance with good clinical practice (GCP) guidelines and standard precautions for working with PC2 material;
- ensuring that participants are instructed on guidelines and protocols such as, decontaminating their urine for 30 days after each treatment and other guidelines outlined in the *Informed Consent and Research Authorization Form;*
- requiring that clinical trial staff handling and/or administering the GM treatment wear and use personal protective clothing and equipment. Further information regarding the conduct of the trial has been declared Confidential Commercial Information (CCI) under Section 185 of the Act. Relevant CCI will be made available to the prescribed experts and agencies that are consulted on the RARMP;
- requiring that any leakage of GMO from the instillation/bladder that will occur during the administration of the GMO, is to be collected with absorbent pads, double contained and disposed of as PC2 infectious clinical waste;
- import, transport and storage of the GMO and the GMO contaminated waste generated at a clinical trial site must be performed in accordance with the current version of the Regulator's *Guidelines for the Transport, Storage and Disposal of GMOs.* Import will be additionally performed in accordance with International Air Transport Association (IATA) guidelines;
- requiring decontamination of materials and equipment that have been in contact with the GMOs at clinical trial sites using effective disinfectants or, disposal and destruction using a certified waste contractor in accordance with standard clinical waste disposal practices, as required by the relevant Australian and state legislation, and
- requiring that all personnel handling the GMO will be trained or informed of contingency plans and spills procedures.

## 2.3. Details of the proposed activities

### *2.3.1. Manufacture of the GMO*

21. The GMO has been manufactured overseas in accordance with good manufacturing practice (GMP). Further information regarding the manufacturing of the GMO has been declared CCI under Section 185 of the Act. Relevant CCI was made available to the prescribed experts and agencies that were consulted on the RARMP.

### *2.3.2. Conduct of the clinical trial*

22. The proposed clinical trial is a phase 3, open-label, single arm trial evaluating the efficacy and safety of the adenovirus treatment in subjects with a persistent NMBIC unresponsive to Bacillus Calmette-Guérin (BCG) therapy.

### *2.3.3. Selection of trial participants and behavioural requirements*

23. In the proposed clinical trial, the GMO would be administered via intravesical (IVE) route to participants with carcinoma *in situ* (CIS) unresponsive to the BCG treatment. In order to be enrolled in

the trial, participants must meet the following (but not limited to) relevant inclusion and exclusion criteria:

- trial participant must be ≥18 years of age on the day of signing consent and be willing to adhere to the requirements of the study and to communicate with the Investigator and understand the requirements of the study;
- trial participant must be judged as suitable by the Investigator, as determined by medical history, physical examination, vital signs and clinical safety laboratory examinations;
- trial participants must not be confirmed or suspected to be immunosuppressed and must not be pregnant or breastfeeding;
- trial participants must have pathologically confirmed CIS, according to current WHO grading system, unresponsive to BCG as confirmed by adequate initial treatment;
- histology of the carcinoma must be confirmed to be predominantly (≥ 50%) TCC and have all the Ta and/or T1 disease resected and all CIS resected or fulgurated;
- trial participants must demonstrate adequate organ function, and
- participants who possess a septic toilet must be excluded from the trial.

24. The applicant indicated that participants will be required to avoid contact with high risk subjects including:

- immunocompromised people for 30 days post treatment;
- children under 12 months old, pregnant, or nursing women, for at least 14 days after each treatment.

25. Staff at a high risk from exposure (including immunosuppressed individuals) must not handle and administer the treatment or provide care for the participants for seven days after each dose.

26. Participants would consent to a number of requirements that would minimise the exposure of other people to the GMO:

- participants must not share utensils that have come into contact with body fluids (such as cutlery) for at least 14 days after each treatment;
- participants must abstain from any intimate and sexual physical contact with others, for at least 14 days after each treatment;
- clothing, bedding, and towels should be laundered in hot water with laundry detergent and addition of undiluted house bleach per wash load for at least 14 days after each administration;
- participants must use double barrier contraceptives until 6 weeks after receiving each dose of the treatment, and
- participants must practice good hand hygiene with soap and warm water or with hand rubs containing at least 60% alcohol.

### *2.3.4. Transport, supply and storage of the GMO*

27. The GMO would be imported according to International Air Transport Association (IATA) UN 3373 (category B) requirements for packaging and labelling.

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

29. Transport of the GMOs within Australia will include transport to and from a third-party storage facility and to the clinical trial sites. During transport, the GMO will be kept frozen on dry ice below - 60 °C. Within the clinical trial site, the GMO will be transported between the pharmacy and the point-of-administration and will be accessed only by appropriately trained personnel. The GMO will be contained in a sealed, leak-proof secondary container, labelled to indicate that it contains GMO, the OGTR licence number and contact details of an appropriate clinical trial staff member in case of loss of containment (who would on-report to Novotech).

30. Collected biological samples may be transported to a third-party testing laboratory within Australia or transported for export to overseas laboratories for analysis. Since the collected samples may contain the GMO, they will be handled using packaging and labelling for Risk Group 2 microorganisms.

31. The GMO will be stored in a secure area with restricted access, both in the third party facility and hospital pharmacy below -60 °C. At a clinical trial site, it will be stored in the freezer and kept physically separated from other drug products and accessed by appropriate site personnel only (e.g., trained and appropriately delegated pharmacists, technicians). Similarly, waste containing the GMO would be transported by trained staff in line with the TSDs.

### *2.3.5. Preparation of the GMO for administration*

32. Staff administering the GMO must be trained, adhere to safe sharps handling and disposal practices, use personal protective (PPE) equipment when appropriate and maintain adequate hand and respiratory hygiene. Further information on preparation of the GMO has been declared CCI under Section 185 of the Act. Relevant CCI was made available to the prescribed experts and agencies that were consulted on the RARMP.

### *2.3.6. Intravesical administration of the GMO*

33. The GMO will be administered by IVE directly into the emptied participant's bladder. After a specified period, the bladder contents will be drained into the drainage bags attached to the catheter through a drainage port.

34. Drainage bags will be treated as clinical waste. Any spillage and leakage from the bladder or catheter, would be collected by absorbent material and disposed of in clinical waste as for other contaminated material (see 2.3.9). Participants would be monitored for at least 1 hour following the procedure for observation. Further information regarding the administration of the GMO has been declared CCI under Section 185 of the Act. Relevant CCI was made available to the prescribed experts and agencies that were consulted on the RARMP.

### *2.3.7. Sample collection and analysis*

35. Samples collected from trial participants will include urine, blood, saliva and tumour tissue extracted during biopsies. These samples may be analysed in Australia or overseas in dedicated third-party testing laboratories.

36. Samples will be collected by medically trained staff at multiple time points according to the schedule specified by the applicant and may contain the GMO.

### *2.3.8. Personal protective clothing*

37. Clinical trial staff performing dealings with the GMO and administration of the GMO to trial participants and clean-up of potential spills would wear a gown, gloves, surgical mask and eye protection (safety glasses or face-shields) and adhere to the spill containment procedures.

38. The applicant advised that participants would wear a disposable gown during the procedure.

### *2.3.9. Decontamination and disposal of the GMOs (including waste contaminated with the GMOs)*

39. All unused GMO and disposable materials used during the preparation, administration or the collection of samples from participants (e.g. surgical masks, gloves, needles, tissues, syringes, infusion bags and absorbent towels) would be disposed of according to clinical waste management procedures. Commercial waste management contractors would be used as arranged by a clinical site. All disposable GMO waste would be destroyed by high-temperature incineration.

40. Reusable PPE (e.g. safety goggles/face shields) would be disinfected by soaking in a solution with ≥0.6% active chloride for at least 10 minutes. Disposable gowns used by participants and staff would be disposed as clinical waste. Gowns and scrubs would be laundered according to clinical site protocols unless direct contact with a spill of ≥50 mL has been made, in which case the gowns are to be incinerated.

41. Spills of the GMO would be decontaminated using a fresh dilution of ≥0.6% active chloride or ≥60% ethanol with a minimum contact time of 10 minutes (Lin et al., 2020), or other disinfectants known to be effective against the GMO.

42. In the proposed study, the applicant intends to provide participants with instructions to add bleach to the toilet following urination and prior to flushing for 30 days following administration of the GMO.

### *2.3.10. Training of clinical trial personnel*

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

44. All clinical trial staff handling the GMO will be trained in GCP and all staff administering the treatment will be medically trained and competent in the procedures involved in preparation and administration of the GMO. These competencies include safe sharps handling and disposal practices and respiratory hygiene.

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

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

47. Couriers would be informed that they are transporting a GMO via the labelling on the outer container of the package. In addition, a copy of the licence would be included in the shipping documentation.

### *2.3.11. Contingency plans*

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

49. Staff cleaning the spill would wear PPE and allow aerosols to settle (30 minutes). The spill would be gently covered with absorbent material and treated with a disinfectant such as 0.6% active chloride with a minimum of 10 minutes contact time. All materials that come into contact with a spill would be contained in a sealed biohazard waste bag and incinerated or disposed of as clinical waste.

50. In the event of exposure of people to the GMO, the GMO would be washed off immediately with soap and water, and all contaminated clothing removed. In case of eye contact, water would be applied to the area for at least 3 minutes. Exposure to broken skin or a needle-stick would be cleaned with soap and water and/or disinfectant.

### *2.3.12. Accountability and Monitoring*

51. Every primary container with the GMO would be accounted for, in line with standard clinical practice.

52. Severe adverse events (SAEs) occurring at any time during the study would be recorded and reported to the OGTR. Reported adverse events (AEs) would also be recorded for each participant for 30 days following the treatment using GMO.

## Section 3 Parent organism – human adenovirus

53. The parent organism is a human adenovirus type 5 (species C). The characteristics of the non-GM parent organism provide a baseline for comparing the potential for harm from dealings with GMOs. As such, the relevant biological properties of human adenovirus will be discussed here.

54. Human adenoviruses (HAdVs) are common pathogens of humans and cause periodical outbreaks of respiratory diseases and continuously cause problems in ocular, gastrointestinal, and genito-urinary systems, and can lead to metabolic disorders (Ismail et al., 2018). Currently, there are 89 different HAdV serotypes (types) that have been classified into seven species (A to G) based on their biology, sequence homology and pathogenicity (Ismail et al., 2018; Dhingra et al., 2019).

55. Overall, adenovirus infections are responsible for around 5% of all respiratory infections in humans (Pond, 2005). Specifically, 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., 2018).

56. A majority of HAdV species have tens of different types (e.g. 57 types have been reported for HAdV-D), but species C comprises only of 5 types, of a higher medical significance with potentially serious manifestations in immunocompromised patients (Dhingra et al., 2019).

57. Outbreaks of adenovirus-associated respiratory disease have been more common in the late winter, spring and early summer, however infections can occur throughout the year. The incubation period of HAdVs ranges from 2 days to 2 weeks, depending on the viral species and type as well as the mechanism of acquisition (Allard and Vantarakis, 2017). The general symptoms are usually mild and may include nasal congestion, cough, tonsillitis and/or conjunctivitis.

58. HAdVs infect over 80% of the human population with selected serotypes responsible for about 5% of upper respiratory tract infections and up to 15% of symptomatic lower respiratory tract infections. HAdVs can persist in selected body tissues for years post-infection and lead to intermittent shedding of infectious virus in faeces (Dhingra et al., 2019). HAdV DNA was previously detected in tumour-infiltrating lymphocytes and also in T cells isolated from the colon.

59. Adenoviruses are generally transmitted by inhalation of aerosol droplets excreted from the respiratory tract, 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). It was reported that human adenoviruses are uncommon in the urine of immunocompetent individuals but can be found in the urine of immunocompromised patients (Echavarría, 2008).

60. It has been shown, that the number of viral copy numbers in stool samples is correlated with a number of infection sites of HAdV infection in humans. Based on HAdV shedding data, (Kosulin et al., 2016) , suggested that there might be multiple locations of viral persistence in human body, including compartments in the gastrointestinal tract (GI) that result in viral shedding into stool reaching $10^{11}$ vp/g of stool (Kosulin et al., 2016).

61. Immunocompromised individuals are a high risk group for development of severe disease following infection with HAdV infection. These include people who have received T-cell suppressive regimens, received allogeneic hematopoietic stem cell transplant (HSCT) (Dhingra et al., 2019), lymphoma patients receiving antiCD52 antibody therapy, and solid-organ transplant recipients (Ljungman, 2004).

62. Adenoviruses are classified as highly immunogenic and their ability to stimulate the host-immune response can be harnessed for cancer immunotherapy purposes. To date, adenoviral vectors have been used in therapeutic gene transfer, vaccines, and oncolytic treatments in cancer gene therapy. The anti-tumour immune response induced by oncolytic adenovirus treatments is critical for their efficacy (Shaw and Suzuki, 2019).

## 3.1. Genome and virion structure of human adenovirus

63. HAdV are non-segmented, non-enveloped, linear double-stranded DNA viruses, with a genome of about 35 kilobases (kb), flanked by inverted terminal repeats (ITRs) (Dhingra et al., 2019).

64. The adenovirus genome is organised into early and late regions, corresponding to the temporal kinetics of transcription of these regions (Figure 2). The early region consists of multiple transcription units, termed E1A, E1B, E2A, E2B, E3 and E4.

65. Early genes (E1A, E1B, E2, E3 and E4) are involved in activating transcription of other viral regions, altering the host cellular environment to enhance viral replication, and replication of viral DNA (Saha and Parks, 2017).

66. E1A transcription unit controls transcription of viral genes and modifies host-cell gene expression to benefit viral reproduction. The E1A gene products are the first proteins expressed from the infecting virus, and are essential for the efficient transactivation of other viral coding regions as they interact with a multitude of cellular proteins (Saha and Parks, 2017). E1B assists in viral replication and is mainly required for the export of viral late mRNA (L1 to L5) from the host-cell nucleus. The E1A and E1B coding regions are necessary for efficient viral gene expression and replication (Saha and Parks, 2017).

67. The products of E2 transcription are mainly involved in viral DNA replication. The E3 transcription unit encodes viral proteins that destabilise host immune responses. For instance, adenovirus E3-19K transmembrane glycoprotein counteracts immunosurveillance of the host immune system. This glycoprotein is localised in the endoplasmic reticulum, which forms a complex with major histocompatibility complex class I antigens and retains them in the endoplasmic reticulum, thereby preventing cytolysis by cytotoxic T lymphocytes (Hermiston et al., 1993; Wold, 1993). The E4 transcription products modulate cellular function and assist with viral DNA replication and RNA processing.

68. The late transcription unit is alternatively spliced to yield five groups of mRNAs termed L1 through L5. These late mRNAs encode structural proteins or contribute to virion production. Additional transcription units such as pIX and IVa2, perform structural functions or play a role in viral packaging.

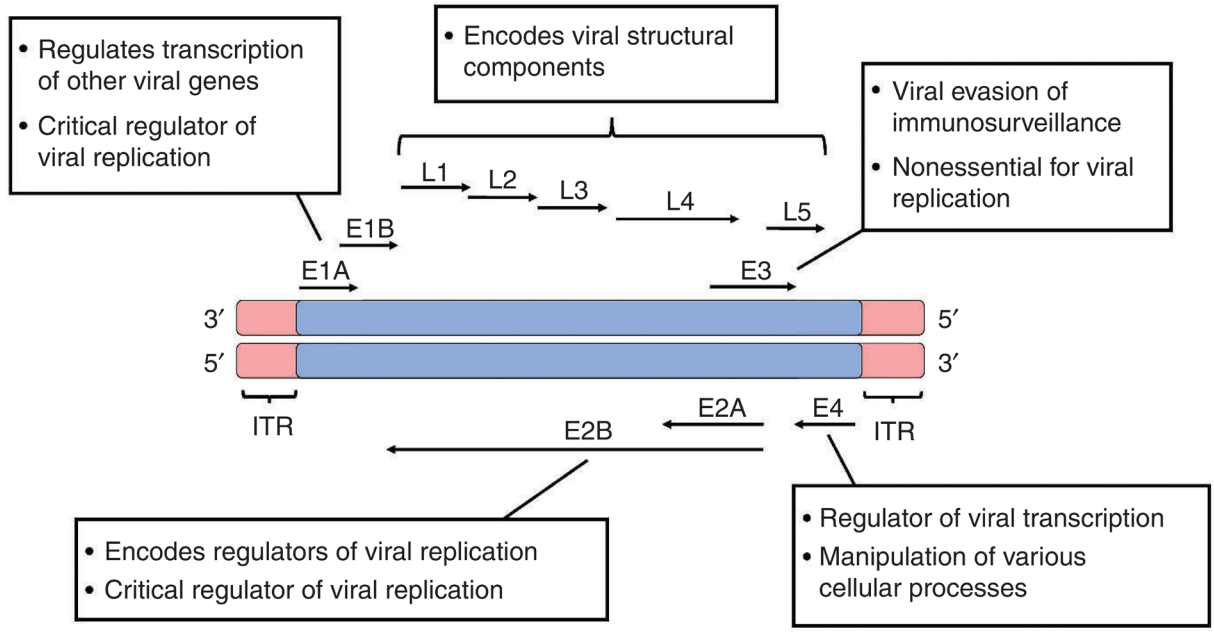


**Figure 2 Functions, organisation and structure of HAdV genome. Image source: Afkhami et al. (2016).**

## 3.2. Viral infection and replication

69. Adenoviruses can infect a wide range of cells and tissues, and replicate efficiently in both dividing and non-dividing cells. Most frequently they infect epithelia of the upper or lower respiratory tract, conjunctiva, gastrointestinal and urinary tract. HAdV-2 and HAdV-5 enter cells by binding to coxsackie and adenovirus receptor (CAR) transmembrane proteins present in heart, brain, epithelial and endothelial cells (Zhang and Bergelson, 2005). The replication of HAdVs takes place in the host-cell's nucleus and relies on host-cell's nuclear machinery for viral gene expression and DNA replication. Following the entry into the host-cell, viral particles are uncoated and the viral genome is transported along microtubules to the nuclear pore complex. The replication cycle begins when viral DNA gets into the nucleus (Charman et al., 2019).

70. Transcription takes place in the nucleus in two phases. The first step of the replication process is the production of regulatory proteins which activate other viral genes and modify the intracellular environment in the host-cell. During this process, the expression of host proteins is altered to prevent premature death of infected cells. The second, late phase of gene expression begins after the

components for DNA replication are ready and viral structural proteins are produced. Finally, the virus is assembled into a virion and host-cell lysis is induced (Waye and Sing, 2010).

71. The DNA replication cycle in HAdV is a very efficient process and can yield ca. one million copies of viral DNA within 40 hours (Hoeben and Uil, 2013). The progeny viruses are assembled from newly synthesised genomes and virion proteins. Post lysis, progeny viruses released from infected cells do not usually spread further than the regional lymph nodes.

## 3.3. Mutation and recombination

72. The within-species genetic recombination in HAdVs has been described between different strains in the regions of high homology (Lukashev et al., 2008). While interspecies recombination is uncommon, it has been reported (Borkenhagen et al., 2019).

73. Homologous recombination plays an important role in the molecular evolution of HAdVs and has been shown in HAdV-A, HAdV-B, HAdV-D. For example, recently Yang et al, isolated at least three, new recombinant lineages of HAdV-C in China (Yang et al., 2019).

74. A study on phylogenetic relationships of HAdV-C field isolates suggested a frequent within-species recombination. For instance, the comparison between DNA sequences among eight HAdV-2 strains revealed 17 positions with nucleotide variations. However, only one of them altered the amino acid composition. Thus, it appears that HAdV-C accumulate predominantly neutral point mutations in their genomes that do not cause substantial modifications. This indicates a high-stability and conservation of protein sequence and justifies a relatively small number of HAdV-C serotypes (Lukashev et al., 2008).

75. Yu et al. (2020) described a possible recombinant HAdV-C strain that was isolated from a person in China. Analysis showed that the strain contained genes from HAdV-1, HAdV-2, and HAdV-5. The recombination events resulted in a virus that contained pTP, 52k, and partial pIIIa genes from HdV2, the E4 gene from HAdV-5 incorporated into the HAdV-1 backbone. The effect of these two recombination events is yet to be investigated.

76. The HAdVs are usually episomal and integration of their DNA with the host-genome is rare (Desfarges and Ciuffi, 2012; Desheva, 2018). Some evidence indicates, that integration of the adenoviral genome is possible through a patchy nucleotide homology of ITR sequences in AdVs, which are homologous to some host-cell repetitive elements. The stable integration of Ad-12 and partial integration of HAd-5 was observed previously *in vitro* in hamster cell lines (Desfarges and Ciuffi, 2012).

## 3.4. Host range

77. Human and non-human adenoviruses 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). Direct transmission of HAdVs from people to animals is not common but there is substantial evidence suggesting that HAdVs can cross species barriers. Several studies suggested AdV transmission between humans and non-human primates can result in serious health consequences due to novel pathogenicity and disease dynamics (Hoppe et al., 2015; Borkenhagen et al., 2019).

78. Even though AdVs have been reported to cross species barriers, others note technical challenges associated with identification of permissive non-human hosts for studies on HAdVs. A comprehensive *in vitro* screening study of primary cell cultures from seven animal species has demonstrated a highly restricted viral replication and release in non-human cells with the exception of porcine cell lines. In pigs which have been experimentally infected with WT HAdV-5, viral DNA was detected in the liver,

lung, and kidneys, as well as serum samples 7 day post-infection (Jogler et al., 2006) suggesting that pigs are at least susceptible to initial HAdV infection.

79. Chronic human infections with HAdVs provide an opportunity for within type homologous recombination during a co-infection. Such events can lead to the increase of within-species genetic diversity and theoretically increase the likelihood of forming highly pathogenic strains (Yang et al., 2019).

80. In general, HAdVs do not cause disease in animals and animal adenoviruses are only pathogenic to the species of origin. However, asymptomatic infections with human adenovirus type 12 have been documented in higher primates, and antibodies to canine, bovine and non-human primate adenoviruses have been detected in humans (Allard and Vantarakis, 2017). Companion animals such as dogs and cats are unlikely to be infected with HAdVs (Borkenhagen et al., 2019).

## 3.5. Environmental stability and decontamination methods for human adenovirus

81. Adenoviruses are quite resistant to chemical or physical decontamination processes and agents. They have been detected in various waters worldwide including sewage, river, ocean and swimming pool water as well as drinking water. They are thought to be more prevalent and persistent in comparison to other common viruses. However, these studies need to be interpreted with caution since most were based on detection of virus nucleic acids in the environment and did not examine whether intact, infectious virus was present.

82. Adenoviruses can persist in extreme pH conditions, are resistant to tertiary and UV treatment of urban wastewater and are very resistant to lipid-disrupting disinfectants. Thus, they can survive outside of a host for up to 8 weeks and may be spread through contaminated surfaces, such as shared towels (Pond, 2005).

83. The persistence of infectious particles of HAdV-41 was shown to be dependent on temperature (the main factor determining viral persistence in the environment) and differed for encapsidated genomes and free nucleic acids. The HAdV remained infectious for at least 25 days in tested temperatures (4 °C, 20 °C, 37 °C) and a maximum of 70 days (4 °C, 20 °C), while encapsidated genomes persisted for over 70 days (Prevost et al., 2016; Allard and Vantarakis, 2017).

84. Adenoviruses can be effectively inactivated using heat treatment or certain disinfectants. Surfaces can be chemically decontaminated with chlorine, formaldehyde or alcohol-based disinfectants. Adenoviruses are sensitive to 70% ethanol with minimum 5 minute contact time (Rutala et al., 2006). Liquid waste may be treated by exposing to bleach with a final concentration of 10%, for 15 minutes (Allard and Vantarakis, 2017).

## 3.6. Shedding of adenovirus from infected hosts

85. HAdV shedding is largely dependent on tissue and infection type. 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). The transmission of adenovirus is facilitated by very high levels of viral particles (100,000-1,000,000/mL) in the sputum or oral secretions of infected adults (Allard and Vantarakis, 2017).

86. HAdV shedding was also evaluated in faecal and oral swabs after the administration of a live, oral vaccine containing two HAdV serotypes (HAdV-4 and HAdV-7). Over half of the vaccine recipients tested positive for adenovirus faecal shedding between 7-28 days following vaccination. No faecal shedding was detected after 28 days following vaccination or at any time point in throat swabs (Allard and Vantarakis, 2017).

87. A bio-distribution study of HAdV-5 and HAdV-35 vectored vaccines administered intramuscularly to rabbits showed that these vectors primarily remained at the site of inoculation in the muscle and skin and only trafficked to the liver (HAdV-5), pelvic lymph nodes (HAdV-35) and spleen but no other distal organs. Vectors were shown to be cleared within 3 months (Sheets et al., 2008).

88. A study of trial participants who received intranasal, intrabroncheal, intramyocardial, intramuscular, or intratumoural injection of replication defective HAdVs-5 demonstrated negligible replication defective and no replication competent virus shedding in pharyngeal, rectal, nasal swabs, urine, and blood samples taken on days 1 and 7 post injection (Crystal et al., 2002; Wold and Toth, 2013).

89. Further, studies that examined shedding using stool, throat or nasal swabs and urine failed to detect any virus following intra-tumoural administration of HAdV-5 into non-lung tumours. However, viral particles were detected in blood or plasma between 8 and 24 hours post-administration, and viral particles were detected at the injection site 30 days following administration (Brandon et al., 2008).

## 3.7. Occurrence in the environment

90. There are multiple reports indicating adenoviral presence in the environment. Most frequently HAdVs are analysed in concentrated environmental samples using polymerase chain reaction (PCR) techniques, which do not provide information on their viability. Their presence in the environment is usually related to contamination by human faeces. Adenoviruses of several types have been detected in high concentrations in domestic sewage and sludge in various countries and have since become potential indicators for the presence of human sewage in waste waters and effluents. Average adenovirus DNA concentrations in sewage can reach over $10^6$ virus particles/L. Various adenovirus types (41, 12, 40, 2, 3) have been isolated from raw sewage, sludge and primary effluents, and various organisms that live in aquatic environments (Allard and Vantarakis, 2017).

91. Adenoviruses have also been detected in surface and ground waters including drinking water sources and recreational water bodies. Surface waters contaminated with HAdV and may contain relatively high concentrations of up to $3 \times 10^9$ adenovirus genome copies per L. Although, some of the data can be misleading since does not include information on the viability of viral particles (Allard and Vantarakis, 2017).

## 3.8. Antiviral treatments for human adenovirus

92. Antiviral drugs are generally used in immunocompromised patients or those with severe viral infection. Despite the prevalence of adenoviruses, there are currently no adenovirus-specific treatments (Waye and Sing, 2010).

93. The antiviral agents commonly used in adenoviral therapy are Cidofovir and Ribavirin. Cidofovir is among the most potent of antivirals for DNA viruses. It inhibits viral replication by mimicking the monophosphate form of nucleotide. Its efficacy has been confirmed in all HAdV types. Ribavirin is a broad spectrum antiviral with a similar mode of action to Cidofovir. However, Ribavirin was shown to be active against HAdV-C isolates and has variable activity in other species (Waye and Sing, 2010; Hoeben and Uil, 2013).

## 3.9. Risk group of human adenovirus

94. The Australian Standard 2243.3:2010 Safety in Laboratories Part 3: Microbiological safety and containment (Standards Australia/New Zealand, 2010) classifies adenovirus as a Risk Group 2 organism.

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

95. The applicant has proposed clinical trials of a GM human adenovirus that preferentially replicates in bladder cancer cells and stimulates the immune system in the tumour.

## 4.1. The genetic modifications

96. The GMO (commercial name CG0070) is an oncolytic adenovirus designed to preferentially replicate in and destroy cancer cells with defects in the retinoblastoma (Rb) signalling pathway. The GMO backbone is HAdV-5 with modifications to the E3-19k gene region and E1a promoter (with the added pA signal on its 5' end) (Figure 3).

### *4.1.1. Substitution of E3-19K with hGM-CSF*

97. The parent organism was firstly modified by substitution of the E3-19K region, a transmembrane glycoprotein that counteracts immunosurveillance in a host-organism, with a human granulocyte macrophage colony-stimulating factor gene (hGM-CSF) (Ramesh et al., 2006). The immunomodulatory hGM-CSF gene is expected to stimulate immune responses against local and distant tumours which will potentially lead to the eradication of the cancer (Burke et al., 2012).

98. The anti-tumour immune response resulting from a local delivery of adenovirus treatment could be achieved by (1) oncolytic lysis of tumour cells and (2) a nonspecific inflammatory reaction generated by the infiltration of neutrophils and eosinophils. GM-CSF-secreting cancer immunotherapies have been evaluated in multiple preclinical and clinical studies and showed specific and potent anti-tumour responses.

99. The product of the hGM-CSF gene is a cytokine primarily produced by activated Th1, type 1 cytotoxic T-cells and activated macrophages. It was identified as the most potent cytokine inducer of specific, long-lasting anti-tumour immunity (Dranoff et al., 1993; Ramesh et al., 2006). It induces the proliferation and differentiation of certain types of immune cells e.g. granulocytes (neutrophils, eosinophils, and basophils) and monocytes. Since, hGM-CSF has a proven role of stimulating anti-tumour immune responses, the local expression of hGM-CSF by the GMO infected cells is expected to induce local inflammatory responses, thereby enhancing the local anti-tumour activity of the vector. Additionally, hGM-CSF secreted by the GMO infected tumour cells can attract antigen presenting cells to the tumour site and lead to the priming of naïve effector T-cells. Thus, in addition to its direct oncolytic effect after local treatment, the GMO may also induce systemic tumour specific immunity such that distant tumour metastases may be affected. In this way, systemic therapy may be achieved with local delivery.

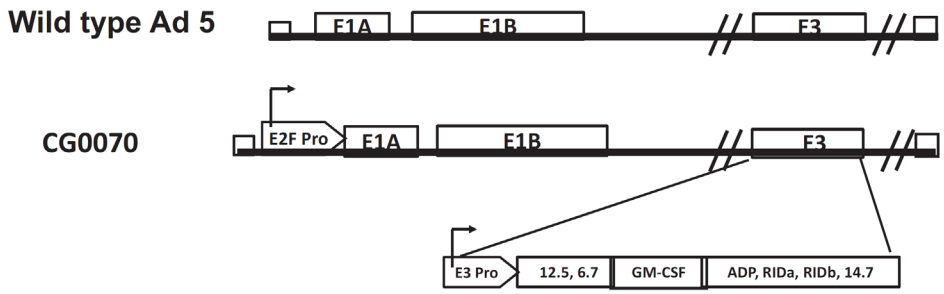


**Figure 3 Schematic diagram of a parental human adenovirus and the GMO (CG0070). Image source: Burke et al. (2012)**

### *4.1.2. Substitution of E1a promoter with hE2F-1 promoter*

100. The parent organism was also modified by substitution of the endogenous E1a promoter with a human hE2F-1 promoter. It is expected that the new promoter will enable conditional replication of the GMO in Rb-pathway defective cells.

101. While the expression of all viral genes is normally under the control of E1a, in the GMO, viral genes and the hGM-CSF transgene are controlled by the tumour specific hE2F-1. This promoter ensures targeted and conditional replication of the virus with minimal damage to normal tissues that have a functional Rb signalling pathway. In addition, a pA signal was inserted 5' of the hE2F-1 promoter to protect it from transcriptional read-through activating E1A expression.

102. Rb is a nuclear phosphoprotein critical for cell-cycle regulation. The ability of Rb to repress the cell cycle is frequently dysregulated in cancer cells, making the defective Rb pathway a hallmark of cancer metabolism and enables the GM HAdV to preferentially replicate in cancer cells. Similar to other proteins from its family, Rb binds to its specific partner protein, in this case hE2F-1. The interaction with partner proteins (such as E2F, MDM2, c Jun and cyclins D, E and A) and thus the function of Rb are partly dependent on phosphorylation of Rb by selected kinases (Denechaud et al., 2017).

103. The E2F transcription factors (E2F-1-8) are a group of proteins classified based on their structures, their interaction partners, and their transcriptional properties. E2F-1 is a cell cycle regulatory protein that works as a key regulator of the progression of the cell-cycle, as well as a mediator of apoptosis. While, E2F-1 has the ability to bind the Rb protein, its activity is dependent on dimerization proteins (DP) and 'pocket proteins' such as Rb protein (Denechaud et al., 2017).

104. During the G1 and S phase of the cell cycle, E2F forms a dimer with a member of the DP family and further binds to E2F-binding motifs, such as the E2F-1 promoter to activate the transcription of target genes (Jakubczak et al., 2003). The E2F-1/DP dimer can bind to unphosphorylated Rb present in the G1 or G0 phase of cell cycle and form a complex which prevents the recruitment of transcriptional co-activators to the promoters of its target genes therefore, acting as a transcription repressor (Denechaud et al., 2017). This mechanism enables the preferential transcription of the GM HAdV viral genes in tumour cells.

105. In principle, E2F-1 protein has to be transcriptionally active (unbound) to enable transcription. In normal cells, phosphorylation of Rb disables its binding sites and frees E2F-1. In tumour cell, the Rb pathway defects or Rb-deficiency is responsible for increased levels of transcriptionally active E2F-1 which results in expression of E2F-1-responsive genes. In conclusion, the increase of free E2F-1 results in activation of E2F-1 promoter in tumour cells with an Rb pathway defect, that usually remains repressed in normal cells (Jakubczak et al., 2003; Bell and Ryan, 2004). The preferentially replicating GM HAdV can indicate that the GM virus preferentially infects and kills cancer cells (oncolytic agent) which may be appropriate for the treatment of a number of solid tumour types.

## 4.2. Characterisation of the GMO

106. The GMO is a replication competent oncolytic adenovirus designed to preferentially replicate in cancer cells with deficiencies or defects in the pRb-signalling pathway. Its ability to replicate in non-cancerous cells is highly repressed due to selectivity conferred by the hE2F-1 promoter (Jakubczak et al., 2003; Ramesh et al., 2006; Jhawar et al., 2017b).

107. The GMO is engineered on the HAdV-5 backbone with the exception of the two transgenes, thus the cell-host recognition in the GMO relies on the same mechanism as the wild-type HAdV-5 and depends on the recognition of CAR which are highly expressed on the surface of cancer cells.

### *4.2.1. Characteristics of the E2F-1 promoter*

108. The human hE2F-1 promoter has been shown to be selectively activated/repressed in tumour cells with a defect in the Rb pathway. For example, Ar6pAE2fE3F and Ar6pAE2fF are oncolytic adenoviral vectors that utilise the hE2F-1 promoter to regulate the expression of the viral E1A transcription unit. Anti-tumour activity of these vectors *in vitro* and *in vivo* is dependent on the hE2F-1 promoter driving expression of viral genes in Rb pathway-defective cells. In the evaluated study, selective destruction of cancer cells by Ar6pAE2fF was dependent on the presence of functional hE2F-1 binding sites in the promoter. Ar6pAE2fF and Ar6pAE2fE3F were also compared with Addl1520 and are reported to be molecularly identical to an E1B-55K deleted vector currently in clinical trials (Jakubczak et al., 2003).

109. A number of adenoviruses have been engineered with exogenous promoters conferring altered tissue tropism. For example, a viral genome has been placed under the prostate-specific antigen promoter which allowed for a conditional replication limited to prostate cancer cells only. Another example is a GM adenovirus (KH901) in which transcription is dependent on human telomerase reverse transcriptase (hTERT) enabling it to conditionally express in actively dividing cells only (Jhawar et al., 2017b).

### *4.2.2. Background information on the hGM-CSF*

110. The hGM-CSF proteins are pleiotropic mammalian cytokines that can stimulate the proliferation, maturation, and activation of a variety of hematopoietic cells. Data from previous studies and clinical trials, as well as from the RARMP for DIR 132 suggests that expression of the hGM-CSF protein is safe and does not cause unwanted effects at high levels. hGM-CSF has also been approved by United States Food and Drug Administration as a pharmaceutical drug.

111. The administration of GMOs expressing hGM-CSF has been extensively tested and found to be safe in people and a variety of animal species (Davis et al., 1990; Baiocchi et al., 2001; Liu et al., 2003; Soiffer et al., 2003). For example, T-VEC (also known as OncoVEX or Imlygic), which is an attenuated herpes simplex virus (HSV) was developed for melanoma cancer immunotherapy. It was engineered with two copies of the hGM-CSF (Jhawar et al., 2017a) and approved by the US Food and Drug Administration for the treatment of advanced melanoma and approved by the Office of the Gene Technology Regulator in 2015 (DIR 132).

112. Another study has shown that the GM HSV virus (with murine GM-CSF) induced significant shrinkage or clearance of tumour cells in contralateral tumours that were not directly injected with the GMO. These results indicate that the GM virus preferentially infects and kills cancer cells (oncolytic agent), and generates a systemic immune response in cancer cells (Liu et al., 2003).

113. Preclinical studies of oncolytic SKL001/SKL004 with a murine and human GM-CSF transgene showed that both human and murine GM-CSF-expressing adenoviruses induced necrosis and mononuclear cell infiltration into the tumour, but only murine GM-CSF induced eosinophil infiltration in cancer cells. These vectors were designed to selectively replicate in Rb pathway–defective tumour cells similarly to the evaluated GMO and generated results aligned with the preclinical data on the GMO. However, the actual mechanisms involved in this immune response need to be further studied (Ramesh et al., 2006; Du et al., 2014).

114. SKL001-mediated GM-CSF expression was observed in all human cancer cell lines examined. At 100 viral particles per cell or higher, production of biologically active GM-CSF exceeded 40 ng $ml^{-1}/10^6$ cells per 24 h. This level is considered 3-20-fold higher than the levels sufficient to induce potent, long-lasting anti-tumour immunity in *in vivo* tumour vaccination models. Therefore, SKL001 has the potential to produce GM-CSF in quantities predicted to be therapeutic. In the immunocompetent

mouse tumour model higher efficacy was presented by the vector carrying murine GM-CSF (Du et al., 2014).

115. Overall, the vectors carrying GM-CSF were 1000-fold more cytotoxic in Rb pathway–defective human tumour cells in comparison with normal human cells. This indicates a potentially low damage to normal, non-cancerous tissues in participants who will be treated with the GMO. In addition, significantly better anti-tumour activity was observed using a species-specific GM-CSF (e.g. mGM-CSF in mice) (Du et al., 2014).

116. In addition, hGM-CSF has been used in other clinical trials and has been found to be safe and effective against several malignancies (Bendandi et al., 1999; Daud et al., 2008; Sato et al., 2008; Spitler et al., 2009).

### *4.2.3. Nonclinical studies on the GMO*

117. The GMO has been evaluated in a preclinical studies *in vitro* and *in vivo*. Both types of studies showed selective replication and destruction of cancer cells, localised GM-CSF production and anti-tumour efficacy of the HAdV treatment in several bladder cancer models (Ramesh et al., 2006; Du et al., 2014). High levels of lysis was observed in cancer-cell lines and tumours but only minimal impact was observed on healthy human cells lines (Ramesh et al., 2006).

118. Cytotoxicity *in vitro*, was analysed using Rb-pathway-defective bladder TCC cell lines (RT4, SW780 and UC14) and normal human cell lines with a well-functioning Rb-pathway. The cytotoxicity assays showed that the GMO reduced the viability of bladder TCC cell lines to 10-20% but in contrast is highly attenuated in normal human cells lines with cell viability of >95% post-infection.

119. The GMO replicated in the Rb pathway–defective human bladder TCC cell lines RT4, SW780, UC14, and 253J B-V cells as efficiently as wild-type HAdV-5 and as a result, produced similar numbers of progeny (3,000-9,000 plaque-forming units per cell). Comparatively, the GMO produced >2 log more progeny in the bladder TCC cell lines in than in normal human cell lines. Considering the GMO is highly attenuated in normal human cells, it can could potentially be used as a targeted oncolytic treatment (Ramesh et al., 2006).

120. The expression of the hGM-CSF gene was quantified using ELISA in bladder TCC cell lines and primary cells post infection with the GMO. hGM-CSF protein expressed in the cell lines was much higher in bladder TCC cell lines in comparison to primary cell culture. The differences in expression levels varied depending on the infection levels but overall the expression of the hGH-CSF was up to 30 to 140-fold higher in TCC cell lines (Ramesh et al., 2006).

### *4.2.4. Clinical studies on the GMO*

121. It is expected, that due to the IVE route of administration of the treatment, the biodistribution of the GMO in participants with a non-traumatic catheterisation and in the absence of a breech in the lining of the bladder or urethra, shedding will be limited. In addition, previous clinical studies with this GMO have not resulted in person-to-person transmission of the GMO.

122. Three clinical studies have been carried out in the USA and Canada (Table 1). Overall, a total of 118 participants have been administered the GMO in (phase I and phase II) clinical trials. The trials have been completed and their results published in peer-reviewed journals (Burke et al., 2012; Packiam et al., 2018).

123. Grading of adverse events was performed according to National Cancer Institute Common Terminology Criteria for Adverse Events (USA). Across the clinical studies, no serious adverse events

(grade 4/5) were reported from the treatment. Based on previous clinical experience with this GMO, the most common treatment-related events were predominantly Grade 1/ Grade 2 localised bladder effects including frequent urination, bladder discomfort, bloody urine, pain during urination, and feeling the need to urinate during the night.

**Table 1 Summary of previous clinical trials using (CG0070) GMO.**

| No. | Study design | No. treated | Main adverse events (AE) | References |
|---|---|---|---|---|
| 1 | Phase I, open-label, dose-escalation trial of IVE administration of the GM treatment in participants with NMIBC who have failed BCG treatment. This study confirmed the anti-tumour activity of the treatment established a safety profile without attaining a maximum tolerated dose of the treatment. | 35 | Grade 1–2 bladder effects including pain during urination (71%), bloody urine and urinary frequency (43%) were the most common. Three participants experienced a total of 6 grade 3 or greater AEs, including frequent urination during the day, reduced white blood cells, pain during urination, urgency and night-time urination in 1 each. | Burke et al, 2012<br>NCT00109655 |
| 2 | Randomised Phase II study of the GM treatment versus investigator's choice in 22 participants with non-muscle invasive bladder CIS disease.* | 16* | No data available. | NCT01438112 |
| 3 | Open-label, single-arm, phase II multicentre study of the safety and efficacy of the GM treatment. | 67 | 66% of participants reported at least one adverse event related to the treatment. All treatment–related AEs were grade 1 to 3 including lower bladder spasms (36%), bloody urine (28%), pain during urination (25%), urgency (22%)<br>Likely immunologic treatment–related events such as flu-like symptoms (12%), fatigue (6%), and low blood pressure (3%). Some participants (7.8%) reported grade 3 pain during urination and low blood pressure. | Packiam et al., 2018<br>NCT02365818 |

*Note. * - denotes data provided by the applicant.*

124. In the clinical trials, virus shedding was assessed by PCR which detected viral fragments in urine and blood samples. However, this method does not discriminate between the presence of genomic DNA fragments and the presence of live adenovirus.

125. In the phase I clinical trial, urine samples were evaluated for the presence of the GMO genome post-IVE administration. Following the initial instillation with a treatment dose of up to $3 \times 10^{13}$ vp, the highest urine levels of the GMO genome was noted on day 5. Mean genome numbers started to decrease after day 5 in all tested participants. Most of the participants (87.9%) had no quantifiable GMO (i.e., ≤1,500 copies/mL) in urine by 29 days post treatment. The presence of the GMO genome was also detected, by PCR, in the plasma of three out of thirty-five participants (9%) (i.e., ≥1,500 copies/mL) (Burke et al., 2012).

126. All participants expressed high levels of hGM-CSF (up to 62,907 pg/ml of urine) two days following the treatment. With subsequent treatments, these levels decreased significantly suggesting accelerated HAdV clearance, potentially as a result of the induction of anti-adenovirus immunity or a decreased number of cancer cells (Burke et al., 2012).

127. In a phase II study, a fixed dose of $1 \times 10^{12}$ vp of the treatment was administered to participants weekly for 6 weeks. The concentration of the GMO genome in urine was highest at 2 hours post treatment and remained above the baseline at the 24 hour sampling point. Mean urine concentration of the GMO genome was $1.6 \times 10^{7}$ genomes/mL, 2 hours after treatment, and $4.3 \times 10^{5}$ genomes/mL 24 hours after treatment (Packiam et al., 2018). The data on urine shedding of the GMO for the interim 2h – 24h was not collected. However, prior to treatment the PCR test detected $4.9 \times 10^{2}$ genomes/mL, likely detecting HAdv genome rather than the GMO.

128. In addition, in the 39 tested participants, the geometric mean values of GMO detected in serum was within the range of $2.5 – 3.1 \times 10^{2}$ genomes/mL. The highest mean GMO values occurred prior to the last (sixth) induction treatment, however, the mean GMO level returned to $2.5 \times 10^{2}$ genomes/mL 2 hours post-treatment (Packiam et al., 2018).

129. Transgenes used in engineering of the GMO were tested for stability in pre-clinical studies and showed stability and good safety profiles in clinical trials (Dranoff et al., 1993; Ragnhammar et al., 1994; Liu et al., 2003; Du et al., 2014). In addition, the GMO (CG0070), has been tested in pre-clinical and clinical trials (phase I and II) and showed low-toxicities, limited and transient shedding in selected tissues and biofluids and a good, stabile safety profile in the participants with the NMIBC (Ramesh et al., 2006; Burke et al., 2012; Packiam et al., 2018).

# Section 5 Receiving environment

130. The receiving environment forms part of the context in which the risks associated with dealings involving the GMOs are assessed. Relevant information about the receiving environment includes the presence of species susceptible to the GMO, the presence of the parent organism and related viral species, and environmental characteristics that may influence the likelihood of the GMOs spreading or persisting outside the site of release, or the harm they may cause.

## 5.1. Trial site

131. The intended primary receiving environment would be the bladder of trial participants as the GMO is to be delivered IVE.

132. The secondary receiving environment would be the room and the clinical trial site where the GMO is dispensed, administered and waste disposed of. All clinical sites involved in the study would be equipped to handle infectious agents and procedures would be conducted in accordance with institutional policies based on Standard Precautions for handling potentially infectious substances and the *Australian Guidelines for the Prevention and Control of Infection in Healthcare* (National Health and Medical Research Council, 2019).

133. The principal route by which the GMO may enter the wider environment is by shedding of the initial viral inoculum in urine from trial participants once they leave the clinical trial site and return home. The tertiary receiving environment includes the trial participants' homes and any places they visit during the period when the GMO is shedding.

## 5.2. Related viral species in the receiving environment

134. Adenoviruses belong into two separated genera: the avian adenoviruses (aviadenoviruses) and the mammalian adenoviruses (mastadenovirus). As such, they are common in animals and humans, and infections occur in both adults and children. AdVs may survive for a long period outside of a host and can cause infections throughout the year (Usman and Suarez, 2020). A more detailed description of adenoviruses present in the environment is discussed in Chapter 1, Sections 3.4 and 3.5.

### 5.3. Presence of the hGM-CSF and hE2F-1 promoter in the environment

135. The hGM-CSF gene encodes the protein human granulocyte-macrophage colony-stimulating factor (hGM-CSF). GM-CSFs are pleiotropic cytokines found in mammals that can stimulate the proliferation, maturation, and activation of a variety of blood cells. GM-CSFs are largely species-specific in their actions. For example, hGM-CSF is not active on murine cells and murine GM-CSF not active in human cells (Lee et al., 1985; Du et al., 2014), however hGM-CSF is active in dog cells and weakly active in bovine cells, indicating that hGM-CSF does not exhibit absolute species specificity (Maliszewski et al., 1988; Mayer et al., 1990).

136. The transcription factor E2F is a central regulator of cell progression and has the ability to induce cell cycle progression and apoptosis. The functionality of the E2F-1 promoter is dependent on the Rb-signalling pathway, usually defective in cancer cells (Efiok and Safer, 2000; Bell and Ryan, 2004). The WT E2F-1 promoters are present in mammalian species but their homologs have been described in flies, worms, frogs and in some plant species (Dimova and Dyson, 2005). In addition, constructs containing this promoter have been widely used in rodent and human models *in vitro*.

## Section 6 Relevant Australian and international approvals

### 6.1. Australian approvals

137. The proposed GM treatment has not been previously trialled in Australia. Approval for general use of the treatment would be required from the TGA. Import of the GMO would also require a permit from DAWE.

138. The Regulator has previously issued a DIR licence for similar dealings involving other GM oncolytic virus. The Regulator issued a DIR licence (DIR 132) for the commercial supply of a tumour-selective genetically modified virus for cancer therapy, declared under the name Talimogene laherparepvec (previously known as oncoVEX in 2015). Furthermore, the Talimogene laherparepvec was developed by Amgen Australia Pty Ltd under the name Imlygic and has been assessed and approved for use in Australia by the TGA and has been registered on the Australian Register of Therapeutic Goods (ARTG) (ARTG ID: 232296) since 21 December 2015.

### 6.2. International approvals and experience

139. Three clinical trials have been approved overseas using the GMO (CG0070) based treatment (Table 2).

**Table 2. Overseas approvals for clinical trials using the GMO (CG0070).**

| Study | Phase | Treatments tested | No. participants | Countries | Identifiers ClinicalTrials.gov |
|---|---|---|---|---|---|
| V-0046 | I | CG0070 | 35 | United States/Canada | NCT00109655 |
| BOND | II | CG0070 | 22 | United States | NCT01438112 |
| BOND2 | II | CG0070 | 67 | United States | NCT02365818 |

140. While GC0070 treatment has not been commercially approved in any country to date, similar treatments were approved for use overseas. The H101 which was developed by Shanghai Sunway Biotech Co. Ltd. was the world first oncolytic virus to be licenced for commercial use, under the name of Oncorine, in November 2005 by China Food and Drug Administration (CFDA) (Pol et al., 2015).

# Chapter 2 Risk Assessment

## Section 1 Introduction

141. The risk assessment identifies and characterises risks to the health and safety of people or to the environment from dealings with GMOs, posed by, or as the result of, gene technology (Figure 4). Risks are identified within the established risk assessment context (Chapter 1), taking into account current scientific and technical knowledge. A consideration of uncertainty, in particular knowledge gaps, occurs throughout the risk assessment process.

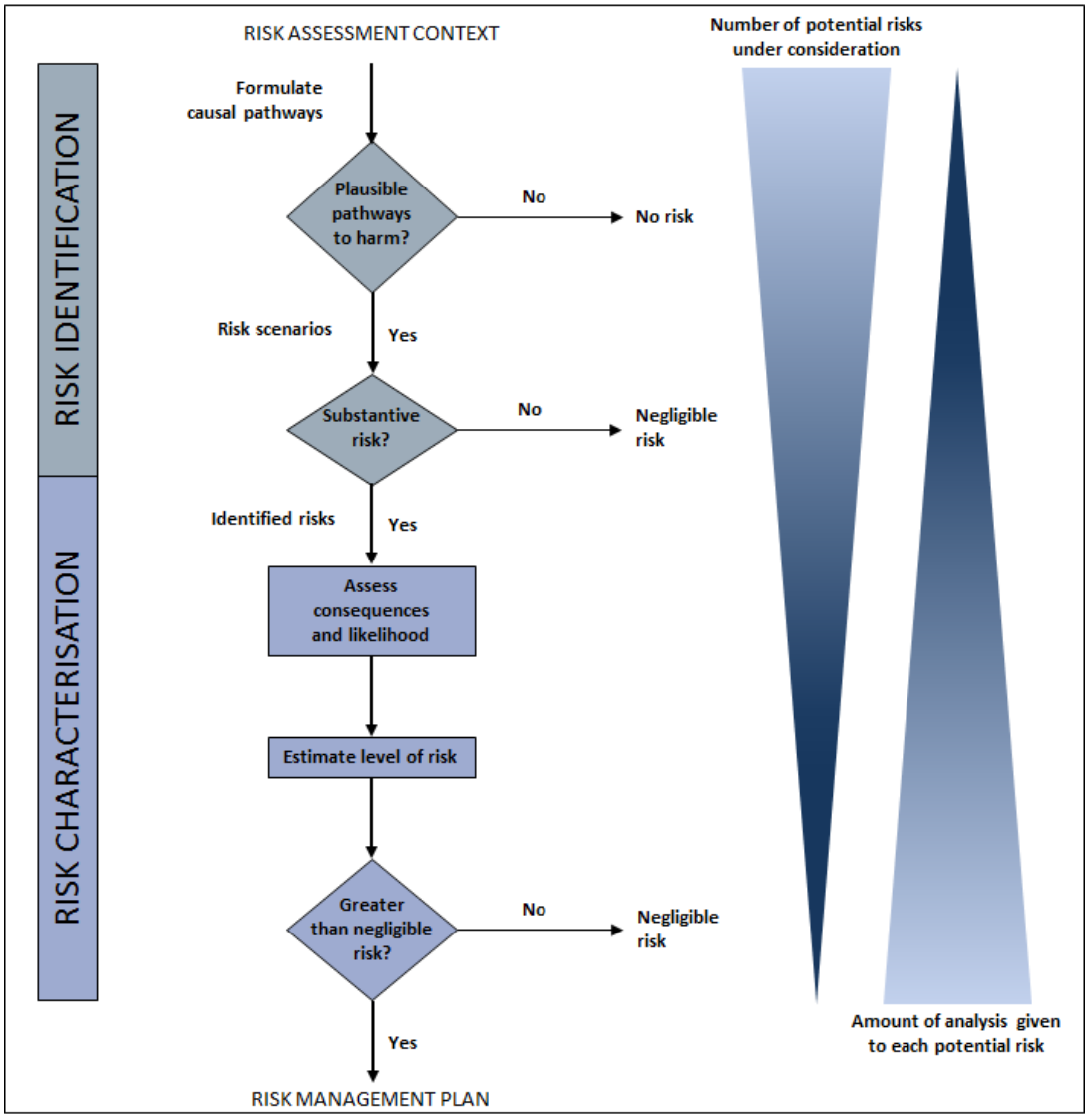


**Figure 4 The risk assessment process.**

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

143. Risk identification first considers a wide range of circumstances in which the GMO, or the introduced genetic material, could come into contact with people or the environment. This leads to postulating causal pathways that may give rise to harm for people or the environment from dealings with a GMO. These are risk scenarios.

144. Risk scenarios are screened to identify substantive risks, which are risk scenarios that are considered to have some reasonable chance of causing harm. Risk scenarios that could not plausibly occur, or do not lead to harm in the long or short term, do not advance in the risk assessment process (Figure 4), i.e. the risk is considered no greater than negligible.

145. 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

146. Postulated risk scenarios are comprised of three components (Figure 5):

i. the source of potential harm (risk source)

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

iii. potential harm to people or the environment.

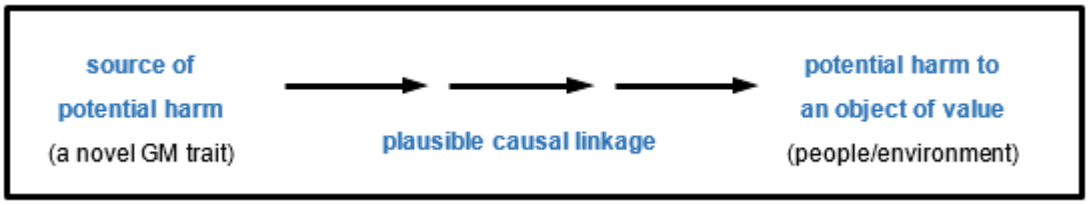


**Figure 5 Components of a risk scenario.**

147. 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

148. The parent organism of the GMO is a common human pathogen, human adenovirus serotype 5 (HAdV-5), responsible for respiratory tract infections in human hosts. Details on the pathogenicity and transmissibility of HAdV-5 are presented in Chapter 1. Infection with the virus is generally a result of an oral-faecal transmission, inhalation of aerosol droplet excreted from the respiratory tract or ocular secretions or of mucosal exposure to the virus. Disease symptoms are usually mild and may include nasal congestion, cough, tonsillitis and/or conjunctivitis.

149. HAdVs can form a latent infection in lymphoid tissues and increase the period of viral persistence in the body. However, the HAdV-5 remains episomal throughout the infection period and does not integrate into the host DNA. Thus, an integration with a host-cell DNA will not be further discussed.

150. Toxicity and allergenicity of the introduced genes and their protein products were not directly considered, but are taken into account in the context of their contribution to ill health.

151. Potential sources of harm can be intended, novel GM traits associated with one or more introduced genetic elements, or unintended effects/traits arising from the use of gene technology. Unintended effects can arise through horizontal gene transfer (HGT), the stable transfer of genetic material from one organism to another without reproduction. All genes within an organism, including those introduced by gene technology, can be transferred to another organism by HGT. A gene transferred through HGT could confer a novel trait to the recipient organism. The novel trait may result in negative, neutral or positive effects on the fitness of the recipient organism. However, HGT rarely occurs from cells to viruses.

152. Recombination, could be a source of new serotypes in HAdVs, however changes arising from this process in HAdV-5 rarely impact whole genes, changing their function or result in gaining/losing a function. HAdVs are species-specific and have a very limited ability to infect other animals so this possibility will not be considered further due to its unlikely occurrence. Recombination between different species of adenoviruses is very unlikely and will not be considered further.

153. The transgene and exogenous promoter introduced to the GMO are of human origin. These changes limit GMO's replication ability and enable it to proliferate in permissive cells with Rb-signalling pathway defects. Potential risks from a recombination between the GMO and the WT HAdV-5 is considered below.

## 2.2. Causal pathway

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

- the proposed dealings;
- the proposed limits including extent and scale of the proposed dealings;
- the proposed controls to limit the spread and persistence of the GMOs;
- routes of exposure to the GMOs, the introduced gene(s) and gene product(s);
- potential effects of the introduced gene(s) and gene product(s) on the properties of the organism;
- potential exposure of other organisms to the 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 environment at the site(s) of the trial;
- 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);
- gene transfer by horizontal gene transfer;
- unauthorised activities, and
- practices during and after administration of the GMOs.

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

not be identified. This is particularly relevant to the hGM-CSF transgene, encoding pleiotropic cytokines found in mammals which stimulate the proliferation, maturation, and activation of a variety of hematopoietic cells. It has been previously as assessed in DIR 132 in a tumour selective GM virus used for cancer therapy. The detailed RARMP for the DIR 132 can be found on the ogtr.gov.au website.

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

157. 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

158. In addition, the following factors are taken into account when postulating relevant risk scenarios for this licence application:

- harm to the health of people or desirable organisms, including disease in humans or animals or adverse immune response
- the potential for establishment of a novel virus in the environment.

## 2.4. Postulated risk scenarios

159. Four risk scenarios were postulated and screened to identify any substantive risks. These scenarios are summarised in Table 3 and examined in detail in Sections 2.4.1-2.4.4.

160. In the context of the activities proposed by the applicant and considering both the short and long term, none of the risk scenarios gave rise to substantive risks.

**Table 3. Summary of risk scenarios from the proposed dealings with the GMOs**

| Risk scenario | Risk source | Causal pathway | Potential harm | Substantive risk? | Reason |
|---|---|---|---|---|---|
| 1 | GM HAdV | Exposure of people to the GMO during<br>a) preparation of the GMO<br>b) administration of the GMO<br>c) transport or storage of the GMO<br>d) disposal of the GMO; or<br>e) collection, transport or analysis of biological samples from participants containing the GMO<br>via oral-faecal, ocular secretions, aerosols, fomites, contact with abraded skin or mucous membranes<br>⬇<br>Transduction of cells<br>⬇<br>Post-infection immune response due to the presence of the virus<br>and/or<br>due to the expression of hGM-CSF in Rb-deficient cells | Illness, local inflammation, flu-like symptoms | No | • Import and transport of the GMO would be in accordance with IATA UN 3373 and/or the Regulator's *Guidelines for Transport, Storage and Disposal of GMOs*<br>• Only trained and/or experienced personnel would conduct dealings with the GMO, using personal protective equipment to minimise potential exposure<br>• GMOs and contaminated waste would be double contained and disposed of as infectious clinical waste<br>• The dose received through accidental exposure during preparation or administration would be substantially less than that administered to trial participants and would not be sufficient to result in a serious adverse reaction in exposed persons<br>• The GMO has limited replication in healthy cells<br>• People are exposed to HAdVs regularly and the genetic modification does not confer any pathogenic advantage over the wild type HAdV<br>• Most of the population has pre-existing immunity to HAdVs<br>• The immune system can clear the virus quickly |
| 2 | GM HAdV | Treatment of a trial participant with the GMO<br>⬇<br>Trial participant discharges unincorporated treatment or sheds GMO progeny with blood or urine<br>⬇<br>Exposure of other people (e.g. sexual contacts, household contacts) including at risk people and pregnant women or animals via:<br>- direct contact with the trial participant;<br>- exposure to aerosolised secretions (e.g. from urine); or<br>- exposure to the GMO in water (e.g. swimming pool, bathtub)<br>- contact with GMO contaminated items (e.g. items the trial participant has touched, or contaminated tissues);<br>⬇<br>Transduction of cells | Illness, local inflammation, flu-like symptoms | No | As in the previous risk scenario and also as the following:<br>• The participant's bladder is drained post-instillation, removing most of the GMO<br>• While shedding is at its peak, urine will be bleached to destroy the GMO<br>• Viral titres shed by trial participants are likely to be decreasing over time due to a smaller number of the GMO permissive cells and immune response<br>• The GMO would not be expected to regain replication competence<br>• The exposure to people at risk (e.g. immunocompromised, pregnant) will be limited<br>• The GMO does not multiply outside of a host<br>• Participants will consent to adequate hygiene and protective practices |

| Risk scenario | Risk source | Causal pathway | Potential harm | Substantive risk? | Reason |
|---|---|---|---|---|---|
| | | ⬇<br>Post-infection immune response due to the presence of the virus<br>and/or<br>due to the expression of hGM-CSF in Rb-deficient cells | | | |
| 3 | GM and WT HAdVs | Trial participant inoculated with the GMO is infected with another HAdV-C<br>⬇<br>Both viruses co-infect the same host-cell<br>⬇<br>GMO and the WT HAdV recombine<br>⬇<br>Replication competent recombinant GMO virus with functional hGM-CSF gene<br>⬇<br>Recombinant GMO infects host and replicates<br>⬇<br>Establishment of viral infection in host<br>⬇<br>Recombinant GMO virus shed<br>⬇<br>Recombinant GMO virus transmitted and infects other hosts | Illness, local inflammation, enhanced immune response | No | As in the previous risk scenarios and also as the following:<br>• There is only a short temporal window when co-infection would be able to occur and the same cell has to be infected with both viruses at the same time<br>• The GMO is only expected to be present in the trial participant for a short time before being cleared by the immune system<br>• The majority of the GMOs are expected to shed for 5 days following the first instillation and within 24 hours after administration<br>• Recombination between different types of HAdVs are uncommon<br>• Overall quantity of the GMO released into the environment is limited |
| 4 | GM HAdV | Treatment of a trial participant with the GMO<br>⬇<br>Trial participant discharges unincorporated treatment or sheds GMO progeny with urine<br>⬇<br>GMO survives sewage treatment<br>⬇<br>GMO is present in the environment and infects other hosts | Local inflammation, flu-like symptoms | No | As in the previous risk scenarios and also as the following:<br>• HAdV-5 infects only human hosts<br>• GMO does not have any advantage or increased pathogenicity over WT HAdVs commonly present in the environment<br>• GMO would not recombine with wild type HAdVs outside of the host<br>• There is a large number of HAdVs in the sewage and water systems<br>• GMO does not infect aquatic animals and persists outside of the host for up to 8 weeks<br>• Overall amount of the GMO released to the environment is limited and would be too low to be infectious<br>• The GMO does not multiply outside of a host |

### *2.4.1. Risk scenario 1*

| | |
|---|---|
| ***Risk source*** | The GMO |
| ***Causal pathway*** | Exposure of people to the GMO during<br>- preparation of the GMO<br>- administration of the GMO<br>- transport or storage of the GMO<br>- disposal of the GMO; or<br>- collection, transport or analysis of biological samples from participants containing the GMO<br>via oral-faecal, ocular secretions, aerosols, fomites, contact with abraded skin or mucous membranes<br>⬇<br>Transduction of cells<br>⬇<br>Post-infection immune response due to the presence of the virus and/or<br>due to the expression of hGM-CSF in Rb-deficient cells |
| ***Potential harm*** | Illness, local inflammation, flu-like symptoms |

**Risk source**

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

**Causal Pathway**

162. HAdVs are easily transmissible via aerosol droplets during respiratory tract infection, contact with mucous membranes, through ocular secretions and through faecal-oral contact. They can also be transmitted when contaminated surfaces, such as hands or tissues, make contact with mucous membranes or are introduced to the respiratory tract.

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

*Exposure during preparation and administration of the GMO*

164. As discussed in Chapter 1, Section 2.3.5 the GMO would be prepared in a hospital pharmacy. During preparation of the treatment, there is a potential for exposure of people involved in the clinical trial via inhalation of aerosols containing the GMO. Additional information relating to the preparation and the administration of the GMO has been declared CCI under Section 185 of the Act. Relevant CCI was made available to the prescribed experts and agencies that were consulted on the RARMP.

165. The unincorporated treatment or GMO progeny are expected to shed with urine for days or weeks following the multi-dose instillation. There is a possibility of creating aerosols during urination and also in the process of flushing the toilet which could lead to exposure of other users of the facility. The GMO is not expected to be present in oral or ocular secretions thus, it is not expected to be transmitted through aerosols generated during coughing or sneezing.

166. Controls proposed by the applicant include providing specific training for clinical trial staff on the procedures associated with handling and administering GMO. The GMO would be prepared and administered by authorised, experienced and trained medical staff i.e. nurses and doctors (Chapter 1, Sections 2.3.5 and 2.3.6). In addition, clinical trial staff preparing and administering the GMO would wear protective equipment including goggles, surgical mask or face shield, gown and gloves. Additional information regarding the proposed controls during the preparation and administration of

the GMO have been declared CCI under Section 185 of the Act. Relevant CCI was made available to the prescribed experts and agencies that were consulted on the RARMP.

167. Preparation and administration of the treatment would be performed in a designated room at the clinical trial site, with restricted access and in the absence of those at risk e.g. immunocompromised people, pregnant or nursing women, or children under the age of 12 months. In addition, the contents of the bladder post-instillation would be drained via catheter into a PVC bag and disposed of as clinical waste. An absorbent pad would be placed underneath the participants during the administration procedure, and the entrance to the urethra will be protected with absorbent material to contain any leaks.

168. Clinical trial staff would be required to follow respiratory hygiene and hand-cleaning hygiene precautions, which would minimise interpersonal spread of the GMOs. Carers, who must not be people at risk, will be provided with detailed guidance around how to look after the participants post-administration.

169. The applicant has also proposed contingency plans in case of spills, eye contact or skin contact with the GMO (Chapter 1, Section 2.3.11). There is a range of disinfecting products that will be available at clinical trial sites, to clean equipment and any potential spills.

170. All personnel working in settings where healthcare is provided are required to comply with the standard precautions for working with potentially infectious material, as described in the Australian Guidelines for the Prevention and Control of Infection in Healthcare (2019). Compliance with these behavioural practices at clinical trial sites will also limit and control exposure of people to the GMO.

171. The above mentioned limits and controls would minimise the potential exposure of people to the GMOs via aerosols during administration of the GMO.

*Exposure during transport, storage and disposal of the GMO*

172. If the GMO was unintentionally spilt or lost during transport or storage, this could result in exposure to people in the area, as aerosol droplets could be formed, leading to aerosol or liquid contact with eyes or mucous membranes and subsequent infection with the GMO.

173. During the transport and storage at clinical trial sites, the GMO would be labelled and double-contained to minimise potential exposure (Chapter 1, Section 2.3.4). Contractors responsible for transport and storage would be informed of the presence of the GMO.

174. The import, transport and storage procedures proposed by the applicant meet the requirements of the Regulator's *Guidelines for the Transport, Storage and Disposal of GMOs* and would limit and control risks of exposure due to spills of the GMO during these dealings. As described in Chapter 1, Section 2.2 the GMO would be imported to Australia according to IATA UN 3373 guidelines.

*Exposure by contact with contaminated materials*

175. If people inadvertently had contact with materials or surfaces contaminated with the GMO, they could be infected with the GMO through hand to mouth transmission. Exposure could occur during disposal of the GMO or materials contaminated with the GMO.

176. The applicant has proposed that unused GMO and waste contaminated with the GMO would be placed in clinical waste containers and disposed of as clinical waste by suitably experienced commercial waste contractors (Chapter 1, Section 2.3.9). It was also proposed that any laundry

contaminated with the GMO would be treated by suitable contractors according to the procedures for infectious substances.

*Exposure during collection, transport or analysis of biological samples from participants containing the GMO*

177. The applicant has proposed that samples would be collected from trial participants after administration of the GMO. Personnel collecting samples from trial participants, transporting or analysing the samples could be exposed to GMOs present in the samples via needle stick/sharps injury or aerosols.

178. Samples collected from the trial participants for the purposes of the clinical trial would be collected by medically trained staff, using standard precautions for the prevention and control of infection in healthcare. Staff would be trained in sharps handling procedures, good hand and respiratory hygiene. This would minimise potential exposure if any GMOs were present in the samples.

179. The applicant advised that samples collected from trial participants may be transported to third-party testing laboratories in Australia according to TSD guidelines, or exported to overseas laboratories for analysis. As discussed above, the packaging requirements under UN 3373 would limit exposure to biological samples potentially containing the GMOs.

180. Analysis of participant samples in Australia would be conducted by personnel in certified analytical and pathology laboratories who are trained and experienced at handling biological samples that may contain other, more dangerous human pathogens. The National Pathology Accreditation Advisory Council (NPAAC) is responsible for developing standards and guidelines for pathology practices which include safety precautions for workers exposed to infectious pathogens (Chapter 1, Section 2.3.9). Waste associated with participant samples would be treated as clinical waste.

181. Taking everything into consideration, the behavioural requirements of healthcare professionals and personnel analysing biological samples and the proposed transport procedures for biological samples would be expected to minimise exposure to the GMO through participant samples, if any GMOs were indeed present.

**Potential harm**

182. If people are exposed to the GMOs via needle stick/sharps injury or aerosols or GMO contaminated waste, they could develop flu-like symptoms or local inflammations for a short period of time before the virus is cleared by the immune system. It is highly unlikely, that exposed people would experience serious adverse events following the exposure unless they are severely immunocompromised.

183. Any dose received through accidental exposure would be substantially less than that administered to trial participants and would not be expected to result in infection as the viral load would be much lower than the immunising dose. In addition, the GMO replicates preferentially in Rb-deficient cells such as tumour cells and is not expected to multiply in other cells. The minimal exposure and transient nature of infection would be expected to result in very mild, or negligible symptoms and would also minimise the potential for an adverse immune response to the treatment.

**Conclusion**

184. Risk scenario 1 is not identified as a substantive risk because exposure is limited by the proposed limits and controls and by the mandatory use of standard precautions for working with potentially

infectious material in all Australian healthcare facilities. Additionally, the GMO has limited replication in normal cells and it is not expected that the GMO will cause illness or an adverse immune response in people who are incidentally exposed. Therefore this risk could not be greater than *negligible* and does not warrant further detailed assessment.

### *2.4.2. Risk scenario 2*

| | |
|---|---|
| ***Risk source*** | The GMO |
| ***Causal pathway*** | Treatment of a trial participant with the GMO<br>⬇<br>Trial participant discharges unincorporated treatment or sheds GMO progeny with blood or urine<br>⬇<br>Exposure of other people (e.g. sexual contacts, household contacts) including at risk people and pregnant women or animals via:<br>- direct contact with the trial participant<br>- exposure to aerosolised secretions (e.g. from urine); or<br>- exposure to the GMO in water (e.g. swimming pool, bathtub)<br>- contact with GMO contaminated items (e.g. items the trial participant has touched, or contaminated tissues)<br>⬇<br>Transduction of cells<br>⬇<br>Post-infection immune response due to the presence of the virus<br>and/or<br>due to the expression of hGM-CSF in Rb-deficient cells |
| ***Potential harm*** | Illness, local inflammation, flu-like symptoms |

**Risk source**

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

**Causal Pathway**

*Trial participant discharges unincorporated inoculum or sheds GMO progeny to the environment*

186. If trial participants discharge unincorporated inoculum or shed GMO progeny, they could contaminate surfaces with the GMO and/or generate aerosols containing the GMO when they urinate or uncontrollably leak urine. This could lead to infection of other people and animal hosts in the environment.

187. The quantity of the GMO shed into the environment from trial participants would be limited. Moreover, any GMO that enters the environment will not multiple further because multiplication selectively occurs in live cancer cells which contain a specific defect and these cells are rare in the environment. Finally, quantity of GMO that has the potential to enter the environment is limited by the small number of people (up to 60) enrolled in the clinical trial in Australia.

188. The applicant has proposed that trial participants would be administered the GMO in hospitals. The majority of the unincorporated treatment will be drained from participants' bladder and disposed into clinical waste according to clinical trial site procedures.

189. Any contaminated materials, such as urinary catheters, gloves, bandages, and dressings used by participants up to 14 days post-administration would be contained in a bag or a sealed container and disposed in regular rubbish bins or returned to the clinic for biohazard bag disposal.

190. Participants will be instructed to follow and comply with good hygiene practices and avoid immuno-compromised people for 30 days following each administration of the GMO. The GMO is not expected to shed through the respiratory tract and only limited number of participants are expected to present detectable levels of the GMO in blood as discussed in Section 3.6. The shedding of large numbers of the GMO in urine is expected in the first 2 hours post-treatment and then to sharply decrease after 5 days for a first instillation or 24 hours for subsequent instillation and over 29 days (Chapter 1, Section 3.6) with the highest amounts shed during the first instillation.

191. Following administration, the participants will have their bladder contents drained into drainage bags, which will be discarded as clinical waste.

192. Trial participants would be required to disinfect their urine following the administration and contain incontinence pads in a plastic bag and place in household rubbish.

193. It is expected that the administered GMO would be localised to the bladder in the majority of cases, and infect tumour-cells in which it will replicate and lyse the cells, clearing its presence from a human host. In the unlikely event of the infection occurring in healthy epithelial cells, the GMO will not be able to replicate and will be quickly cleared by the immune system. This would inhibit the virus from going into a latent phase and shedding for extended periods.

194. Shedding of the GMO by trial participants would not negatively impact the environment. It is estimated that 80% of human population has been exposed to HAdVs that are continuously present in the environment. Furthermore, people infected with HAdVs shed large numbers of viral particles through the respiratory or gastro-intestinal (GI) tracts. It is estimated that domestic sewage contains $10^6$ HAdV vp/L. Most of the data on the presence and shedding of HAdVs is based on the detection of nucleic acids not infectious virions in environmental samples. Under the most favourable conditions HAdVs can survive for up to 8 weeks in the environment. However, this GMO does not have a competitive advantage compared with the WT HAdV as the GMO preferentially replicates in cancer cells and, therefore is unlikely to persist in the environment.

195. The applicant is proposing precautions to exclude subjects who have septic toilets at home and imposes a condition to eliminate interaction with people at risk such as young children (less than 12 months of age), people with immunosuppressive conditions or pregnant/nursing women. The applicant proposed that participants and their family/close contacts would avoid direct contact with the participants' urine for 14 days post-administration. To decrease the potential risk arising from shedding, participants would also be advised not to share cooking utensils or other objects that have come into contact with body fluids (e.g. towels, cutlery, toothbrush), and abstain from intimate physical contact with others for at least 14 days after each treatment. Washing of clothing and bedding and other commonly used items which are likely to contain viral particles will be performed in hot water with laundry detergent and approximately one cup of undiluted house bleach per wash load, for at least 14 days after each treatment.

**Potential harm**

196. The GMOs would be expected to be shed from trial participants for a limited period of time, limiting exposure of other people or animals, such as pets, to the GMOs. Since healthy cells do not support replication of the virus they are not destroyed by the treatment and are therefore viral particles are not shed in large quantities (Ramesh et al., 2006). The minimal exposure and transient nature of infection would be expected to result in very mild, or negligible symptoms and would also minimise the potential for an adverse immune response to the GMO.

197. If immunosuppressed people or animal hosts were exposed to and infected by the GMO, it would be expected to replicate preferentially in tumour cells and would be unable to cause disease.

Although they are likely to have some pre-existing immunity to HAdVs, people or animals with immunosuppression may take longer to clear the GMO virus.

198. Only severely immunocompromised people could experience a more severe infection but they are excluded from contact with the participant during the treatment

**Conclusion**

199. Risk scenario 2 is not identified as a substantive risk. Trial participants may discharge the administered unincorporated GMO or its progeny, however this would be expected to occur for a relatively short period of time. Release of the GMO into the environment will be limited by the decontamination of the participants' urine while shedding of the GMO is at its peak. Additionally, discharged GMO is unlikely to persist in the environment. The applicant proposed limits and controls would also assist in minimising the exposure of people and animals to the GMOs. Harm is unlikely because the GMO replicates preferentially in tumour cells. Therefore this risk could not be greater than *negligible* and does not warrant further detailed assessment.

### *2.4.3. Risk scenario 3*

| | |
|---|---|
| ***Risk source*** | The GMO and WT HAdV-C |
| ***Causal pathway*** | Trial participant inoculated with the GMO is infected with another HAdV from species C<br>⬇<br>Both viruses co-infect the same host cell<br>⬇<br>GMO and the WT HAdV recombine<br>⬇<br>Replication competent recombinant GMO virus with functional hGM-CSF gene<br>⬇<br>Recombinant GMO infects host and replicates<br>⬇<br>Establishment of viral infection in host<br>⬇<br>Recombinant GMO virus shed<br>⬇<br>Recombinant GMO virus transmitted and infects other hosts |
| ***Potential harm*** | Disease in humans |

**Risk source**

200. The sources of potential harm for this postulated risk scenario are the GMO and WT HAdV-C.

**Causal pathway**

201. Recombination between human adenoviruses belonging to the same species could occur if two different viruses of the same type or species infect a host cell at the same time. It is unlikely that a pre-existing WT virus would be in the same tissue as the GM HAdV. In this rare case of co-infection, an unlikely recombination event between different types of HAdV-C or, a more frequent recombination between the HAdVs of the same type, could take place. However, the majority of the recombination events occur in the DNA regions of high homology and usually lead to neutral changes in the DNA as previously described in Section 3.3.

202. Two different GM recombinants could be generated through the process of recombination of the GMO with the WT-HAdV. Firstly, the WT could receive the hGM-CSF gene and gain immuno-stimulatory function. Secondly, the GMO could regain its E1a promoter and thus, its full replication competence. In both cases, the recombinant adenoviruses, would be able to replicate in infected cells unconditionally and express hGM-CSF. These new viruses could then be shed from the host and transmitted to other hosts in the environment.

203. Co-infection in trial participants could occur if the trial participant has an asymptomatic persistent or latent infection with another human adenovirus species C (Ad1, Ad2, Ad5, Ad6 and Ad57) and due to difficult/open wound catheterisation, the GM HAdV infiltrated other tissues/cells hosting a WT Ad. Alternatively, the GMO could be reintroduced into the participant orally or transmitted with fomites or unclean hands.

204. The applicant suggested that participants undergoing treatment will need to follow the procedures specified in the *Informed Consent and Research Authorization Form* that will prevent or at least significantly limit the possibility of concurrent infection in participants. For example, participants who will notice signs of a respiratory illness with a fever, ocular disease, or diarrhoea will be instructed to avoid direct physical contact with any potentially contaminated material (e.g. urine-soiled clothing), avoid touching eyes and nose especially after coughing. When symptoms of respiratory illness are present, participants would be wearing a mask when around other people, and not share commonly used household and personal hygiene items. Limits and controls discussed in previous risk scenarios are also applicable here.

205. Recombination could also take place in a secondary host who could get infected with the GMO while having another HAdV infection. However, as discussed in previous scenarios, due to limited shedding of the GMO and limits and controls proposed by the applicant the transmission and concurrent infection in other hosts is very unlikely.

206. The data obtained in phase 2 clinical trials indicates that shedding of the virus into the blood following the IVE is uncommon and happens only in participants in whom the instillation was difficult and generated open wounds. However, the data obtained in previous studies does not distinguish the GMO from WT HAdV infection nor, provides the information on the virulence of the virus. The applicant has now developed a GMO-specific method that will facilitate its specific detection and quantification.

207. In the event of recombination, resultant recombinants would be replication competent and carry an hGM-CSF gene. Ability to express, hGM-CSF in the host-cell, would activate systemic immune response and clear the virus from the infected tissue.

208. Viral infection and expression of hGM-CSF could pose a risk to severely immunodeficient people or those in high risk group. However, people who could potentially experience serious reactions to the GMO or the recombinant GMO, are unlikely to be present in public areas.

209. In risk scenarios 1 and 2, the proposed controls for the clinical trial would minimise the likelihood of exposure of people to the GM virus. Additionally, as noted for Scenario 1, the GM virus is a preferentially replicating virus with a transient presence in the environment, making it less likely to recombine with other viruses.

**Potential harm**

210. Replication competent recombinants containing the hGM-CSF would be expected to have a better ability to stimulate host-immune response and generate local inflammation, which would likely eliminate virus quicker than the WT. This modification, could have negative effects only in people at

risk who would not have pre-existing immunity against HAdV pathogens. In a very limited number of severely immunocompromised people, the untreated infection with a recombinant virus, could have severe adverse effects. However, the infection would be expected to be treatable with antiviral pharmacological treatment (Chapter 1, Section 3.8). In addition, as described in Section 6.1 the hGM-CSF has been approved under the licence DIR 132 for a commercial release of the oncolytic treatment in 2015 and has been approved by USFDA, TGA and the OGTR.

**Conclusion**

211. Risk scenario 3 is not identified as a substantive risk. Co-infection of a host-cell with the GMO and a WT HAdV would be highly unlikely. Recombinant viruses would not be more virulent, and would be expected to be cleared from the host-organism due to an enhanced immune-response. The only people that could be affected by the recombinant strain are those in a high-risk group, but those would be very unlikely to be exposed due to a small number of trial participants and implemented controls. Therefore, the risk could not be greater than *negligible* and does not warrant further detailed assessment.

### *2.4.4. Risk scenario 4*

| | |
|---|---|
| ***Risk source*** | The GMO |
| ***Causal pathway*** | Treatment of a trial participant with the GMO<br>⬇<br>Trial participant discharges unincorporated treatment or sheds GMO progeny with urine<br>⬇<br>GMO survives sewage treatment<br>⬇<br>GMO is present in the environment and infects other hosts |
| ***Potential harm*** | Disease in humans or animals |

Risk source

212. The sources of potential harm for this postulated risk scenario is the GMO.

**Causal pathway**

213. Trial participants will discharge unincorporated inoculum or shed GMO progeny during urination. If urine is not disinfected, the GMO will end up in the sewage treatment plant. As described in Section 3.5, HAdVs can survive the sewage treatment for up to 8 weeks and are frequently identified in the effluent. Thus, for this period, the GMO may persist and be found in surface waters such as rivers, oceans and lakes. This could potentially lead to infection of other human and animal hosts in the environment.

214. HAdVs are widely spread in the environment, especially in the surface waters (Chapter 1, Section 3.7). Most methods used for the detection of the HAdVs in the environment have been designed to detect nucleic acids without the confirmation of their virulence. Therefore, the consideration of the number of viral particles present in the waters or shed with urine is assuming that all of the detected virus remains virulent.

215. In risk scenario 2, infection of other hosts by the GMO has been already considered and the same aspects apply in this risk scenario. In addition to the above, HAdVs are not known to infect aquatic animals with the exception of aquatic mammals such as water rats.

**Potential harm**

216. Potential harms in this risk scenario would be the same as considered in risk scenario 2 presented above. In addition, the GMOs would be considered less likely to infect a host after being discharged with the sewage due to its small quantity in a large volume of liquid waste or water and time taken between shedding and a potential new infection.

217. HAdVs could infected other species and potentially cause an illness in animals. However, HAdVs are not known to infect aquatic animals, with the possible exception of water-dwelling mammals, and the amount present in the sewage would be considered too small to be infectious.

218. As in Risk Scenarios 2 and 3, exposure of susceptible animals, such as pigs, would be expected to be minimal and the viral load they would be exposed to would be significantly less than that administered to the participants. Additionally, interspecies recombination of adenoviruses is unlikely, as discussed in Chapter 2, Section 2.1.

**Conclusion**

219. Risk scenario 4 is not identified as a substantive risk. Trial participants may discharge the administered unincorporated GMO or its progeny, however the number of viral particles in the sewage is expected to be very small and its presence in the environment would be transient. The GMO is not known to infect marine animals. Therefore this risk could not be greater than negligible and does not warrant further detailed assessment.

## Section 3 Uncertainty

220. Uncertainty is an intrinsic part of risk analysis[2]. 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.

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

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

[2] 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.

222. 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.

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

224. For DIR 177, uncertainty is noted in relation to shedding and persistence of GMO in the environment as well as the likelihood of potential secondary infection and recombination with other HAdVs. Existing studies utilising oncolytic treatments suggest that they are safe to use and their shedding in the environment is limited and not known to cause any harm. Shedding data collected during the clinical trials with this GMO indicates that the majority of unincorporated inoculum and GMO progeny is shed within 24 hours to 5 days post-administration. Considering all of the suggested precautions and unlikely occurrence of meaningful recombination between HAdVs, it is unlikely that the transgenes would be transferred to other strains. This was taken into account in estimating the level of risk.

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

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

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

## Section 4 Risk evaluation

228. 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.

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

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

230. Four risk scenarios were postulated whereby the proposed dealings might give rise to harm to people or the environment. In the context of the proposed clinical trial sites, limits and controls proposed by the applicant, and considering both the short and long term consequences, none of these scenarios were identified as substantive risks. The principal reasons for these conclusions are summarised in Table 3 and include:

- the GMO has been altered to replicate preferentially in tumour cells which helps prevent it from multiplying in other cells

- the GMO will be shed for a relatively short time from a maximum of 60 trial participants
- the GMO has a limited opportunity to regain replication competence through recombination
- adenoviruses are widely present in the environment and most people have a pre-existing immunity
- recombination of HAdVs does not happen outside of a host

231. Due to the suitability of the limits and controls proposed by the applicant, and those imposed by the licence, risks to the health and safety of people, or the environment, from the proposed clinical trial of the GMO into the environment are considered to be negligible. The *Risk Analysis Framework*, which guides the risk assessment and risk management process, defines negligible risks as risks of no discernible concern with no present need to invoke actions for mitigation. Therefore, no 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

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

233. 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.

234. 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.

235. 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.

236. Licence conditions are discussed and summarised in this Chapter and listed in detail in the licence.

## Section 2 Risk treatment measures for substantive risks

237. 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 of the GM HAdV treatment. These risk scenarios were in the context of the scale of the proposed clinical trial (Chapter 1, Section 2.3), the proposed containment measures (Chapter 1, Sections 2.1-2.2), and the receiving environment (Chapter 1, Section 5), and considering both the short and the long term. The risk evaluation concluded that no specific risk treatment measures are required to treat these negligible risks. Limits and controls proposed by the applicant and other general risk management measures are discussed below.

## Section 3 General risk management

238. The limits and controls proposed in the application were important in establishing the context for the risk assessment and in reaching the conclusion that the risks posed to people and the environment are negligible. Therefore, to maintain the risk context, licence conditions have been imposed to limit the size, location and duration of the clinical trial, and to restrict the spread and persistence of the GMO and its genetic material in the environment. The conditions are discussed and summarised in this Chapter and detailed in licence.

## 3.1. Limits and controls on the clinical trial

239. Sections 2.1 and 2.2 in Chapter 1 list the limits and controls proposed by Novotech (Australia) Pty Limited. Many of these are discussed in the four risk scenarios considered in Chapter 2. The appropriateness of the limits and controls is considered further in the following sections.

### *3.1.1. Consideration of limits and controls proposed by Novotech (Australia) Pty Limited*

240. The proposed clinical trial would involve a maximum of 60 participants within Australia. Preparation, administration and disposal of the GMO would take place in hospitals, while biological samples potentially containing the GMO would be collected at clinical trial sites and analysed at certified analytical and pathology laboratories. Activities that would occur outside of these facilities include transport, storage and disposal of the GMOs (risk scenario 1) and shedding of the GMO inoculum or its progeny outside of the proposed clinical trial sites (risk scenario 2 and 4). The applicant has proposed to complete the study within 5 years of commencement. The GMO would be administered to participants who fulfil inclusion criteria and consent to conditions included in the *Informed Consent and Research Authorization Form*. These conditions will limit the spread of shed GMO and limit the chance of the recombination event between the GMO and other HAdVs (risk scenario 3). Conditions maintaining the risk context and proposed limits of the trial such as the maximum number of trial participants, duration of the study and the permitted timing of treatments have been included in the licence.

241. The applicant advised that import and transport of the GMO and waste containing the GMO would be in accordance with IATA shipping classification UN 3373 [Category B] and/or the Regulator's *Guidelines for the Transport, Storage and Disposal of GMOs*. These are standard protocols for the handling and minimising exposure to the GMOs. Once at the clinical trial site, access to the GMO would be restricted to appropriately trained personnel. These proposed transport conditions are suitable for the GMO. Therefore, the draft licence details the minimum requirements for packaging and labelling the GMO and waste contaminated with the GMO for transport and storage within a clinical trial site, as well as transport of the GMO for export. These measures would limit the exposure of people and the environment to the GMOs (risk scenario 1).

242. The trial participants are limited to participants with NMIBC unresponsive to BCG and relevant proposed inclusion and exclusion criteria are outlined in Chapter 1, Section 2.3.3. The inclusion and exclusion criteria for trial participants would be subject to approval by a HREC, who would consider the safety of the individuals involved in the trial.

243. The applicant advised that the GMO would be disposed as clinical waste at the clinical trial site. The applicant has also proposed that clinical staff would wear personal protective equipment including disposable gowns, gloves face shields and/or goggles. Participants would also be wearing disposable gowns during the procedure and placed over an absorbent pad that will contain all the spills. These and other safe work practices listed in Section 2.2 and in the application would minimise the exposure of people handling and administering the GMOs (risk scenario 1) and have been included in the licence conditions.

244. 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 at the clinical trial site are decontaminated by autoclaving, chemical treatment or by high-temperature incineration. Licence conditions require that the licence holder must ensure that the GMO, or material or waste that has been in contact with the GMO, that is to be destroyed by external service providers, is through a clinical waste stream. This is considered satisfactory, provided that the licence holder is only permitted to engage persons who can adhere to appropriate standards to conduct the dealings, as described in paragraph 248. The licence also requires waste disposal by external service providers to be by

autoclaving or incineration. These measures would limit the exposure of people or other animals to the GMOs (risk scenario 1).

245. The applicant has proposed that trial participants and their caregiver(s) would be required to consent to the precautionary measures and guidelines aimed to minimise interpersonal spread and shedding of the GMOs to the environment as specified in the *Informed Consent and Research Authorization Form*. Trial participants would remain at the trial site for 1 hour following instillation.

246. Data from previous studies has shown that HAdVs should be cleared from participants in under 29 days following the IVE administration of the treatment with the majority of the GMO being discharged within 5 days of the first instillation and 24 hours of subsequent instillations (Chapter 1, Section 4.2.4). The applicant initially proposed that participants would decontaminate their urine using bleach for 30 days post-administration. This application was initially submitted as a dealing not involving the release of a GMO into the environment. As this application was assessed as a dealing involving the limited and controlled release into the environment (DIR), the shedding profile of the GMO was examined in depth.

247. Considering that the shedding of the GMO and its progeny happens at the highest rates within the first 5 days of the first instillation and subsequently the first 24 hours after each additional instillation (Burke et al., 2012), licence conditions require that the urine be decontaminated only during these times. This condition ensures that the shedding of the GMO into the environment is limited.

248. Implemented measures would minimise interpersonal transmission and thus, limit the potential of recombination with other HAdVs. In the very unlikely event that other people were unintentionally exposed to the GMO, they would be expected to have very mild to no symptoms for a short time period due to pre-existing immunity in the human population as well as immunogenic properties of the GMO (risk scenario 2). Therefore, the conditions imposed in the licence requiring trial participants and their caregivers/people in their immediate environment to follow behavioural requirements would be sufficient to limit or control transmission of the GMOs.

249. A standard condition is included in the licence requiring the licence holder to ensure that dealings are conducted so as to ensure containment of the GMO, not compromise the health and safety of people and minimise unintentional exposure to the GMO. A note written under the condition explains that compliance may be achieved by only engaging persons who are required to adhere to appropriate standards to conduct the dealings.

250. 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.

251. Further conditions have also been imposed in the licence to ensure that a Compliance Management Plan is in place for each clinical trial site before administration of the GMOs commences at that site. The Compliance Management Plan must detail how the licence holder intends to comply with the licence conditions, including listing persons responsible for site management, proposed reporting structures, staff training procedures and transport and disposal processes.

### *3.1.2. Summary of licence conditions to be imposed to limit and control the clinical trial*

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

- limit the trial to vaccination of up to 60 trial participants at clinical trial sites, for a period of 5 years;
- 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-contaminated materials and equipment that have been in contact with the GMO at clinical trial sites using effective disinfectants or disposal using a certified waste contractor in accordance with standard clinical waste disposal practices, as required by the relevant Australian and state legislation;
- require trial participants to consent to appropriate decontamination procedures and practices imposed to limit the spread of the GMO after leaving the clinical trial site;
- require trial participants to eliminate direct contact with people at risk and limit the possibility of interpersonal transmission by following the guidelines listed in the *Informed Consent and Research Authorization Form, and*
- transport and store the GMO and samples from GMO-treated participants in accordance with IATA requirements shipping classification UN 3373 [Category B] and/or the minimum requirements for packaging, and labelling as detailed in the licence.

## 3.2. Other risk management considerations

253. 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 purposes of monitoring for compliance.

### *3.2.1. Applicant suitability*

254. 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.

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

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

### *3.2.2. Contingency Plans*

257. Novotech (Australia) Pty Limited is required to submit a Contingency plan to the Regulator before commencing dealings with the GMOs. This plan will detail measures to be undertaken in the event of:

- the unintended release of the GMOs, including spills
- exposure of, or transmission to persons other than trial participants
- a person exposed to the GMOs developing a serious adverse response.

### *3.2.3. Identification of the persons or classes of persons covered by the licence*

258. The persons covered by the licence would be the licence holder and employees, agents or contractors of the licence holder and other persons who are, or have been, engaged or otherwise authorised by the licence holder to undertake any activity in connection with the dealings authorised by the licence. Prior to dealings with the GMOs, Novotech (Australia) Pty Limited is required to provide a list of people and organisations that are covered by the licence, or the function or position where names are not known at the time.

### *3.2.4. Reporting requirements*

259. The licence requires the licence holder to immediately report any of the following to the Regulator:

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

260. A number of written notices are also required under the licence regarding dealings with the GMO, to assist the Regulator in designing and implementing a monitoring program for all licensed dealings. The notices include:

- identification of the clinical trial sites where trial participants would be inoculated
- expected date of inoculation with the GMOs for each clinical trial site
- cease of inoculation with the GMOs for each clinical trial site.

### *3.2.5. Monitoring for Compliance*

261. 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.

262. 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.

263. In cases of non-compliance with licence conditions, the Regulator may instigate an investigation to determine the nature and extent of non-compliance. The Act provides for criminal sanctions of large fines and/or imprisonment for failing to abide by the legislation, conditions of the licence or directions from the Regulator, especially where significant damage to the health and safety of people or the environment could result.

## Section 4 Issues to be addressed for future clinical trials or commercial release

264. Additional information has been identified that may be required to assess an application for a commercial release of the GMO, or to justify a reduction in limits and controls. This includes information and data that would address the uncertainties noted in Chapter 2, Section 3.

## Section 5 Conclusions of the RARMP

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

266. 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.

# References

Afkhami, S., Yao, Y., and Xing, Z. (2016). Methods and clinical development of adenovirus-vectored vaccines against mucosal pathogens. Molecular Therapy Methods & Clinical Development *3*, 16030.

Allard, A., and Vantarakis, A. (2017). Adenoviruses. (Michigan State University, MI: UNESCO).

Baiocchi, R.A., Ward, J.S., Carrodeguas, L., Eisenbeis, C.F., Peng, R., Roychowdhury, S., Vourganti, S.*, et al.* (2001). GM-CSF and IL-2 induce specific cellular immunity and provide protection against Epstein-Barr virus lymphoproliferative disorder. Journal of Clinical Investigation *108*, 887-894.

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

Bell, L.A., and Ryan, K.M. (2004). Life and death decisions by E2F-1. Cell Death & Differentiation *11*, 137-142.

Bendandi, M., Gocke, C.D., Kobrin, C.B., Benko, F.A., Sternas, L.A., Pennington, R., Watson, T.M.*, et al.* (1999). Complete molecular remissions induced by patient-specific vaccination plus granulocyte-monocyte colony-stimulating factor against lymphoma. Nature Medicine *5*, 1171-1177.

Borkenhagen, L.K., Fieldhouse, J.K., Seto, D., and Gray, G.C. (2019). Are adenoviruses zoonotic? A systematic review of the evidence. Emerging Microbes & Infections *8*, 1679-1687.

Brandon, E.F.A., Tiesjema, B., Eijkeren, J.C.H.v., and Hermsen, H.P.H. (2008). Effect of administration route on biodistribution and

shedding of replication-deficient viral vectors used in

gene therapy. Report No. 320001001/2008. (Office for Genetically Modified Organisms of the National Institute for Public Health and the Environment ).

Burke, J.M., Lamm, D.L., Meng, M.V., Nemunaitis, J.J., Stephenson, J.J., Arseneau, J.C., Aimi, J.*, et al.* (2012). A first in human phase 1 study of CG0070, a GM-CSF expressing oncolytic adenovirus, for the treatment of nonmuscle invasive bladder cancer. Journal of Urology *188*, 2391-2397.

Charman, M., Herrmann, C., and Weitzman, M.D. (2019). Viral and cellular interactions during adenovirus DNA replication. FEBS Letters *593*, 3531-3550.

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

Crystal, R.G., Harvey, B.-G., Wisnivesky, J.P., O'Donoghue, K.A., Chu, K.W., Maroni, J., Muscat, J.C.*, et al.* (2002). Analysis of risk factors for local delivery of low-and intermediate-dose adenovirus gene transfer vectors to individuals with a spectrum of comorbid conditions. Human Gene Therapy *13*, 65-100.

Daud, A.I., Mirza, N., Lenox, B., Andrews, S., Urbas, P., Gao, G.X., Lee, J.H.*, et al.* (2008). Phenotypic and functional analysis of dendritic cells and clinical outcome in patients with high-risk melanoma treated with adjuvant granulocyte macrophage colony-stimulating factor. Journal of Clinical Oncology *26*, 3235-3241.

Davis, T.A., Monroy, R.L., Skelly, R.R., Donahue, R.E., and MacVittie, T.J. (1990). Differential augmentation of in vivo natural killer cytotoxicity in normal primates with recombinant human interleukin-1 and granulocyte-macrophage colony-stimulating factor. Clinical and Experimental Immunology *79*, 436-442.

Denechaud, P.-D., Fajas, L., and Giralt, A. (2017). E2F1, a novel regulator of metabolism. Frontiers in Endocrinology *8*.

Desfarges, S., and Ciuffi, A. (2012). Viral integration and consequences on host gene expression. In Viruses: essential agents of life (Dordrecht: Springer), pp. 147-175.

Desheva, Y. (2018). Introductory chapter: Human adenoviruses. In Adenoviruses (IntechOpen).

Dhingra, A., Hage, E., Ganzenmueller, T., Böttcher, S., Hofmann, J., Hamprecht, K., Obermeier, P*., et al.* (2019). Molecular evolution of human adenovirus (HAdV) species C. Scientific Reports *9*, 1039.

Dimova, D.K., and Dyson, N.J. (2005). The E2F transcriptional network: old acquaintances with new faces. Oncogene *24*, 2810-2826.

Dranoff, G., Jaffee, E., Lazenby, A., Golumbek, P., Levitsky, H., Brose, K., Jackson, V*., et al.* (1993). Vaccination with irradiated tumor cells engineered to secrete murine granulocyte-macrophage colony-stimulating factor stimulates potent, specific, and long-lasting anti-tumor immunity. Proceedings of the National Academy of Sciences *90*, 3539-3543.

Du, T., Shi, G., Li, Y.M., Zhang, J.F., Tian, H.W., Wei, Y.Q., Deng, H*., et al.* (2014). Tumor-specific oncolytic adenoviruses expressing granulocyte macrophage colony-stimulating factor or anti-CTLA4 antibody for the treatment of cancers. Cancer Gene Therapy *21*, 340-348.

Echavarría, M. (2008). Adenoviruses in immunocompromised hosts. Clinical Microbiology Reviews *21*, 704-715.

Efiok, B.J.S., and Safer, B. (2000). Transcriptional regulation of E2F-1 and eIF-2 genes by α-Pal: a potential mechanism for coordinated regulation of protein synthesis, growth, and the cell cycle. Biochimica et Biophysica Acta (BBA) - Molecular Cell Research *1495*, 51-68.

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

Hermiston, T.W., Tripp, R.A., Sparer, T., Gooding, L.R., and Wold, W.S. (1993). Deletion mutation analysis of the adenovirus type 2 E3-gp19K protein: identification of sequences within the endoplasmic reticulum lumenal domain that are required for class I antigen binding and protection from adenovirus-specific cytotoxic T lymphocytes. Journal of Virology *67*, 5289.

Hoeben, R.C., and Uil, T.G. (2013). Adenovirus DNA Replication. Cold Spring Harbor Perspectives in Biology *5*, a013003.

Hoppe, E., Pauly, M., Gillespie, T.R., Akoua-Koffi, C., Hohmann, G., Fruth, B., Karhemere, S*., et al.* (2015). Multiple cross-species transmission events of human adenoviruses (HAdV) during Hominine evolution. Molecular Biology and Evolution *32*, 2072-2084.

Huh, K., Kim, I., Jung, J., Lee, J.E., Jhun, B.W., Gu, S.H., Song, D.H*., et al.* (2019). Prolonged shedding of type 55 human adenovirus in immunocompetent adults with adenoviral respiratory infections. European Journal of Clinical Microbiology & Infectious Diseases *38*, 793-800.

Ismail, A.M., Cui, T., Dommaraju, K., Singh, G., Dehghan, S., Seto, J., Shrivastava, S*., et al.* (2018). Genomic analysis of a large set of currently—and historically—important human adenovirus pathogens. Emerging Microbes & Infections *7*, 1-22.

Jakubczak, J.L., Ryan, P., Gorziglia, M., Clarke, L., Hawkins, L.K., Hay, C., Huang, Y*., et al.* (2003). An oncolytic adenovirus selective for retinoblastoma tumor suppressor protein pathway-defective tumors. Cancer Research *63*, 1490.

Jhawar, S., Goyal, S., Bommareddy, P.K., Zloza, A., and Kaufman, H. (2017a). Talimogene laherparepvec (T-VEC) as cancer immunotherapy. Journal for ImmunoTherapy of Cancer *6*, 53.

Jhawar, S.R., Thandoni, A., Bommareddy, P.K., Hassan, S., Kohlhapp, F.J., Goyal, S., Schenkel, J.M*., et al.* (2017b). Oncolytic viruses—Natural and genetically engineered cancer immunotherapies. Frontiers in Oncology *7*.

Jogler, C., Hoffmann, D., Theegarten, D., Grunwald, T., Überla, K., and Wildner, O. (2006). Replication properties of human adenovirus in vivo and in cultures of primary cells from different animal species. Journal of Virology *80*, 3549-3558.

Kosulin, K., Geiger, E., Vécsei, A., Huber, W.D., Rauch, M., Brenner, E., Wrba, F.*, et al.* (2016). Persistence and reactivation of human adenoviruses in the gastrointestinal tract. Clinical Microbiology and Infection *22*, 381.e381-381.e388.

Lee, F., Yokota, T., Otsuka, T., Gemmell, L., Larson, N., Luh, J., Arai, K.*, et al.* (1985). Isolation of cDNA for a human granulocyte-macrophage colony-stimulating factor by functional expression in mammalian cells. Proceedings of the National Academy of Sciences *82*, 4360-4364.

Lin, Q., Lim, J.Y.C., Xue, K., Yew, P.Y.M., Owh, C., Chee, P.L., and Loh, X.J. (2020). Sanitizing agents for virus inactivation and disinfection. *1*, e16.

Liu, B., Robinson, M., Han, Z., Branston, R., English, C., Reay, P., McGrath, Y.*, et al.* (2003). ICP34. 5 deleted herpes simplex virus with enhanced oncolytic, immune stimulating, and anti-tumour properties. Gene Therapy *10*, 292-303.

Ljungman, P. (2004). Treatment of adenovirus infections in the immunocompromised host. European Journal of Clinical Microbiology and Infectious Diseases *23*, 583-588.

Lukashev, A.N., Ivanova, O.E., Eremeeva, T.P., and Iggo, R.D. (2008). Evidence of frequent recombination among human adenoviruses. Journal of General Virology *89*, 380-388.

Maliszewski, C.R., Schoenborn, M.A., Cerretti, D.P., Wignall, J.M., Picha, K.S., Cosman, D., Tushinski, R.J.*, et al.* (1988). Bovine GM-CSF: molecular cloning and biological activity of the recombinant protein. Molecular Immunology *25*, 843-850.

Mayer, P., Werner, F.J., Lam, C., and Besemer, J. (1990). In vitro and in vivo activity of human recombinant granulocyte-macrophage colony-stimulating factor in dogs. Experimental Hematology *18*, 1026-1033.

National Health and Medical Research Council (2019). Australian Guidelines for the Prevention and Control of Infection in Healthcare. (Canberra: Australian Government).

National Health and Medical Research Council, Australian Research Council, and Universities Australia (2018). National Statement on Ethical Conduct in Human Research 2007 (Updated 2018). (Canberra: Commonwealth of Australia).

OGTR (2013). Risk Analysis Framework 2013, 4th edn (Canberra: Office of the Gene Technology Regulator).

Packiam, V.T., Lamm, D.L., Barocas, D.A., Trainer, A., Fand, B., Davis III, R.L., Clark, W.*, et al.* (2018). An open label, single-arm, phase II multicenter study of the safety and efficacy of CG0070 oncolytic vector regimen in patients with BCG-unresponsive non–muscle-invasive bladder cancer: Interim results. Urologic Oncology *36*, 440-447.

Pol, J., Kroemer, G., and Galluzzi, L. (2015). First oncolytic virus approved for melanoma immunotherapy. Oncoimmunology *5*, e1115641-e1115641.

Pond, K. (2005). Water recreation and disease: plausibility of associated infections: acute effects, sequelae, and mortality (World Health Organization).

Prevost, B., Goulet, M., Lucas, F., Joyeux, M., Moulin, L., and Wurtzer, S. (2016). Viral persistence in surface and drinking water: Suitability of PCR pre-treatment with intercalating dyes. Water Research *91*, 68-76.

Ramesh, N., Ge, Y., Ennist, D.L., Zhu, M., Mina, M., Ganesh, S., Reddy, P.S.*, et al.* (2006). CG0070, a conditionally replicating granulocyte macrophage colony-stimulating factor–armed oncolytic adenovirus for the treatment of bladder cancer. Clinical Cancer Research *12*, 305.

Rutala, W.A., Peacock, J.E., Gergen, M.F., Sobsey, M.D., and Weber, D.J. (2006). Efficacy of hospital germicides against adenovirus 8, a common cause of epidemic keratoconjunctivitis in health care facilities. Antimicrob Agents Chemother *50*, 1419-1424.

Saha, B., and Parks, R.J. (2017). Human adenovirus type 5 vectors deleted of early region 1 (E1) undergo limited expression of early replicative E2 proteins and DNA replication in non-permissive cells. PLOS ONE *12*, e0181012.

Sato, T., Eschelman, D.J., Gonsalves, C.F., Terai, M., Chervoneva, I., McCue, P.A., Shields, J.A.*, et al.* (2008). Immunoembolization of malignant liver tumors, including uveal melanoma, using granulocyte-macrophage colony-stimulating factor. Journal of Clinical Oncology *26*, 5436-5442.

Shaw, A.R., and Suzuki, M. (2019). Immunology of adenoviral vectors in cancer therapy. Molecular Therapy Methods & Clinical Development *15*, 418-429.

Sheets, R.L., Stein, J., Bailer, R.T., Koup, R.A., Andrews, C., Nason, M., He, B.*, et al.* (2008). Biodistribution and toxicological safety of adenovirus type 5 and type 35 vectored vaccines against human immunodeficiency virus-1 (HIV-1), Ebola, or Marburg are similar despite differing adenovirus serotype vector, manufacturer's construct, or gene inserts. Journal of Immunotoxicology *5*, 315-335.

Soiffer, R., Hodi, F.S., Haluska, F., Jung, K., Gillessen, S., Singer, S., Tanabe, K.*, et al.* (2003). Vaccination with irradiated, autologous melanoma cells engineered to secrete granulocyte-macrophage colony-stimulating factor by adenoviral-mediated gene transfer augments antitumor immunity in patients with metastatic melanoma. Journal of Clinical Oncology *21*, 3343-3350.

Spitler, L.E., Weber, R.W., Allen, R.E., Meyer, J., Cruickshank, S., Garbe, E., Lin, H.-Y.*, et al.* (2009). Recombinant human granulocyte-macrophage colony-stimulating factor (GM-CSF, sargramostim) administered for 3 years as adjuvant therapy of stages II (T4), III, and IV melanoma. Journal of Immunotherapy *32*, 632-637.

Standards Australia/New Zealand (2010). Safety in laboratories - Microbiological safety and containment AS/NZS 2243.3:2010, AS/NZS 2243.3:2010 edn (Standards Australia/New Zealand).

Usman, N., and Suarez, M. (2020). Adenoviruses. StatPearls.

Waye, M.M.Y., and Sing, C.W. (2010). Anti-viral drugs for human adenoviruses. Pharmaceuticals *3*, 3343-3354.

Wold, W.S.M. (1993). Adenovirus genes that modulate the sensitivity of virus-infected cells to lysis by TNF. Journal of Cellular Biochemistry *53*, 329-335.

Wold, W.S.M., and Toth, K. (2013). Adenovirus vectors for gene therapy, vaccination and cancer gene therapy. Current Gene Therapy *13*, 421-433.

Yang, J., Mao, N., Zhang, C., Ren, B., Li, H., Li, N., Chen, J.*, et al.* (2019). Human adenovirus species C recombinant virus continuously circulated in China. Scientific Reports *9*, 9781.

Yu, J., Zhao, S., and Rao, H. (2020). Whole genomic analysis of a potential recombinant human adenovirus type 1 in Qinghai plateau, China. Virology Journal *17*, 111.

Zhang, Y., and Bergelson, J.M. (2005). Adenovirus Receptors. Journal of Virology *79*, 12125-12131.

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

| Submission | Summary of issues raised | Comment |
|---|---|---|
| 1 | Does not have a formal position on the use of GM technologies in the medical field. | Noted. |
| 2 | Does not have relevant expertise in this area to provide comment, but would appreciate being kept informed on this application. | Noted. |
| 3 | No specific comment on the proposal as the specialist health nature of this trial falls beyond the expertise and responsibilities of local government. Note the restrictions of the trial and that the conditions are designed to ensure there is no impact on the local community from trial or beyond. | Noted. |
| 4 | Agrees with the overall conclusions of the RARMP and that the risk assessment identifies all plausible risk scenarios. Did not identify any additional information that should be considered and agrees with the proposed exclusions and measures for limiting contact with at risk people. | Noted. |
| 5 | Agrees that the GM virus is unlikely to pose environmental risks due to the small and contained nature of the trial. While there is not likely to be environmental harm, it is recommended that in preparing the RARMP, additional discussion is included (detailed below) to support any risk assessment or conclusions regarding shedding, persistence, host range, and recombination potential between wild-type virus and the GM virus in the vaccinated individual. | Noted. |
| | **Shedding**<br>The RARMP would benefit from additional discussion of factors that are relevant to the risk assessment of shedding such as administration route, biodistribution, excreta routes, and persistence. | An additional reference regarding shedding data has been added. Chapter 1, Section 4.2.4 includes clinical data on shedding of the GMO in plasma. Post-administration controls to reduce the potential of the GMO to be released into the environment due to shedding are discussed in Chapter 2 Section 2.4.2 (Risk Scenario 2). |
| | **Persistence**<br>The RARMP should clarify that adenoviruses may persist in the environment for up to 8 weeks. | Paragraphs 193 and 195 have been reworded for clarification. |
| | **Host range**<br>The RARMP should clarify that adenoviruses other than human adenovirus 5 are found in animals and discuss potential exposure and replication in animal species. | More information has been added to Chapter 1, Section 3.4 and Chapter 2 Section 2.4.4 (Risk Scenario 4) regarding adenovirus infections in susceptible animals. |

| Submission | Summary of issues raised | Comment |
|---|---|---|
| | **Recombination**<br>The RARMP should include recent data on recombination of human adenovirus 5. | Background regarding HAdV-5 recombination has been added in Chapter 1, Section 3.3. |

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

| Submission | Summary of issues raised | Comment |
|---|---|---|
| 1 | God help the patients | Noted. |
| 2 | I would like to make it very clear that | |
| | 1) I recognise the importance of genetic engineering in science. | Noted |
| | 2) The utilisation of genetically engineered treatments taken with the knowledge of the patients of their mode of manufacturing I support. | Noted. |
| | 3) What I believe is that individuals should have the choice to select alternatives if they are available. | Participation in the clinical trial is voluntary and approval by a Human Research Ethics Committee is a requirement of the clinical trial. |
| | 4) That genetically engineered product content should be clearly labelled as such to enable such choice. | Containers containing the GMO are required to be labelled as such for the clinical trial. |
| | Congratulations on your potential new treatment | Noted. |