

**Risk Assessment and Risk Management Plan**  
for

**DIR 181** – Clinical trial of a genetically modified  
Herpes virus for the treatment of cystic  
fibrosis

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

---

PAGE INTENTIONALLY LEFT BLANK

# Summary of the Risk Assessment and Risk Management Plan

## for

### Licence Application No. DIR 181

#### ***Decision***

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

The applicant, Novotech (Australia) Pty Limited (Novotech) proposes to conduct a clinical trial of a genetically modified (GM) *Herpes simplex virus-1* (HSV-1) as a gene therapy treatment for adult patients with cystic fibrosis. The clinical trial is proposed to take place at hospitals within Australia over a period of up to three years. Up to 15 people with cystic fibrosis would receive one of three courses of treatment with the GMO, delivered by inhalation, with the aim of evaluating the safety and efficacy of the treatment.

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

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

## The application

<b>Project Title</b>	Clinical trial of a genetically modified Herpes virus for the treatment of cystic fibrosis <sup>1</sup>
<b>Parent organism</b>	<i>Herpes simplex virus-1 (HSV-1)</i>
<b>Genetic modifications</b>	The GMO: <ul style="list-style-type: none"> <li>• has been modified such that it cannot replicate; and</li> <li>• expresses two copies of the full length <i>human cystic fibrosis transmembrane conductance regulator (CFTR)</i> gene. These are intended to replace the dysfunctional CFTR gene in people with cystic fibrosis</li> </ul>
<b>Principal purpose</b>	The trial is an initial study of genetically modified (GM) HSV-1 expressing human cystic fibrosis transmembrane conductance regulator (CFTR), intended to assess safety and efficacy in a small group of people with cystic fibrosis.
<b>Previous clinical trials</b>	This is a first-in-human study of this GMO
<b>Limits and controls proposed by applicant</b>	
Proposed duration	3 years
Proposed release size	Up to 15 participants will be enrolled into the trial
Proposed location/s	Clinical trials will be conducted at hospitals within Australia. The number of sites and specific locations are yet to be determined.
Proposed controls	<ul style="list-style-type: none"> <li>• The GMO will be administered to trial participants in a hospital setting</li> <li>• The GMO will be administered in a closed room</li> <li>• Staff administering the GMO will wear personal protective equipment</li> <li>• Waste that may contain the GMO will be disposed of via the clinical waste stream</li> <li>• Persons with discernible oral Herpes lesions will be excluded from participating in the trial</li> <li>• Trial participants will be asked to implement hygiene measures intended to minimise exposure of caregivers and other close contacts</li> <li>• Trial participants will be monitored for signs of active HSV-1 infection and treated with oral antiviral medication if this occurs</li> </ul>

## Risk assessment

The risk assessment concludes that the proposed clinical trial poses negligible to moderate risks to human health and safety and negligible risks to the environment, but that these risks can be managed by imposing conditions on the conduct of the trial.

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

Credible pathways to potential harm that were considered include exposure of other people or animals to the GMO, expression of CFTR in transduced cells, and the potential for complementation

<sup>1</sup> The title of the application submitted by Novotech was 'KB407 for the treatment of Cystic Fibrosis'

by, and recombination with, wild-type HSV-1. Potential harms that were considered in relation to these pathways included discomfort or ill health due to inappropriate CFTR expression.

Important factors in reaching the conclusions of the risk assessment included that the GM HSV-1 is replication defective, but also that wild type HSV-1 is highly prevalent in the human population, the route of administration brings the GMO into contact with a common site of HSV-1 infection, and HSVs are known to recombine readily. As a consequence, replication competent recombinant virus carrying the CFTR transgene may be generated.

As risks to the health and safety of people have been assessed as negligible to moderate, and risks to the environment as negligible, the Regulator considers that the dealings involved in the proposed trial of the GM *HSV-1* can be managed so they do not pose a significant risk to people and 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, including the following. These measures are considered sufficient to manage the identified risks.

<b>Limits and controls in addition to those proposed by the applicant</b>	
Additional controls imposed by the licence	<ul style="list-style-type: none"> <li>• Trial participants must be seronegative for HSV-1</li> <li>• After treatment, trial participants must be tested weekly for primary HSV 1 infection by diagnostic laboratory testing and offered oral anti-viral medication if they acquire an infection</li> <li>• Clinical trial staff must be protected from aerosol exposure</li> <li>• A plan to limit exposure of people other than trial participants must be developed in consultation with each clinical trial site</li> <li>• Hygiene measures implemented by trial participants to minimise exposure of close contacts must be in place for 48 hours</li> </ul>

Since this is a clinical trial, the licence also includes limits on the number of trial participants, types of facility where the trial may be conducted, and on the duration of the trial. There are also several general conditions relating to ongoing licence holder suitability, auditing and monitoring, and reporting requirements which include an obligation to report any unintended effects.

## Table of contents

<b>Summary of the Risk Assessment and Risk Management Plan .....</b>	<b>I</b>
<b>The application .....</b>	<b>II</b>
<b>Risk assessment .....</b>	<b>II</b>
<b>Risk management plan .....</b>	<b>III</b>
<b>Table of contents .....</b>	<b>IV</b>
<b>Abbreviations.....</b>	<b>VI</b>
<b>Chapter 1 Risk assessment context.....</b>	<b>1</b>
Section 1 Background.....	1
1.1 Interface with other regulatory schemes .....	2
1.2 Cystic fibrosis.....	3
Section 2 The proposed dealings .....	4
2.1 The proposed limits of the trial (duration, location, scale, people) .....	4
2.2 The proposed controls to restrict the spread and persistence of the GMOs in the environment.....	4
2.3 Details of the proposed dealings .....	5
Section 3 Parent organism – <i>Herpes simplex virus-1</i> .....	10
3.1 Classification and genome characteristics .....	10
3.2 Cell tropism .....	11
3.3 Host range .....	11
3.4 Transmission .....	12
3.5 HSV-1 shedding .....	12
3.6 Recombination.....	13
3.7 Treatment of HSV-1 infection .....	14
3.8 Environmental stability .....	14
3.9 Risk group classification of HSV-1 .....	15
Section 4 The GMO – nature and effect of the genetic modifications .....	15
4.1 Modification of the HSV-1 backbone .....	15
4.2 The introduced gene – cystic fibrosis transmembrane conductance regulator .....	15
4.3 Transduction, functional activity and biodistribution of the GMO.....	16
4.4 Pre-clinical safety data .....	17
4.5 Human safety studies involving similar HSV-1-based viral vectors .....	17
Section 5 The receiving environment.....	18
5.1 Clinical trial participants.....	18
5.2 Clinical trial sites.....	19
5.3 Relevant environmental factors.....	20

5.4	Related viral species in the receiving environment.....	20
5.5	Presence of the introduced genes and encoded proteins in the environment.....	21
Section 6	Relevant Australian and international approvals.....	21
6.1	Australian approvals.....	21
6.2	International approvals.....	21
<b>Chapter 2</b>	<b>Risk assessment .....</b>	<b>22</b>
Section 1	Introduction.....	22
Section 2	Risk identification .....	23
2.1	Risk source .....	23
2.2	Causal pathway .....	23
2.3	Potential harm .....	24
2.4	Postulated risk scenarios.....	24
	Table 2. Summary of risk scenarios from dealings with GM HSV-1 .....	25
Section 3	Risk characterisation .....	30
3.1	Risk scenario 2.....	30
Section 4	Uncertainty.....	35
Section 5	Risk evaluation.....	36
<b>Chapter 3</b>	<b>Risk management plan .....</b>	<b>38</b>
Section 1	Background.....	38
Section 2	Risk treatment measures for substantive risks .....	38
Section 3	General risk management .....	39
3.1	Limits and controls on the clinical trial .....	39
3.2	Other risk management considerations .....	43
Section 4	Issues to be addressed for future releases.....	44
Section 5	Conclusions of the RARMP .....	44
	<b>References.....</b>	<b>45</b>
	<b>Appendix A: Summary of submissions from prescribed experts, agencies and authorities on the consultation RARMP.....</b>	<b>55</b>

## Abbreviations

AHSSQA	Australian Health Service Safety and Quality Accreditation
AICIS	Australian Industrial Chemical Introduction Scheme
APA	Approved Pathology Authority
APL	Accredited Pathology Laboratory
APP	Approved Pathology Practitioner
APVMA	Australian Pesticides and Veterinary Medicines Authority
CCI	Confidential Commercial Information
CDC	Centers for Disease Control and Prevention
CFTR	Cystic fibrosis transmembrane conductance regulator
CTA	Clinical Trial Approval
CTN	Clinical Trial Notification
DAWE	Department of Agriculture, Water and the Environment
DIR	Dealings involving Intentional Release
DNA	Deoxyribonucleic acid
FSANZ	Food Standards Australia New Zealand
GM	Genetically modified
GMO	Genetically modified organism
kb	kilobase
hCMV	Human cytomegalovirus
HREC	Human Research Ethics Committee
HSV	<i>Herpes simplex virus</i>
IATA	International Air Transport Association
ICP	Infected-cell polypeptide
IE	Immediate early
IBC	Institutional Biosafety Committee
ICH-GCP	<i>Guidelines for Good Clinical Practice</i> of the International Council for Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use
MCC	Mucociliary clearance
NHMRC	National Health and Medical Research Council
NPAAC	National Pathology Accreditation Advisory Council
NSQHS	National Safety and Quality Health Service
OGTR	Office of the Gene Technology Regulator
PCR	Polymerase chain reaction
PFU	Plaque-forming units
PPE	Personal protective equipment
qPCR	Quantitative polymerase chain reaction
RARMP	Risk Assessment and Risk Management Plan
Regulations	Gene Technology Regulations 2001
Regulator	Gene Technology Regulator



---

TGA	Therapeutic Goods Administration
the Act	<i>Gene Technology Act 2000</i>
TSD	Regulator’s Guidelines for the Transport, Storage and Disposal of GMOs
USA	United States of America
WHO	World Health Organization

# Chapter 1 Risk assessment context

## Section 1 Background

1. An application has been made under the *Gene Technology Act 2000* (the Act) for Dealings involving the Intentional Release (DIR) of genetically modified organisms (GMOs) into the Australian environment.
2. The Act and the Gene Technology Regulations 2001 (the Regulations), together with corresponding State and Territory legislation, comprise Australia’s national regulatory system for gene technology. Its objective is to protect the health and safety of people, and to protect the environment, by identifying risks posed by or as a result of gene technology, and by managing those risks through regulating certain dealings with GMOs.
3. Section 50 of the Act requires that the Gene Technology Regulator (the Regulator) must prepare a Risk Assessment and Risk Management Plan (RARMP) in response to an application for release of GMOs into the Australian environment. Sections 50, 50A and 51 of the Act and Sections 9 and 10 of the Regulations outline the matters which the Regulator must take into account and who must be consulted when preparing the RARMP.
4. The *Risk Analysis Framework* (OGTR, 2013) explains the Regulator’s approach to the preparation of RARMPs in accordance with the Act and the Regulations. The Regulator has also developed operational policies and guidelines that are relevant to DIR licences. These documents are available from the Office of the Gene Technology Regulator ([OGTR website](#)).
5. Figure 1 shows the information that is considered, within the regulatory framework, in establishing the risk assessment context. This information is specific for each application. Potential risks to the health and safety of people or the environment posed by the proposed release are assessed within this context. Chapter 1 provides the specific information for establishing the risk assessment context for this application.

<b>RISK ASSESSMENT CONTEXT</b>	
<p><b>The GMO</b> Modified genes Novel traits</p> <p><b>Parent organism (comparator)</b> Origin and taxonomy Cultivation and use Biology</p> <p><b>Receiving environment</b> Environmental conditions: abiotic and biotic factors Production practices Related organisms Similar genes and proteins</p>	<p><b>Proposed GMO dealings</b> Activities Limits Controls</p> <p><b>Previous releases</b> Australian approvals International approvals</p>

**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. Section 52 of the Act requires the Regulator to seek comment on the consultation RARMP from agencies - the Gene Technology Technical Advisory Committee (GTTAC), State and Territory Governments, Australian Government authorities or agencies prescribed in the Regulations, Australian local councils and the Minister for the Environment - and from the public. The advice from the

prescribed experts, agencies and authorities, and how it was taken into account, is summarised in Appendix A. No public submissions were received.

### 1.1 Interface with other regulatory schemes

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

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

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

10. The International Council for Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use – Guidelines for Good Clinical Practice (ICH-GCP) is an international ethical and scientific quality standard for designing, conducting, recording and reporting trials that involve the participation of human subjects (ICH, 2016). The guideline was developed with consideration of the current good clinical practices of the European Union, Japan, and the United States of America (USA), as well as those of Australia, Canada, the Nordic countries and the World Health Organization (WHO). The TGA has adopted the *Integrated addendum to ICH E6(R1): Guideline for good clinical practice E6(R2) (Therapeutic Goods Administration)*, which provides overarching guidance for conducting clinical trials in Australia which fall under TGA regulation.

11. The National Health and Medical Research Council (NHMRC) has issued the National Statement on Ethical Conduct in Human Research (National Health and Medical Research Council et al., 2018). This document sets the Australian standard against which all research involving humans is reviewed. The Therapeutic Goods Act 1989 requires that the use of a therapeutic good in a clinical trial must be in accordance with the ethical standards set out in this document.

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

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

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

15. The National Pathology Accreditation Advisory Council (NPAAC) advises Commonwealth, State and Territory health ministers on matters relating to the accreditation of pathology laboratories. NPAAC plays a key role in ensuring the quality of Australian pathology services and is responsible for the development and maintenance of standards and guidelines for pathology practices. The standards include safety precautions to protect the safety of workers from exposure to infectious microorganisms in pathology laboratories. While compliance with NPAAC standards and guidelines is not mandatory, there is a strong motivation for pathology services to comply, as Medicare benefits are only payable for pathology services if conducted in an appropriate Accredited Pathology Laboratory (APL) category, by an Approved Pathology Practitioner (APP) employed by an Approved Pathology Authority (APA). Accreditation of pathology services is overseen by Services Australia (formerly Department of Human Services), and currently, the only endorsed assessing body for pathology accreditation is the National Association of Testing Authorities (NATA).

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

## 1.2 Cystic fibrosis

17. Cystic fibrosis is a life-limiting genetic disorder with 70 000-100 000 people affected globally (Cystic Fibrosis Worldwide). It is most common in people of northern European descent, for whom it is the most common lethal inherited disease, and accordingly is prevalent in Europe, North America and Australia (Elborn, 2016). In 2017, the Australian Cystic Fibrosis Data Registry recorded a total of 3151 cystic fibrosis patients, corresponding to 0.012% of the Australian population (Ruseckaite et al., 2019).

18. Cystic fibrosis develops when inactivating mutations occur in both copies of the gene encoding a chloride-conducting transmembrane channel called the cystic fibrosis transmembrane conductance regulator (CFTR). CFTR regulates anion transport across epithelial surfaces and affects the respiratory, digestive, circulatory and reproductive systems. Defects in ion-water transport across epithelia produce an abnormally thick sticky mucus that restricts organ function. The main clinical symptoms are elevated sweat chloride concentration, impaired pancreatic secretion of digestive enzymes, male infertility and progressive obstructive lung disease, with the last being the major cause of complications and death. Mucus plaques and plugs in the airways obstruct airflow and are foci for inflammation and infection. Narrower airways (bronchioles) progressively fill with mucus and become irreversibly damaged by ensuing inflammation. Once a part of the airway becomes non-functional, air cannot pass through it; it and its descendant bronchioles are no longer available for gas exchange. People with cystic fibrosis eventually face severe breathing problems with episodes of worsened

respiratory symptoms including shortness of breath, increased cough, and sputum production. Progressive lung disease ultimately causes irreversible damage and death due to respiratory failure (Elborn, 2016; Flume et al., 2009; Turcios, 2020; Zarei et al., 2012).

19. Development and implementation of various symptomatic treatments since the 1970s have greatly improved survival beyond childhood, with the life expectancy of newborns with cystic fibrosis now over 50 years (Brodie et al., 2015). In Australia, 53.7% of cystic fibrosis patients are adult, with the majority aged under 35. Fifty percent of adult patients for whom marital status was reported are in a formal or informal marriage relationship (Ruseckaite et al., 2019).

## Section 2 The proposed dealings

20. Novotech (Australia) Pty Limited (Novotech) has proposed a Phase 1 clinical trial of a GM *Herpes simplex virus-1* (KB407), modified such that it cannot replicate in host cells. The GMO expresses two functional copies of the CFTR gene, which is defective in people with cystic fibrosis (see Section 1.2). The primary purpose of the trial is to assess the safety and tolerability of the GMO, with assessment of efficacy a secondary objective.

21. The GMO will be manufactured in the USA and imported into Australia. It will be administered to adult cystic fibrosis patients of any gender who meet specific disease-related criteria, and delivered directly to lung tissue by inhalation. Samples that may contain the GMO will be collected from trial participants for analysis in laboratories within Australia or exported overseas.

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

- (a) import the GMO;
- (b) conduct the following experiments with the GMO:
  - i. administer the GMO to clinical trial participants by inhalation;
  - ii. collect biological samples from trial participants;
  - iii. analyse the samples described in 22(b)ii;
- (c) transport the GMO;
- (d) dispose of the GMO;

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

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

23. A timeframe of three years is requested.
24. The clinical trial would take place at hospitals within Australia.
25. Up to 15 trial participants with cystic fibrosis would receive the GMO.
26. 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 in the environment

27. 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 that:

- the GMO will be administered to trial participants in a hospital setting;
- the GMO will be administered to participants in a closed room;
- staff administering the GMO will wear personal protective equipment;
- waste that may contain the GMO will be disposed of via the clinical waste stream;

- persons with discernible oral Herpes lesions will be excluded from participating in the trial;
- trial participants will be asked to implement hygiene measures intended to minimise exposure of caregivers and other close contacts; and
- trial participants will be monitored for signs of active HSV-1 infection and treated with oral antiviral medication if this occurs.

## 2.3 Details of the proposed dealings

### 2.3.1 Overview of the clinical trial

28. The international sponsor for the trial (the Sponsor) is Krystal Biotech, Inc, located in the USA. Novotech is applying for authorisation to conduct the proposed clinical trial in Australia and will be referred to as the Applicant throughout this document. If the licence is approved, Novotech will be the Contract Research Organisation responsible for managing the trial and ensuring that licence conditions are met.

29. The trial is a Phase 1 open-label dose-escalation study that will evaluate the safety and efficacy of KB407 when administered via three dosing regimens. Three successive cohorts will receive one of three different courses of treatment with the GMO delivered directly to the lungs by inhalation. All cohorts will receive a dose on Day 0, with subsequent dosing specific to the treatment group. The clinical dose delivered on each occasion will be the same and is still to be determined based on pre-clinical toxicology studies.

30. All participants will return to the clinical trial site for a number of follow up visits, with further monitoring for an additional year.

31. Site visit procedures will include a review of adverse events, a physical examination, and collection of blood samples for safety and efficacy testing. Samples will also be collected for assessment of viral shedding, defined as excretion/secretion of viral particles that could be transmitted to other individuals. **Selection and management of trial participants**

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

- be of any gender aged at least 18 years;
- have a confirmed cystic fibrosis diagnosis; and
- be willing to attend all study visits and complete all procedures required by the protocol.

33. Relevant exclusion criteria include:

- an active oral herpes infection at either the Screening or Study Day 0 visit; and
- the subject is known to be noncompliant or is unlikely to comply with the requirements of the study protocol, in the opinion of the Investigator.

34. Although the presence of an active oral herpes infection is an exclusion criteria only at Screening and Day 0, the Applicant has stated that all participants treated with the GMO will be monitored for development of herpes-like lesions, indicative of an active HSV-1 infection. Should these appear, the participant will be treated with an oral antiviral.

### 2.3.3 Manufacture and import of the GMO

35. The GMO will be manufactured in the USA in accordance with Good Manufacturing Practice (GMP) guidelines and packaged into breakage-resistant pharmaceutical vials sealed with a flexible stopper. Each vial will contain the GMO supplied as a frozen liquid. The concentration has not been specified but will be stated on each vial.

36. Imported shipments will be received at a third party storage facility offering logistical services and distribution of clinical supplies. Product from this facility will be transported to clinical trial sites at a later time.

### 2.3.4 Transport of the GMO

37. Transport during import will be contracted to a specialised courier company such as World Courier. The GMO will be packed to meet the requirements of International Air Transport Association (IATA) shipping classification UN 3245 (GMOs that are not classified as category A or B infectious substances). This classification applies to GMOs not meeting the definition of ‘infectious substance’ (i.e. substances known or reasonably expected to contain microorganisms that can cause disease in humans or animals). For international transport, packing and labelling must be in accordance with IATA packing instruction 959<sup>2</sup>.

38. The Applicant stated that individual product vials (primary container) will be packed in water-tight cryo-boxes (secondary container) containing absorbent material. Cryo-boxes will be further sealed in a CO<sub>2</sub>-protective bag, then packed in dry ice in an IATA-approved shipping carton. The outer carton will be labelled to indicate that the package contains GMOs, with contact details of the licence holder and instructions for what to do in the event of a spill. This meets the packaging requirements stipulated in the Regulator’s *Guidelines for the Transport, Storage and Disposal of GMOs* for transport of PC2 GM microorganisms.

39. Other required transport includes.

- Transport from the storage facility to clinical trial sites, which will again be undertaken by a specialised courier company experienced in the transport of GMOs. The Applicant anticipates that the number of GMO vials needed by the clinical site will be removed from storage and repacked in accordance with IATA UN 3245. The outer container will be labelled with:
  - the address of the receiving clinical trial site;
  - a statement that the shipment contains GMOs;
  - the DIR licence number and contact details of the licence holder; and
  - instructions on what to do in the event of a spill.
- Transport within the clinical trial site, from the storage location to the treatment area for administration to trial participants. The Applicant will assess each site’s capacity for compliance with the Regulator’s *Guidelines for the Transport, Storage and Disposal of GMOs* (TSDs) as part of the site selection process.
- Waste containing the GMO will be placed in clinical waste bins and transported from the clinical trial site to the site of destruction by an external service provider (see Section 2.3.10).
- Samples collected from participants will be transported to analytical facilities within the clinical trial site, and potentially to third party analytical facilities within Australia or exported overseas. All samples will be treated as though they contain the GMO. They will be packaged for transport in two levels of containment: a suitable specimen container (e.g. a vacutainer for blood) and a sealed, unbreakable, leak-proof outer container. The outer container will be labelled to indicate it contains GMO, with the DIR licence number and contact details of a relevant clinical study staff member.

<sup>2</sup> For liquid substances, this specifies:

- a leak proof primary container, a secondary packaging and a rigid outer packaging;
- sufficient absorbent material placed between the primary container and secondary packaging to absorb the entire contents of the primary containers;
- that multiple fragile primary receptacles placed within a single secondary package are individually wrapped or separated so as to prevent contact between them;
- that dry ice must be placed outside the secondary packaging;
- a rigid outer packaging that is strong enough for its capacity, weight and intended use; and
- labelling must include the name and address of the shipper and consignee.

### **2.3.5 Storage of the GMO**

40. Once received at the third party storage facility, staff will inspect the package for damage. Vials may be stored in the original secondary packaging or repackaged into comparable containers. Individual vials may also be relabelled to comply with Australian regulatory requirements. Secondary containers will be stored in a freezer whose access is restricted to appropriate staff, and placed on a shelf or in a box dedicated to the trial. The freezer and/or boxes will be labelled as containing biohazardous material.

41. Once received at a clinical trial site, vials will be unpacked and inspected prior to storage in a freezer that is either lockable or has access restricted in some other way. As clinical sites have not been selected, details of storage location, labelling and security are not available for assessment against TSD requirements. However, the Applicant will assess the site's capacity for compliance with the Regulator's guidelines as part of the site selection process (see paragraph 132).

### **2.3.6 Preparation and administration of the GMO, and post-administration activities**

42. The GMO will not need dilution or other preparation, and vials as supplied by the Sponsor will be transported to the clinical area for dosing. A delegated staff member will remove the required number of vials from the freezer and allow them to thaw and equilibrate to room temperature. This step will take a maximum of one hour.

43. The GMO will be administered by inhalation using a nebuliser. As the GMO may be released during the procedure, this will take place in a closed room. The Applicant has not proposed any requirements as to air exchange rate but stated that the treatment room should contain no unnecessary equipment or furniture so as to limit surfaces on which aerosolised GMO might settle or accumulate. Only the administrator and trial participant will be in the room during the procedure. Both will wear PPE as described in Section 2.3.8. Participants will be assigned their own nebuliser. The Applicant does not anticipate requiring that the room be dedicated to this clinical trial for the duration of the dosing period, thus it may be used for other purposes and by other hospital staff and/or patients when trial participants are not being treated.

44. The GMO solution will be converted to a fine mist using a nebuliser. GMO solution will be loaded into a covered medication chamber and the aerosol delivered via a simple mouth piece. Mist production is constant while the device is switched on, however can be easily switched on and off as needed. The instruction manual indicates that during use, some aerosol is emitted from the back of the mouthpiece assembly. The device does not capture or filter breath exhaled from the mouth, and participants will also be able to exhale through their nose.

45. The GMO will be administered by a delegated administrator, for example, the Primary Investigator, Sub-Investigator, a respiratory therapist or nurse. Immediately before dosing, the administrator will use a needle and syringe fitted with a safety lock to transfer GMO solution from sealed vials into the medication chamber of the nebuliser. A fresh needle/syringe will be used for each transfer, after which they will engage the safety lock and dispose of both needle/syringe and vial into a sharps container. Instructions for this procedure and for safe handling of the GMO will be provided in the pharmacy manual, which is not yet available.

46. For inhalation of the GMO, the instruction manual directs users to place the mouthpiece between their teeth, sealing their lips around it, then breathe normally through their mouth. Participants will switch on aerosol production only once the mouthpiece is in place, and dosing will take up to 30 minutes. Participants are expected to keep the mouthpiece in place for the full period, but should they need a break, can switch off the device until ready to resume.

47. After inhaling the GMO, both participant and administrator will remain in the room for a further 60 minutes, during which several non-invasive tests will be completed. Testing will require the participant to exhale forcefully into a testing device; the Applicant has stated that all device parts that go into the mouth will be disposed of into a biohazard container after each use. It is not known



whether clinical sites will take additional measures such as using disposable in-line bacterial/viral filters to protect tubing and the device interior from airborne contamination.

48. Before leaving the room, all work surfaces and equipment potentially exposed to the GMO (e.g. the nebuliser and other testing devices) are to be cleaned using a disinfectant effective against the GMO, such as sodium hypochlorite. Both administrator and participant will remove their PPE when leaving the room and place it in biohazard containers located adjacent to the room exit.

### **2.3.7 Sample collection and analysis**

49. Samples will be collected from trial participants subsequent to each treatment with the GMO. Samples will be collected at the clinical trial site, with staff such as phlebotomists, nurses and doctors carrying out the procedures. Staff with sufficient training to conduct routine blood draws will carry out blood collection procedures requiring the use of sharps. Samples may be analysed in Australia or overseas.

50. All types of sample will be used to assess shedding of the GMO and will be treated as though the GMO is present. Samples will be labelled to indicate they contain a GMO, and those requiring transport from the clinical trial site will be shipped by a specialised courier as described in Section 2.3.4. Within the clinical trial site and analytical facilities, the Applicant proposes to use standard practices appropriate for handling of infectious materials.

### **2.3.8 Personal protective equipment and other precautions**

51. No special handling procedures have been proposed for handling the sealed vials of GMO at the storage facility or clinical trial sites. The Applicant stated that standard clinical/laboratory best practices will be observed, i.e. wearing gloves or a lab coat or other removable garment that covers the arms.

52. The person delegated to administer the GMO will wear PPE that includes a face shield, mask (e.g. N95) covering the mouth and nose, disposable full-length isolation gown, gloves and shoe covers. The Applicant indicated that gowns will be made from an impermeable material. Trial participants will also wear a full length gown, gloves and shoe covers during the nebulisation procedure.

53. The enrolment criteria exclude prospective participants with an active oral herpes infection at screening or Study Day 0. Participants accepted into the trial will undergo a physical examination during each visit to the clinical trial site and any open wounds or areas of compromised skin will be covered with PPE before the GMO is administered.

54. The person administering the GMO will not be required to protect any compromised skin not covered by PPE. They are also not subject to restrictions based on discernibly active oral herpes infections.

### **2.3.9 Behavioural requirements**

55. Participants will be asked to minimise exposure of caregivers or other close contacts for at least 24 hours after treatment. They will be asked to wear a standard surgical mask, avoid direct or indirect oral contact with others e.g. kissing, sharing utensils, cups and plates, and to avoid coughing or sneezing in close proximity to others. They will also be asked to implement standard hygiene practices including thoroughly hand washing after coughing, sneezing or touching nasal secretions, and avoiding touching their eyes or mouth until this is done. These expectations will be clearly explained at the time of consent and before discharge following each administration of the GMO, and supported by written materials to remind participants of the requirements.

56. For 24 hours after the procedure, trial participants will also be asked to collect their nasal secretions and place contaminated tissues in a biohazard waste container, to be supplied by the clinical site. These are to be returned to the site for disposal as biohazardous waste at their next visit.

57. Trial participants will be informed that they are receiving a GM therapy and that they should not donate blood, tissues or organs for 90 days post-treatment.

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

58. Any waste or disposable items that come into contact with the GMO will be disposed of as infectious clinical waste. Clinical waste disposal will be managed according to clinical site procedures and contracted to specialised waste disposal companies, with destruction by high-temperature incineration. The Applicant has proposed *not* to label the waste bins as containing GMOs on the basis that the clinical waste disposal pathway, which is generally accepted as suitable for infectious human pathogens, is also sufficient for the GMO.

59. Work surfaces and equipment in the treatment room will be decontaminated using a disinfectant effective against the GMO. The Applicant has specified sodium hypochlorite as suitable.

60. Nebulisers will be decontaminated after each use and destroyed at the end of the trial.

61. Any unused vials of GMO will also be destroyed at the end of the trial, following institutional guidelines for infectious materials. Disposal or destruction will be documented.

**2.3.11 Relevant training and experience of clinical trial personnel**

62. As described in Section 2.3.6, persons administering the GMO will be required to use sharps while loading the nebuliser with GMO solution. Sharps will also be used to collect blood samples during return visits to the clinical site (see Section 2.3.1). The Applicant has stated that, as the trial will be conducted in hospitals, all staff who will use sharps when handling the GMO will be clinically trained and very experienced in safe needle handling techniques relevant to preparing and administering medicines, and collecting patient samples.

**2.3.12 Contingency plans**

63. In the event of a spill, the Applicant proposes that institutional cleaning procedures be followed.

64. In case of unintended exposure of people, such as those administering the GMO, the applicant has proposed that:

- affected eyes or mucous membranes should be flushed with copious amounts of clean water for at least 15 minutes;
- in the event of exposure to broken skin or a needle stick, the site should be thoroughly cleaned with soap and water or an appropriate skin disinfectant.

The occupational health physician of the hospital should also be contacted and will monitor for signs of infection. Acyclovir or other similar antiviral drugs may be administered to anyone exposed to the GMO to minimise the extent or duration of possible infection.

65. The Applicant stated that reporting on incidents and accidents to regional or national authorities must be performed according to relevant regulations.

**2.3.13 Informing persons covered by the licence about licence conditions**

66. The Applicant proposes to inform the various classes of person who will conduct dealings of licence conditions applicable to them as follows.

- Staff directly involved in handling the GMO during preparation and administration to participants will be trained in licence conditions during the site initiation visit (SIV). Any staff joining the trial at a later date will be trained by staff who attended the SIV. All training will be documented in training logs kept at the clinical site.
- Laboratory staff in pathology laboratories will be informed of licence conditions via a letter sent to the laboratory manager. Conditions relevant to laboratory staff will be outlined in the letter and a copy of the licence conditions included with this communication.
- External service providers involved in transporting the GMO, other than those transporting for the purposes of disposal, will be informed that they are doing so via labelling on the outermost container. In addition, a copy of the licence will be included in the shipping documentation.

67. The Applicant does not propose to inform contractors collecting pathological waste of licence conditions, stating that stringent measures appropriate for managing infectious agents in these contexts are suitable for the GMO.

#### **2.3.14 Accountability and monitoring**

68. All documentation related to GMO receipt, authorisation for use, dispensing and destruction will be filed at each clinical site and made available for inspection by the licence holder and Sponsor, who will monitor each site to ensure compliance with ICH GCP requirements and DIR licence conditions.

69. The Applicant stated that all required reporting to the Regulator will occur within the timeframes stipulated in the licence or, if timeframes are not specified, as soon as the licence holder becomes aware of the reportable event.

### **Section 3 Parent organism – *Herpes simplex virus-1***

70. HSV-1 is a widespread human pathogen with approximately 76% seroprevalence in Australian adults (Cunningham et al., 2006). HSV-1 causes direct cell death through cytolysis which stimulates an inflammatory response. HSV-1 initially infects epithelia and then spreads to sensory neurons innervating the site. The virus is transported along nerve axons to the neural ganglia where it may establish a latent infection and persist until such time as it receives a reactivation stimulus. Once reactivated, HSV-1 particles are transported back to peripheral sites where lytic replication and viral shedding ensues (Luker et al., 2002; Shivkumar et al., 2016; Watson et al., 2012).

71. Oral herpes infection is mostly asymptomatic, and most people with HSV-1 are unaware they are infected (World Health Organisation, 2020). Where symptomatic, common signs of primary infection include painful inflammation of the gums and lips, sore throat and tonsillitis. Intermittent reactivations may be clinical, causing typical herpetic oral-facial lesions (cold sores), corneal ulcerations and rare, but life-threatening encephalitis. More frequently, reactivation is subclinical, causing asymptomatic viral shedding (Glezen et al., 1975; Mark et al., 2008; Ribes et al., 2001). HSV-1 is also responsible for a growing proportion of genital herpes infections, which again may be asymptomatic or cause painful ulcerative lesions.

#### **3.1 Classification and genome characteristics**

72. Herpes simplex viruses are member of the *Simplexvirus* genus of the family *Herpesviridae*, subfamily *Alphaherpesvirinae*. There are two types of Herpes simplex virus: *herpes simplex virus 1* (HSV-1) and *herpes simplex virus 2* (HSV-2), which share extensive genome and protein homology.

73. HSV-1 is an enveloped virus with a linear double stranded DNA genome approximately 152 kilobases (kb) in length, and containing approximately 84 unique protein coding genes and 94 putative open reading frames (Rajcáni and Durmanová, 2000). These genes encode the majority of the proteins of the mature virion (virus particle), including those involved in forming the capsid, viral matrix and envelope, as well as proteins controlling viral replication and infectivity. The HSV-1 genome is non-integrating, meaning it does not integrate into host cell chromosomes.

74. The HSV-1 genome consists of two unique regions – unique long (U<sub>L</sub>) and unique short (U<sub>S</sub>), and two distinct inverted repeat elements which flank the unique segments. During productive (lytic) infection, viral genes are expressed in three temporal phases: *immediate-early* (IE), *early* and *late*. IE gene expression is activated by a protein component of the virus particle. IE gene products then regulate expression of both early and late genes, which encode proteins involved in DNA replication and assembly of the virus particle (Lim et al., 2013). HSV-1 genes are also categorised as essential or non-essential for replication in tissue culture. Of the five IE genes, Infected Cell Polypeptide (ICP) 4 and ICP27 are essential for entry in the lytic replication cycle.

75. Details about the HSV-1 strain that the GMO is based on are under consideration to be declared as Confidential Commercial Information (CCI) under Section 185 of the Act. This information was made

available to the prescribed experts and agencies consulted on this application. CCI is not available to the public.

### 3.2 Cell tropism

76. HSV-1 is well known to infect epithelial and neuronal cells, and use of HSV-based oncolytic viruses to treat many types of cancer suggests that a wide range of epithelial lineages are susceptible. Productive infection of T cells and human gingival fibroblasts has been demonstrated *in vitro* (Zuo et al., 2019), as has infection of type I alveolar epithelial cells and certain myeloid cell populations when virus was delivered intranasally to mice (Lawler et al., 2015; Shivkumar et al., 2016). It is unclear from the scientific literature whether HSV-1 has tropism for other cell types, both in terms of the ability to enter cells and then to undergo lytic infection.

### 3.3 Host range

77. Herpesviruses have co-evolved with their hosts and show high species-specificity (Epstein and Price, 2009; Stalder et al., 2015; Tischer and Osterrieder, 2010). They usually do not cause serious infection in healthy members of the natural host species. While most cross-species infections are likely abortive, some herpesviruses produce serious or lethal infections when transmitted to a non-natural host species (Eberle and Jones-Engel, 2017). In the case of HSV-1, humans are the natural reservoir and natural infection of other animals is rare, although has been reported in some non-human primates (NHPs), rabbits, several rodent species and pygmy hedgehogs. Such ‘zooanthroponotic’ infections most often affect captive or domesticated animals with close human contact (Epstein and Price, 2009).

78. HSV-1 infection of marmosets and other New World monkeys commonly results in serious illness and death, with deaths of both captive and wild marmosets reported (Casagrande et al., 2014; Epstein and Price, 2009; Imura et al., 2014) (Longa et al., 2011; Mätz-Rensing et al., 2003). Fatal infections have also been reported in the European rabbit (de Matos et al., 2014; Grest et al., 2002; Janovitz et al., 1998; Muller et al., 2009; Weissenbock et al., 1997), chinchilla (Wohlsein et al., 2002) and in European and African pygmy hedgehogs (Allison et al. 2002; Riley & Chomel 2005). In contrast, sequelae following natural infection of Old World monkeys such as gorillas, chimpanzees, macaques bonobos and gibbons appear similar to human disease; infection remains localised in mucocutaneous tissues and does not usually become systemic (Aravantinou et al., 2017; Fan et al., 2017; Mätz-Rensing et al., 2003).

79. Case reports suggest that transmission to animals requires close contact with a human who has an active HSV-1 infection, including via contaminated food or physical objects (fomites). A captive marmoset colony had contact with a caretaker exhibiting a herpes labialis-like illness a few days before the outbreak occurred, and in other cases of captive NHPs, visitors, caretakers or students may have caused infection by passing partly eaten food into the enclosure (Mätz-Rensing et al., 2003). A group of wild marmosets were regularly fed by human residents of an apartment complex (Longa et al., 2011). Three case reports involving pet rabbits noted in one that the person with closest contact was a child frequently affected with herpes labialis (Weissenbock et al., 1997), in another that the owner had an episode of cold sores before the rabbit became sick (de Matos et al., 2014), and in the third that the owner had a severe labial and facial herpesvirus infection five days before the rabbit became sick and reported ‘intensive’ nose-to-nose and mouth-to-nose contact with the rabbit (Muller et al., 2009). Given the high prevalence of HSV-1 in the human population and its pathogenicity in these species, the small number of case reports suggest that human to animal transmission may be infrequent or does not always cause disease.

80. Rabbits and mice can also be infected experimentally (Public Health Agency of Canada, 2011) and are used as animal models of HSV-1 infection (Luker et al., 2002; Luker et al., 2006). Reliable infection with HSV strains of varying virulence is induced by invasive techniques such as corneal scarification, and intraocular or intracerebral inoculation (Webre et al., 2012; Weissenbock et al., 1997).

81. A literature search conducted to establish the likelihood of HSV-1 infection in other species interacting with humans found no evidence of naturally occurring HSV-1 infection in dogs, cats, horses, cows or other common domesticated animals.

82. At least ten herpesvirus species commonly infect a range of Australian native marsupials, including kangaroos, wallabies, wombats, koalas and Tasmanian Devils, with prevalence rates in free-living animals between 25-45%. Associated disease outbreaks and mortality have predominately been reported in captive animals. This is presumed due to the close proximity in which these animals are housed, allowing for increased rates of transmission, transmission to species other than the natural host, and higher rates of stress (Stalder et al., 2015). No reports were located regarding HSV-1 infection in native Australian animals.

### 3.4 Transmission

83. HSV-1 is highly contagious. Primary infection is generally considered to be via an oral route (Bello-Morales et al., 2020; World Health Organisation); (World Health Organisation, 2020), however it has been argued that an intranasal route is also possible. In a mouse model, intranasal infection was efficient and targeted the olfactory neuroepithelium, progressing to the trigeminal ganglia (Shivkumar et al., 2013).

84. Transmission, including autoinoculation, can occur through direct contact with lesions, or via infected secretions, particularly saliva. Fomite transmission of HSV-1 is well documented: people often put their hands to their mouth and then often touch objects which are touched by others, for example, taps, door handles and coins, which have been demonstrated to support viable HSV-1 for at least two hours (Bardell, 1990, 1993, 1994).

85. Inoculation of virus at susceptible sites such as the oropharynx, eyes, genital mucosa or small cracks in skin is required for infection (Ribes et al., 2001; Shivkumar et al., 2013). The cornea may be infected via droplet transmission or direct inoculation (Agelidis and Shukla, 2015; Ahmad and Patel, 2020; Public Health Agency of Canada, 2011) (Public Health Agency of Canada, 2011). Infection can also occur via non-physiological routes, such as intra-tumoural or intravenous delivery of HSV-based oncolytic viruses to tumours.

86. Aerosols are not known as a primary transmission pathway for HSVs. However, their occupational relevance is noted in the dental context where routine use of high-speed handpieces and ultrasonic scalers on patients with active infection may generate an aerosolised saliva/herpes mix. Recommended risk management strategies to minimise occupational exposure include use of PPE to protect from aerosols, avoiding elective treatment of patients with symptomatic infection and providing anti-viral medication prior to dental treatment (Browning and McCarthy, 2012; Kelsch, 2013; Lewis, 2004).

### 3.5 HSV-1 shedding

87. HSV-1 is shed during active infections – both primary and after reactivation, but not during viral latency. Both symptomatic and asymptomatic infection lead to shedding and consequent transmission. In the context of the closely related HSV-2, transmission during short asymptomatic shedding episodes is more common than from contact with symptomatic lesions (Mertz, 2008), likely due to their frequency and lack of precautions taken by the infected person who is unaware they are shedding.

88. Many studies have examined the frequency and duration of asymptomatic HSV-1 shedding in the oral cavity (Da Silva et al., 2005; Gilbert, 2006; Kaufman et al., 2005; Liljeqvist et al., 2009; Mark et al., 2008; Miller and Danaher, 2008; Ramchandani et al., 2016; Ramchandani et al., 2019; van Velzen et al., 2013). In the most thorough study reported, 18 HSV-1 seropositive immunocompetent adults collected oral swabs at six hourly intervals for sixty days (Mark et al., 2008). HSV-1 reactivation was both frequent and rapidly cleared:

- 83% of subjects produced at least one HSV-1 positive sample over the study period, with a median of 3 reactivation episodes per person (range 1-4 reactivations). In contrast, only one person experienced a symptomatic herpes lesion;
- HSV-1 shedding from any participant was detected on 12% of the days assessed;
- shedding duration ranged from 4 hours to 12 days (median 24 hours), with 39% of episodes lasting 12 hours or less and 46% lasting over 24 hours.

89. A later study of eight seropositive adults with a history of cold sores reported more frequent HSV-1 shedding over a five week period, with DNA detected on 26.5% of the days examined. Again, most episodes were asymptomatic, with asymptomatic shedding detected on 27.1% of days where no herpes lesions were present. The oral area was the most common site of HSV-1 reactivation and shedding, with viral DNA collected from sites throughout the oral cavity (e.g. lips, tongue, palate and throat). Few shedding episodes were associated with the nose and eyes (Ramchandani et al., 2016).

90. Reports of viral reactivation and shedding in the eyes and nose are mixed. HSV-1 DNA has been detected in human corneas at rates from 0-36% (Hill and Clement, 2009). Three studies using relatively insensitive culture techniques to detect virus particles found no shedding from the eye (Kaye et al., 1990), or a low incidence of shedding (one positive sample each in four of eleven people sampled daily over 20 days (Kaufman et al., 1967) and one occurrence in a six month study of ten people (Okinaga, 2000)). In the small study described above, more sensitive PCR detection of viral DNA still found few shedding episodes associated with the eyes (two of eight subjects; 0.85% of swabs) and nostrils (six of eight subjects, 3% of swabs) (Ramchandani et al., 2016). In contrast, Kaufman et al. sampled 50 subjects twice daily and found that 46 people (92%) excreted HSV DNA in tears at least once during the 30 day study period, with 33.5% of tear samples positive. Shedding was intermittent, with the number of episodes ranging from 0-11 per person, and DNA copy numbers from 50-75 (34%) to >120,000 (21%) per tear sample (Kaufman et al., 2005). The sensitivity of detection may have contributed to the higher shedding rate observed in this study.

91. Studies of HSV-2 infection indicate that reactivation episodes are resolved by tissue resident T cells – a T cell subset which persists within solid tissues and provides immune surveillance at sites of prior infection (Roychoudhury et al., 2020; Schiffer and Corey, 2013; Schiffer et al., 2013; Schiffer et al., 2018).

### 3.6 Recombination

92. HSV-1 is considered highly recombinogenic, with genome analyses suggesting that most wild-type HSV-1 strains have recombinant mosaic genomes. Recombination is linked mechanistically to HSV's mode of genome replication and requires that cells be infected by multiple (different) virions (Law et al., 2018; Lee et al., 2015). A number of studies have shown that humans can harbour natural infections by more than one HSV-1 strain (Bowen et al., 2016; Bower et al., 1999; Greninger et al., 2018; Lewis et al., 1984; Liljeqvist et al., 2009; Umene et al., 2007), and multiple strains have been detected in the same latently infected ganglia (references in (Tenser and Edris, 1987; van Velzen et al., 2013)) – both being prerequisites for same cell co-infection.

93. Recombination has been shown experimentally by co-infecting cultured cell lines or mouse ocular tissues (*in vivo*) with two distinct HSV-1 strains at a 1:1 ratio and examining viral progeny. Co-infection experiments with genetically tagged HSV-1 genomes showed extensive recombination, apparent from 6 hours post infection *in vitro*. Consistent with the need for both parent genomes to infect the same cell, increasing concentrations of input virus produced more recombinant progeny, up to a saturating level (~30%<sup>3</sup>) likely reflecting a limit on the cells' capacity to support viral replication.

---

<sup>3</sup> Recombinant phenotypes reported in this study underestimate total recombination of the two parent genomes, which each incorporated one of two fluorescent reporter genes at different loci. Progeny genomes containing both or neither reporter gene were counted as recombinants. Recombinants in which the reporter genes did not participate in the recombination event(s), or those in which a double cross-over event switched

The increase was logarithmic, with recombination at the lowest multiplicity of infection<sup>4</sup> tested (0.1) being relatively high (~7.6% of progeny virions). The distribution of recombinants was maintained across three sequential rounds of infection and replication, without selection pressure and with no single phenotype overtaking the population. Recombination occurred in both epithelial cells and neurons (Law et al., 2018).

94. The same study found that recombination occurred to a similar extent in culture and in the mouse eye, but increased as the infection spread via nerve axons into the brain, with recombinants exceeding 50% of the virus population in multiple animals (Law et al., 2018). Kintner *et al* also observed an increasing percentage of recombinants as a mixture of two HSV-1 strains progressed from the mouse cornea (59.1%) to the trigeminal ganglia (74.1%) (Kintner et al., 1995).

95. Recombination break points were distributed throughout the genome, favoured GC-rich regions, and showed a very strong bias towards the inverted repeat regions and a less strong but pronounced bias towards intergenic regions and against coding regions (Lee et al., 2015).

96. Two examples, one experimental and one occurring in the field, demonstrate that *in vivo* recombination can enhance virulence in attenuated herpesvirus strains. Two weakly neuroinvasive HSV-1 strains generated neuroinvasive recombinants when mice were inoculated with a 1:1 mixture (Sedarati et al., 1988). Recombination of two live attenuated alphaherpesvirus vaccines for Marek's Disease used by the Australian poultry industry also restored virulence, generating two novel infectious strains with mortality rates up to 18% (Lee et al., 2012).

### 3.7 Treatment of HSV-1 infection

97. Antiviral medications such as acyclovir, famciclovir and valacyclovir are the most effective treatments available for people infected with HSV. These can help to reduce the severity and frequency of symptoms but cannot cure the infection (World Health Organisation).

### 3.8 Environmental stability

98. As an enveloped virus, HSV-1 is sensitive to desiccation when outside of a host. Infectious titres of droplets containing HSV-1 decline markedly as drying occurs (Bardell, 1990, 1993, 1994; Firquet et al., 2015; Gerhardtts et al., 2016; Turner et al., 1982).

99. In a dried state at room temperature, HSV-1 can survive on glass surfaces for at least 8 weeks at low relative humidity (7%) and at least one day (but less than a week) at higher humidity (55%) (Condair, 2018; Mahl and Sadler, 1975). Optimum indoor humidity is 40-60%, although Australia has no building standards that require this (Condair, 2018; Victorian Trades Hall Council Occupational Health and Safety Unit, 2020).

100. After the initial loss of infectivity associated with drying, viral titre on plastic was maintained for at least another day, was significantly reduced by the end of day 2 and returned to baseline by the end of day 3 (Firquet et al., 2015). Infectivity of HSV-1 dried onto cotton gauze showed a steady decline at room temperature and was close to the assay detection limit after 24 hours in one study (Gerhardtts et al., 2016), but persisted at least 72 hours in another (Larson and Bryson, 1985). In a third study, infectious virus remained viable on cloth only to three hours and was undetectable at four hours (Turner et al., 1982).

101. Infectious virus was recovered from plastic door handles and chrome-plated washbasin taps over a 2 h period, with the titre declining over time. Infectious virus was isolated from skin after touching contaminated doorknobs and taps at all test times over the same period (Bardell, 1990,

---

both reporter genes from one genome to the other were indistinguishable from non-recombinants and so were not counted.

<sup>4</sup> Multiplicity of infection (MOI) is the ratio of infectious agents to targets (e.g. of virus particles to cells).

1993). HSV-1 also persisted on the surface of United States coins for the same two hour period, but their metallic nature contributed to a loss in titre over this time (Bardell, 1994).

102. HSV-1 was detected on the hands of people with virus-positive oral herpes lesions in six of nine cases (Turner et al., 1982). When experimentally applied to human skin, HSV-1 showed the initial drop in titre associated with drying (paragraph 98) but survived for at least two hours (the maximum time examined). Touching dried virus with a dry finger led to virus transfer on 40% of attempts, whereas transfer occurred 100% of the time if the finger was first moistened with water or saliva. Within the 2 hour test period, transfer to dry skin was random and unrelated to how long the virus had remained in the dry state (Bardell, 1989).

### **3.9 Risk group classification of HSV-1**

103. According to the criteria listed in the Australian Standard 2243.3:2010 Safety in Laboratories Part 3: Microbiological safety and containment (Standards Australia/New Zealand 2010), HSV-1 can be classified as a Risk Group 2 micro-organism. PC2 containment and work practices are therefore appropriate when working with the unmodified virus.

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

### **4.1 Modification of the HSV-1 backbone**

104. The GMO was engineered to be replication-incompetent and to constitutively express two copies of the human CFTR gene. Other modifications are intended to increase safety of the product. Details of the genetic modifications are under consideration to be declared as CCI. This information was made available to the prescribed experts and agencies that were consulted on the RARMP.

105. When administered to trial participants, the GMO is expected to infect susceptible cells (epithelial cells and associated sensory neurons (Fink et al., 2011)) which will constitutively express the encoded CFTR protein. Vector DNA will not integrate into the host cell genome but will be maintained extra-chromosomally and may be lost over time by cell turnover. The Applicant expects ongoing CFTR expression to compensate for the deficiencies in CFTR expression and/or function in people with cystic fibrosis who receive the treatment.

### **4.2 The introduced gene – cystic fibrosis transmembrane conductance regulator**

#### **4.2.1 CFTR and cystic fibrosis**

106. Defects in the cystic fibrosis transmembrane conductance regulator (CFTR) gene are responsible for cystic fibrosis. The gene is located on chromosome 7 and encodes a protein of 1480 amino acids which is expressed primarily on the outer surface of epithelial cells that line ducts and cavities within the body. It is found in functionally diverse tissues including the lungs, pancreas, gastrointestinal tract, male reproductive tract and sweat ducts.

#### **4.2.2 CFTR – additional functions**

107. The CFTR anion channel plays a major role in regulating both secretion and absorption in a diverse range of epithelial tissues. In contrast to cystic fibrosis, caused by defects in CFTR function, hyper-activation of the CFTR ion channel causes excessive fluid secretion. CFTR hyper-activation by bacterial enterotoxins is responsible for fluid secretion in life-threatening secretory diarrhoeas such as cholera. CFTR dysfunction is also implicated in the pathogenesis of acute pancreatitis, chronic obstructive pulmonary disease and asthma (Saint-Criq and Gray, 2017; Thiagarajah and Verkman, 2012; Zhang et al., 2012).

108. Additional functions for CFTR have been described. CFTR modulates other ion channels and transporters, regulate cellular processes through protein-protein interactions, and is associated with programmed cell death in proximal renal failure (references in (Hou et al., 2016)). In addition to expression in secretory epithelia, it is found in cardiac muscle, vascular endothelium, immune cells,



red blood cells, arterial smooth muscle and skeletal muscle, osteoblasts and neurons (Krishnan et al., 2017; Marcocelles et al., 2014; Robert et al., 2007; Yeh et al., 2019).

109. Chloride currents are known to modify cardiac electrical activity. GM mice overexpressing CFTR predominantly in heart tissue appeared outwardly normal but exhibited a stress-related ‘sudden death’ phenotype, e.g. in response to fighting or mating. This was associated with conduction abnormalities and arrhythmia; the authors highlighted the importance of target tissue specificity in cystic fibrosis gene therapy (Ye et al., 2011).

110. CFTR is expressed in the nervous system and linked to its correct functioning. A study of CFTR<sup>-/-</sup> pigs identified primary defects in nerve cell structure and function, including reduced nerve conduction velocity (Reznikov et al., 2013). Functional studies demonstrate a role for CFTR in regulating cytosolic chloride levels, for example in chick retinal neurons, the rat lumbar spinal cord and neonatal rat spinal motor neurons. Cytosolic Cl<sup>-</sup> concentration is important in determining the outcome - excitation or inhibition - of certain synaptic signals (Krishnan et al., 2017; Ostroumov et al., 2011; Ostroumov et al., 2007). In a rat model of rapid-eye-movement (REM) sleep, brain stem motoneurons were found to express CFTR. Activation of this chloride channel contributed to the post-synaptic inhibition of motoneurons associated with atonia (muscle paralysis) that occurs during this sleep phase (Morales et al., 2011).

111. A recent study linked CFTR to transmission of nerve signals. CFTR is highly expressed in the nervous system of the gastrointestinal (GI) tract, particularly in motor neurons that control the smooth muscle contractions required for transport of food and faecal matter. It was found to have a functional role in mediating release of the neurotransmitter acetylcholine, which transmits the excitatory signal from neuron to muscle cell, possibly through regulating the movement of calcium ions. Low CFTR expression was linked to slow transit constipation, a chronic gut motility disorder characterised by prolonged colonic transit (Yeh et al., 2019).

112. CFTR plays an important role in epithelial cell migration, differentiation and wound healing. CFTR deficiency promotes cell proliferation while overexpression shifts the balance towards epithelial differentiation. CFTR expression increases during the wound healing process, and activating or overexpressing it enhances cell migration and wound closure. In contrast, CFTR inhibitors impede wound healing (Chen et al., 2016; Chiu et al., 2019; O'Grady, 2017).

113. CFTR is also associated with cancer, with differing outcomes depending on tissue/organ. Deficiency or down-regulation of CFTR is linked to gastrointestinal, lung, breast, pancreatic, prostate, nasopharyngeal and other head and neck cancers (Tu et al., 2016), with tumour suppressor function demonstrated in colorectal cancer models (Anderson et al., 2019; Liu et al., 2020; Than et al., 2016). In contrast, high expression is associated with more aggressive features in glioma and cancers of the female reproductive system (cervical, ovarian and endometrial). CFTR has a functional role in cellular behaviours associated with malignancy and acts in glioma by upregulating an anti-apoptotic pathway (Huang et al., 2017; Peng et al., 2012; Royse et al., 2014; Wu et al., 2013; Xia et al., 2017; Xu et al., 2016; Xu et al., 2015; Zhao et al., 2020). Its exact role in these cancers is unclear and a causative effect on cancer initiation has not been demonstrated.

### 4.3 Transduction, functional activity and biodistribution of the GMO

114. *In vitro* data demonstrate that the GMO transduces lung cells isolated from cystic fibrosis patients, induces expression of functional and correctly localised CFTR, and can be nebulised without loss of activity (Krystal Biotech Inc., 2020a).

115. The Sponsor used an *in vitro* model of stratified human airway epithelium to assess the ability of the GMO to penetrate the mucus layer characteristic of cystic fibrosis epithelia. This model reproduces the air liquid interface and the cells secrete a mucus layer typical of the donor from whom they were obtained (Epithelix, 2021). When applied to cultures derived from a CF patient biopsy, there was no significant difference in the ability of KB407 to transduce cells with an intact mucus layer and those from which the mucus layer was washed off (Krystal Biotech Inc., 2020a). This suggests that the thick

mucus characteristic of cystic fibrosis will not prevent transduction by the GMO. Subsequent CFTR transcription was higher in washed cells, however.

116. *In vivo* studies in wild-type mice showed that the GMO effectively transduced lung tissue. Two days after treatment with aerosolised GMO, qPCR analysis showed vector genomes and CFTR mRNA expression disseminated throughout the lungs. Minimal levels of DNA or transcripts were detected in the trachea or bronchi. No KB407 DNA was detected in blood samples, suggesting the GMO is limited to the site of exposure (Krystal Biotech Inc., 2020a).

117. Transduction was higher in CFTR-deficient mice than in wild-type counterparts. Low but detectable DNA and transcript levels were present in the bronchi. In some lung samples, CFTR transcript levels were higher in wild-type than in CFTR-deficient mice but it is unclear whether the difference was statistically significant (Krystal Biotech Inc., 2020a).

118. An experiment with a single macaque confirmed localisation to lung tissue and that the GMO was not distributed systemically via the blood. The animal was sacrificed two days after receiving a second dose of nebulised GMO. Vector genomes and CFTR mRNA transcripts were detected only in lung tissue. No vector DNA was detected in blood samples, liver, heart, kidney, brain, spleen or lymph nodes (Krystal Biotech Inc., 2020a).

119. The Applicant stated that there are no viral shedding data for the GMO.

#### 4.4 Pre-clinical safety data

120. In the preclinical studies described above, the GMO was assessed as safe and well tolerated. There were no toxicity or significant adverse findings after a single or repeat dose, noting that observations were limited to two days post treatment.

- Mice were observed and vital signs monitored for two days after treatment with the GMO, with no adverse findings. A certified pathologist found no immune cell infiltration or immune activation, fibrosis or necrosis in any examined lung tissues. Quantitative assessment of broncho-alveolar lavage fluid also showed no statistically significant changes in immune cell infiltration (Krystal Biotech Inc., 2020a).
- The treated macaque displayed no abnormal signs over the course of the study and no gross findings were noted at the time of necropsy. There were no significant changes in haematology or clinical chemistry after dosing with the GMO (Krystal Biotech Inc., 2020a).

#### 4.5 Human safety studies involving similar HSV-1-based viral vectors

121. Three small gene therapy trials using HSV-1-based vectors have been conducted by the Sponsor and other researchers and are summarised in Table 1. The Sponsor's products KB103 and KB105 (rows #2-3) are based on the same HSV-1 vector proposed for this study. The routes of administration differed, as these products were delivered intra-dermally or topically to compromised skin. For comparison, details of the proposed clinical trial are included in row 4.

122. Comparative details of the vectors are under consideration to be declared as CCI. This information was made available to the prescribed experts and agencies that were consulted on the RARMP. Transgenes in all three studies expressed therapeutic proteins and were not intended to induce an immune response. As the proposed GMO expresses a 'self' protein, any vector-mediated toxicity is expected to be similar to that observed in these studies.

123. In study 1, seven of ten subjects received an intradermal injection of  $1 \times 10^8$  or  $1 \times 10^9$  pfu GM HSV-1, and results have been published. Over a four month period, there were no treatment-related serious adverse events (SAEs), however the majority of participants reported transient redness and itching at the injection site, considered mild in severity (Fink et al., 2011).

124. Summaries of studies 2 and 3 provided by the Sponsor noted that KB103 (nine subjects) and KB105 (three subjects) did not produce any SAEs, severe product-related adverse events (AEs) or significant immunogenicity (Krystal Biotech Inc., 2021).

**Table 1. Comparison of human gene therapy trials involving similar or identical HSV-1-based vectors with the proposed study**

Study	Study type	Number of Subjects	Administration route	Dose	Total AEs (SAEs)
1 <sup>1</sup>	Phase I	10	Intra-dermal (10 sites)	1x10 <sup>7</sup> , 1x10 <sup>8</sup> , 1x10 <sup>9</sup> pfu total	72 (0)
2 <sup>2</sup> (KB103)	Phase I/II	9	Topical to compromised skin	1-8x10 <sup>8</sup> pfu/site	0 (0)
3 <sup>3</sup> (KB105)	Phase I/II	3	Topical to compromised skin	2x10 <sup>9</sup> pfu/site	0 (0)
4 <sup>4</sup> (KB407)	Phase I	15	Inhalation	To be determined	N/A

<sup>1</sup> First-in-human study of an HSV-1 vector expressing human preproenkephalin (PENK) in patients with intractable focal pain caused by cancer. The vector was intended to deliver a gene expression cassette that carrying a gene encoding opioid peptides to neurons via skin inoculation. Specific neural structures can be targeted by injection into associated skin regions (Fink et al., 2011).

<sup>2</sup> Human trial of B-VEC (KB103), an HSV-1 vector expressing collagen type 7 (COL7) for treatment of Dystrophic Epidermolysis Bullosa. Undertaken by Krystal Biotech Inc. Details from (Krystal Biotech Inc., 2021).

<sup>3</sup> Human trial of KB105, an HSV-1 vector expressing transglutaminase-1 (TGM1) for treatment of Autosomal Recessive Congenital Ichthyosis. Undertaken by Krystal Biotech Inc. Details from (Krystal Biotech Inc., 2020b).

<sup>4</sup> For comparison, comparable information pertaining to the proposed dealings.

## Section 5 The receiving environment

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

### 5.1 Clinical trial participants

126. The primary receiving environment will be the respiratory tract of people with cystic fibrosis who participate in the trial. This includes the oral cavity and nasopharyngeal region (nose, throat and back of mouth), tracheobronchial region (trachea, bronchi and larger bronchioles) and pulmonary region (very small bronchioles and the alveolar sacs of the lung). Each participant will inhale the GMO in the form of aerosolised particles and the Sponsor expects these to reach the lungs.

127. In a healthy person, inhaled particles are continually cleared from the lower airways by mucociliary clearance (MCC). MCC relies on hairlike motile cilia that line the airways and beat continuously in a rhythmic wavelike motion, overlaid by mucus that entraps microorganisms and debris. The ciliated epithelium is bathed in a low viscosity periciliary fluid that lubricates the surface and allows the cilia to beat rapidly. This coordinated beating pushes the mucus layer upwards and propels entrapped particles and pathogens out of the airways to the back of the throat, where it is either swallowed or expelled by coughing. It is estimated that a virion trapped in the mucus layer

would be cleared from the lower respiratory tract in less than 12 minutes (Bustamante-Marin and Ostrowski, 2017; Kuek and Lee, 2020; Quirouette et al., 2020).

128. The water content of the mucus layer is regulated by epithelial secretion of chloride ions through the CFTR and calcium-activated chloride channels, and by uptake of sodium ions (Bustamante-Marin and Ostrowski, 2017). This balance is impaired in people with cystic fibrosis and the resulting thick dehydrated mucus disrupts mucociliary clearance and antimicrobial defences (Whitsett, 2018).

129. The build up of thick mucus in the airways and lungs of people with cystic fibrosis also reduces lung capacity. This would limit the airway volume available to the GMO and mucus may also form a barrier, impeding its ability to reach the epithelial surface. The Applicant has provided *in vitro* data demonstrating that the GMO effectively penetrates a mucus layer produced by cystic fibrosis -derived epithelial cells (Section 4.3), but no corresponding *in vivo* data.

130. Chronic congestion in cystic fibrosis leads to symptoms including a persistent cough that produces sputum (thick mucus), repeated lung infections and sinusitis, and inflamed nasal passages (stuffy nose). Due to the risk of serious lung infection, people with cystic fibrosis are advised to take ongoing precautions to reduce the likelihood of acquiring infections. In Australia, this advice includes practicing good hand hygiene, not sharing utensils or toothbrushes, washing hands after touching shared objects (e.g. keyboards, pens, gym equipment), avoiding sick people, maintaining a 2m distance from people not living in the same household and a 4m distance from other people with cystic fibrosis (Cystic Fibrosis Australia, 2015; Cystic Fibrosis WA, 2017a). In the school context, advice is that children should also avoid sharing utensils, cups and drink bottles (Cystic Fibrosis WA, 2017b).

## 5.2 Clinical trial sites

131. The secondary receiving environment will be the clinical trial sites where the GMO will be received, administered and waste disposed of. Specific sites are yet to be identified, however the application indicates that the trial will take place in hospitals. As such, clinical sites will be equipped to handle infectious agents and conduct procedures in accordance with the *Australian Guidelines for the Prevention and Control of Infection in Healthcare* (National Health and Medical Research Council, 2019).

132. The Applicant has stated that sites will be selected following a feasibility assessment and site selection visit, as is standard practice in accordance with ICH-GCP guidelines (see paragraph 10). ICH GCP requirements include that participating Investigators/Institutions agree to conduct the trial in compliance with GCP guidelines and other applicable regulatory requirements. Accordingly, as the investigational product is a GMO, site selection will include an assessment to determine whether sites can perform a clinical trial in accordance with the Regulator's *Guidelines for the Transport, Storage and Disposal of GMOs* (OGTR 2011) and are prepared to adhere to the conditions of a DIR licence. The Applicant will also take prior experience dealing with GM investigational products into consideration.

133. As the GMO will be administered in aerosolised form, the treatment room forms part of the receiving environment. Clinical sites have not yet been engaged, so treatment room size and usual purpose is not known. Each room will be closed while treatment is in progress, but the Sponsor has not proposed that it be negatively pressured or dedicated exclusively to the study. The Sponsor has directed that it should contain no unnecessary equipment or furniture; required equipment will include a nebuliser and other equipment needed for post treatment tests. As the participant and administrator will be in the room for up to 90 minutes, furnishings are expected to include a work surface and two chairs at a minimum. The room will remain closed for 60 minutes following the treatment, allowing time for aerosolised particles to settle.

134. In the health care setting, AS/NZS 1668.2:2012 (The use of ventilation and air conditioning in buildings) requires ventilation for infection control only in (a) protective isolation rooms, operating rooms, and sterile store and set-up rooms, and (b) infectious isolation rooms, post-anaesthetic

recovery rooms, dirty utility rooms<sup>5</sup> and autopsy rooms. Group (a) rooms serve to protect the occupants/contents from the surrounding environment, while group (b) rooms protect the environment from activities within the room. Air from group (b) rooms may not be recirculated to any other enclosure type and must be mechanically exhausted to atmosphere at minimum rates starting at six air changes per hour (for infectious isolation rooms). Infectious isolation rooms, dirty utility rooms and autopsy rooms must also have a room air pressure lower than that of surrounding areas. Dirty utility rooms and autopsy rooms would be unsuitable for administering the GMO to trial participants, and it cannot be assumed that infectious isolation rooms would be available for this purpose.

135. The principal routes by which the GMO could enter the wider environment are by:

- by exposure and infection of clinical site staff, particularly those involved in administering the GMO, and subsequent users of the treatment room;
- escape of aerosolised GMO from the treatment room, or exposure of subsequent users to residual GMO that remains in the air or on surfaces in the treatment room; and
- egress or shedding of the GMO, or any complemented or recombinant derivative thereof, from treated trial participants once they leave the clinical trial site and return home.

136. The tertiary receiving environment includes trial participants' homes and any places they visit during any period when they are capable of shedding the GMO or a complemented or recombinant derivative.

### 5.3 Relevant environmental factors

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

138. Humans are susceptible to infection with HSV-1 and are expected to be present in environments where the GMO or recombination products could be shed by trial participants and exposed clinical trial staff (e.g., the clinical trial site, patient's homes, work places, public transport etc).

139. Of the animal species known to be susceptible to natural HSV-1 infection, feral European rabbits (*Oryctolagus cuniculus*) are widespread in the Australian environment and domesticated rabbits may be kept as pets in all states/territories except Queensland. There are no wild NHP species in Australia. Captive NHPs may only be imported for eligible non-commercial purposes e.g. research, or exhibition in zoos or wildlife parks, and cannot be kept as pets by private owners. Chinchillas and pygmy hedgehogs are not permitted within Australia.

### 5.4 Related viral species in the receiving environment

140. Wild-type HSV-1 and the closely related HSV-2 are endemic in the human population. A study using data from 1999 reported that 76.5% of Australian adults were seropositive for HSV-1 and 12% for HSV-2. HSV-2 seroprevalence was higher in women (16%) than men (8%), and in large cities (13%) than in rural populations (9%) (Cunningham et al., 2006). HSV-1 is increasingly identified as the cause of genital herpes in Australians; it was identified in the anogenital area of only 3% of the population in 1980, but had risen to 41% in 2001 (Haddow et al., 2006).

---

<sup>5</sup> Dirty utility rooms are enclosures in which contaminated linen, utensils and instruments are located in preparation for cleaning.

## **5.5 Presence of the introduced genes and encoded proteins in the environment**

141. The inserted CFTR gene is already present in the human genome. Therefore, all humans without certain forms of cystic fibrosis already express and are immunologically exposed to the CFTR protein expressed by the GMO.

## **Section 6 Relevant Australian and international approvals**

### **6.1 Australian approvals**

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

143. The Regulator recently issued DNIR-631 authorising the clinical trial of a SARS-CoV-2 vaccine based on the same replication-defective HSV-1 viral vector. The aim of this study is to assess the safety and efficacy of the GM vaccine candidate against disease caused by SARS-CoV-2.

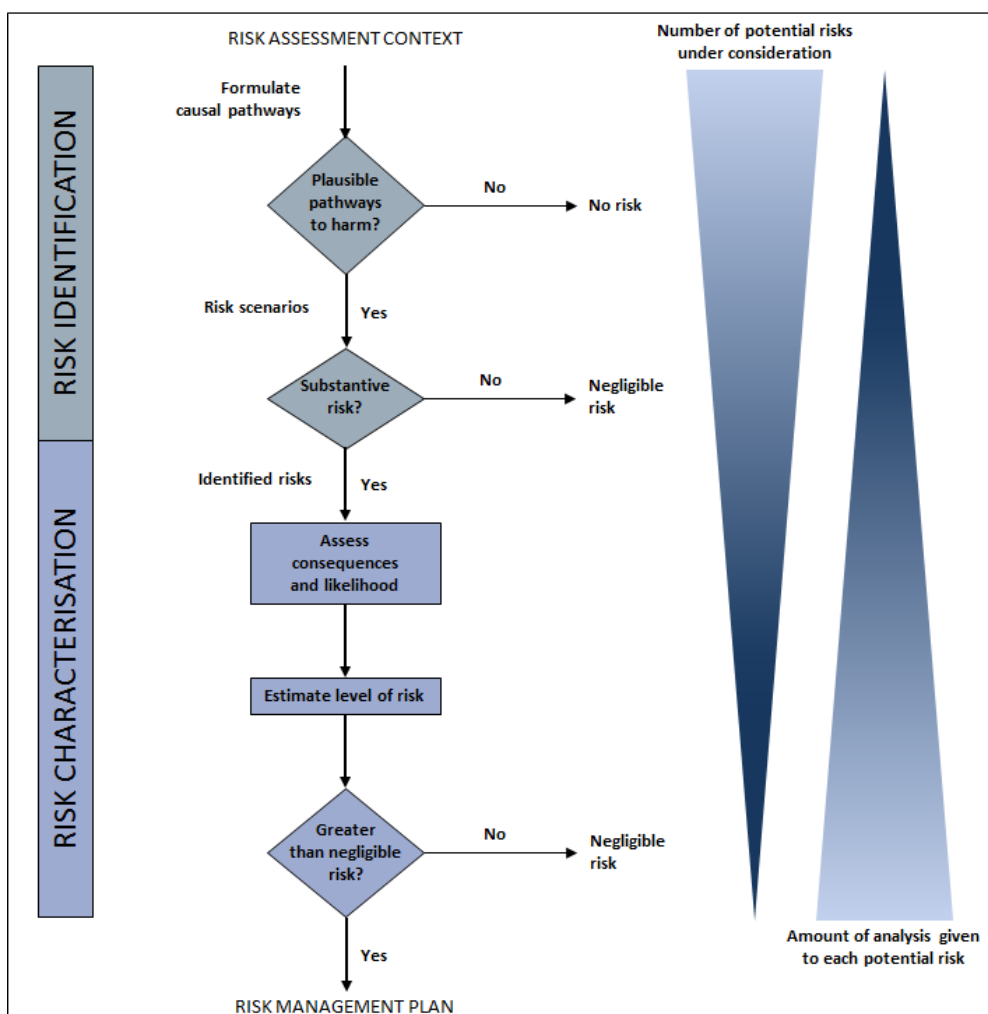
### **6.2 International approvals**

144. The proposed trial is a first-in-human study and there are no relevant international approvals at this time.

## Chapter 2 Risk assessment

### Section 1 Introduction

145. The risk assessment identifies and characterises risks to the health and safety of people or to the environment from dealings with GMOs, posed by or as the result of gene technology (Figure 2). Risks are identified within the established risk assessment context (Chapter 1), taking into account current scientific and technical knowledge. A consideration of uncertainty, in particular knowledge gaps, occurs throughout the risk assessment process.



**Figure 2. The risk assessment process**

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

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

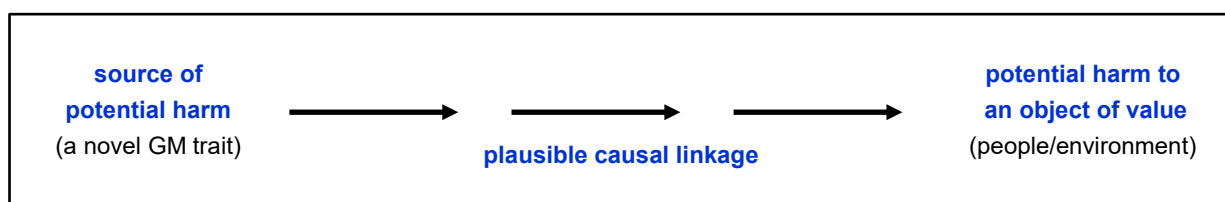
148. Risk scenarios are screened to identify those that are considered to have a reasonable chance of causing harm in the short or long term. Pathways that do not lead to harm, or those that could not plausibly occur, do not advance in the risk assessment process (Figure 3) i.e. the risk is considered to be no greater than negligible.

149. 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

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

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



**Figure 3. Components of a risk scenario**

151. 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

152. The parent organism of the GMO is HSV-1. Details on the pathogenicity and transmissibility of HSV-1 are located in Chapter 1, Section 3.

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

154. The introduction of a constitutively expressed gene encoding human CFTR, intended to replace defective copies of this gene in the lungs of trial participants, is considered as a potential source of harm.

155. Expression of the introduced CFTR gene is controlled by exogenous regulatory sequences. Regulatory sequences are naturally present in all organisms and the introduced sequences are DNA that is not expressed as proteins, and are expected to operate in similar ways to endogenous sequences. Potential harms from regulatory sequences will be considered only to the extent that the promoter is expected to drive high level constitutive expression of the inserted CFTR gene in cells transduced by the GMO.

### 2.2 Causal pathway

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

- potential effects of the introduced gene and gene product on the properties of the parent organism;



- the proposed dealings;
- proposed limits, including the extent and scale of the proposed dealings;
- proposed controls to limit the spread and persistence of the GMO;
- practices during and after administration of the GMO;
- unauthorised activities;
- routes of exposure to the GMO, the introduced gene and gene product;
- the release environment;
- potential exposure of other people to the GMO in the wider environment;
- spread and persistence of the GMO (e.g. dispersal pathways and establishment potential);
- environmental stability of the GMO (tolerance to temperature, UV irradiation and humidity);  
and
- gene transfer by horizontal gene transfer.

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

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

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

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

### 2.3 Potential harm

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

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

### 2.4 Postulated risk scenarios

162. Two risk scenarios were postulated and screened to identify substantive risks, and are summarised in Table 2. In the context of limits and control measures proposed by the applicant, one risk scenario was identified as posing substantive risks which warranted further assessment. More detail on the scenario not identified as a substantive risk is provided in Section 2.4.1, while the substantive risk is characterised in Section 3.1.

**Table 2. Summary of risk scenarios from dealings with GM HSV-1**

Risk Scenario				Substantive risk?	Reasons
#	Risk source	Causal Pathway	Potential harm		
<b>Section 2.1 : Risks to people from exposure to GMO</b>					
1	GM HSV-1	i. Exposure of people and animals to the GMO by various routes. ↓ ii. Transduction of susceptible cells ↓ iii. CFTR expression in transduced cells	Discomfort or ill health due to inappropriate CFTR expression in infected cells	No	<ul style="list-style-type: none"> <li>Only trained and experienced personnel will administer and analyse the GMO. These personnel will be experienced in use and disposal of sharps.</li> <li>The GMO will be administered in a closed room with a single staff member present and minimal furniture and equipment.</li> <li>Use of PPE (e.g. gown, gloves, mask covering nose and mouth) will limit exposure of staff present during nebulisation.</li> <li>Trial participants will wear PPE to limit dispersal of GMO from the treatment room. The work area and equipment will be decontaminated when vacating the room.</li> <li>Trial participants will implement hygiene measures intended to minimise exposure of others.</li> <li>The GMO is replication-incompetent.</li> <li>Accidental exposure in the context of these controls would involve a relatively small quantity of GMO relative to the therapeutic dose.</li> </ul>
2	GM HSV-1	i. Trial participant with asymptomatic HSV-1 infection is treated with nebulised GMO, and/or Treatment administrator with active HSV-1 infection is exposed to the GMO ↓ ii. Transduction of epithelial cells. ↓ iii. Co-infection of transduced epithelial	Discomfort or ill health due to inappropriate CFTR expression in infected cells	Yes	<ul style="list-style-type: none"> <li>Trial participants and clinical trial staff administering the treatment will be exposed to aerosolised GM HSV-1 over a prolonged period.</li> <li>HSV-1 has high prevalence in humans, and the GMO will contact facial mucosa that are common sites of HSV-infection.</li> <li>Co-infection of susceptible cells with GMO and wild-type HSV-1 could lead to recombination and generation of replication</li> </ul>

Risk Scenario				Substantive risk?	Reasons
#	Risk source	Causal Pathway	Potential harm		
		<p>cells with wild-type HSV-1.</p> <p style="text-align: center;">↓</p> <p>iv. Complementation of disrupted replicative gene functions by wild-type HSV-1 in co-infected cells, permitting:</p> <ul style="list-style-type: none"> <li>• GMO replication and release of replication defective GM virus particles, together with HSV-1 virus particles; and/or</li> <li>• recombination with HSV-1, generating products that include replication competent HSV-1 carrying a copy of the human CFTR gene</li> </ul> <p style="text-align: center;">↓</p> <p>v. Shedding and transmission to other people or animals of:</p> <ul style="list-style-type: none"> <li>• GMO and ‘helper’ HSV-1; and/or</li> <li>• novel replication competent GMO</li> </ul>			<p>competent progeny carrying the CFTR transgene</p> <ul style="list-style-type: none"> <li>• Recombinants could be transmitted to close contacts and over time, spread within the general population.</li> <li>• See Section 3.1 for risk characterisation.</li> </ul>

### 2.4.1 Risk scenario 1

<b>Risk source</b>	GM HSV-1
<b>Causal pathway</b>	i. Exposure of people and animals to the GMO by various routes. ↓ ii. Transduction of susceptible cells ↓ iii. CFTR expression in transduced cells
<b>Potential harm</b>	Discomfort or ill health due to inappropriate CFTR expression in transduced cells

#### Risk source

163. The source of harm for this postulated risk scenario is the GMO, expressing the CFTR transgene

#### Causal Pathway

164. This scenario applies to people conducting dealings at clinical trial, storage or analytical facilities, to subsequent users of the treatment room (staff and/or patients), and close contacts of trial participants, both human and animal. Each group may be exposed to the GMO via a distinct route, as discussed below, culminating in transduction of susceptible cells and expression of the CFTR transgene.

#### Exposure of people conducting dealings

165. People handling the GMO at clinical trial sites, storage facilities or analytical facilities could be exposed to aerosols or airborne droplets, by direct contact with the GMO solution or via sharps injury. As discussed in Chapter 1, Section 2.3.6, people administering the GMO to trial participants would have the greatest opportunity for exposure, and to the highest GMO concentrations.

##### *Inhalation of aerosolised GMO or airborne droplets*

166. Clinical site staff treating participants with aerosolised GMO could inadvertently inhale it themselves. Loading the nebuliser will involve transfer of liquid from a syringe fitted with a needle into an open container - a process likely to generate aerosols or airborne droplets. The GMO will then be nebulised over a period up to 30 minutes. A portion of nebulised medication is typically released into the surrounding area and can lead to bystander inhalation (McGrath et al., 2019). The nebuliser to be used in this study may release aerosolised GMO from the back of the mouthpiece assembly and GMO will also be exhaled by the trial participant (paragraph 44). The administrator will remain in a closed room with airborne material for up to 90 minutes.

167. The Applicant anticipates bystander exposure to aerosolised GMO and has proposed controls to reduce this. Attending staff will be limited to one person who will wear a mouth and nose covering, such as a mask with an N95 or P2 rating. N95/P2 masks intended for the healthcare setting are single-use respiratory protective devices designed to achieve a very close facial fit and efficient filtration of airborne particles ( $\geq 95\%$  filtration efficiency at  $0.3 \mu\text{m}$ ). The edges of the mask should form a seal around the nose and mouth. The effectiveness of these control measures are discussed in Chapter 3.

##### *Exposure of facial mucosa or compromised skin via a splash or direct contact with aerosols*

168. HSV-1 can cause infection if it comes into contact with susceptible mucosal sites such as the oropharynx, conjunctivae and cornea, or with small cracks in the skin. While loading the nebuliser, expelling GMO solution from a syringe offers potential for a direct splash. As discussed above, aerosolised GMO will be released into the treatment room. The administrator will wear PPE that includes a mask and face shield, protecting the oral mucosa, nose and eyes from splashes and the mouth and nose from aerosol contact. Most non-facial skin will be covered by the combination of clothing, a long-sleeved isolation gown made from impervious material, and gloves.

169. Analytical facility staff could be exposed to splashes and aerosols during sample analysis. However, sample testing will be conducted by qualified personnel in pathology or other testing laboratories, which are required to adhere to national standards for handling of infectious substances, which will minimise their likelihood of exposure to the GMO.

*Exposure via needle stick or other sharps injury*

170. The proposed dealings present several opportunities for sharps injury. Staff unpacking GMO shipments after import or transport could encounter a broken vial. The nebuliser will be loaded using a syringe fitted with a needle. Biological samples, including blood, will be collected from participants throughout the trial and some may contain the GMO.

171. Controls proposed by the Applicant will minimise the likelihood of exposure. Staff receiving GMO shipments will inspect the package for damage before handling vials. Hospital staff administering the GMO and taking blood samples will have clinical training and be highly experienced with safe needle handling techniques relevant to preparing/administering medicines and collecting patient samples. The administration protocol does not require reuse of the needle/syringe assembly, nor will contaminated needles be recapped or removed from the syringe. Empty needle/syringe assemblies will be placed in a sharps container immediately after use.

*Exposure via hand contamination and transfer to susceptible sites*

172. Staff at storage facilities, clinical sites and analytical facilities will handle GMO vials during relabelling or repackaging, storage, administration, sample collection and sample analysis. Contamination of a person's hands could lead to exposure and transduction of high contact areas, such as the face and genital area, which are susceptible to HSV-1 infection. Compromised skin could be similarly affected.

173. Mitigating factors include that the product will be supplied in sealed pharmaceutical vials and, prior to administration, no one will directly handle the GMO. Nonetheless, the Applicant expects that staff will follow standard clinical/laboratory practices when handling vials, including wearing gloves and a protective garment that covers the arms. The administrator will be required to wear gloves and a long sleeved isolation gown, and will remove and dispose of them when leaving the treatment room. Gloves would also be standard PPE while collecting and analysing biological samples. Correct use of disposable gloves will minimise the opportunity for hand contamination with the GMO and subsequent transfer to susceptible tissues.

***Exposure of people not conducting dealings - Subsequent users of treatment room and surrounding areas***

174. It is anticipated that aerosolised GMO will deposit on surfaces in the treatment room. It could also spread to adjacent rooms through the ventilation system or when the door is opened at the end of the procedure. Subsequent users of the room or connected areas may touch contaminated surfaces while not wearing gloves and transfer GMO to susceptible epithelia through hand to face/genital contact. Transduction and expression of the CFTR protein may follow.

175. To minimise exposure of people not conducting the dealings, the Applicant proposes that the door be kept closed during the nebulisation procedure and for 60 minutes afterwards, to allow aerosols to settle out and limit the immediate spread of the GMO. Clinical trial sites be also instructed to decontaminate work surfaces and equipment potentially exposed to the GMO following treatment. The effectiveness of these control measures are discussed in Chapter 3.

***Exposure of people not conducting dealings – Close contacts of trial participants***

176. Patients with cystic fibrosis receiving the GMO are likely to have thick mucus build up in the airways, which could slow the transduction process, and free GMO could persist in the airways for some time after treatment. Trial participants may expose close contacts to the GMO by coughing, sneezing or transferring saliva to another person directly (e.g. by kissing) or indirectly (e.g. by sharing food or utensils), leading to transduction of susceptible epithelia and expression of CFTR protein. They could similarly expose susceptible animals, such as pet rabbits, e.g. by feeding them leftover food scraps (see Chapter 1, Section 3.3).

177. As a mitigation measure, the Applicant has proposed that trial participants be asked to minimise exposure of close contacts for 24 hours following the treatment by containing any egress of GMO that is not rapidly taken up by host cells. Behavioural measures include wearing a surgical mask, avoiding coughing or sneezing near other people, avoiding both direct and indirect oral contact, and implementing standard hygiene practices. They will also be asked to collect nasal secretions for disposal at the clinical site. The effectiveness of these control measures are discussed in Chapter 3.

### **Potential harm**

178. If people or susceptible animals are exposed to the GMO such that it contacts and transduces susceptible epithelial cells, these cells may express the CFTR protein. Given the neurotropic nature of HSV, the GMO may also enter sensory nerve termini innervating the affected epithelia and express CFTR within neurons. This is analogous to the intended delivery mechanism for several replication defective HSV-1-based vectors targeting the peripheral nervous system, which are taken up and delivered to neural ganglia after intradermal or subcutaneous inoculation (Fink et al., 2011; Fink and Wolfe, 2011; Glorioso and Fink, 2004; Goss et al., 2002; Mata et al., 2008).

179. Transgene expression could lead to an adverse immune response, however the GMO is replication defective and would transduce only a limited number of cells in the vicinity of exposure. Furthermore, CFTR is a self-protein and should be ignored by the immune system.

180. Transgene expression at an unintended site also offers potential for adverse consequences. As described in Chapter 1, Section 4.2.2, CFTR has a wide range of possible effects and functions. CFTR is naturally expressed in the epithelia and neurons of many tissues, but regulation of gene expression is complex and tightly controlled. There is limited information regarding consequences of inappropriate expression. However, as the GMO is replication defective any effect would be limited to the small number of cells accessible from the initial site of exposure.

### **Conclusion**

181. Risk scenario 1 is not identified as a substantive risk because potential exposure routes to the GMO will be mitigated by the proposed controls, and the GMO is replication defective with its effect limited to the area initially exposed. Therefore, this risk could not be greater than negligible and does not warrant further detailed assessment.

## Section 3 Risk characterisation

### 3.1 Risk scenario 2

<b>Risk source</b>	GM HSV-1 carrying the CFTR transgene	
<b>Causal pathway</b>	<b>A</b>	<b>B</b>
	i. Trial participant with asymptomatic HSV-1 infection is treated with nebulised GMO. ↓ ii. Transduction of oral epithelial cells. ↓ iii. Co-infection of transduced oral epithelial cells with wild-type HSV-1. ↓	Treatment administrator with active HSV-1 infection is exposed to the GMO. ↓ Transduction of wild-type HSV-1-infected cells. ↓
	iv. Complementation of deleted replicative gene functions by wild-type HSV-1 in co-infected cells, permitting: <ul style="list-style-type: none"> <li>• GMO replication and release of replication defective GM virus particles, together with HSV-1 virus particles; and/or</li> <li>• recombination with HSV-1, generating products that include replication competent HSV-1 carrying a copy of the human CFTR gene</li> </ul> ↓ v. Shedding and transmission to other people or animals of: <ul style="list-style-type: none"> <li>• GMO and ‘helper’ HSV-1; and/or</li> <li>• novel replication competent GMO</li> </ul>	
<b>Potential harm</b>	Discomfort or ill health due to inappropriate CFTR expression in infected cells.	

182. Two risk scenarios were postulated and evaluated, as summarised in Table 2. In the context of control measures proposed by the applicant, the second risk scenario was identified as posing a substantive risk which warrants further assessment. This section provides more detail on the evaluation of this scenario.

183. Trial participants and clinical trial staff administering the treatment will be exposed to aerosolised GM HSV-1 over a prolonged period. Co-infection of susceptible cells with GMO and wild-type HSV-1 could lead to recombination and generation of replication competent progeny carrying the CFTR transgene. Such recombinants could be transmitted to close contacts and over time, spread within the general population.

#### 3.1.1 Risk source

184. The source of harm for this postulated risk scenario is the GMO, expressing the CFTR transgene.

#### 3.1.2 Causal Pathway description and likelihood assessment

##### 3.1.2.1 Causal pathway 2A – co-infection following intentional exposure of trial participants to the GMO

185. The oral mucosa is considered a site commonly infected after both transmission and reactivation of wild-type HSV-1. During treatment, a trial participant’s entire oral cavity will be exposed to nebulised GMO for up to 30 minutes. This is more extensive contact than required for natural transmission of the parent organism, and the genetic modifications do not impact on host cell entry mechanisms. As the first tissue exposed, the GMO will be at its highest concentration. Particles depositing in the tracheobronchial region may be subject to mucociliary clearance, depending on mucus viscosity in the airway, but this does not apply to oral tissues (see Chapter 1, Section 5.1). As aerosolised GMO will be in contact with the entire oral cavity, transduction could be extensive and uniform across oral tissues.

186. The Sponsor has not assessed the potential for transduction of oral epithelia. Murine studies showed minimal viral DNA in the bronchi and trachea of wild type mice after inhalation of the GMO, and very low uptake in the bronchi of CFTR<sup>-/-</sup> mice (Chapter 1, Section 4.3). As these structures are subject to rapid mucociliary clearance (Chapter 1, Section 5.1), these data are not relevant to oral transduction and the potential for transducing human oral epithelia during treatment is unknown.

187. Based on population data, around 76% of trial participants may be seropositive for HSV-1 (Cunningham et al., 2006), although this figure could be lower given hygiene measures encouraged in people with cystic fibrosis (paragraph 130). Should latent HSV-1 reactivate before or during the trial, epithelia transduced by the GMO may be co-infected with wild-type virus. The Applicant has proposed measures to minimise this.

188. Firstly, individuals with discernible herpes lesions at Screening and Day 0 will be excluded from participating in the trial. Excluding individuals with symptomatic infection would eliminate part of the seropositive population, potentially lowering the overall percentage of seropositive participants.

189. Secondly, treated participants will be monitored for development of herpes-like lesions. Should these occur, the participant will be treated with an oral anti-viral medication, which would prevent any potential consequences of co-infection.

190. These measures reduce the potential for co-infection. However, many studies have documented that HSV-1 reactivation and infection of the oral mucosa is frequent and often asymptomatic (Chapter 1, Section 3.5). Asymptomatic infection in trial participants would not be detectable, either at Screening, Day 0 or after the person has been treated. Trial participants could thus have an active oral HSV-1 infection when treated with aerosolised GMO, and the GMO could transduce epithelial cells in which wild-type HSV-1 is actively replicating. As an alternative pathway to co-infection, trial participants could develop an asymptomatic oral herpes infection, new or reactivated, during the time transduced cells persist after treatment.

191. The oral epithelium is stratified, with cell division restricted to the basal layer. Daughter cells differentiate as they move upwards through the epithelium and are eventually sloughed off the surface (Jones and Klein, 2013; Papagerakis et al., 2014). Reported median turnover times for differentiated oral epithelia range from 14-24 days, depending on location within the mouth (Squier and Kremer, 2001), yielding a window of at least several weeks during which transduced epithelial cells could become co-infected with wild-type HSV-1. Should long-lived stem and transit amplifying cells in the basal layer be transduced they would be expected to persist for longer periods or permanently, however would be less accessible to the GMO and multiple rounds of cell division should dilute out the non-replicating viral vector.

192. The 15 trial participants will collectively receive multiple treatments, with some individuals receiving more than one. Based on a median 14-24 day lifetime for oral epithelial cells<sup>6</sup> and taking into account the timing of GMO doses, transduced cells could be expected to be present in any of the participants for 385-535 person-days. With up to 76% (293-407) of these days associated with seropositive participants (Cunningham et al., 2006) and an observed 12% frequency of asymptomatic HSV-1 shedding on any given day as reported by (Mark et al., 2008), the trial could generate 35-49 days on which transduced cells and active HSV-1 infection are both present in oral epithelia, and co-infection could occur.

193. If susceptible cells are co-infected by both replicating HSV-1 and the GMO, proteins expressed by the wild-type virus may complement the GMO, facilitating genome replication and production of replication-defective progeny.

194. *In vivo* complementation of a defective HSV-1 gene (thymidine kinase) by intact virus has been demonstrated. When applied to the mouse cornea, TK<sup>-</sup> HSV-1 mutants replicated in ocular tissues but were unable to establish acute or latent infection in the trigeminal ganglia. When mixed with standard TK<sup>+</sup> HSV-1, however, TK<sup>-</sup> mutants readily infected the trigeminal ganglia and established latency (Tenser and Edris,

---

<sup>6</sup> This overestimates the persistence time of surface epithelial cells already close to the end of their lifetime, but as reported turnover times are median values, also underestimates the persistence time of cells at the upper end of the range.



1987; Tenser et al., 1981). In another study, a mixture of two avirulent viruses – a TK<sup>-</sup> HSV-2 mutant and a non-neuroinvasive HSV-1 strain – complemented one another to display a highly neurovirulent phenotype in mice (Nishiyama et al., 1991). A number of replication defective animal viruses are complemented by related or unrelated helper viruses that supply the missing function, including adeno-associated virus, hepatitis D virus and acutely transforming avian sarcoma viruses. By analogy, wild-type HSV-1 and the GMO may actively replicate as a pair and express CFTR in epithelia and associated neurons, establish latency in neurons and reactivate at later times. As reactivated HSV-1 can re-establish latency in the nervous system (Kennedy et al., 2015), a GMO/HSV-1 virus pair may do likewise, providing repeated opportunities to transduce neurons in the initial host. It could also be transmitted as a co-replicating pair to new hosts, at the time of treatment or at a later time, allowing the GMO to spread and persist in the human population.

195. With wild-type HSV-1 supplying the missing proteins and facilitating GMO replication, there is also potential for co-infecting genomes to recombine (Chapter 1, Section 3.6) and produce replication competent progeny expressing the CFTR gene. Details on how this could occur are under consideration to be declared as CCI and were made available to the prescribed experts and agencies that are consulted on the RARMP. Replication-competent recombinants could then be transmitted to other people.

196. The simplest pathway to this particular outcome would be the acquisition of one CFTR copy by wild-type HSV-1. The CFTR expression cassette is not expected to destabilise the recombinant, however neither is it expected to provide any selection advantage. Genomic stability of the transgene is unknown and it may not persist long-term, however several studies document retention of transgenes not related to virus function. HSV-1 genomes carrying fluorescent reporter genes were maintained through serial rounds of infection and replication *in vitro*, and following neuroinvasive spread into the mouse brain, with no tendency for recombinants lacking the expression cassettes to dominate the population (Law et al., 2018). HSV-based vectors with replication capacity and reliant on continued transgene expression have also been developed for cancer therapy (Li et al., 2020; Menotti and Avitabile, 2020), and transgene stability of a GM HSV-2 expressing human granulocyte-macrophage colony-stimulating factor was demonstrated over 20 generations in tissue culture (Wang et al., 2018).

197. Should replication competent HSV-1 carrying the CFTR gene be produced, it could be further transmitted to close contacts of the individual in whom it arose and spread within the human population or to susceptible animals living in close proximity to humans. There is also a theoretical potential for transmission of complementing GMO/HSV-1 pairs, which could recombine in any person or susceptible animals they infect.

#### 3.1.2.2 Likelihood assessment for pathway 2A

198. The oral cavity of a trial participant will be uniformly exposed to the GMO for a sustained period, is lined with cells susceptible to HSV-1 infection and there is no data regarding transduction of these cells. Although opportunities for oral transduction and co-infection will be limited by enrolling only 15 participants, excluding people with symptomatic herpes lesions, and treating with anti-viral medication if obvious lesions develop after treatment, there is potential for asymptomatic co-infection in every participant who is seropositive for HSV-1. Active HSV-1 infection and GMO-transduced cells may be jointly present in the oral cavity on an estimated 35-49 days over the course of the trial, providing opportunities for co-infection and recombination. *In vivo* complementation of replication defective viruses has been demonstrated, and HSV recombination occurs readily.

199. The local immune response is likely to provide some mitigation. Most HSV-1 reactivations are of relatively short duration (hours to days), and studies of asymptomatic HSV-2 episodes show their extent is limited by tissue resident T cells (Chapter 1, Section 3.5). It is expected that the local immune response would similarly restrict the period of co-replication in a trial participant with co-infected cells. However, recombination is likely even within this context, as a maximum percentage of recombinants has been observed within six hours of co-infection (Law et al., 2018). Resolution of the infection would limit the period in which infection of nerve cells or transmission to a new host could occur.

200. Generation and persistence of a specific set of recombinants - those that are replication competent and carry a copy of the CFTR transgene - is another limiting step in this scenario. Recombination is expected to be random and the frequency of any particular outcome is unknown. However, as noted in Chapter 1,

Section 3.6, when an experiment was designed to detect, but not select for, the transfer of functional genes between co-infecting HSV-1 genomes, the desired recombinants were readily observed.

201. Scenario 2A culminates in transmission of replication competent GMOs or co-replicating GMO/HSV-1 pairs from the trial participant to other people or to susceptible animals.

#### ***Transmission to people***

202. For co-infection occurring at the time of treatment, the behavioural measures the Applicant has proposed (Chapter 1, Section 2.3.9) would minimise transmission to people during the first 24 hours after treatment, a period over which many asymptomatic HSV-1 infections resolve (Mark et al., 2008). However, these measures would not prevent transmission of recombinants or co-replicating GMO/HSV-1 pairs that arise at later times. As participants will be monitored for the development of herpes-like lesions, *symptomatic* co-infections arising after the GMO treatment would be curtailed by the proposed treatment with oral anti-viral medication once clinical trial staff are aware of the lesion. However, this does not address the potential for transmission subsequent to *asymptomatic* co-infection. As discussed in paragraph 191, the proposed trial offers opportunities for short-lived asymptomatic HSV-1 infections to coincide with diffusely distributed transduced epithelial cells, with potential for co-infection, complementation and recombination between GM and HSV-1 genomes. Excluding days on which behavioural measures will be in place, there are still 32-46 days on which co-infection and recombination could occur with no limit on transmission to third parties.

203. For these reasons, the events described in Pathway 2A, involving complementation of the GMO by, and recombination with, wild-type HSV-1, followed by transmission to other people, appear possible and are considered to fall *between unlikely* (harm could occur in some limited circumstances) and *likely* (harm could occur in many circumstances) (OGTR, 2013).

#### ***Transmission to animals***

204. It is expected that transmission of replication competent GMOs or co-replicating GMO/HSV-1 pairs to animals would be limited to those animals susceptible to natural infection with HSV-1 and living in close proximity to humans. Considering relevant species that are present in Australia (Chapter 1, Section 5.3), these could include pet rabbits, as well as rabbits or NHPs confined to research or exhibition facilities. Approximately 0.8% of Australian households keep pet rabbits (Newgate Research, 2019), and few people regularly handle animals in research or exhibition facilities. Given the small number of trial participants, it is **highly unlikely** that a trial participant who developed a transmissible form of the GMO would subsequently pass it to a susceptible animal.

#### **3.1.2.3 Causal pathway 2B - co-infection following inadvertent exposure of persons administering the GMO**

205. As discussed in paragraphs 166-168, the person administering the treatment will be exposed to aerosolised GMO released during the nebulisation process, and potentially also to splashes while loading the nebuliser. As these staff will remain in the room while the full amount of GMO is nebulised and for 60 minutes afterwards, they will be exposed to a potentially high dose of fugitive aerosols each time.

206. As with trial participants, 76% of clinical trial staff can be expected to be seropositive for HSV-1 (Cunningham et al., 2006). Having a discernible herpes infection will not restrict their involvement in the trial, and as discussed earlier, asymptomatic infection is common. These staff members could therefore have an active HSV infection - symptomatic or asymptomatic – in the oral or ocular mucosa during any administration procedure, creating the potential for the GMO to transduce facial epithelial cells already infected with wild-type HSV-1.

207. Co-infection of susceptible cells with both replicating HSV-1 and the GMO could then initiate the events described above in paragraphs 193-197.

#### **3.1.2.4 Likelihood assessment for Pathway 2B**

208. In relation to its likelihood, Pathway 2B differs from Pathway 2A in three major respects: clinical trial staff will be exposed to a lesser amount of aerosolised GMO than trial participants will receive, but

consideration of their HSV-1 ‘active infection status’, or implementing barriers to their transmitting recombinants to third parties (human or animal), were not proposed.

209. Any staff exposure to the GMO would involve fugitive aerosols rather than the full GMO dose, but potentially with multiple exposures. As clinical sites have not been identified, it is not known whether the total exposure time will be divided amongst a number of staff. Should a single site be used, one person could administer all the doses and receive all the exposure. PPE as proposed by the Applicant will reduce contact with cells that could harbour an active HSV-1 infection. The proposed mouth and nose covering, provided an N95/P2 or better mask is used, offers protection from aerosol exposure to the mouth and nose, while a face shield would protect the mask fabric and eyes from splashes. A face shield would not protect the staff member’s eyes from aerosolised GMO, however, and the incidence of HSV-1 DNA in the cornea is relatively high, with asymptomatic shedding documented (Chapter 1, Section 3.5). Furthermore, the effectiveness of an N95/P2 mask would be contingent on fit testing to match the model/size to each individual’s face shape, followed by fit checking before each use. Fit testing was not discussed in the application and is not widely applied in Australian hospitals (Department of Health, 2020).

210. It is uncertain whether this level of exposure would be sufficient to cause infection, given the infectious dose for HSV is not known and aerosols are not a primary transmission route for HSV (see paragraph 86). If infection does ensue, co-infection of cells would be possible as aerosols would contact exposed tissues uniformly, and HSV-1 seropositive staff could have an active infection – either asymptomatic or symptomatic - at the time of dosing, or develop one during the time that transduced cells persist.

211. Should co-infection and recombination occur, producing replication-competent progeny that carry the CFTR gene, there would be no barrier to their transmission as clinical trial staff will not be subject to any behavioural requirements following the procedure.

#### ***Transmission to people***

212. In view of the reduced GMO exposure relative to trial participants and uncertainty as to infectious dose, countered by the greater likelihood of active HSV-1 infection and capacity for transmission of any recombinants that do form, Pathway 2B overall is considered **unlikely** to occur.

#### ***Transmission to animals***

213. Compared with trial participants, clinical trial staff administering the GMO are likely to interact with pet rabbits and exhibition animals at a similar low rate. They could potentially have more contact with research animals, however such animals would be housed in hygienic conditions with good laboratory practices observed during handling and feeding. Given the small size of the trial, it is again considered **highly unlikely** that a staff member who developed a transmissible form of the GMO would subsequently pass it to a susceptible animal.

#### **3.1.3 Consequence assessment**

214. If either a GMO/HSV-1 co-replicating pair is established, or a novel replication competent GM virus expressing CFTR is produced, this could affect both the person in whom it is generated and other people or susceptible animals to whom it is transmitted. Tissues likely to be affected are those commonly associated with HSV-1 infection – i.e. the oral mucosa and surrounding area, eyes and genitals, and associated sensory neurons. CFTR may then be expressed in these cells under control of the heterologous promoter, i.e. in a constitutive manner.

215. After infecting sensory neurons, HSV-1 may re-commence lytic replication or establish a long-lived latent state in which the genome persists as a non-replicating episomal element and most gene expression is repressed. HSV-1-based vectors have nonetheless been developed for gene delivery to the nervous system and strong constitutive promoters can achieve gene expression *in vivo* in the order of weeks (Fink et al., 2011; Goss et al., 2011; Goss et al., 2014). Similarly, CFTR expression may be maintained for at least some weeks in sensory neurons.

216. In the nervous system, CFTR regulates intracellular Cl<sup>-</sup> levels and has functional roles in synaptic signalling. In varying circumstances, activation of Cl<sup>-</sup> channels in sensory neurons can be either excitatory or

inhibitory (Goss et al., 2011), thus dysregulation of tightly controlled CFTR expression may be deleterious. Over- or mis-timed expression could interfere with the nervous system and have unexpected functional effects. As an example, activating a ligand-regulated Cl<sup>-</sup> channel expressed in neurons innervating tissue exposed to painful stimuli reduced the perception of pain (Goss et al., 2011). Failure to perceive and respond to noxious stimuli could have harmful consequences. In addition to pain, sensory neurons transmit information about many aspects of the environment from the periphery to the brain, e.g. physical stimuli (pressure, vibration and stretch), temperature, taste and smell (Betts et al., 2017). Inappropriate expression of CFTR could therefore impact on sensory perception more generally.

217. The consequences for humans of generating replication-competent HSV-1 expressing the CFTR gene are considered to range from **marginal** (minimal or no increase in illness/injury to people) to **intermediate** (significant increase in illness/injury to people that requires specialised treatment). On the one hand, there is uncertainty as to the consequences of expressing CFTR protein in sensory neurons, its expression during any viral reactivation episode is likely to last only a few weeks, and symptomatic reactivations could be treated using antiviral drugs. However, no drugs are effective against HSV in its latent form, thus infection with the GMO and the potential for its periodic reactivation would be lifelong. Considering long term outcomes, the infectious nature of HSV creates potential for replication-competent recombinants to spread widely in the human population.

218. The extent to which similar events could occur in animals susceptible to HSV-1 infection is uncertain. However, homology between the human CFTR gene and that of other mammalian species ranges from 91-92% for rabbits, pigs, ferrets and sheep, to 78% for mice. Regions important in maintaining structural and functional integrity are highly conserved (Liu et al., 2017). Human CFTR can rescue phenotypic abnormalities in *Cftr*-knockout mice, indicating functional similarity between the mouse and human proteins (Gawenis et al., 2019; Koehler et al., 2003). It is possible therefore, that susceptible animals such as rabbits, present in Australia and whose *Cftr* gene shares greater homology with human *Cftr*, could be negatively impacted by infection with replication competent HSV-1 expressing human CFTR.

219. Any impact on susceptible species would be limited by the need for close contact with humans, thus infection with replication competent virus would not spread beyond the individual animals or small groups, and could not disseminate more broadly through animal-to-animal contact.

220. Given the uncertainty as to individual consequence and minimal capacity to spread within animal populations without human agency, the consequences with respect to animals are considered **marginal** (minimal or no increase in harm to desirable components of the environment).

#### 3.1.4 Risk estimate

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

222. For humans, the potential consequences of exposure to and infection by the GMO are considered to range from **marginal** to **intermediate**, with probabilities between **unlikely** and **likely** for pathway 2A, and **unlikely** for pathway 2B. The overall risk is therefore estimated to lie between **negligible** (risk is of no discernible concern and there is no present need to invoke actions for mitigation) and **moderate** (risk is of marked concern and will necessitate actions for mitigation that need to be demonstrated as effective).

223. For animals, the potential consequences of exposure to and infection by the GMO are considered **marginal**, and are **highly unlikely** to occur. The overall risk is therefore estimated to be **negligible** (risk is of no discernible concern and there is no present need to invoke actions for mitigation).

## Section 4 Uncertainty

224. Uncertainty is an intrinsic part of risk analysis<sup>7</sup>. 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.

---

<sup>7</sup> 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.

In relation to risk management, there can be uncertainty about the effectiveness, efficiency and practicality of controls, and there are several types of uncertainty in risk analysis (Bammer and Smithson, 2008; Clark and Brinkley, 2001; Hayes, 2004). These include:

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

225. 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.

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

227. For DIR-181, uncertainty is noted in relation to a number of points, including:

- the potential for aerosolised GMO to transduce oral epithelial cells during treatment or other facial epithelia in the event of exposure, as these points were not assessed in pre-clinical studies;
- the potential for wild-type HSV-1 to complement the GMO, assist its replication and establish a co-infection;
- the capacity for propagation and transmission of the GMO and wild-type HSV-1 as a co-replicating pair is unknown;
- the potential for CFTR to mediate adverse effects. Few studies address its role in nerve cell function and synaptic transmission, and there is little data exploring the consequences of over-expression. Some aspects of CFTR function are tissue dependent, so the consequences of overexpression may also vary with the type of cell in which it is overexpressed; and

228. In this case the uncertainties outlined above have been accommodated by taking a conservative approach to the risk analysis.

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

## Section 5 Risk evaluation

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

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

- risk criteria
- level of risk
- uncertainty associated with risk characterisation

- interactions between substantive risks.

232. Two risk scenarios were postulated whereby the proposed dealings might give rise to harm to people or the environment. This included consideration of the potential for: expression of the introduced genes and genetic modifications to impact on the disease burden caused by the GM virus; infection of at-risk individuals; and infection of animals.

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

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

235. The risk due to recombination with wild-type HSV-1 in both trial participants and clinical trial staff, with potential for transmission of replication competent progeny to other people or to animals resulting in discomfort or ill health, was estimated as posing a low to moderate risk to human health and safety and a negligible risk to the environment.

236. The applicant has proposed some control measures related to these risks. Additional treatment measures to mitigate the identified risks should be applied, and are considered in Chapter 3.

## Chapter 3 Risk management plan

### Section 1 Background

237. 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.

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

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

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

### Section 2 Risk treatment measures for substantive risks

241. The risk identification process led to identification of one substantive risk, involving GMO recombination with wild-type HSV-1 in trial participants and/or clinical trial staff, with potential for transmission of replication competent progeny to other people or to susceptible animals resulting in discomfort or ill health. This risk was characterised in Chapter 2, Section 3, and risk evaluation proposed that this risk should be treated.

242. Regarding trial participants, the applicant has proposed to reduce the possibility of co-infection by excluding individuals with discernible herpes lesions at Screening or Day 0, and to monitor treated participants for development of herpes-like lesions. Any participants who develop lesions would be treated with an oral anti-viral medication. However, active HSV-1 infection occurs frequently in seropositive individuals and is often asymptomatic. Without regular diagnostic testing, asymptomatic outbreaks could not be discerned or halted by the proposed anti-viral treatment. To further reduce the likelihood of coinfection and recombination with wild-type HSV-1, only persons who are seronegative for HSV-1 should be included in the trial. Excluding people with latent HSV-1 infection will minimise the opportunity for coinfection of oral epithelia with the GMO and wild-type HSV-1 both at the time of treatment and during the period that transduced epithelial cells persist. There is then little chance that replication competent recombinants could form, minimising the likelihood of their transmission to other people or to susceptible animals.

243. Seronegative trial participants could still acquire a primary infection with HSV-1 subsequent to GMO treatment, providing an opportunity for co-infection and recombination should this occur within the turnover time of GMO-transduced oral epithelial cells. To manage this risk, participants should be screened (by diagnostic testing) for evidence of oral HSV-1 infection at follow up visits to the clinical site. If primary infection is confirmed, and as proposed by the Applicant, participants should be offered oral anti-viral treatment.

244. The measures described in paragraphs 242-243 may impact on the pool of cystic fibrosis patients eligible for the trial but are considered straightforward and effective, and have been imposed as licence conditions.

245. Regarding clinical trial staff administering the GMO and present in the room while aerosols are being generated, the applicant proposed PPE that would afford protection to the mouth and nose. This is provided N95/P2 masks are used, are correctly fit tested and staff are trained in their correct use, including how to perform fit-checking and safe removal. However, the eyes are also a site of asymptomatic HSV-1 infection and reactivation, and proposed eye protection (a face shield) is not effective against aerosol exposure.

246. Multiple approaches can be envisaged for reducing the potential for transduction of ocular tissues already infected with HSV-1. The trial participant could be isolated from the staff member during the nebulisation process and until most aerosols have settled out of the air, either in a mist tent or a closed room with communication via a glass partition or webcam. If the staff member must be in the room while aerosols are present, more effective eye protection could be used. Adding eye protection such as tight-fitting goggles to the proposed mask is not ideal, as this may interfere with effective sealing of the mask. Powered air-purifying respirators (PAPRs) are widely available and offer effective and comfortable aerosol protection to all of the mouth, nose and eyes, particularly where respiratory protection is needed for an extended period.

247. As clinical trial sites are likely to have different facilities and equipment available, an approach tailored to each location is likely to provide optimal risk management. Given that sites have not yet been selected and such factors cannot be considered at this time, licence conditions require that the licence holder prepare, for each clinical trial site, a plan for aerosol protection of staff administering the GMO. Each plan must document effective protection of the mouth, nose and eyes, and must be approved in writing by the Regulator before administration of the GMO at the corresponding site can commence. The licence holder must also ensure that clinical trial staff implement this plan when carrying out the procedure.

248. Minimising the potential for trial participants who receive the GMO from being co-infected with HSV-1, and for clinical trial staff who could be infected with HSV-1 being exposed to the GMO, are sufficient to manage the negligible to moderate risks associated with transmission of replication competent progeny to other people. The risks posed to animals were assessed as negligible, but will also be minimised by this strategy.

## **Section 3 General risk management**

249. The limits and controls proposed in the application were important in establishing the context for the risk assessment and in reaching the conclusions regarding risks posed to people and the environment. Therefore, to maintain the risk context, licence conditions have been imposed to limit the number of trial participants and duration of the trial, and to restrict the location to hospitals. As the application is for a limited and controlled release of the GMO, A range of additional controls have been imposed to limit the spread and persistence of the GMO in the environment. The conditions are discussed and summarised in this Chapter and listed in detail in the licence.

### **3.1 Limits and controls on the clinical trial**

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

251. The proposed clinical trial involves a maximum of 15 adult cystic fibrosis patients within Australia, with most dealings to take place in storage facilities, hospitals and analytical facilities. Activities that will occur elsewhere include transport and disposal of the GMO. The Applicant has proposed to complete the study within three years of commencement. Conditions maintaining the proposed limits of the trial, such as



the duration of the study, and the maximum number, age range and disease status of trial participants, are included in the licence.

252. Proposed criteria for selecting participants include those listed in Chapter 1, Section 2.3.2. These will be subject to approval by a HREC, who will consider safety of the individuals involved in the trial. Of these criteria, the licence requires that trial participants be willing to complete all procedures required by the protocol. These procedures include behavioural measures that participants will be asked to undertake to limit exposure of caregivers and close contacts. Required measures are discussed further in paragraph 266, and will reduce the potential for the GMO to spread and persist within the community (risk scenario 1).

253. The applicant advised that import and transport of the GMO would be in accordance with IATA shipping classification UN 3245 (GMOs that are not classified as category A or B infectious substances) and/or the Regulator's *Guidelines for the Transport, Storage and Disposal of GMOs*. These provide standard protocols for handling and minimising exposure to the GMOs. Once at storage facilities or clinical trial sites, 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 for transport and storage within a storage facility or clinical trial site, as well as transport of the GMO for export. These measures limit the exposure of people and the environment to the GMOs.

254. Conditions are included in the draft licence requiring the licence holder to ensure that all GMOs, including material or waste that has been in contact with the GMO, within the clinical trial site, are decontaminated by autoclaving, chemical treatment or by high-temperature incineration. Draft licence conditions require that the licence holder ensure that the GMO, or material or waste that has been in contact with the GMO, that is to be destroyed by external service providers, is via the clinical waste stream.

255. The Industry Code of Practice for the Management of Clinical and Related Wastes details requirements for clinical waste including waste segregation, packaging, labelling, storage, transport and accountability (Biohazard Waste Industry, 2010). The clinical waste stream typically involves destruction of infectious waste by incineration or autoclaving, which are considered appropriate for disposal of the GMO. Given that HSV-1 can persist for some time in the environment (Chapter 1, Section 3.8), disposal measures such as maceration could lead to exposure of staff at waste disposal facilities. Therefore, the draft licence also requires waste disposal by external service providers to be by autoclaving or high-temperature incineration. These measures would limit the exposure of people to the GMO.

256. A range of people will handle the GMO in addition to those administering it to trial participants. The Applicant has proposed that all such staff will wear PPE that includes gloves, which will reduce opportunities for auto-inoculation of susceptible sites (e.g. the face and genitals). Accordingly, the licence requires that persons handling the GMO must wear protective clothing including gloves.

#### 3.1.1.1 Controls to minimise exposure of clinical trial staff administering the GMO, subsequent users of treatment room and users of surrounding areas

257. Participants will inhale the GMO using a nebuliser, under the direction of staff at clinical trial sites. The Applicant has proposed controls that adequately protect the staff member from splashes to the face and from needle stick injury that could occur while loading the nebuliser with GMO solution. Licence conditions therefore require that PPE include a splash barrier in front of the face, and that staff are appropriately qualified to carry out procedures.

258. The specific nebuliser proposed is likely to allow significant release of aerosolised GMO into the room, through a combination of aerosol released during the patient's exhalation cycle, aerosol that is inhaled and then exhaled by the patient, and aerosol released from the back of the unit during operation (see paragraph 166). The Applicant has proposed a number of controls to minimise exposure of bystanders to the GMO, both in aerosolised form and GMO that may remain on surfaces following the procedure.

259. Controls to limit the administrator's exposure to aerosolised GMO were discussed in Section 2 of this chapter. Additional controls to minimise exposure of other bystanders include that:

- the GMO will be administered in a closed room, and the door will remain closed for 60 minutes after the procedure, limiting aerosol spread to adjoining spaces; and
- a single staff member will be present during the procedure, limiting the number of people exposed.

260. Settling rates for particles in the expected size range suggest that 60 minutes may not be sufficient for all aerosols to settle out (Baron, 2010). Exhaled particles are smaller than those initially generated (McGrath et al., 2019), and suspended particles are also expected to reduce in size through evaporation. Both factors would extend the time needed for aerosolised GMO to fully settle on surfaces in the room. GMO may therefore enter adjoining rooms or corridors when the room is opened. It may also spread to other areas through the ventilation system, as observed in an exposure study of a nebulised adeno-associated viral vector (Croteau et al., 2004). People using these spaces may therefore be exposed to the GMO.

261. Given the variation in facilities, layout and ventilation anticipated at different clinical trial sites, licence conditions require that the licence holder prepare, in consultation with clinical trial sites, an exposure management plan for each site documenting how exposure of people not involved in the trial will be minimised. Suitable strategies could include using a negatively pressured, HEPA-filtered environment, such as a mist tent, isolation room or suitably ventilated laboratory; limiting use of adjoining rooms or corridors during and after the procedure; considering air circulation between rooms via the ventilation system; and allowing sufficient time for aerosolised GMO to settle out before opening the treatment room door. Where applicable, these plans should be agreed to by the Institutional Biosafety Committee (IBC) associated with the site.

262. Controls to minimise exposure of subsequent users of the treatment room to contaminated surfaces include that:

- The room will contain a minimum of furniture and equipment, limiting surfaces on which GMO will be able to settle;
- the staff member and participant will both wear PPE that includes a disposable gown made from impermeable material, gloves and shoe coverings. These will be removed and disposed of before leaving the room; reducing contamination of clothing and skin;
- after the procedure, work surfaces and equipment potentially exposed to the GMO will be cleaned using a disinfectant such as sodium hypochlorite.

263. All GMO that is not inhaled or drawn into the ventilation system is expected to eventually settle on surfaces. HSV-1 in a dried state can persist on surfaces such as plastic and cotton for as long as 48-72 hours, and is expected to remain on frequently touched surfaces in addition to those that the Applicant proposed to decontaminate – for example, the door handle, chairs and walls. Dried HSV-1 is reliably transferred to moistened skin and more sporadically to dry skin (Chapter 1, Section 3.8). To minimise exposure of people *not* conducting the dealings to residual GMO, licence conditions require decontamination of all frequently touched surfaces in the treatment room, including chairs and door handles, before the room is used for another purpose. Alternatively, the room may be left unused until the GMO is expected to become inactive; a 72 hour period is considered sufficient.

264. Biological particles that have settled on flooring can be resuspended by foot traffic (Nazaroff, 2014), and subsequent users of the treatment room could be exposed to airborne GMO released from the floor. To address this exposure pathway, licence conditions require that the floor also be decontaminated or left unused for 72 hours before the room is used for another purpose.

265. Humans release bioaerosols from their skin and clothing during bodily movement (Nazaroff, 2014). The proposed impermeable gown, gloves and shoe coverings to be worn by people present during nebulisation and disposed of as biohazardous waste before they leave the room, will reduce the quantity of GMO available for release in other locations. In addition to this PPE, licence conditions require an impermeable hair covering, to prevent hair becoming a vehicle for transporting GMO from the room.

### ***3.1.1.2 Controls to minimise exposure of close contacts of trial participants***

266. The Applicant has proposed that trial participants be asked to minimise exposure of close contacts for 24 hours following the treatment by containing any egress of GMO that is not rapidly taken up by host cells. However, it is not certain that 24 hours is sufficient to ensure the airways will be free of extracellular GMO. Licence conditions therefore require that behavioural measures be implemented for at least 48 hours. As certain animals are susceptible to infection with HSV-1, participants should avoid close contact with relevant species (e.g. pet rabbits) as well as with humans.

### ***3.1.1.3 Additional controls***

267. Biodistribution studies in mice and macaques suggest that the GMO does not move beyond the site of transduction and is unlikely to be found in the blood. However, this is a first-in-human study and will generate data regarding behaviour of the GMO in humans. Using the conservative timeframe of 90 days, as proposed by the Applicant, abstinence from blood or organ donation would minimise the potential for transmission of transducing viral particles to other people by this route. Therefore, the criteria included in the draft licence are that the licence holder must obtain written agreement from the trial participant that they will not donate blood or organs for 90 days after the last dose of the GMO.

268. A standard condition is included in the draft 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.

269. Other conditions included in the licence are standard conditions that state that only people authorised by the licence holder are covered by the licence, and that the licence holder must inform all people dealing with the GMOs, other than external service providers, of applicable licence conditions.

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

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

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

- limit the trial to 15 participants, who are seronegative for HSV-1
- require the trial to be conducted at clinical trial sites, defined as hospitals located in Australia and notified in writing to the Regulator for the purposes of conducting this clinical trial
- restrict access to the GMO
- ensure personnel involved in the trial are appropriately trained and follow appropriate behavioural requirements
- restrict personnel permitted to administer the GMO
- ensure appropriate PPE is used by both staff, and by trial participants during administration of the GMO
- ensure that behavioural requirements are communicated to trial participants and their agreement obtained
- require decontamination of the GMO and materials and equipment that have been in contact with the GMO at clinical trial sites using effective disinfectants or disposal using a certified waste contractor in accordance with standard clinical waste disposal practices, as required by the relevant Australian and state legislation
- transport and store the GMO and samples from GMO-treated participants in accordance with IATA shipping classification UN 3245 (GMOs that are not classified as category A or B infectious)

substances) and/or the minimum requirements for packaging, and labelling as detailed in the draft licence.

- destroy untreated GMO and GMO-related waste via the clinical waste stream, with disposal by autoclaving or high temperature incineration to be used by external service providers

### **3.2 Other risk management considerations**

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

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

#### **3.2.1 Applicant suitability**

273. 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.

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

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

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

276. The persons covered by the licence will be the licence holder and employees, agents or contractors of the licence holder and other persons who are, or have been, engaged or otherwise authorised by the licence holder to undertake any activity in connection with the dealings authorised by the licence. Prior to dealings with the GMOs, Novotech is required to keep a record of people and organisations that are covered by the licence.

#### **3.2.3 Reporting requirements**

277. The licence will require the licence holder to immediately report any of the following to the Regulator:

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

278. 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:

279. identification of clinical trial sites where the GMO will be administered to trial participants

280. expected date of first administration of the GMO for each clinical trial site

281. date of final administration of the GMO for each clinical trial site

### **3.2.4 Monitoring for compliance**

282. 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.

283. 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.

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

## **Section 4 Issues to be addressed for future releases**

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

- data regarding transduction of HSV-1-susceptible epithelia at different locations in the oral cavity and other areas of the face likely to be exposed to aerosolised GMO e.g. nasal cavity and eyes, as this was not assessed in pre-clinical studies;
- the potential for wild-type HSV-1 to complement the GMO, assist its replication and establish a co-infection. While a theoretically plausible proposition, this has not been assessed experimentally; and
- the potential for recombination between the GMO and wild-type HSV-1.

## **Section 5 Conclusions of the RARMP**

286. The risk assessment concludes that the proposed clinical trial poses negligible to moderate risks to the health and safety of people, and a negligible risk to the environment, as a result of gene technology. These negligible to moderate risks require specific risk treatment measures.

287. The risk management plan concludes that the identified negligible to moderate risks can be managed so as to protect the health and safety of people and the environment by imposing risk treatment measures. Licence conditions are imposed to limit the release in size, locations and duration, and to restrict the spread and persistence of the GMO and its genetic material in the environment, as these were important considerations in establishing the context for assessing the risks. Specific risk treatment measures have also been imposed to manage the identified negligible to moderate risks.

## References

- Agelidis, A.M., and Shukla, D. (2015). Cell entry mechanisms of HSV: what we have learned in recent years. *Future Virol* 10, 1145-1154.
- Ahmad, B., and Patel, B.C. (2020). Herpes Simplex Keratitis. In *StatPearls* (Treasure Island (FL)).
- Anderson, K.J., Cormier, R.T., and Scott, P.M. (2019). Role of ion channels in gastrointestinal cancer. *World J Gastroenterol* 25, 5732–5772.
- Aravantinou, M., Mizenina, O., Calenda, G., Kenney, J., Frank, I., Lifson, J.D., Szpara, M., Jing, L., Koelle, D.M., Teleshova, N., *et al.* (2017). Experimental Oral Herpes Simplex Virus-1 (HSV-1) Co-infection in Simian Immunodeficiency Virus (SIV)-Infected Rhesus Macaques. *Front Microbiol* 8, 2342.
- Bammer, G., and Smithson, M. (2008). *Uncertainty and risk: Multidisciplinary perspectives* (London: Earthscan).
- Bardell, D. (1989). Hand-to-hand transmission of herpes simplex virus type 1. *Microbios* 59, 93-100.
- Bardell, D. (1990). Survival of herpes simplex virus type 1 on some frequently touched objects in the home and public buildings. *Microbios* 63, 145-150.
- Bardell, D. (1993). Survival of herpes simplex virus type 1 in saliva and tap water contaminating some common objects. *Microbios* 74, 81-87.
- Bardell, D. (1994). Studies on the survival and inactivation of herpes simplex virus type 1 on coins. *Microbios* 77, 161-166.
- Baron, P. (2010). *Generation and behaviour of airborne particles (aerosols)* (Centers for Disease Control and Prevention).
- Bello-Morales, R., Andreu, S., and Lopez-Guerrero, J.A. (2020). The Role of Herpes Simplex Virus Type 1 Infection in Demyelination of the Central Nervous System. *Int J Mol Sci* 21, 1-19.
- Betts, J.G., DeSaix, P., Johnson, E., Johnson, J.E., Korol, O., Kruse, D.H., Poe, B., Wise, J.A., Womble, M., and Young, K.A. (2017). *Anatomy and Physiology* (Houston, Texas: OpenStax).
- Biohazard Waste Industry (2010). *Industry Code of Practice for the Management of Clinical and Related Wastes*, 6 edn.
- Bowen, C.D., Renner, D.W., Shreve, J.T., Tafuri, Y., Payne, K.M., Dix, R.D., Kinchington, P.R., Gatherer, D., and Szpara, M.L. (2016). Viral forensic genomics reveals the relatedness of classic herpes simplex virus strains KOS, KOS63, and KOS79. *Virology* 492, 179-186.
- Bower, J.R., Mao, H., Durishin, C., Rozenbom, E., and Detwiler, M. (1999). Intrastrain variants of herpes simplex virus type 1 isolated from a neonate with fatal disseminated infection differ in the ICP34.5 gene, glycoprotein processing, and neuroinvasiveness. *J Virol* 73, 3843–3853.
- Brodlie, M., Haq, I.J., Roberts, K., and Elborn, J.S. (2015). Targeted therapies to improve CFTR function in cystic fibrosis. *Genome Med* 7, 101-116.
- Bustamante-Marin, X.M., and Ostrowski, L.E. (2017). Cilia and Mucociliary Clearance. *Cold Spring Harb Perspect Biol* 9, 1-17.

- Casagrande, R.A., Pannuti, C.S., Kanamura, C., Freire, W.S., Grespan, A., and Matushima, E.R. (2014). Fatal *Human herpesvirus 1* (HHV-1) infection in captive marmosets (*Callithrix jacchus* and *Callithrix penicillata*) in Brazil: clinical and pathological characterization. *Pesquisa Veterinária Brasileira* 34, 1109-1114.
- Chen, J., Chen, Y., Chen, Y., Yang, Z., You, B., Ruan, Y.C., and Peng, Y. (2016). Epidermal CFTR Suppresses MAPK/NF-κB to Promote Cutaneous Wound Healing. *Cellular Physiology and Biochemistry* 39, 2262-2274.
- Chiu, W.T., Tran, T.V., Pan, S.C., Huang, H.K., Chen, Y.C., and Wong, T.W. (2019). Cystic Fibrosis Transmembrane Conductance Regulator: A Possible New Target for Photodynamic Therapy Enhances Wound Healing. *Adv Wound Care (New Rochelle)* 8, 476-486.
- Clark, A.J., and Brinkley, T. (2001). Risk management: for climate, agriculture and policy (Canberra: Commonwealth of Australia), pp. 1-62.
- Condair (2018). Humidification for hospital and healthcare environments, C. plc, ed. (Littlehampton, West Sussex).
- Croteau, G.A., Martin, D.B., Camp, J., Yost, M., Conrad, C., Zeitlin, P.L., and Heald, A.E. (2004). Evaluation of exposure and health care worker response to nebulized administration of tgAAVCF to patients with cystic fibrosis. *Ann Occup Hyg* 48, 673-681.
- Cunningham, A.L., Taylor, R., Taylor, J., Marks, C., Shaw, J., and Mindel, A. (2006). Prevalence of infection with herpes simplex virus types 1 and 2 in Australia: a nationwide population based survey. *Sex Transm Infect* 82, 164-168.
- Cystic Fibrosis Australia (2015). CF Facts - Pseudomonas, Cystic Fibrosis Australia, ed. (Baulkham Hills NSW).
- Cystic Fibrosis WA (2017a). CF Facts - Common infections (Nedlands WA: Cystic Fibrosis WA).
- Cystic Fibrosis WA (2017b). CF Facts - Infection control in schools (Nedlands WA: Cystic Fibrosis WA).
- Cystic Fibrosis Worldwide. What is Cystic Fibrosis? (available online at <https://www.cfwv.org/>).
- Da Silva, L.M., Guimarães, A.L.S., Victória, J.M.N., Gomes, C.C., and Gomez, R.S. (2005). <2005 - da Silva - HSV-1 shedding in the oral cavity of seropositive patients - Abstract.pdf>. *Oral Dis* 11, 13-16.
- de Matos, R., Russell, D., Van, A.W., and Miller, A. (2014). Spontaneous fatal Human herpesvirus 1 encephalitis in two domestic rabbits (*Oryctolagus cuniculus*). *J Vet Diagn Invest* 26, 689-694.
- Eberle, R., and Jones-Engel, L. (2017). Understanding primate herpesviruses. *J Emerg Dis Virol* 3, 1-24.
- Elborn, J.S. (2016). Cystic fibrosis. *The Lancet* 388, 2519-2531.
- Epithelix (2021). MUCILAIR™ : in vitro 3D human upper airway epithelium (available online at <https://www.epithelix.com/products/mucilair>).
- Epstein, J.H., and Price, J.T. (2009). The significant but understudied impact of pathogen transmission from humans to animals. *Mt Sinai J Med* 76, 448-455.
- Fan, S., Cai, H., Xu, X., Feng, M., Wang, L., Liao, Y., Zhang, Y., He, Z., Yang, F., Yu, W., *et al.* (2017). The Characteristics of Herpes Simplex Virus Type 1 Infection in Rhesus Macaques and the Associated Pathological Features. *Viruses* 9.
- Fink, D.J., Wechuck, J., Mata, M., Glorioso, J.C., Goss, J., Krisky, D., and Wolfe, D. (2011). Gene therapy for pain: results of a phase I clinical trial. *Ann Neurol* 70, 207-212.

- Fink, D.J., and Wolfe, D. (2011). Gene Therapy for Pain: A Perspective. *Pain Manag* 1, 379-381.
- Firquet, S., Beaujard, S., Lobert, P.E., Sane, F., Caloone, D., Izard, D., and Hober, D. (2015). Survival of Enveloped and Non-Enveloped Viruses on Inanimate Surfaces. *Microbes Environ* 30, 140-144.
- Flume, P.A., Mogayzel, P.J., Jr., Robinson, K.A., Goss, C.H., Rosenblatt, R.L., Kuhn, R.J., Marshall, B.C., and Clinical Practice Guidelines for Pulmonary Therapies, C. (2009). Cystic fibrosis pulmonary guidelines: treatment of pulmonary exacerbations. *Am J Respir Crit Care Med* 180, 802-808.
- Gawenis, L.R., Hodges, C.A., McHugh, D.R., Valerio, D.M., Miron, A., Cotton, C.U., Liu, J., Walker, N.M., Strubberg, A.M., Gillen, A.E., *et al.* (2019). A BAC Transgene Expressing Human CFTR under Control of Its Regulatory Elements Rescues Cftr Knockout Mice. *Sci Rep* 9, 11828.
- Gerhardt, A., Bockmühl, D., Kyas, A., Hofmann, A., Weide, M., Rapp, I., and Höfer, D. (2016). Testing of the Adhesion of Herpes Simplex Virus on Textile Substrates and Its Inactivation by Household Laundry Processes. *Journal of Biosciences and Medicines* 04, 111-125.
- Gilbert, S.C. (2006). Oral shedding of herpes simplex virus type 1 in immunocompetent persons. *J Oral Pathol Med* 35, 548-553.
- Glezen, W.P., Fernald, G.W., and Lohr, J.A. (1975). Acute respiratory disease of university students with special reference to the etiologic role of Herpesvirus hominis. *Am J Epidemiol* 101, 111-121.
- Glorioso, J.C., and Fink, D.J. (2004). Herpes vector-mediated gene transfer in treatment of diseases of the nervous system. *Annu Rev Microbiol* 58, 253-271.
- Goss, J.R., Cascio, M., Goins, W.F., Huang, S., Krisky, D.M., Clarke, R.J., Johnson, J.W., Yokoyama, H., Yoshimura, N., Gold, M.S., *et al.* (2011). HSV delivery of a ligand-regulated endogenous ion channel gene to sensory neurons results in pain control following channel activation. *Mol Ther* 19, 500-506.
- Goss, J.R., Goins, W.F., Lacomis, D., Mata, M., Glorioso, J.C., and Fink, D.J. (2002). Herpes Simplex-Mediated Gene Transfer of Nerve Growth Factor Protects Against Peripheral Neuropathy in Streptozotocin-Induced Diabetes in the Mouse. *Diabetes* 51, 2227-2232.
- Goss, J.R., Krisky, D., Wechuck, J., and Wolfe, D. (2014). Herpes simplex virus-based nerve targeting gene therapy in pain management. *J Pain Res* 7, 71-79.
- Greninger, A.L., Roychoudhury, P., Xie, H., Casto, A., Cent, A., Pepper, G., Koelle, D.M., Huang, M.L., Wald, A., Johnston, C., *et al.* (2018). Ultrasensitive Capture of Human Herpes Simplex Virus Genomes Directly from Clinical Samples Reveals Extraordinarily Limited Evolution in Cell Culture. *mSphere* 3, 1-12.
- Grest, P., Albicker, P., Hoelzle, L., Wild, P., and Pospischil, A. (2002). Herpes simplex encephalitis in a domestic rabbit (*Oryctolagus cuniculus*). *J Comp Pathol* 126, 308-311.
- Haddow, L.J., Dave, B., Mindel, A., McPhie, K.A., Chung, C., Marks, C., and Dwyer, D.E. (2006). Increase in rates of herpes simplex virus type 1 as a cause of anogenital herpes in western Sydney, Australia, between 1979 and 2003. *Sex Transm Infect* 82, 255-259.
- 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, CSIRO, ed. (Tasmania: CSIRO Division of Marine Research), pp. 1-73.
- Hill, J.M., and Clement, C. (2009). Herpes simplex virus type 1 DNA in human corneas: what are the virological and clinical implications? *J Infect Dis* 200, 1-4.



- Hou, Y., Guan, X., Yang, Z., and Li, C. (2016). Emerging role of cystic fibrosis transmembrane conductance regulator - an epithelial chloride channel in gastrointestinal cancers. *World J Gastrointest Oncol* 8, 282-288.
- Huang, W., Jin, A., Zhang, J., Wang, C., Tsang, L.L., Cai, Z., Zhou, X., Chen, H., and Chan, H.C. (2017). Upregulation of CFTR in patients with endometriosis and its involvement in NFκB-uPAR dependent cell migration. *Oncotarget* 8, 66951–66959.
- ICH (2016). Integrated addendum to ICH E6(R1): Guideline for good clinical practice, I.C.F.H.O.T.R.F.P.F.H.U. (ICH), ed., pp. 1-66.
- Imura, K., Chambers, J.K., Uchida, K., Nomura, S., Suzuki, S., Nakayama, H., and Miwa, Y. (2014). Herpes simplex virus type 1 infection in two pet marmosets in Japan. *J Vet Med Sci* 76, 1667-1670.
- Janovitz, E.B., Fishman, C., Zimmerman, S., and Thompson, S. (1998). Fatal Human Herpes Simplex Virus Encephalitis in a Domestic Rabbit. In *Indiana Animal Disease Diagnostic Laboratory Newsletter* (West Lafayette, IN), pp. <https://www.addl.purdue.edu/newsletters/1998/summer/1998sum.pdf>.
- Jones, K.B., and Klein, O.D. (2013). Oral epithelial stem cells in tissue maintenance and disease: the first steps in a long journey. *Int J Oral Sci* 5, 121-129.
- Kaufman, H.E., Azcuy, A.M., Varnell, E.D., Sloop, G.D., Thompson, H.W., and Hill, J.M. (2005). HSV-1 DNA in tears and saliva of normal adults. *Invest Ophthalmol Vis Sci* 46, 241–247.
- Kaufman, H.E., Brown, D.C., and Ellison, E.M. (1967). Recurrent Herpes in the Rabbit and Man. *Science* 156, 1628-1629.
- Kaye, S.B., Madan, N., Dowd, T.C., Hart, C.A., McCarthy, K., and Patterson, A. (1990). Ocular shedding of herpes simplex virus. *Br J Ophthalmol* 74, 114-116.
- Kennedy, P.G., Rovnak, J., Badani, H., and Cohrs, R.J. (2015). A comparison of herpes simplex virus type 1 and varicella-zoster virus latency and reactivation. *J Gen Virol* 96, 1581-1602.
- Kintner, R.L., Allan, R.W., and Brandt, C.R. (1995). Recombinants are isolated at high frequency following in vivo mixed ocular infection with two avirulent herpes simplex virus type 1 strains. *Arch Virol* 140, 231–244.
- Koehler, D.R., Sajjan, U., Chow, Y.-H., Martin, B., Kent, G., Tanswell, A.K., McKerlie, C., Forstner, J.F., and Hu, J. (2003). Protection of Cftr knockout mice from acute lung infection by a helper-dependent adenoviral vector expressing Cftr in airway epithelia. *PNAS* 100, 15364-15369.
- Krishnan, V., Maddox, J.W., Rodriguez, T., and Gleason, E. (2017). A role for the cystic fibrosis transmembrane conductance regulator in the nitric oxide-dependent release of Cl(-) from acidic organelles in amacrine cells. *J Neurophysiol* 118, 2842-2852.
- Krystal Biotech Inc. (2020a). KB407 for the treatment of cystic fibrosis (available online at <https://ir.krystalbio.com/index.php/static-files/d9cf10d4-7ab3-41f8-9682-c66dfd50b181>).
- Krystal Biotech Inc. (2020b). Krystal Biotech Announces Positive Interim Results from Phase 1/2 Clinical Trial of KB105 in Patients with TGM1-related Autosomal Recessive Congenital Ichthyosis (ARCI) (available online at <https://ir.krystalbio.com/news-releases/news-release-details/krystal-biotech-announces-positive-interim-results-phase-12>).
- Krystal Biotech Inc. (2021). Krystal. The leader in redosable gene therapy for rare disease (available online at <https://ir.krystalbio.com/index.php/static-files/6345b187-aaef-4f32-9b02-0b755c2424e3>).

- Kuek, L.E., and Lee, R.J. (2020). First contact: the role of respiratory cilia in host-pathogen interactions in the airways. *Am J Physiol Lung Cell Mol Physiol* *319*, L603-L619.
- Larson, T., and Bryson, Y.J. (1985). Fomites and herpes simplex virus. *J Infectious Dis* *151*, 746-747.
- Law, G.A., Herr, A.E., Cwick, J.P., and Taylor, M.P. (2018). A New Approach to Assessing HSV-1 Recombination during Intercellular Spread. *Viruses* *10*, 1-16.
- Lawler, C., Milho, R., May, J.S., and Stevenson, P.G. (2015). Rhadinovirus host entry by co-operative infection. *PLoS Pathog* *11*, e1004761.
- Lee, K., Kolb, A.W., Sverchkov, Y., Cuellar, J.A., Craven, M., and Brandt, C.R. (2015). Recombination Analysis of Herpes Simplex Virus 1 Reveals a Bias toward GC Content and the Inverted Repeat Regions. *J Virol* *89*, 7214-7223.
- Lee, S.-W., Markham, P.F., Coppo, M.J.C., Legione, A.R., Markham, J.F., Noormohammadi, A.H., Browning, G.F., Ficorilli, N., Hartley, C.A., and Devlin, J.M. (2012). Attenuated Vaccines Can Recombine to Form Virulent Field Viruses. *Science* *337*, 188-188.
- Lewis, M.E., Leung, W.C., Jeffrey, V.M., and Warren, K.G. (1984). Detection of multiple strains of latent herpes simplex virus type 1 within individual human hosts. *J Virol* *52*, 300–305.
- Li, L., Liu, S., Han, D., Tang, B., and Ma, J. (2020). Delivery and Biosafety of Oncolytic Virotherapy. *Front Oncol* *10*, 1-15.
- Liljeqvist, J.A., Tunback, P., and Norberg, P. (2009). Asymptomatically shed recombinant herpes simplex virus type 1 strains detected in saliva. *J Gen Virol* *90*, 559-566.
- Lim, F., Khalique, H., Ventosa, M., and Baldo, A. (2013). Biosafety of gene therapy vectors derived from herpes simplex virus type 1. *Curr Gene Ther* *13*, 478-491.
- Liu, C., Song, C., Li, J., and Sun, Q. (2020). CFTR Functions as a Tumor Suppressor and Is Regulated by DNA Methylation in Colorectal Cancer. *Cancer Manag Res* *12*, 4261-4270.
- Liu, F., Zhang, Z., Csanady, L., Gadsby, D.C., and Chen, J. (2017). Molecular Structure of the Human CFTR Ion Channel. *Cell* *169*, 85-95 e88.
- Longa, C.S., Bruno, S.F., Pires, A.R., Romijn, P.C., Kimura, L.S., and Costa, C.H. (2011). Human herpesvirus 1 in wild marmosets, Brazil, 2008. *Emerg Infect Dis* *17*, 1308-1310.
- Luker, G.D., Bardill, J.P., Prior, J.L., Pica, C.M., Piwnicka-Worms, D., and Leib, D.A. (2002). Noninvasive bioluminescence imaging of herpes simplex virus type 1 infection and therapy in living mice. *J Virol* *76*, 12149-12161.
- Luker, K.E., Schultz, T., Romine, J., Leib, D.A., and Luker, G.D. (2006). Transgenic reporter mouse for bioluminescence imaging of herpes simplex virus 1 infection in living mice. *Virology* *347*, 286-295.
- Mahl, M.C., and Sadler, C. (1975). Virus survival on inanimate surfaces. *Can J Microbiol* *21*, 819-823.
- Marcorelles, P., Friocourt, G., Uguen, A., Lede, F., Ferec, C., and Laquerriere, A. (2014). Cystic fibrosis transmembrane conductance regulator protein (CFTR) expression in the developing human brain: comparative immunohistochemical study between patients with normal and mutated CFTR. *J Histochem Cytochem* *62*, 791-801.
- Mark, K.E., Wald, A., Magaret, A.S., Selke, S., Olin, L., Huang, M.L., and Corey, L. (2008). Rapidly cleared episodes of herpes simplex virus reactivation in immunocompetent adults. *J Infect Dis* *198*, 1141-1149.

- Mata, M., Chattopadhyay, M., and Fink, D.J. (2008). Gene Therapy for the Treatment of Diabetic Neuropathy. *Curr Diab Rep* 8, 431–436.
- Mätz-Rensing, K., Jentsch, K.D., Rensing, S., Langenhuyzen, S., Verschoor, E., Niphuis, H., and Kaup, F.-J. (2003). Fatal Herpes simplex Infection in a Group of Common Marmosets (*Callithrix jacchus*). *Vet Pathol* 40, 405-411.
- McGrath, J.A., O'Sullivan, A., Bennett, G., O'Toole, C., Joyce, M., Byrne, M.A., and MacLoughlin, R. (2019). Investigation of the Quantity of Exhaled Aerosols Released into the Environment during Nebulisation. *Pharmaceutics* 11, 1-9.
- Menotti, L., and Avitabile, E. (2020). Herpes Simplex Virus Oncolytic Immunovirotherapy: The Blossoming Branch of Multimodal Therapy. *Int J Mol Sci* 21, 1-26.
- Mertz, Gregory J. (2008). Asymptomatic Shedding of Herpes Simplex Virus 1 and 2: Implications for Prevention of Transmission. *The Journal of Infectious Diseases* 198, 1098-1100.
- Miller, C.S., and Danaher, R.J. (2008). Asymptomatic shedding of herpes simplex virus (HSV) in the oral cavity. *Review Oral Surg Oral Med Oral Pathol Oral Radiol Endod* 105, 43-50.
- Morales, F.R., Silveira, V., Damian, A., Higgin, R., and Pose, I. (2011). The possible additional role of the cystic fibrosis transmembrane regulator to motoneuron inhibition produced by glycine effects. *Neuroscience* 177, 138-147.
- Muller, K., Fuchs, W., Heblinski, N., Teifke, J.P., Brunberg, L., Gruber, A.D., and Klopffleisch, R. (2009). Encephalitis in a rabbit caused by human herpesvirus-1. *J Am Vet Med Assoc* 235, 66-69.
- National Health and Medical Research Council (2019). Australian Guidelines for the Prevention and Control of Infection in Healthcare (Canberra: Commonwealth of Australia, available online <https://www.nhmrc.gov.au/about-us/publications/australian-guidelines-prevention-and-control-infection-healthcare-2019#block-views-block-file-attachments-content-block-1>).
- National Health and Medical Research Council, Australian Research Council, and Universities Australia (2018). National Statement on Ethical Conduct in Human Research 2007 (Updated 2018) (Canberra: Commonwealth of Australia, available online <https://www.nhmrc.gov.au/about-us/publications/national-statement-ethical-conduct-human-research-2007-updated-2018>).
- Nazaroff, W.W. (2014). Indoor bioaerosol dynamics. *Indoor Air* 26, 61-78.
- Newgate Research (2019). Pets in Australia: A national survey of pets and people (Barton, ACT).
- Nishiyama, Y., Kimura, H., and Daikoku, T. (1991). Complementary lethal invasion of the central nervous system by nonneuroinvasive herpes simplex virus types 1 and 2. *J Virol* 65, 4520–4524.
- O'Grady, S.M. (2017). CFTR Involvement in Cell Migration and Epithelial Restitution. In *Progress in Understanding Cystic Fibrosis*, D. Sriramulu, ed. (London, UK: IntechOpen Limited), pp. 1-24.
- OGTR (2013). Risk Analysis Framework 2013, 4th edn (Canberra: Office of the Gene Technology Regulator, available online: <http://www.ogtr.gov.au/internet/ogtr/publishing.nsf/Content/raffinal5-toc>).
- Okinaga, S. (2000). Shedding of herpes simplex virus type 1 into tears and saliva in healthy Japanese adults. *Kurume Med J* 47, 273-277.

- Ostroumov, A., Simonetti, M., and Nistri, A. (2011). Cystic fibrosis transmembrane conductance regulator modulates synaptic chloride homeostasis in motoneurons of the rat spinal cord during neonatal development. *Dev Neurobiol* *71*, 253-268.
- Ostroumov, K., Grandolfo, M., and Nistri, A. (2007). The effects induced by the sulphonylurea glibenclamide on the neonatal rat spinal cord indicate a novel mechanism to control neuronal excitability and inhibitory neurotransmission. *Br J Pharmacol* *150*, 47-57.
- Papagerakis, S., Pannone, G., Zheng, L., About, I., Taqi, N., Nguyen, N.P., Matossian, M., McAlpin, B., Santoro, A., McHugh, J., *et al.* (2014). Oral epithelial stem cells - implications in normal development and cancer metastasis. *Exp Cell Res* *325*, 111-129.
- Peng, X., Wu, Z., Yu, L., Li, J., Xu, W., Chan, H.C., Zhang, Y., and Hu, L. (2012). Overexpression of cystic fibrosis transmembrane conductance regulator (CFTR) is associated with human cervical cancer malignancy, progression and prognosis. *Gynecol Oncol* *125*, 470-476.
- Public Health Agency of Canada (2011). Pathogen Safety Data Sheets: Infectious Substances – Herpes simplex virus, Public Health Agency of Canada, ed. (Public Health Agency of Canada,).
- Quirouette, C., Younis, N.P., Reddy, M.B., and Beauchemin, C.A.A. (2020). A mathematical model describing the localization and spread of influenza A virus infection within the human respiratory tract. *PLoS Comput Biol* *16*, e1007705.
- Rajcáni, J., and Durmanová, V. (2000). Early expression of herpes simplex virus (HSV) proteins and reactivation of latent infection. *Folia Microbiol (Praha)* *45*, 7-28.
- Ramchandani, M., Kong, M., Tronstein, E., Selke, S., Mikhaylova, A., Magaret, A., Huang, M.L., Johnston, C., Corey, L., and Wald, A. (2016). Herpes Simplex Virus Type 1 Shedding in Tears and Nasal and Oral Mucosa of Healthy Adults. *Sex Transm Dis* *43*, 756-760.
- Ramchandani, M.S., Jing, L., Russell, R.M., Tran, T., Laing, K.J., Magaret, A.S., Selke, S., Cheng, A., Huang, M.L., Xie, H., *et al.* (2019). Viral Genetics Modulate Orolabial Herpes Simplex Virus Type 1 Shedding in Humans. *J Infect Dis* *219*, 1058-1066.
- Reznikov, L.R., Dong, Q., Chen, J.H., Moninger, T.O., Park, J.M., Zhang, Y., Du, J., Hildebrand, M.S., Smith, R.J., Randak, C.O., *et al.* (2013). CFTR-deficient pigs display peripheral nervous system defects at birth. *Proc Natl Acad Sci U S A* *110*, 3083-3088.
- Ribes, J.A., Steele, A.D., Seabolt, J.P., and Baker, D.J. (2001). Six-year study of the incidence of herpes in genital and nongenital cultures in a central Kentucky medical center patient population. *J Clin Microbiol* *39*, 3321-3325.
- Robert, R., Savineau, J.P., Norez, C., Becq, F., and Guibert, C. (2007). Expression and function of cystic fibrosis transmembrane conductance regulator in rat intrapulmonary arteries. *European Respiratory Journal* *30*, 857-864.
- Roychoudhury, P., Swan, D.A., Duke, E., Corey, L., Zhu, J., Dave, V., Spuhler, L.R., Lund, J.M., Prlic, M., and Schiffer, J.T. (2020). Tissue-resident T cell-derived cytokines eliminate herpes simplex virus-2-infected cells. *J Clin Invest* *130*, 2903-2919.
- Royse, K.E., Zhi, D., Conner, M.G., Clodfelder-Miller, B., Srinivasasainagendra, V., Vaughan, L.K., Skibola, C.F., Crossman, D.K., Levy, S., and Shrestha, S. (2014). Differential Gene Expression Landscape of Co-Existing Cervical Pre-Cancer Lesions Using RNA-seq. *Front Oncol* *4*, 1-9.

- Ruseckaite, R., Ahern, A., Ranger, T., Dean, J., Gardam, M., Bell, S., and Burke, N. (2019). The Australian Cystic Fibrosis Data Registry Annual Report, 2017 (Monash University, Department of Epidemiology and Preventive Medicine), pp. 1-44.
- Saint-Criq, V., and Gray, M.A. (2017). Role of CFTR in epithelial physiology. *Cell Mol Life Sci* 74, 93-115.
- Schiffer, J.T., and Corey, L. (2013). Rapid host immune response and viral dynamics in herpes simplex virus-2 infection. *Nat Med* 19, 280-290.
- Schiffer, J.T., Swan, D., Al Sallaq, R., Magaret, A., Johnston, C., Mark, K.E., Selke, S., Ocbamichael, N., Kuntz, S., Zhu, J., *et al.* (2013). Rapid localized spread and immunologic containment define Herpes simplex virus-2 reactivation in the human genital tract. *Elife* 2, e00288.
- Schiffer, J.T., Swan, D.A., Prlic, M., and Lund, J.M. (2018). Herpes simplex virus-2 dynamics as a probe to measure the extremely rapid and spatially localized tissue-resident T-cell response. *Immunol Rev* 285, 113-133.
- Sedarati, F., Javier, R.T., and Stevens, J.G. (1988). Pathogenesis of a lethal mixed infection in mice with two nonneuroinvasive herpes simplex virus strains. *J Virol* 62, 3037–3039.
- Shivkumar, M., Lawler, C., Milho, R., and Stevenson, P.G. (2016). Herpes Simplex Virus 1 Interaction with Myeloid Cells In Vivo. *J Virol* 90, 8661-8672.
- Shivkumar, M., Milho, R., May, J.S., Nicoll, M.P., Efstathiou, S., and Stevenson, P.G. (2013). Herpes simplex virus 1 targets the murine olfactory neuroepithelium for host entry. *J Virol* 87, 10477-10488.
- Squier, C.A., and Kremer, M.J. (2001). Biology of the oral mucosa and esophagus. *J Natl Cancer Inst Monogr* 29, 7-15.
- Stalder, K., Vaz, P.K., Gilkerson, J.R., Baker, R., Whiteley, P., Ficorilli, N., Tatarczuch, L., Portas, T., Skogvold, K., Anderson, G.A., *et al.* (2015). Prevalence and Clinical Significance of Herpesvirus Infection in Populations of Australian Marsupials. *PLoS One* 10, e0133807.
- Tenser, R.B., and Edris, W.A. (1987). Trigeminal ganglion infection by thymidine kinase-negative mutants of herpes simplex virus after in vivo complementation. *Journal of Virology* 61, 2171-2174.
- Tenser, R.B., Ressel, S., and Dunstan, M.E. (1981). Herpes simplex virus thymidine kinase expression in trigeminal ganglion infection: correlation of enzyme activity with ganglion virus titer and evidence of in vivo complementation. *Virology* 112, 328-341.
- Than, B.L., Linnekamp, J.F., Starr, T.K., Largaespada, D.A., Rod, A., Zhang, Y., Bruner, V., Abrahante, J., Schumann, A., Luczak, T., *et al.* (2016). CFTR is a tumor suppressor gene in murine and human intestinal cancer. *Oncogene* 35, 4179-4187.
- Thiagarajah, J.R., and Verkman, A.S. (2012). CFTR inhibitors for treating diarrheal disease. *Clin Pharmacol Ther* 92, 287-290.
- Tischer, B.K., and Osterrieder, N. (2010). Herpesviruses - a zoonotic threat? *Vet Microbiol* 140, 266-270.
- Tu, Z., Chen, Q., Zhang, J.T., Jiang, X., Xia, Y., and Chan, H.C. (2016). CFTR is a potential marker for nasopharyngeal carcinoma prognosis and metastasis. *Oncotarget* 7, 76955-76965.
- Turcios, N.L. (2020). Cystic Fibrosis Lung Disease: An Overview. *Respir Care* 65, 233-251.
- Turner, R., Shehab, Z., Osborne, K., and Hendley, J.O. (1982). Shedding and Survival of Herpes Simplex Virus from 'Fever Blisters'. *Pediatrics* 70, 547-549.

- Umene, K., Yamanaka, F., Oohashi, S., Koga, C., and Kameyama, T. (2007). Detection of differences in genomic profiles between herpes simplex virus type 1 isolates sequentially separated from the saliva of the same individual. *J Clin Virol* 39, 266–270.
- van Velzen, M., Ouwendijk, W.J., Selke, S., Pas, S.D., van Loenen, F.B., Osterhaus, A.D., Wald, A., and Verjans, G.M. (2013). Longitudinal study on oral shedding of herpes simplex virus 1 and varicella-zoster virus in individuals infected with HIV. *J Med Virol* 85, 1669-1677.
- Victorian Trades Hall Council Occupational Health and Safety Unit (2020). Offices: Temperature and humidity - what are the 'rules'? (available online at <https://www.ohsrep.org.au/offices temperature and humidity - what are the rules>).
- Wang, Y., Jin, J., Wu, Z., Hu, S., Hu, H., Ning, Z., Li, Y., Dong, Y., Zou, J., Mao, Z., *et al.* (2018). Stability and anti-tumor effect of oncolytic herpes simplex virus type 2. *Oncotarget* 9, 24672-24683.
- Watson, G., Xu, W., Reed, A., Babra, B., Putman, T., Wick, E., Wechsler, S.L., Rohrmann, G.F., and Jin, L. (2012). Sequence and comparative analysis of the genome of HSV-1 strain McKrae. *Virology* 433, 528-537.
- Webre, J.M., Hill, J.M., Nolan, N.M., Clement, C., McFerrin, H.E., Bhattacharjee, P.S., Hsia, V., Neumann, D.M., Foster, T.P., Lukiw, W.J., *et al.* (2012). Rabbit and mouse models of HSV-1 latency, reactivation, and recurrent eye diseases. *J Biomed Biotechnol* 2012, 1-18.
- Weissenbock, H., Hainfellner, J.A., Berger, J., Kasper, I., and Budka, H. (1997). Naturally occurring herpes simplex encephalitis in a domestic rabbit (*Oryctolagus cuniculus*). *Vet Pathol* 34, 44-47.
- Whitsett, J.A. (2018). Airway Epithelial Differentiation and Mucociliary Clearance. *Ann Am Thorac Soc* 15, S143-S148.
- Wohlsein, P., Thiele, A., Fehr, M., Haas, L., Henneicke, K., Petzold, D.R., and Baumgartner, W. (2002). Spontaneous human herpes virus type 1 infection in a chinchilla (*Chinchilla lanigera f. dom.*). *Acta Neuropathol* 104, 674-678.
- World Health Organisation (2020). Herpes simplex virus (available online at <https://www.who.int/news-room/fact-sheets/detail/herpes-simplex-virus>).
- Wu, Z., Peng, X., Li, J., Zhang, Y., and Hu, L. (2013). Constitutive activation of nuclear factor kappaB contributes to cystic fibrosis transmembrane conductance regulator expression and promotes human cervical cancer progression and poor prognosis. *Int J Gynecol Cancer* 23, 906-915.
- Xia, X., Wang, J., Liu, Y., and Yue, M. (2017). Lower Cystic Fibrosis Transmembrane Conductance Regulator (CFTR) Promotes the Proliferation and Migration of Endometrial Carcinoma. *Med Sci Monit* 23, 966-974.
- Xu, J., Lin, L., Yong, M., Dong, X., Yu, T., and Hu, L. (2016). Adenovirus-mediated overexpression of cystic fibrosis transmembrane conductance regulator enhances invasiveness and motility of serous ovarian cancer cells. *Mol Med Rep* 13, 265-272.
- Xu, J., Yong, M., Li, J., Dong, X., Yu, T., Fu, X., and Hu, L. (2015). High level of CFTR expression is associated with tumor aggression and knockdown of CFTR suppresses proliferation of ovarian cancer in vitro and in vivo. *Oncol Rep* 33, 2227-2234.
- Ye, L., Zhu, W., Backx, P.H., Cortez, M.A., Wu, J., Chow, Y.H., McKerlie, C., Wang, A., Tsui, L.C., Gross, G.J., *et al.* (2011). Arrhythmia and sudden death associated with elevated cardiac chloride channel activity. *J Cell Mol Med* 15, 2307-2316.

Yeh, K.M., Johansson, O., Le, H., Rao, K., Markus, I., Perera, D.S., Lubowski, D.Z., King, D.W., Zhang, L., Chen, H., *et al.* (2019). Cystic fibrosis transmembrane conductance regulator modulates enteric cholinergic activities and is abnormally expressed in the enteric ganglia of patients with slow transit constipation. *J Gastroenterol* 54, 994-1006.

Zarei, S., Mirtar, A., Rohwer, F., Conrad, D.J., Theilmann, R.J., and Salamon, P. (2012). Mucus distribution model in a lung with cystic fibrosis. *Comput Math Methods Med* 2012, 1-8.

Zhang, W., Fujii, N., and Naren, A.P. (2012). Recent advances and new perspectives in targeting CFTR for therapy of cystic fibrosis and enterotoxin-induced secretory diarrheas. *Future Med Chem* 4, 329-345.

Zhao, M., Zhang, J., Huang, W., Dong, J., Guo, J., U, K.P., Weng, Z., Liu, S., Chan, H.C., Feng, H., *et al.* (2020). CFTR promotes malignant glioma development via up-regulation of Akt/Bcl2-mediated anti-apoptosis pathway. *Journal of Cellular and Molecular Medicine* 24, 7301-7312.

Zuo, Y., Whitbeck, J.C., Haila, G.J., Hakim, A.A., Rothlauf, P.W., Eisenberg, R.J., Cohen, G.H., and Krummenacher, C. (2019). Saliva enhances infection of gingival fibroblasts by herpes simplex virus 1. *PLoS One* 14, e0223299.

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

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

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

Submission	Summary of issues raised	Comment
1	The Regulator should further consider controls to limit exposure of people other than trial participants.	<p>Additional controls are discussed in Chapter 3, Sections 2 and 3.1 and imposed via licence conditions. These include that:</p> <ul style="list-style-type: none"> <li>• Trial participants must be seronegative for HSV 1 at the start of the trial, regularly monitored for acquisition of a primary infection thereafter, and offered oral anti-viral medication if they acquire an infection</li> <li>• The licence holder must provide the Regulator with detailed plans, specific to each clinical trial site, for: <ul style="list-style-type: none"> <li>➢ aerosol protection of staff administering the GMO; and</li> <li>➢ limiting exposure to the GMO of people not involved in the clinical trial</li> </ul> </li> <li>• Readily accessible surfaces in the treatment room must be decontaminated before it is reused for a different purpose.</li> <li>• Hygiene measures implemented by trial participants to minimise exposure of close contacts must be in place for 48 hours, and applied to both people and susceptible animals</li> </ul>
	The Regulator should consider whether further information about overexpression of the CFTR gene could be included in the RARMP	Additional information about conditions associated with CFTR overexpression and hyper-activation has been included in Section 4.2 of the RARMP.
	The Committee agrees that all plausible risk scenarios have been identified.	Submission has been noted.
2	The Council has no objection to the proposed clinical trial. They note that their Regional Landfill is licenced to receive Category 1 and Category 2 Regulated Waste. Transport of such waste may require Waste Tracking and the use of a licenced Transporter; these would be the responsibility of the waste producer.	Submission has been noted.



Submission	Summary of issues raised	Comment
3	Overall, the application poses negligible risks to the health and safety of people and the environment. We are satisfied that the measures taken to manage the short and long term risks from the proposal are adequate.	Submission has been noted.
4	<p>The Council has no objections to the licence being issued. Comments from members related to risk included that:</p> <ul style="list-style-type: none"> <li>• It is a very new and effectively untried approach to gene therapy, and as such has risk potential that may not be apparent in the absence of previous clinical trials</li> </ul> <hr/> <ul style="list-style-type: none"> <li>• An aerosol is being produced with minimal aerosol controls at the time of administration. <ul style="list-style-type: none"> <li>– a nebuliser is proposed for administration possibly over a long time period (30 minutes for GMO administration);</li> <li>– wiping down surfaces post-procedure does not appear to be an effective control of the aerosolised GMO;</li> <li>– the ‘closed room’ implies it is a regular room with no special sealing treatments or furnishings.</li> </ul> </li> </ul> <p>Additional control through negative air pressure with HEPA filtration to remove the GMO from room air, and requiring a number of air changes before use of the room for other purposes (possibly accomplished during the 60 minutes post administration). Without additional controls it appears to me the agent could leak from the room into the surrounds exposing others unwittingly.</p>	Submission has been noted.
5	The Committee noted that the GMO is replication-defective and the proposed limits and controls would minimise unintended exposure. They support the conclusion that the dealings pose negligible risk of harm to human health and the environment.	Submission has been noted.
6	In relation to the proposal to monitor trial participants for signs of active HSV-1 infection and treat with oral antiviral medication if this occurs, does the applicant not know whether or not administering the GMO will result in	Administering the GMO is not expected to result in active HSV-1 infection, as the GMO is replication-defective. The intent of this precaution is to monitor for obvious signs of active wild-type HSV-1 infection, which could lead to HSV-1/GMO recombination. This has been clarified in Section 2.3.2.

Submission	Summary of issues raised	Comment
	<p>patients presenting with an active infection of HSV-1?</p> <p>Regarding the proposal to administer the GMO by nebulisation, this should be undertaken in room with negative pressure, such as a PC2 laboratory.</p>	<p>Discussion of the effectiveness of proposed controls has been included in Chapter 3. Additional licence conditions require that licence holder provide the Regulator with a detailed plan, specific to each clinical trial site, for aerosol protection of staff administering the GMO. Relevant staff must follow this plan.</p> <p>The licence holder must also develop, in consultation with each clinical trial site, a plan to limit exposure to the GMO of people not involved in the clinical trial.</p>
	<p>Regarding recombination, the clear risk is that the GMO recombines with wild-type virus in the people who receive the treatment. This is acknowledged by the proposal to exclude individuals with discernible herpes lesions at Screening and Day 0 from the trial, however many herpesvirus reactivation events are asymptomatic i.e. virus is actively produced (replicating) and shed without visible lesions. In view of this information, the submission poses the following questions:</p>	
	<ul style="list-style-type: none"> <li>• Does the GMO have an active thymidine kinase (TK) gene? TK renders herpesviruses susceptible to nucleoside analogue drugs, which can be used topically and systemically. If a TK negative GMO were to regain replication competence through recombination, it would be resistant to nucleoside analogue drugs.</li> </ul>	<p>Submission has been noted.</p>
	<ul style="list-style-type: none"> <li>• Has the applicant considered assessing the HSV-1 status of enrolling patients using diagnostic testing, rather than visual assessments? Noting that many reactivation events are subclinical (i.e. asymptomatic), using lesions as an indicator of reactivation seems a very cursory assessment. A qPCR test (or similar) on a swab would enable a more accurate assessment of current infection – the highest risk for recombination occurring between the GMO and wild-type viruses.</li> </ul>	<p>Limiting the trial to seronegative participants was proposed in the consultation RARMP and included in draft licence conditions. Diagnostic testing and exclusion of people seropositive for HSV-1 is imposed in the licence.</p>
	<ul style="list-style-type: none"> <li>• Will immunocompromised patients be excluded from the trial? This is less of an issue than if the GMO were replication competent. However, immune compromised patients would have higher viral loads of wild-type</li> </ul>	<p>Licence conditions require that HSV-1 seropositive patients be excluded from the trial. Further exclusion of immune-compromised patients is not considered necessary as no participants should have a reservoir of latent HSV-1.</p>

Submission	Summary of issues raised	Comment
	virus, and low immune function is a risk for viral reactivation, hence creates a higher risk of recombination.	
7	<p>The RARMP states that bio-distribution studies indicate the GMO does not travel beyond the site of transduction and is unlikely to enter the blood. However, bio-distribution studies of intra-tumoural administration of a similar GM HSV-1 treatment in humans (DIR 132, issued in 2015) observed viral DNA in blood and urine with clearance within two weeks.</p> <p>Any relevant data on shedding and biodistribution of HSV-1 from the commercial release of DIR 132 should be included in the RARMP and the uncertainty should be noted (Chapter 2, section 3) regarding shedding duration, potential increased shedding or different bio-distribution due to the administration route and whether 24 hours is sufficient to minimise shedding and transmission to close contacts.</p> <p>Behavioural controls proposed by the applicant may be insufficient to prevent transmission of any replication recombinants that form shortly after treatment with the GMO. The host range, exposure and transmission to animals should be discussed in risk scenario 2 and the uncertainty regarding both domesticated and native animals should be noted in Chapter 2, Section 3.</p> <p>There are multiple reports of fatal HSV-1 infections in several non-human animal species. While it is agreed that certain domesticated animals (cats, dogs, horses) are not at risk, other animals (rabbits and rodents) may be infected and it is unknown whether native animals can be infected. Animals such as pet rabbits and rodents, and native animals, may be close contacts of trial participants.</p> <p>Recommend extending controls intended to minimise contact with and transmission to close contacts to include animals that may be in close contact with trial participants.</p> <p>Viral recombination can lead to altered characteristics such as increased virulence and altered host range. When</p>	<p>The GMO authorised by DIR-132 is an attenuated HSV-1 modified to selectively replicate in rapidly dividing cells, such as those found in tumours. This selectivity directs the GMO's cytotoxic action to tumours, where it replicates and causes cell lysis, releasing viral progeny into the surrounding tissue and circulation. In contrast, the GMO described in this application is replication defective and is not expected to spread beyond the tissues initially exposed and transduced. Data on shedding and biodistribution of the GMO licenced under DIR-132 are therefore not relevant to this application.</p> <p>The risks discussed in scenario 2 have been managed by requiring that trial participants be seronegative for HSV-1 at the start of the trial, and regularly monitored for acquisition of a primary infection thereafter. Oral anti-viral medication is to be offered should this occur. In the absence of wild-type HSV-1, there is minimal likelihood of recombinants forming and impacting on other individuals of any species.</p> <p>Additional information regarding HSV-1 infection of animals has been included in Chapter 1, Section 3.4 and the risk scenarios. The risks to animals posed by recombination between the GMO and wild-type HSV-1 are discussed and estimated in risk scenario 2. As noted above, risk treatment imposed to manage the risks arising from this scenario will protect both humans and animals. Controls intended to minimise trial participant contact with and transmission to close contacts will also protect susceptible animals.</p> <p>These reports have been included in Chapter 1, Section 3.7 as examples of reconstitution of virulence through recombination.</p>

Submission	Summary of issues raised	Comment
	<p>discussing recombination risk, the RARMP should include reports concerning two avirulent HSV strains that generated lethal recombinants in mice, and recombination of two attenuated poultry vaccines that restored virulence and produced a fitness advantage with severe consequences.</p>	
	<p>It is unclear if participants will be tested for seronegative status using blood antibody tests or just screened for visible lesions and then assumed to be seronegative. The absence of lesions will not suffice to conclude seronegativity as asymptomatic HSV can occur in seropositive people. Considering the small number of trial participants, it is recommended that they are tested using a blood antibody test to confirm seronegativity and the absence of wild type HSV1 in potentially asymptomatic participants.</p>	<p>Draft licence conditions in the consultation RARMP required that participants be seronegative for HSV-1 at the start of the trial, and regularly monitored thereafter for acquisition of a primary infection. The licence has been amended to clarify that seronegativity must be demonstrated by diagnostic antibody testing, and that trial participants must be assessed by diagnostic testing for the presence of HSV-1 primary infection at follow-up visits to the clinical trial site.</p>