Australian Government
Department of Health and Ageing
Office of the Gene Technology Regulator

Risk Assessment and Risk Management Plan for **DIR 116**

Limited and controlled release of genetically modified live viral vaccines against prostate cancer

Applicant: PPD Australia Pty Ltd

5 October 2012

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Executive Summary

Introduction

The Gene Technology Regulator (the Regulator) has made a decision to issue a licence in respect of licence application (DIR 116) from PPD Australia Pty Ltd (PPD). The licence authorises dealings involving the limited and controlled release of genetically modified (GM) live viral vaccines against prostate cancer.

The *Gene Technology Act 2000* (the Act), the Gene Technology Regulations 2001 and corresponding state and territory law govern the comprehensive and highly consultative process undertaken by Gene Technology Regulator (the Regulator) before making a decision whether to issue a licence to deal with a genetically modified organism (GMO).

The decision is based upon a Risk Assessment and Risk Management Plan (RARMP) prepared by the Regulator in accordance with requirements of the legislation. RARMPs apply the *Risk Analysis Framework* and are finalised following consultation with a wide range of experts, agencies and authorities, and the public[1].

The application

PPD has applied for a licence for dealings involving the intentional release into the Australian environment of two genetically modified (GM) vaccines for the treatment of prostate cancer on a limited scale and under controlled conditions.

The GM candidate vaccines are based on *Vaccinia virus* vaccine strain New York City Board of Health (NYCBH) and *Fowlpox virus* vaccine strain POXVAC-TC, which have each been modified to contain the same four human genes. Expression of these genes is expected to induce immune responses against the *prostate-specific antigen* (PSA) and to stimulate the immune system to attack and destroy cancer cells expressing PSA.

The trial in Australia would form part of an international clinical trial involving 1200 patients in approximately 22 countries. The purpose of the trial is to evaluate the effectiveness of the viral vaccines in treating prostate cancer. The trial is proposed to take place in specified hospitals and health care facilities in ACT, NSW, QLD, SA, VIC and WA. Once underway the trial is expected to be completed within five years.

The applicant proposed a number of control measures to restrict exposure to the GM vaccines that were considered during the evaluation of this application.

Confidential Commercial Information

Some information, including details of the genetic construct used to create the GMOs and unpublished data from previous clinical have been declared Confidential Commercial Information (CCI) under section 185 of the Act. The confidential information was made available, in accordance with section 187 of the Act, to the prescribed experts and agencies consulted on the RARMP for this application.

Risk assessment

The risk assessment took into account information in the application (including proposed containment measures), relevant previous approvals and current scientific knowledge. Advice

[1] More information on the process for assessment of licence applications to release a genetically modified organism (GMO) into the environment is available from the Office of the Gene Technology Regulator (OGTR) (Free call 1800 181 030 or at <http://www.ogtr.gov.au/>), and in the Regulator's *Risk Analysis Framework* (OGTR 2009) at <http://www.ogtr.gov.au/internet/ogtr/publishing.nsf/Content/riskassessments-1>.

relating to risks to human health and safety and the environment provided in submissions received during consultation on the RARMP was also considered. No new risks to people or the environment were identified from the advice received on the consultation RARMP.

Initially, potential pathways that might lead to harm to people or the environment as a result of gene technology are postulated (risk scenarios), and those that warrant detailed characterisation are determined. This process is described as risk identification.

Seven risk scenarios were postulated, including consideration of whether or not expression of the introduced genes could result in products that are toxic or allergenic to people or other organisms; alter characteristics that may impact on the disease burden due to the GM viruses; or produce unintended changes in viral characteristics. The opportunity for gene flow to other organisms, and its effects if it were to occur, was also assessed.

A risk is only identified for further assessment when a risk scenario is considered to have some chance of causing harm. Pathways that do not lead to an adverse outcome, or could not reasonably occur, do not advance in the risk assessment process.

The characterisation of the seven risk scenarios in relation to both the seriousness and likelihood of harm, in the context of the control measures proposed by the applicant and considering both the short and the long term, did not give rise to any identified risks that required further assessment.

Any risks of harm to the health and safety of people, or the environment, from the proposed release of the GM viral vaccines into the environment are assessed to be negligible.

Risk management plan

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 evaluates and treats identified risks, evaluates controls and limits proposed by the applicant, and considers general risk management measures. The risk management plan is given effect through licence conditions.

As none of the seven risk scenarios characterised in the risk assessment give rise to an identified risk that requires further assessment, the level of risk from the proposed dealings is assessed to be negligible. The Regulator's *Risk Analysis Framework* defines negligible risks as insubstantial, with no present need to invoke actions for their mitigation in the risk management plan. However, conditions have been imposed to restrict exposure to the GMOs and its genetic material in the environment and to limit the trial to the size and locations proposed in the application as these were important considerations in establishing the context for assessing the risks.

The licence conditions require PPD to limit the dealings to suitable adult male participants at clinical facilities between October 2012 and December 2017. The control measures include administration of the GM vaccines by trained staff, containment provisions at the clinical site, educating trial participants in injection site bandaging and care, destroying GM vaccines not required for further studies; transporting the GM vaccines in accordance with the Regulator’s transport guidelines and other specific conditions.

Conclusions of the RARMP

The risk assessment concluded that the limited and controlled release of GM virus to take place in hospitals in ACT, NSW, QLD, SA, VIC and WA, involving up to 1200 trial participants and expected to run for up to five years, poses negligible risks to the health and safety of people or the environment as a result of gene technology.

The risk management plan concluded that these negligible risks do not require specific risk treatment measures. However, licence conditions have been imposed to limit the trial in size, locations and duration, and to require controls in line with those proposed by the applicant, as these were important considerations in establishing the context for assessing the risks.

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Abbreviations

ACT	Australian Capital Territory
the Act	*Gene Technology Act 2000*
APVMA	Australian Pesticides and Veterinary Medicines Authority
BHK21	immortalised baby hamster kidney cell line number 21
CCI	Confidential Commercial Information as declared under section 185 of *the Gene Technology Act 2000*
CD	Cluster of Differentiation
CTLA-4	Cytotoxic T-Lymphocyte Antigen 4 (also known as CD152)
CTN	Clinical trial notification
CTX	Clinical trial exemption
DAFF	Department of Agriculture, Fisheries and Forestry
DIR	Dealings Involving intentional Release
DNIR	Dealings Not Involving intentional Release
DNA	Deoxyribonucleic Acid
EEV	external enveloped virus
fowlpox	*Fowlpox virus*
FSANZ	Food Standards Australia New Zealand
GM	Genetically Modified
GM-CSF	Granulocyte macrophage-colony stimulating factor
GMO	Genetically Modified Organism
GTTAC	Gene Technology Technical Advisory Committee
HGT	Horizontal Gene Transfer
HIV	*Human immunodeficiency virus*
HREC	Human Research Ethics Committee
IATA	International Air Transport Authority
ICAM-1	Intercellular adhesion molecule-1 (also known as CD54)
ICH	International Conference on Harmonisation
ICH-GCP	International Conference on Harmonisation Good Clinical Practice standard.
IL	Interleukin
IMV	internal mature virus
ITR	inverted terminal repetitions
kb	kilobase(s)
LFA-1	Leukocyte function associated antigen-1 (also known as CD11a/CD18)
LFA-3	Leukocyte function associated antigen-3 (also known as CD58)
LGA	Local government area
Mac-1	macrophage adhesion ligand-1 (also known as Integrin alpha M (ITGAM), complement receptor 3 (CR3) and CD11b/CD18)
ml	Millilitre
mRNA	Messenger Ribonucleic Acid
NHMRC	National Health and Medical Research Council
NSW	New South Wales
NICNAS	National Industrial Chemicals Notification and Assessment Scheme
NYCBH	New York City Board of Health
OGTR	Office of the Gene Technology Regulator
PC2	Physical containment level 2
PCR	Polymerase Chain Reaction
PPD	PPD Australia Pty Ltd

PROSTVAC-F	Recombinant *Fowlpox virus* that expresses human genes encoding B7.1, LFA-3, ICAM-1 and modified PSA (also referred to in this document as GM fowlpox)
PROSTVAC-V	Recombinant *Vaccinia virus* that expresses human genes encoding B7.1, LFA-3, ICAM-1 and modified PSA (also referred to in this document as GM vaccinia)
PSA	prostate-specific antigen
QLD	Queensland
Q-PCR	Quantitative Polymerase Chain Reaction
RARMP	Risk Assessment and Risk Management Plan
the Regulations	Gene Technology Regulations 2001
the Regulator	Gene Technology Regulator
REV	*Reticuloendotheliosis virus*
RNA	Ribonucleic acid
SA	South Australia
TGA	Therapeutic Goods Administration
TNFα	Tumour necrosis factor alpha
TRICOM	Triad of co-stimulatory molecules
USA	United States of America
UV	Ultra violet
VIC	Victoria
VIG	Vaccinia hyperimmune gamma-globulin
vaccinia	*Vaccinia virus*
WA	Western Australia
WHO	World Health Organisation

Technical Summary

Introduction

The Gene Technology Regulator (the Regulator) has made a decision to issue a licence in respect of licence application (DIR 116) from PPD Australia Pty Ltd (PPD). The licence authorises dealings involving the limited and controlled release of genetically modified (GM) live viral vaccines against prostate cancer.

The *Gene Technology Act 2000* (the Act), the Gene Technology Regulations 2001 and corresponding state and territory law govern the comprehensive and highly consultative process undertaken by the Gene Technology Regulator (the Regulator) before making a decision whether or not to issue a licence to deal with a genetically modified organism (GMO).

The decision is based upon a Risk Assessment and Risk Management Plan (RARMP) prepared by the Regulator in accordance with requirements of the legislation. RARMPs apply the *Risk Analysis Framework* and are finalised following consultation with a wide range of experts, agencies and authorities, and the public[2].

The application

PPD has applied for a licence for dealings involving the intentional release into the Australian environment of two genetically modified (GM) vaccines for the treatment of prostate cancer on a limited scale and under controlled conditions.

The GM vaccines are based on *Vaccinia virus* vaccine strain New York City Board of Health (NYCBH) and *Fowlpox virus* vaccine strain POXVAC-TC, which have each been modified to contain the same four human genes. Expression of these genes is expected to induce immune responses against the *Prostate-Specific Antigen* (PSA) and to stimulate the immune system to attack and destroy cancer cells expressing PSA.

The trial in Australia would form part of an international clinical trial involving 1200 patients in approximately 22 countries. The purpose of the trial is to evaluate the effectiveness of the viral vaccines in treating prostate cancer. The trial is proposed to take place in hospitals and health care facilities in ACT, NSW, QLD, SA, VIC and WA. Once underway the trial is expected to be completed within five years.

The applicant proposed a number of control measures to restrict exposure of non-trial participants to the GM virus. These controls have been considered during the evaluation of the application.

Confidential Commercial Information

Some details, including vector maps and unpublished results from related clinical trials have been declared Confidential Commercial Information (CCI) under section 185 of the Act. The confidential information was made available, in accordance with section 187 of the Act, to the prescribed experts and agencies consulted on the RARMP for this application.

Risk assessment

The risk assessment took into account information in the application (including proposed containment measures), relevant previous approvals and current scientific/technical knowledge. Advice relating to risks to human health and safety and the environment provided in submissions

[2] More information on the process for assessment of licence applications to release a genetically modified organism (GMO) into the environment is available from the Office of the Gene Technology Regulator (OGTR) (Free call 1800 181 030 or at <http://www.ogtr.gov.au/>), and in the Regulator's *Risk Analysis Framework* (OGTR 2009) at <http://www.ogtr.gov.au/internet/ogtr/publishing.nsf/Content/riskassessments-1>.

received during consultation on the RARMP was also considered. No new risks to people or the environment were identified from the advice received on the consultation RARMP.

Initially, potential pathways that might lead to harm to people or the environment as a result of gene technology are postulated (risk scenarios), and those that warrant detailed characterisation are determined. This process is described as risk identification.

Seven risk scenarios were postulated, including consideration of whether or not expression of the introduced genes could result in products that are toxic or allergenic to people or other organisms; alter characteristics that may impact on the disease burden due to the GM virus; or produce unintended changes in its characteristics. The opportunity for gene transfer to other organisms, and its effects if this were to occur, was also assessed.

A risk is only identified for further assessment when a risk scenario is considered to have some chance of causing harm. Pathways that do not lead to an adverse outcome, or could not reasonably occur, do not advance in the risk assessment process.

The characterisation of the seven risk scenarios in relation to both the seriousness and likelihood of harm, in the context of the control measures proposed by the applicant and considering both the short and the long term, did not give rise to any identified risks that required further assessment. The principal reasons for this include:

- Transmission to the environment of the two GM viruses via viral shedding during the trial will be minimised through:
  - the participant exclusion criteria; the route of inoculation (subcutaneous); bandaging of the injection site and appropriate training of both healthcare workers and patients (in the case of vaccinia); and
  - the nature of the virus (in the case of fowlpox).
- No increase in disease severity due to the introduction of the four human genes has been observed in previous clinical trials.
- The products of the four introduced genes are not expected to be toxic to humans or other animals, due to their widespread presence in the environment.

Risks to the health and safety of people, or the environment, from the proposed release of the GM virus into the environment are assessed to be negligible.

Risk management plan

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 evaluates and treats identified risks, evaluates controls and limits proposed by the applicant, and considers general risk management measures. The risk management plan is given effect through licence conditions.

As none of the seven risk scenarios characterised in the risk assessment give rise to an identified risk that requires further assessment, the level of risk from the proposed dealings is assessed to be negligible. The Regulator's *Risk Analysis Framework* defines negligible risks as insubstantial, with no present need to invoke actions for their mitigation in the risk management plan. However, a range of licence conditions have been imposed to restrict exposure to the GMO, to limit the proposed trial size and locations proposed in the application, as these were important considerations in establishing the context for assessing the risks.

Licence conditions

The Regulator has imposed a number of licence conditions, including requirements to:

- limit the trial to a maximum of 1200 trial participants inoculated with the GM viruses at designated clinical facilities

- restrict exposure of at-risk individuals by specific exclusion criteria
- restrict trial participation to people who have previously received a vaccinia vaccination
- restrict the method of inoculation of GM Vaccinia to subcutaneous inoculation
- ensure that inoculations be performed by trained nurses and/or physicians at clinical facilities in accordance with standard universal precautions and ICH-GCP[3], and that appropriate personal protective equipment is worn.
- store and transport all GM vaccines in accordance with relevant regulations and guidelines[4]
- dispose of all waste generated in the clinic, as well as patient waste following GM Vaccinia inoculation, in accordance with standard clinical waste disposal practices.

Other regulatory considerations

Australia's gene technology regulatory system operates as part of an integrated legislative framework that avoids duplication and enhances coordinated decision making. The Regulator is responsible for assessing risks to the health and safety of people and the environment associated with the use of gene technology. However, dealings conducted under a licence issued by the Regulator may also be subject to regulation by other agencies that also regulate GMOs or GM products including Food Standards Australia New Zealand (FSANZ), Australian Pesticides and Veterinary Medicines Authority (APVMA), Therapeutic Goods Administration (TGA), National Industrial Chemicals Notification and Assessment Scheme (NICNAS) and Department of Agriculture, Fisheries and Forestry (DAFF) Biosecurity[5].

TGA is responsible for human safety assessment of the participants in clinical trials. The applicant has notified the TGA of the trial. Each trial site will also notify the TGA through the Clinical Trial Notification (CTN) Scheme. The Regulator sought advice from TGA during the assessment of this licence application.

Identification of issues to be addressed for future releases

Additional information has been identified that may be required to assess an application for a large scale or commercial release of the GM vaccines, or to justify a reduction in containment conditions. This includes the potential shedding of GM vaccinia from trial subjects.

Conclusions of the RARMP

The risk assessment concluded that this limited and controlled release of GM vaccines to take place in hospitals in ACT, NSW, QLD, SA, VIC and WA, involving up to 1200 trial participants and expected to run for up to five years, poses negligible risks to the health and safety of people or the environment as a result of gene technology.

The risk management plan concluded that these negligible risks do not require specific risk treatment measures. However, licence conditions have been imposed to limit the trial in size, locations and duration, and to require controls in line with those proposed by the applicant, as these were important considerations in establishing the context for assessing the risks.

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[3] The International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human use, guidelines for good clinical practice (ICH 1996), as annotated by TGA (http://www.tga.gov.au/industry/clinical-trials-note-ich13595.htm).

[4] The Gene Technology Regulator's *Guidelines for the Transport, Storage and Disposal of GMOs;* IATA Transportation Regulations

[5] More information on Australia's integrated regulatory framework for gene technology is contained in the *Risk Analysis Framework* (OGTR 2009) available from the Office of the Gene Technology Regulator (OGTR). Free call 1800 181 030 or at <http://www.ogtr.gov.au/internet/ogtr/publishing.nsf/Content/riskassessments-1>.

Chapter 1 Risk context

Section 1 Background

1. This chapter describes the parameters within which risks to the health and safety of people or the environment by the proposed release are assessed (Figure 1).

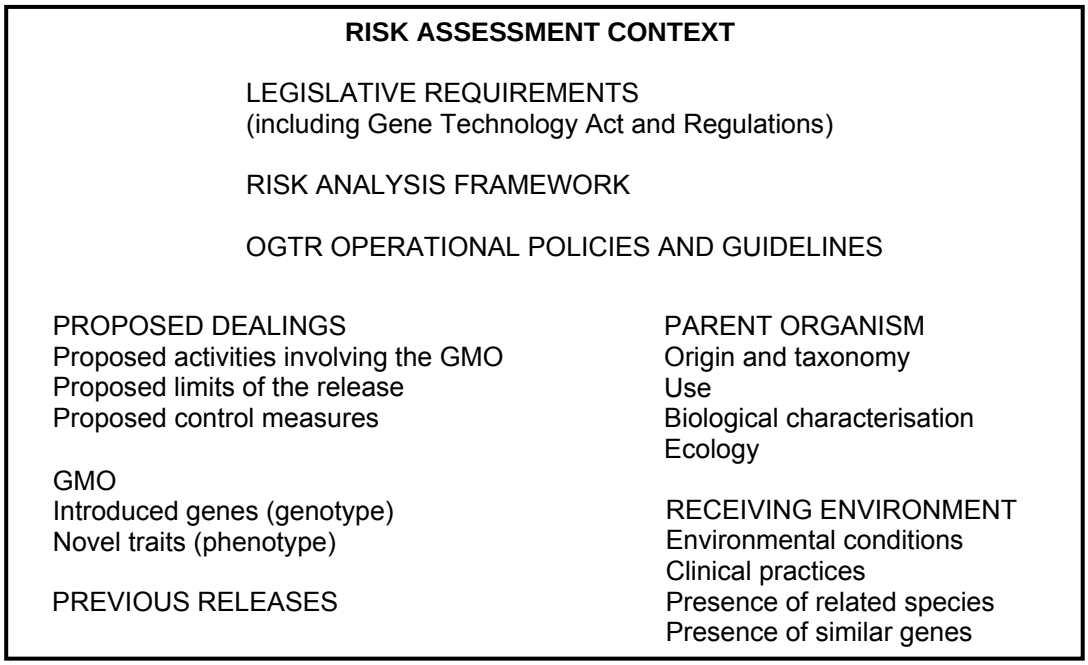

Figure 1. Parameters used to establish the risk assessment context

2. The risk context is developed within the framework of the *Gene Technology Act 2000* (the Act) and Gene Technology Regulations 2001 (the Regulations, Section 2), the Regulator's *Risk Analysis Framework* (OGTR 2009) and operational policies and guidelines available at the OGTR website <http://www.ogtr.gov.au>

3. In addition, establishing the risk assessment context for this application includes consideration of:

- scope and boundaries – interaction with other regulatory schemes (Section 3)
- the proposed dealings (Section 4)
- the parent organism (Section 5)
- the genetically modified organisms (GMOs), nature and effect of the genetic modification (Section 6)
- the receiving environment (Section 7)
- previous releases of these or other GMOs relevant to this application (Section 8)

Section 2 The legislative requirements

4. Sections 50, 50A and 51 of the Act outline the matters which the Gene Technology Regulator (the Regulator) must take into account, and with whom he must consult, in preparing the Risk Assessment and Risk Management Plans (RARMPs) that form the basis of his decisions on licence applications. In addition, the Regulations outline matters the Regulator must consider when preparing a RARMP.

5. In accordance with section 50A of the Act, the Regulator considered information provided in the application and was satisfied that its principal purpose is to enable the applicant to conduct experiments. In addition, limits have been proposed on the size, locations and duration of the trial

and controls have been proposed by the applicant to restrict spread and persistence of the GMO that could lead to exposure of people and the environment to the GMO. Those limits and controls are such that the Regulator considered it appropriate not to seek the advice referred to in subsection 50(3) of the Act. Therefore, this application is considered to be a limited and controlled release and the Regulator has prepared a RARMP for this application.

1. Section 52 of the Act requires the Regulator to seek comment on the RARMP from the States and Territories, the Gene Technology Technical Advisory Committee (GTTAC), Commonwealth authorities or agencies prescribed in the Regulations, the Minister for the Environment, local council(s) where the trial is proposed to take place, and the public. The advice from the prescribed experts, agencies and authorities, and how it was taken into account, is summarised in Appendix A. A summary of the submission received from a member of the public, and how it was taken into account, is at Appendix B.

6. Section 52(2)(ba) of the Act requires the Regulator to decide whether one or more of the proposed dealings may pose a 'significant risk' to the health and safety of people or to the environment, which then determines the length of the consultation period as specified in section 52(2)(d). The decision is provided in Chapter 2, Section 3 of this RARMP.

Section 3 Scope and boundaries

7. Gene technology legislation operates in conjunction with other regulatory schemes in Australia. 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 registered on the Australian Register of Therapeutic Goods. The Therapeutic Goods Administration (TGA) is responsible for administering the provisions of this legislation. Clinical trials usually involve the use of therapeutic products that are experimental and under development, prior to a full evaluation and assessment, and require approval from TGA through the Clinical Trial Exemption (CTX) scheme or the Clinical Trial Notification (CTN) scheme.

8. Where the clinical trial may involve a GMO, TGA has primary regulatory responsibility for patient safety. However, authorisation is also required under gene technology legislation. In order to avoid duplication of regulatory oversight the Regulator is responsible for assessing risks posed to other people who may be involved in the dealings and risks to the environment.

9. The International Conference on 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. The guideline was developed with consideration of the current good clinical practices of the European Union, Japan, and the United States, as well as those of Australia, Canada, the Nordic countries and the World Health Organization (WHO). TGA has adopted the ICH-GCP in principle as *Note for Guidance on Good Clinical Practice* (designated CPMP/ICH/135/95). It provides overarching guidance for conducting clinical trials in Australia.

10. The NHMRC has issued the *National Statement on the Ethical Conduct in Research Involving Humans*. This document sets the Australian standard against which all research involving humans is reviewed.

11. Approval by a Human Research Ethics Committee (HREC) is also a fundamental requirement of a clinical trial. HRECs conduct both an ethical and a 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.

Section 4 The proposed dealings

12. PPD Australia Pty Ltd (PPD) proposes to release two viral vaccines which have each been modified to contain the same four human genes. The two vaccines will be used for the treatment of prostate cancer. Expression of the genes by the vaccines is expected to induce cell mediated immune responses against the prostate-specific antigen (PSA) and is intended to stimulate the immune system to attack and destroy cancer cells expressing PSA.

13. This proposed trial would form part of an international clinical trial involving 1200 patients in approximately 22 countries. The title of the clinical trial is '*A Randomized, Double-blind, Phase 3 Efficacy Trial of PROSTVAC-V/F ± GM-CSF in Men With Asymptomatic or Minimally Symptomatic Metastatic, Castrate-Resistant Prostate Cancer*'. The purpose of the trial is to evaluate the effectiveness of the GM viral vaccines in treating prostate cancer. PPD is seeking approval for dealings associated with the Australian arm of the trial.

14. The dealings involved in the proposed intentional release would include:

- importing the GMOs
- conducting experiments with the GMOs
- transporting the GMOs
- disposing of the GMOs

and possession, storage, supply or use of the GMOs for the purposes of any of the above.

15. These dealings are described in more detail throughout the remainder of the current chapter.

16. Some details of the application including vector maps and unpublished results from related clinical trials have been declared Confidential Commercial Information (CCI) under section 185 of the Act. This information was considered during the preparation of the RARMP and was made available to the prescribed expert groups and authorities consulted on the application.

4.1 The proposed activities

17. The applicant has stated that the objective of the proposed clinical trial is to investigate the efficacy of the vaccines when used alone or in conjunction with a purified protein, granulocyte macrophage-colony stimulating factor (GM-CSF). Secondary objectives of the study are to investigate safety and tolerability of the GM vaccines.

18. The GM vaccines proposed for release would be imported from the USA and transported to a central storage and distribution site at Flinders Clinical Trials Services, Adelaide, South Australia, before being transported to the clinical trial sites.

19. Each patient will receive seven separate inoculations, consisting of an initial inoculation with the GM *Vaccinia virus* (GM vaccinia) followed by six inoculations with the GM *Fowlpox virus* (GM fowlpox), over a period of five months. The inoculations would involve subcutaneous injections of a 0.5 ml dose drawn immediately before use from a single dose vial.

20. All of the inoculations will be conducted by trained staff and be undertaken at clinical facilities. The applicant has yet to finalise the trial site locations. A list of seven sites has been provided in Table 1, although more sites may be added later.

Table 1 Proposed localities for release of the GM vaccines

Clinical Facility	Local Government Area	Locality
Ashford Cancer Centre Research	City of West Torrens	Kurralta Park, SA
Calvary Mater Newcastle	Newcastle City Council	Waratah, NSW
The Geelong Hospital	Greater Geelong City Council	Geelong, VIC
Princess Alexandra Hospital	Brisbane City Council	Woolloongabba, QLD
Redcliffe Hospital	Moreton Bay Regional Council	Redcliffe, QLD
St John of God Hospital	City of Subiaco	Subiaco, WA
Sydney Haematology and Oncology Clinics - Sydney Adventist Hospital	The Council of the Shire of Hornsby	Wahroonga, NSW

21. Once a trial participant has completed the vaccination regime, they will be expected to return to the clinical facility for follow up once every six months, until twelve months after the final patient enrolled in the clinical trial has received all seven inoculations.

4.2 The proposed limits of the dealings (size, location and duration)

22. The trial is proposed to take place at seven clinical facilities located in the local government areas listed in the table above, from October 2012 until the final patient has received the full course of vaccinations (seven injections over five months; estimated to be December 2017). The applicant intends to enrol a total of 1200 men world wide of which one third would receive both vaccines, one third would receive both vaccines in combination with GM-CSF and the final third would be assigned to control group which would receive a placebo consisting of seven doses of the parent *Fowlpox virus* in combination with GM-CSF placebo.

4.3 The proposed controls to restrict the spread and persistence of the GMOs and their genetic material in the environment

23. The applicant has proposed a number of controls to restrict exposure to the GM vaccines and the introduced genetic material including:

- excluding participants who have not previously been inoculated with the *Vaccinia virus* as a smallpox vaccine
- excluding patients who have the following conditions or who will have close household contact with people with the following conditions, for a period of 21 days after the initial (GM vaccinia) inoculation:
  - persons with a history of, or active eczema or other eczematoid skin disorders;
  - persons with other acute, chronic or exfoliative skin conditions (e.g. burns, impetigo, chicken pox, severe acne or other open rashes or wounds) until the condition resolves;
  - pregnant or nursing women;
  - children less than three years of age; and
  - immunodeficient or immunosuppressed persons (by disease or therapy), including those with HIV infection
- excluding clinical staff who have an immunodeficiency, are taking immunosuppressive drugs, have active or chronic eczema or skin conditions that cause skin damage, or those that are pregnant or breastfeeding
- ensuring that clinical staff involved in the study are qualified by education, training and experience, and are encouraged to follow the guidelines set forth by the United States' Centers for Disease Control as contained in the Study Protocol
- assuming responsibility for the proper conduct of the trial according to the guidelines outlined in ICH-GCP
- instructing clinical staff responsible for administering the GMO, collection of clinical specimens, or clinical evaluation of study participants, to follow the World Health

Organisation *Universal precautions for the prevention of transmission of infectious agents in healthcare settings* (World Health Organisation 2007)

- storing the GM vaccines in an outer package in a secure location with access limited to clinical staff participating in the study
- transporting the vaccine to the clinical site according to the Regulator's *Guidelines for the Transport, Storage and Disposal of GMOs*
- placing used study vaccine syringes into locked containers or sealed bags immediately after use and retaining them for accountability
- destroying used study vaccine syringes after reconciliation at the clinical site following institutional procedures for the disposal of biohazardous material
- discarding clinical waste generated during the study into appropriate biohazard containers and disposing of the waste at the clinical site following institutional procedures for the disposal of biohazardous material
- instructing patients on how to change the inoculation site dressing following the GM vaccinia injection. This includes depositing waste in sealable biohazard bags that will be returned to the clinic for destruction following institutional procedures for the disposal of biohazardous material
- exporting unused study vaccine to the USA (returned to the suppliers) or disposing of it at the clinical site following institutional procedures for the disposal of biohazardous material.

24. An overarching document, the Investigator's Brochure, details procedures and practices, inclusion and exclusion criteria, informed consent, monitoring, auditing, reporting and recordkeeping and other governance and administrative requirements for the study. The Principal Investigator and clinical staff at each site would be responsible for recording clinical information regarding the trial, including the location and date where the GM vaccines were administered.

25. Written informed consent from each trial participant would be required for participation in the trial. This would be monitored by the relevant HREC.

26. The study would be monitored on a regular basis throughout the study period by a Safety Monitoring Committee, including compliance with procedures and record keeping, the study protocol, handling of the vaccine and clinical samples, collection of informed consent and safety reporting according to HREC requirements.

27. These controls and the limits outlined in Chapter 1, Section 4.2 have been taken into account in establishing the risk assessment context (this chapter), and their suitability for containing the proposed release is evaluated in Chapter 3, Section 4.1.2.

Section 5 The parent organisms

28. The GM vaccines are based on *Vaccinia virus* vaccine strain New York City Board of Health Vaccine (NYCBH) and *Fowlpox virus* vaccine strain POXVAC-TC; which have each been modified to contain the same four human genes. These attenuated vaccine strains were developed through artificial selection processes and do not exist naturally in the environment. As such, a discussion of the parent organisms carried out at the species level, rather than strain level, is required to inform the risk assessment.

5.1 *Vaccinia virus* and *Fowlpox virus* taxonomy

29. The parent organisms *Vaccinia virus* (vaccinia) and *Fowlpox virus* (fowlpox) are double stranded, DNA viruses of the Family *Poxviridae* (ICTV 2009). The taxonomy of vaccinia and fowlpox are outlined in Table 2. The family *Poxviridae* includes two subfamilies, *Chordopoxvirinae* (pox viruses of vertebrate animals) and *Entomopoxvirinae* (pox viruses of invertebrates), and eleven genera which are primarily differentiated by their host species.

Table 2 Taxonomy of Vaccinia virus and Fowlpox virus

	Vaccinia virus	*Fowlpox virus*
Family	*Poxviridae*	*Poxviridae*
Subfamily	*Chordopoxvirinae*	*Chordopoxvirinae*
Genus	Orthopoxvirus	Avipoxvirus
Species	*Vaccinia virus*	*Fowlpox virus*
Strain	*Vaccinia virus* strain NYCBH	*Fowlpox virus* strain POXVAC-TC

30. The genus *Orthopoxvirus* includes the human pathogens *Variola virus* (smallpox) and *Vaccinia virus*, as well as *Monkeypox virus, Cowpox virus* (cowpox), *Ectromelia virus* (mousepox) and others. The genus *Avipoxvirus* includes bird pathogens such as *Fowlpox virus, Canarypox virus, Pigeonpox virus, Quailpox virus, Turkeypox virus* and others. However, it should be noted that the human pathogen known as Chickenpox (or chicken pox) is caused by the *Varicella zoster virus* and is not a member of the *Poxviridae*.

31. *Fowlpox virus* was the first *Avipoxvirus* to be identified and is considered the prototype virus for the genus. Early scientific literature often used the term fowlpox to refer to all pox-viruses of birds, rather than the species of virus now known as *Fowlpox virus* (Buller & Palumbo 1991). Therefore, care needs to be taken when examining the early references to fowlpox to determine whether the information presented is general to all *Avipoxvirus*, specific to fowlpox or concerns an unspecified bird pox-virus. This is especially important when determining the host specificity of fowlpox.

5.2 Distribution and transmission

5.2.1 Vaccinia virus

32. *Vaccinia virus* is a mild human pathogen that was used extensively in the smallpox eradication program. The natural host and origins of vaccinia are not known and it is also unknown as to when and how vaccinia replaced cowpox as the vaccine for smallpox (Henderson et al. 2008; Lefkowitz et al. 2006; Marennikova et al. 2005; Smith 2007).

33. Vaccinia was first identified in 1939 (Downie 1939) and was initially thought to be a variant of cowpox that was modified through serial transmission in humans. However, sequence analysis has shown that they are distinct, though closely related species (Hendrickson et al. 2010). Vaccinia was also thought to be a strain of smallpox which had been attenuated through propagation in bovine tissue samples (in parallel with the cowpox strains) during the smallpox vaccine production process. Phylogenetic sequence analysis suggests smallpox is closer to *Camelpox virus* and *Taterapox virus* (isolated from a west African rodent, Kemp's gerbil) (Hughes et al. 2010), whereas vaccinia is more closely related to cowpox (Hendrickson et al. 2010). Another early theory was that vaccinia is a naturally occurring hybrid between smallpox and cowpox. Artificial recombinants of smallpox and cowpox were produced that had characteristics similar to vaccinia (Bedson & Dumbell 1964a; Bedson & Dumbell 1964b). However, this hypothesis is not supported by the sequence analysis of the three viruses.

34. Vaccinia can infect and cause disease in a range of mammals including humans, mice, rabbits, cattle, horses and buffalo. Outbreaks of pox virus in buffalos (buffalopox) have been seen following smallpox vaccination campaigns in India and Brazil (Baxby & Hill 1971). Sequence analysis of the causative agent suggests that it is a variant of vaccinia. Buffalopox is now recognised as a subspecies of vaccinia. Buffalopox has continued to circulate after the smallpox vaccination campaign stopped, and there is no record of buffalopox occurring as a disease in buffalos prior to the smallpox vaccination campaigns. Buffalopox can cause mild disease in humans similar to vaccinia (Campos et al. 2011; Singh et al. 2006; Yadav et al. 2010). Several vaccinia-like isolates have also been isolated from mice and other rodents in the same areas. It has yet to be determined whether the buffalo is the primary host of buffalopox or whether they are acquiring the disease from rodent populations (da Fonseca et al. 2002). Similarly, vaccinia-like viruses have also been isolated from horses (Campos et al. 2011) and cattle in Brazil (de Souza Trindade et al. 2003; de Souza

Trindade et al. 2007; Leite et al. 2012). *Cantagalo virus*, an emerging poxvirus disease effecting cattle handlers in Rio de Janeiro, has also been shown to have significant sequence similarity to vaccinia and may represent another instance of vaccinia becoming endemic following the smallpox eradication campaign (Damaso et al. 2000).

35. Vaccinia is transmitted between humans through direct contact with a pustule or inoculation site (including sexual and sporting contacts) or contact with something that has been in direct contact with the inoculation site (e.g. towels, sheets, clothes, bandages) (Centers for Disease Control and Prevention 2004; 2009; Egan et al. 2004; Ferreira et al. 2008; Isaacs 2004; Lewis et al. 2006; MacNeil et al. 2009; Moussatche et al. 2003; Neff et al. 2002; Sepkowitz 2003; Young et al. 2011).

36. Average transmission rates from historical smallpox vaccination campaigns are reported as two to six per one hundred thousand vaccinations (Neff et al. 2002). However, the majority of contacts potentially exposed at that time would have been immune or have had previous exposure to vaccinia due to ongoing vaccination campaigns. Therefore, not every exposure would have resulted in observable infection. While it would be reasonable to expect a higher transmission rate today among the predominantly unvaccinated population (Neff et al. 2002), a recent report estimates that, among people vaccinated between 2003 and 2011, the rate of transmission from vaccinees to non-vaccinees was 5.4 per 100,000 vaccinees (Wertheimer et al. 2012). Reports of accidental infection (which includes self-infection at sites other than the site of inoculation prior to immune seroconversion) show transmission rates could be as high as one in one thousand (Andreev et al. 1969).

5.2.2 Fowlpox virus

37. *Fowlpox virus* is a pathogen of chickens (*Gallus gallus*), but may also infect turkeys (*Meleagris gallopavo*) and cause an asymptomatic infection in pigeons (family *Columbidae*) (Barthold et al. 2011; Siddique et al. 2011). *Avipoxvirus* showing high sequence homology to fowlpox has recently been isolated in New Zealand from the New Zealand Variable Oystercatcher (*Haematopus unicolor*), North Island Saddleback (*Philesturnus carunculatus rufusater*), and Shore Dotterel or Shore Plover (*Thinornis novaeseelandiae*) (Ha et al. 2011).

38. *Fowlpox virus* is currently considered endemic in Australia (Boyle et al. 1997; Diallo et al. 1998; French & Reeves 1954) along with other avipox viruses (Annuar et al. 1983; Harrigan et al. 1975). Commercial chicken flocks are usually vaccinated at the first sign of an outbreak, but may be vaccinated soon after hatching in areas where outbreaks are common.

39. Fowlpox is transmitted via mechanical vectors, primarily by species of mosquitoes. Transmission occurs when the mosquito feeds on an infected bird and then feeds on a susceptible uninfected bird. Fowlpox does not replicate inside the mosquito, instead virus particles contained in the blood meal, or on the mosquitoes proboscis, remain viable and can be transmitted for more than a fortnight after feeding on an infected bird (French & Reeves 1954; Kligler & Ashner 1929; Kligler et al. 1929).

40. Fowlpox can also be transmitted by direct contact between infected and susceptible birds. The virus is transmitted through abraded or broken skin or the conjunctiva (mucous membrane covering the anterior surface of the eyeball). Indirect transmission of fowlpox can also occur via ingestion when food and water sources, feeders, perches, cages, or clothing are contaminated with virus-containing scabs shed from the lesions of an infected bird. Indirect transmission can also occur via inhalation of pox-virus infected dander, feather debris and air-borne particles (Barthold et al. 2011; Boyle 2007; Tripathy 2008).

41. Replication of fowlpox in mammalian cells has been investigated in detail. Fowlpox viral particles may enter mammalian cells but do not result in a productive viral infection. Non-productive infection was demonstrated in monkey and human cells *in vitro*; as well as in cat, dog, rabbit, rat and cattle, *in vivo* (Taylor et al. 1988). Investigation of the molecular pathways involved

in the infection of monkey and human cultured cells demonstrated that viral early gene expression and DNA replication were able to occur, but late gene expression was reduced and the production of viral particles stalled (Somogyi et al. 1993).

42. There has been a single report of fowlpox replication in the mammalian cell line known as Baby Hamster Kidney 21 (BHK21) (Weli et al. 2005). Although this is suggestive of the ability to replicate in mammalian cells, this cell line is well known to be very susceptible to viral infection and is used for the culture of a number of avian viruses which do not otherwise replicate in mammalian cells (Folk et al. 1981; Huhtamo et al. 2007; Macpherson & Stoker 1962; Otsuki et al. 1979). Therefore, this can be attributed to unique properties of the immortalised cell line, and should not be taken as representative of mammalian cells in general.

43. Similarly there have been reports of fowlpox being isolated from a pox lesion on a rhinoceros. The rhinoceros was known to be terminally ill, and was suffering from other opportunistic pathogens at the time, indicating it was immunocompromised. It is also reported that the rhinoceros was being medicated with cortisone which may have further interfered with its immune system. Characterisation of the virus was unable to determine whether it was fowlpox or another, then unidentified, *Avipoxvirus* (Grunberg & Burtscher 1968; Mayr & Mahne 1970).

5.3 *Vaccinia virus* and *Fowlpox virus* genomic organisation

44. The genomes of vaccinia and fowlpox consist of a double stranded DNA molecule of between 165 and 213 kilobases (kb) and 266 to 289 kb in length respectively, encoding between 200 and 300 genes (Viral Bioinformatics Resource Center 2012). There appears to be considerable variation in genome length for both viruses with isolates found to contain large genomic deletions, multi-gene families made up of varying numbers of closely related genes, as well as areas of repeated sequences of up to 10kb in size. Genes in both viruses are encoded on both the positive and negative strand and in multiple open reading frames. Generally speaking, poxvirus genes tend to occur in blocks and are transcribed in the direction of the nearest end of the genome. Typically the more conserved genes, those involved in vital virus functions, are found towards the centre of the genome, while more variable genes, such as those involved in host interactions, are found towards the ends of the genome (Moss 2007). Around fifty genes have been identified that are present in all poxviruses sequenced so far, and another forty or so are present in all members of the *Chordopoxvirinae* (Lefkowitz et al. 2006).

45. Both poxviruses have inverted terminal repetitions (ITRs), which consist of identical, but oppositely oriented sequences at the two ends of the genome. These ITRs include:

- an A/T rich hairpin loop that connects the two DNA strands;
- a highly conserved region involved in DNA replication;
- variable lengths of short tandemly repeated sequences; and
- open reading frames.

46. Both poxvirus genomes contain a number of genes involved in modifying the virus-host interaction. This includes receptors for, and homologues of, host immunomodulatory genes (Johnston & McFadden 2003; Johnston & McFadden 2004; Spriggs 1996).

5.4 Poxvirus life cycle

47. The life cycle of a virus involves the transmission of infective viral particles to a host organism, recognition, attachment and entry into the host cells and then replication of viral nucleic acid and protein production, followed by assembly and release of infective virus (see Figure 2). Viruses have co-evolved with their host species and are generally specific for that host organism and infect only certain tissue types within that organism.

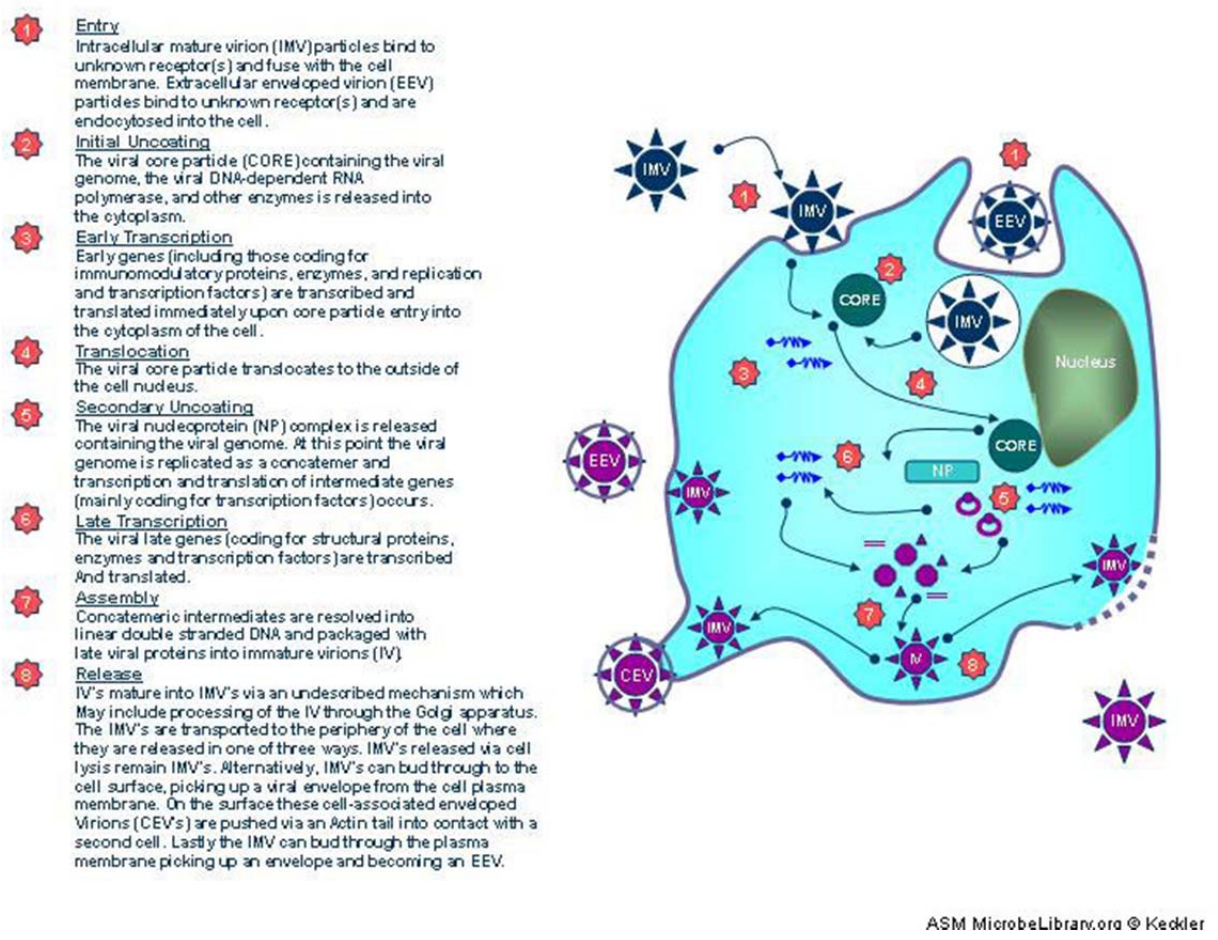

Figure 2. Steps in the replication cycle of poxviruses (Shannon Keckler - American Society for Microbiology Microbe Library - Creative Commons)

48. Poxviruses replicate entirely within the cytoplasm of the infected cell. This means they are unable to use host replication enzymes and therefore must encode their own enzymes for RNA synthesis including their own multi-subunit RNA polymerase and gene-specific transcription factors.

49. Genes are expressed in three temporal classes, early, intermediate and late genes which have their own associated transcription factors. The viral core contains the entire machinery to start transcription of early genes, whereas expression of intermediate and late genes occurs post-DNA replication and needs *de novo* RNA and protein synthesis. Transcription factors for intermediate genes have early promoters and transcription factors for late genes have intermediate promoters while late promoters control expression of early transcription factors which are then packaged into the mature virus particle for use following entry into a new cell. Such a cascade mechanism allows temporal regulation of the gene expression pattern.

50. Poxvirus early genes can be detected within minutes of virus entry into the cell and are primarily involved in DNA replication and other host-virus interactions. Expression of intermediate genes begins around the time that the expression of the early genes reaches their peak. The majority of intermediate genes characterised so far are transcription factors needed for expression of the late genes. Expression of the late genes typically starts around two and a half to three hours after infection and focuses on the remaining genes necessary for virus production and assembly. There is considerable overlap in expression of the intermediate and late genes, with some intermediate genes continuing to be expressed throughout the late phase (reviewed in Moss 2007).

5.5 Pathology of viral infection

5.5.1 Vaccinia virus

51. Vaccination with *Vaccinia virus* typically occurs through scarification with a two-pronged needle dipped in virus. The skin is mildly abraded, or scratched, in order to break the skin surface and allow a small number of virus particles to penetrate. Infection typically results in the formation of a single pustule (pock) at the vaccination site around three to five days later, accompanied by low grade fever and mild swelling and tenderness in the draining lymph node. Symptoms may also include headache, muscle pain, chills and nausea. This pustule reaches its maximum size after eight to ten days. A virus filled scab forms over the pustule and falls off after 14 to 21 days leaving a recognisable vaccine scar.

52. A number of adverse reactions to vaccinia vaccination have been recorded. These include eczema vaccinatum (approximately thirteen cases per million), progressive vaccinia (approximately one case per million), generalised vaccinia (approximately forty cases per million) and postvaccinal encephalitis (approximately three cases per million) (Aragon et al. 2003). These are discussed further below.

53. Eczema vaccinatum, in which pre-existing eczema or dermatitis conditions have reduced the effectiveness of the skin barrier in protecting against vaccinia infection. The skin becomes widely infected with vaccinia, possibly from a viremia or direct contact. The pustules typically follow the same progression as the primary vaccination site. However, confluent or erosive lesions can occur, accompanied by fever and swelling and tenderness in the draining lymph node, and affected persons are frequently systemically ill. Prior to the availability of vaccinia hyperimmune gamma-globulin (VIG) (purified human antibodies to vaccinia), this condition had a high mortality; establishing the diagnosis early and treating with VIG is crucial in reducing mortality.

54. Progressive vaccinia, which occurs when the immune system is unable to resolve the initial infection due to immunosuppression or immunodeficiency. This can lead to secondary lesions on the body; the lesions can become necrotic, secondary infection may ensue, and the patient can become septic. The condition is considered rare, severe and is often fatal.

55. Generalised vaccinia, in which the viral infection becomes systemic, and pustules appear in locations other than the vaccination site, but is resolved by the immune system in the usual 21 day time frame.

56. Postvaccinal encephalitis typically develops between eight and fifteen days after vaccination and is characterised by fever, vomiting, headache, malaise, and anorexia. This is followed by disorientation, drowsiness and may result in convulsions, coma and death in up to 28% of postvaccinal encephalitis cases. Survivors may experience long term neurological sequelae.

57. In an attempt to avoid the above known adverse advents, contraindications for vaccination include:

- those with existing or previous eczema, atopic dermatitis, or other skin conditions;
- immune deficiency disorders or immunosuppression;
- existing disorders of the central nervous system;
- allergies to the components of the vaccine; and
- pregnant women.

58. Accidental infection can occur when virus is transmitted from the vaccination site to other parts of the body or to another individual through physical contact with the patient or with an item used by the patient to tend to their vaccination site. In most cases this results in an infection course similar to intentional vaccination. However, as the amount and location of inoculation is not controlled the number of pustules formed can vary (reviewed in Henderson et al. 2008; Kretzschmar et al. 2006; Vellozzi et al. 2005).

59. Treatments for vaccinia infection can include VIG and the antiviral drug Cidofovir (1(S)[3-hydroxy-2-(phosphonomethoxy)propyl]cytosine) (De Clercq 2002; Quenelle et al. 2004).

60. Vaccinia, and particularly buffalopox, can also cause disease in buffalo, horses and cattle. In the mild form, lesions are localized on the udder, teats and groin, and the base and inner surface of the ear and eyes. In the severe form, the lesions are generalized and can be found anywhere on the skin surface. However, generalized forms of the disease are infrequent these days as the lesions are mostly confined to the udder, teats, and sometimes on the thighs and hindquarters of the affected animals. Infection in milk animals leads to mastitis, frequently due to secondary bacterial infections, which contributes to reduction in milk yield and the working capacity of draft animals. Severe cases of mastitis can result in a permanent reduction in milk yield (Singh et al. 2007).

5.5.2 Fowlpox virus

61. Fowlpox is a commercially significant disease of chickens and turkeys. The disease can take two forms, which typically result from the mode of transmission.

62. Where transmission occurs through mechanical transfer, such as direct contact with lesions, pecking, fighting, or insect bite, the viral infection is usually concentrated in the skin and forms infectious lesions or papules on the comb, wattles, around the beak and occasionally on the legs and feet. This disease is known as the cutaneous form (dry pox), is rarely lethal and is usually resolved in around three weeks. However, it can affect the bird's laying ability and predispose the bird to other infections.

63. Where transmission occurs through the inhalation of infectious droplets, the resulting viral infection is usually concentrated in the mucous membranes of the mouth, pharynx, larynx and sometimes in the trachea. This is known as the diptheritic form (wet pox) and can result in significant mortality (up to 50%) where the lesions coalesce to form a necrotic pseudo-membrane which can restrict breathing resulting in asphyxiation (Barthold et al. 2011; Boyle 2007; Tripathy 2008).

64. Integration of *Reticuloendotheliosis virus* (REV) sequences has been observed in the genome of *Fowlpox virus*. While most field strains of fowlpox contain REV provirus, most vaccine strains have only remnants of long terminal repeats. Virulence is enhanced by the presence of REV provirus in the genome of field strains of fowlpox virus (Awad et al. 2010; Diallo et al. 1998; Hertig et al. 1997; Tripathy 2008).

5.6 Poxvirus environmental stability

65. Poxviruses are well known for their ability to persist in the environment. Clothes, bedding and personal effects from smallpox patients are known to have remained contagious after several years of storage or use. *Vaccinia virus* particles contained in dried samples such as scabs, skin flakes and dried blood have been shown to remain viable for more than 35 weeks at 4°C with no loss of infectivity. Survival times decrease at higher temperatures or high humidity. However, vaccinia in scabs remains viable for more than eight weeks at 35°C. Vaccinia can also persist for more than two weeks on food samples in the fridge (4°C) and more than 166 days is storm water. One in one thousand virus particles stored frozen (-20°C) remain viable after 15 years (reviewed in Essbauer et al. 2007; Rheinbaben et al. 2007). Vaccinia has also been shown to be shed in mouse faeces where it can remain viable for 20 days or more (Abrahão et al. 2009).

66. Purified samples of virus are less stable than those found in association with host cells and proteins. Purified samples of fowlpox and vaccinia are inactivated within 1 minute when using the following disinfectants: 70% ethanol, 50% isopropyl alcohol, 0.5% sodium hypochlorite, 30% formaldehyde, 10% benzalkonium chloride, a mixture of 6.67% cetyltrimethylammonium chloride and 3.33% benzalkonium chloride, and a mixture of 1.75% iodine and 10% polyethyleneglycol nonylphenyl ether (Chambers et al. 2009). However, scabs containing vaccinia placed in a chemical disinfecting suspension were decontaminated after 90 minutes with glutaraldehyde 2%,

formaldehyde 2%, Lysoformin 2% or 3%, phenol 5% and chloramine T 2%, and 3 hours treatment with some alcohols (ethylalcohol 80%, isopropylalcohol 7%, n-propylalcohol 60%), Amocid 5% and formaldehyde 1%. Vaccinia samples on hands were disinfected by chloramine T (1.5%) or isopropylalcohol (70%) in 2 to 5 minutes (Schumann & Grossgebauer 1977), and showed 99.99% reduction in titre from a 30 second hand wash in disinfectants containing greater than 75% ethanol (Kampf et al. 2007).

67. Vaccinia is also susceptible to UV irradiation. However, in dried scabs and blood smears a small population (10% or less) of the total viral population shows significant resistance to inactivation, remaining detectable at low titres for many months (Sagripanti & Lytle 2011). Vaccinia also appears to be relatively resistant to iodine, temperature, drying and pH (reviewed in Rheinbaben et al. 2007).

5.7 *Vaccinia virus* vaccine strain NYCBH

68. NYCBH is a strain of vaccinia which was chosen by the New York City Board of Health as the smallpox vaccine to be used in the United States of America which was developed from seed virus from England in 1856. Marketed by Wyeth as Dryvax™, it was given to approximately fourteen million people per year during the smallpox eradication program, consisting of children, international travellers, health care workers and the military (Parrino & Graham 2006).

69. Examination of historical data suggests the NYCBH had the lowest rate of adverse events of all the strains used in the smallpox eradication program, with a death rate of around 1.4 per million vaccinations (Kretzschmar et al. 2006).

70. Analysis of Dryvax™ revealed a mixed population of vaccinia strains (Osborne et al. 2007). It is not known whether the initial NYCBH strain was also a mixed pool, or whether the variation has resulted from the derivation and manufacturing process (Kretzschmar et al. 2006; Nalca & Zumbrun 2010).

71. After inoculation NYCBH induces a brief, self-limiting infection as characterised in Chapter 1, Section 5.5.1.

5.8 *Fowlpox virus* vaccine strain POXVAC-TC

72. The parental fowlpox virus used for the GM fowlpox was plaque-purified from a vial of a poultry vaccine, POXVAC-TC, which was manufactured by Schering-Plough Corporation (which has since been acquired by Intervet Pty Ltd, now known as MSD Animal Health). POXVAC-TC was marketed as suitable for wing web inoculation of day old chicks. It is unclear whether any of the fowlpox vaccines currently registered by Intervet Australia Pty Ltd (MSD Animal Health Australia) are the same as the POXVAC-TC strain acquired from Schering-Plough Corporation.

73. The starting material for the production of POXVAC-TC was a vial of Vineland Laboratories' chicken embryo origin Fowlpox vaccine. The virus was passaged twice on the chorioallantoic membrane of chicken eggs to produce a master seed virus. The master seed virus was passaged 27 additional times in chicken embryo fibroblasts to prepare the POXVAC-TC master seed. To prepare virus stocks for the generation of POXVAC-TC product lots, the POXVAC-TC master seed was passaged twice on chicken embryo fibroblasts.

Section 6 The GMOs, nature and effect of the genetic modification

74. The adaptive immune system (consisting of white blood cells and antibodies) is capable of responding to new and novel microbial pathogens, as well as remembering pathogens it is has seen in the past. It does this by recognising small pieces of nucleic acid, polysaccharides or proteins known as antigens, which are found on (or in) the invading pathogen, or on the surface of cells infected with the pathogen.

75. The adaptive immune system must also be able to ignore antigens that belong to the host. This is known as self-tolerance. Self-tolerance is necessary so that a person’s immune system doesn’t

attack their own cells in a process known as autoimmunity. Self-tolerance is also one of the reasons why the immune system doesn't automatically attack tumours, as they are usually covered only in self-antigens.

76. The target antigen encoded by the two GM vaccines is the human protein prostate-specific antigen (PSA), which is found naturally in the prostate and is highly expressed in prostate cancer. As this protein is naturally found in humans, the immune system ignores it as a self-antigen. The GM vaccines, and the vaccine regime, have been designed specifically to try and break self-tolerance to this antigen. In the absence of self-tolerance the immune system would attack cells expressing this protein, and therefore, attack the prostate cancer cells.

77. 'Prime Boost' is a process whereby the same antigen (in this case PSA) is presented to the immune system in two different ways (in this case in vaccinia then fowlpox). This leads to an immune response that is specific to the common antigen (PSA) and is much greater than that produced by showing the immune system the antigen in either of the vaccines alone.

78. Additionally, both GM vaccines include the human proteins B7.1 (CD80), intercellular adhesion molecule-1 (ICAM-1 or CD54), and leukocyte function-associated antigen-3 (LFA-3 or CD58). These proteins are known as co-stimulatory molecules and help with the development of an immune response. When present on a cell's surface, these molecules help an antigen presenting cell to bind to an immune cell. This aids in the process known as activation whereby a previously inactive, or naïve, immune cell changes to become one which actively seeks out and destroys cells expressing its target antigen.

79. The initial inoculation with GM vaccinia leads to an active infection whereby the GM virus invades the host's cells and replicates within them. During replication the introduced human genes are expressed. The four human proteins are then present on the surface of the infected cell, along with some virus proteins. As the infection progresses, more cells are infected resulting in the presentation of high levels of antigen (both vaccinia specific proteins and PSA) to the immune system over a period of one to two weeks, substantially increasing the potential for immune stimulation. The immune response specific to vaccinia then eliminates the GM virus and the infected cells.

80. As mentioned above, fowlpox does not replicate in mammalian cells, but some gene expression does occur. Infection with fowlpox results in the infected cells producing the four human proteins, increasing the potential immune response to PSA. However, the immune response specific to vaccinia is not triggered as no vaccinia proteins are present in the GM fowlpox. As fowlpox does not actively replicate it does not generate a strong fowlpox specific immune response, and so the GM vaccine can be administered multiple times.

81. By treating the patients with vaccinia and then six carefully timed doses of fowlpox, all encoding PSA, it is anticipated that the GM vaccines will lead to an increased immune response to PSA.

6.1 Introduction to the GMOs

82. As discussed above, the GM vaccine viruses are based on *Vaccinia virus* and *Fowlpox virus* that have been genetically modified by the introduction of a gene encoding human PSA, which is intended to act as an antigenic target for the immune response. The PSA introduced into the two GM vaccines has been intentionally modified by the change of one amino acid (at position 155) from isoleucine to leucine. This was done to enhance the ability of the primary antigen in this protein to bind one of the most common T cell receptors. This has the effect of increasing the immunogenicity of the protein and its ability to induce high levels of T cell activation (Terasawa et al. 2002).

83. The GM vaccines have also been genetically modified to encode three human immunological molecules B7.1, ICAM-1 and LFA-3. These molecules are intended to attract immune cells to the

site of infection and stimulate the specific type of immune response necessary for the effective clearance of prostate cancer tumour cells. *Vaccinia virus* promoters will drive expression of all four introduced human genes in both viruses. Table 3 (below) lists the genes inserted into the parent organism.

Table 3 The genes used to alter the antigenic properties of the poxviruses

Gene	Full name	Function of protein	Intended purpose
PSA	Prostate-Specific Antigen	Liquefies semen allowing sperm to swim freely	Elicit an immune response against tumour cells expressing PSA
B7.1 (CD 80)	-	Provides a costimulatory signal necessary for T cell activation and survival	Enhance the immune response to PSA
ICAM-1 (CD 54)	Intercellular Adhesion Molecule-1	Aids in the binding of an immune cell to an antigen presenting cell	Enhance the immune response to PSA
LFA-3 (CD 58)	Leukocyte Function-Associated Antigen-3	Increases adhesion between T cells and antigen presenting cell and is involved in the regulation of T cell responses	Enhance the immune response to PSA

6.2 The introduced genes, their encoded proteins and their associated effects

84. Four full gene sequences have been used for the genetic modifications. The purpose of these modifications is to stimulate an immune response against the human protein PSA, which is expressed at a high level in prostate cancer cells. If successful, this will enable the immune system to target and attack prostate cancer cells.

6.2.1 Prostate-Specific Antigen

85. Prostate-Specific Antigen (PSA) is a glycoprotein secreted by the epithelial cells of the prostate gland. PSA is a neutral serine protease with biochemical attributes that are similar to the proteases involved in blood clotting. PSA is produced for the ejaculate, where it liquefies semen in the seminal coagulum and allows sperm to swim freely. It is also believed to be instrumental in dissolving cervical mucus, allowing the entry of sperm into the uterus (Balk et al. 2003).

86. PSA is found in female ejaculate at concentrations roughly equal to that found in male semen (Zaviacic & Ablin 2000). Other than semen and female ejaculate, the greatest concentrations of PSA in biological fluids are detected in breast milk and amniotic fluid (Yu & Diamandis 1995). Low concentrations of PSA have been identified in the urethral glands, endometrium, normal breast tissue and salivary gland tissue (Diamandis & Yu 1997). PSA also is found in the serum of women with breast, lung, or uterine cancer and in some patients with renal cancer (Black et al. 2000; Clements et al. 1997).

6.2.2 Intercellular Adhesion Molecule-1

87. Intercellular Adhesion Molecule 1 (ICAM-1) also known as CD54 (Cluster of Differentiation 54) is continuously present in low concentrations in the membranes of T cells and cells that line blood vessels. ICAM-1 expression can be induced by inflammatory cytokines released early on in the immune response.

88. ICAM-1 binds to macrophage adhesion ligand-1 (Mac-1), leukocyte function associated antigen-1 (LFA-1), and fibrinogen. When expressed at high levels ICAM-1 can facilitate the migration of T cells out of the blood vessels and towards the site of an infection. As such ICAM-1 is an important regulator of the immune response (Damle et al. 1992; Dustin et al. 1986; Long 2011; Rothlein et al. 1986).

6.2.3 Leukocyte Function-Associated Antigen-3

89. Leukocyte Function-Associated Antigen-3 (LFA-3), also known as CD58, is expressed widely on blood cells and various other cells such as cells that line blood vessels, smooth muscle cells and connective tissue cells (Krensky et al. 1983). LFA-3 binds to the cell surface marker CD2,

and mediates cell adhesion (Dustin et al. 1987; Selvaraj et al. 1987). Binding of LFA-3 to CD2 has been shown to enhance antigen-specific activation of T cells.

90. LFA-3 is highly expressed in some tumour cells including and in a variety of malignant neoplasms, including chronic B-cell leukaemia, neoplastic T cells, ReedSternberg cells, myeloma, and myeloid leukaemia and could be used as a marker for cancer development (Lee et al. 2005).

91. LFA-3 has been implicated in multiple sclerosis, with allelic variants linked with the risk of developing the condition. High level expression of a functional gene in peripheral blood mononuclear cells is thought to be linked with delays in onset of the disease and periods of remission (De Jager et al. 2009). Statistical analysis of patient data suggests that LFA-3 may also be a rheumatoid arthritis risk factor (Raychaudhuri et al. 2009).

6.2.4 B7.1

92. B7.1 also known as CD80 is a cell surface glycoprotein which is found exclusively on the surface of cells able to stimulate T cell proliferation. The receptor for B7.1 on T cells is known as CD28. Binding of B7.1 to CD28 initiates T cell activation and proliferation (Lenschow et al. 1996). Alternatively, binding of B7.1 to Cytotoxic T-Lymphocyte Antigen 4 (CTLA-4) (a protein structurally similar to CD28 and expressed after T cell activation) limits T cell proliferation and therefore attenuates the potential immune response (Greenwald et al. 2004; Lohr et al. 2004a).

93. Binding of B7.1 to CD28 also induces expression of a cytokine called interleukin-2 (IL-2) and stabilises its gene product. IL-2 promotes the development of a naïve T cell into an armed effector cell which is capable of rapid proliferation and does not require any further signals to act. In the absence of IL-2, binding of an antigen to a naïve T cell will result in T cell inactivation (anergy) and tolerance (Greenwald et al. 2004; Lohr et al. 2004b).

6.2.5 Toxicity/allergenicity of the proteins/end products associated with the introduced genes

94. All four introduced genes are human genes therefore allergic reactions are not expected to occur.

95. There is no evidence of toxicity resulting from over-expression of the four genes. Clinical trials with the GMOs and with related GMOs expressing the genes singly or in combination have produced no evidence of a toxic response (for example Arlen et al. 2006; Arlen et al. 2007; DiPaola et al. 2006; Doehn & Jocham 2002; Doehn et al. 2007; Eder et al. 2000; Gulley et al. 2008a; Gulley et al. 2008b; Kantoff et al. 2010; Lou et al. 2006; Lubaroff et al. 2009; Madan et al. 2007; Madan et al. 2009; Sanda et al. 1999).

6.3 The regulatory sequences

96. Promoters are nucleotide sequences that are required in order to allow RNA polymerase to bind and initiate correct transcription.

97. Expression of three of the four introduced genes in both GM vaccines is driven by promoters which were isolated from vaccinia. The vaccinia 40K transcriptional promoter (Rosel et al. 1986) is used for expression of the PSA gene. The vaccinia 30K transcriptional promoter (Goebel et al. 1990) is used for expression of the LFA-3 gene. The vaccinia I3 transcriptional promoter (Schmitt & Stunnenberg 1988) is used for expression of the ICAM-1 gene.

98. Expression of the final gene, B7.1, is controlled by a synthetic early/late (sE/L) transcriptional promoter (Chakrabarti et al. 1997) which is based on common vaccinia promoter sequences (Hodge et al. 1999).

6.4 Method of genetic modification

99. Both GMOs were produced by homologous recombination. Cells were infected with the parent virus and a plasmid containing the introduced sequences flanked by the relevant viral

sequences. The viral sequences on the plasmid then bound to the complementary sequences in the viral genome which allowed the genes to be transferred. Different viral sequences were required for the two viruses. However, the DNA encoding the four introduced human genes and their promoters was identical.

100. The resulting GM viruses were then screened to confirm that the four human genes had been integrated into the genomes of the GM viruses. For the GM vaccinia the four human genes were inserted into the intergenic region between open reading frames F12L and F13L. For the GM fowlpox the four human genes were inserted into the fowlpox FPV426 gene. As a result, no viral genes were altered in the GM vaccinia. However, the FPV426 gene can no longer be expressed in the GM fowlpox. The absence of this gene, which has homology to the ankyrin repeat gene family, is not predicted to have an effect on the properties of fowlpox virus. No other plasmid sequences were integrated into the GMOs; only the four human genes, together with the poxviral regulatory sequences, are present in the final GM viruses.

6.5 Characterisation of the GMOs

6.5.1 Stability and molecular characterisation

101. The genome of the working seed virus and the entire genome of one production lot of each GM virus have been sequenced. In addition, for each production lot, identity is confirmed by PCR, Western blot, and restriction site analysis.

102. Identity: Confirmation of the identity and genomic structure of each of the recombinant viruses is accomplished by PCR amplification of inserted DNA regions and flanking regions.

103. Introduced Gene Expression: Western blot analysis using antibodies specific for PSA, B7.1, ICAM-1 and LFA-3, is used to examine the molecular weight and identity of these polypeptides expressed by the GM viruses in human cell lines.

104. Genetic Purity: Quantitative Polymerase Chain Reaction (Q-PCR) is used to analyse for cross contamination with other vaccinia based products produced by Bavarian Nordic or between the two GM viruses covered by this application. In this assay, DNA from the virus preparation is isolated and used as template in Q-PCR tests using virus strain-specific probes.

105. Restriction Site Analysis: Confirmation of the genetic integrity of the recombinant viral genome is established by generating DNA restriction fragment patterns after digestion with restriction endonucleases. The resulting DNA fragment patterns are compared with that of the reference standard.

106. Additionally, the GM viruses have been designed and manufactured in accordance with international standards for drug design and manufacture as well as the international guidance for vaccinia production: *Note for Guidance on the development of vaccinia virus based vaccines against smallpox* (European Commission Enterprise and Industry 2002) and *Recommendations for the production and quality control of smallpox vaccine, revised 2003* (World Health Organisation 2003).

6.5.2 Characterisation of the phenotype of the GM vaccines

107. The phenotype of the GM vaccines has been characterised in model animals such as mice and non-human primates, as well as in human clinical trials (DiPaola et al. 2006; Kantoff et al. 2010; Madan et al. 2009). These studies demonstrate that all four introduced genes are expressed in cells infected with the GM viruses.

6.6 Results of previous clinical trials with the GM vaccines

108. The GM vaccines have been tested in clinical trials and demonstrated an acceptable safety profile with no medically significant vaccine-related adverse events when administered to ten patients with prostate cancer (DiPaola et al. 2006). A second phase I trial involving 15 patients with similar prostate cancer profiles also showed no medically significant adverse events. This trial also

examined viral shedding in four patients. Viral DNA was detected in all four patients (at the inoculation site, in serum and in peripheral blood mononuclear cells) but viable vaccinia virus was detected only in one patient, at the inoculation site. Virus was detected on days 7 and 14 following inoculation, but was no longer detectable by day 28. This patient was also the only one of the four patients to develop a pock at the injection site (Arlen et al. 2007). In both trials mild adverse events were reported including injection site reactions and a subset of patients experiencing associated systemic adverse events such as fatigue, fever and nausea.

109. In a Phase II clinical trial involving 125 patients, 82 of which received the GM vaccines, there was a single significant adverse event associated with thrombotic thrombocytopenic purpura (extensive microscopic blood clots) and myocardial infarction. The case was reported as possibly related to treatment, however, thrombotic thrombocytopenic purpura has not been reported in association with vaccinia immunization (Kantoff et al. 2010).

110. The GM viruses are currently being evaluated in a worldwide study that has been approved in a number of countries (see Section 8.2).

Section 7 The receiving environment

111. The receiving environment forms part of the context in which the risks associated with dealings involving the GMOs are assessed. This includes the geographic regions where the trial would occur and any relevant properties of these locations; the intended clinical practices, including those that may be altered in relation to normal practices; other relevant GMOs already released; and any particularly vulnerable or susceptible entities that may be specifically affected by the proposed trial.

112. The proposed dealings involve inoculating men with prostate cancer at clinical facilities listed in Table 1. The handling of the GM vaccine and inoculation of trial participants would be performed in accordance with the guidelines outlined in the International Conference on Harmonisation (ICH) E6 - *Good Clinical Practices* (ICH 1996), and this is expected to ensure safe receipt, storage, handling, dispensing and disposal.

113. As vaccination with GM vaccinia may result in pustule (pock) formation, and subsequent shedding of the GM vaccinia, the receiving environment would also include the homes where the trial participants reside following inoculation as well as any places that they attend during the period where the GM vaccinia continues to replicate and be shed.

7.1 Relevant environmental factors

114. Environmental factors relevant to the potential persistence or spread of the GM viruses are the presence of susceptible host organisms and any physical conditions that may aid or restrict transmission to these hosts.

115. As smallpox vaccination is no longer ongoing, the majority of people under forty years of age in Australia would not be expected to have any significant levels of immunity to vaccinia. Therefore, children and younger adults are likely to be susceptible to infection with GM vaccinia if exposed to a sufficiently large inoculating dose. People who were vaccinated against smallpox more than forty years ago may be less susceptible to infection, or infection may be asymptomatic or result in less severe symptoms (Cohen 2001; Hatakeyama et al. 2005).

116. Animals that are able or may be able to be infected with the GMOs, such as rodents, chickens and cattle may be present in the environment where the GM viruses may be shed.

117. Physical conditions such as the presence of biological contaminants that prolong the survival of virus outside of the host may assist the transmission of the GM vaccines between trial participants and other susceptible hosts. For clinical facilities the applicant states that the World Health Organisation *Standard Precautions in Health Care* (World Health Organisation 2007) would

be followed, in addition to clinical practices listed below, to ensure hygiene and control any risks to people undertaking the dealing.

118. Following immunisation with the GM vaccinia, the inoculation site will be covered by a sterile non-adherent dressing, and patients will receive instructions regarding dressing care, proper hand hygiene, bathing etc. As the inoculation site is the most likely place for viral shedding to occur, these steps should minimise the likelihood of viral transmission and persistence in the environment.

119. As fowlpox cannot replicate in mammalian cells, GM fowlpox will likely be shed only from the inoculation site, and only for a limited time following vaccination. The use of an adhesive dressing over this site means there is limited opportunity for the GM virus to be transmitted to a susceptible host.

120. For locations outside of the clinical facilities the physical environmental factors influencing the possibility of transmission cannot be fully characterised. However, the presence of hosts potentially predisposed towards known severe adverse events in the environment would be controlled by the exclusion of potential trial participants that may come into contact with immunocompromised people, people with active or chronic skin conditions and pregnant women as discussed in Chapter 1, Section 4.3.

7.2 Presence of related viruses in the receiving environment

121. Vaccinia is not considered endemic in Australia and it is not expected that trial participants would be exposed to wild type vaccinia during the trial.

122. Fowlpox is common in Australian chickens, but is unlikely to be present within the immediate environment of the trial participants.

7.3 Presence of the introduced genes, similar genes and encoded proteins in the environment

123. The introduced genes are isolated from humans. The three immunomodulatory genes, *ICAM-1*, *LFA-3* and *B7.1* are continuously expressed at low levels, and expression is increased during immune responses. *PSA* is expressed by prostate epithelial cells during semen production and also in female ejaculatory fluid and breast tissue. *PSA* is also present in uterine fluid and human breast milk. Therefore, both children and adults would have been exposed to these genes and their gene products.

Section 8 Australian and international approvals

8.1 Australian approvals of GM vaccinia and fowlpox vaccines

8.1.1 Previous releases approved by the Regulator

124. The Regulator has issued a number of licences for Dealings Not involving an Intentional Release (DNIR) with GM vaccinia and GM fowlpox. These include licences for the development of GM vaccines, basic research into fowlpox and vaccinia biology, as well as one licence for a clinical trial of GM *fowlpox virus*.

125. This is the first DIR licence for a clinical trial involving GM vaccinia and GM fowlpox. The Regulator has issued one other licence for a clinical trial of a human GM vaccine which involved the intentional release of a GMO into the environment; DIR 097 - *Limited and controlled release of a genetically modified vaccine for prevention of selected childhood respiratory diseases*. PPD is the licence holder for DIR 097.

8.1.2 Approvals by other government agencies

126. The Regulator is responsible for assessing risks to the health and safety of people and the environment posed by or as a result of gene technology. Other government regulatory requirements may also have to be met in respect of release of GMOs, including those of the Therapeutic Goods

Administration (TGA) and Department of Agriculture, Fisheries and Forestry (DAFF) Biosecurity. This is discussed further in Chapter 3.

127. TGA is the agency with oversight for the experimental use of therapeutic products that are not entered in the Australian Register of Therapeutic Goods, under the Clinical Trial Notification (CTN) or Clinical Trial Exemption (CTX) scheme. The applicant has notified the TGA of the trial. Each trial site will also notify the TGA through the Clinical Trial Notification (CTN) Scheme.

8.1.3 Other Australian approvals

128. Ethical approval is required prior to the commencement of research involving human subjects. Location-specific HREC approval will be sought prior to commencement of the trial at any of the clinical sites.

8.2 International approvals of GM poxvirus vaccines against prostate cancer

129. A number of Phase I and Phase II clinical trials with the two GM viruses have been conducted in the United States and trial participants continue to be actively recruited in that country.

130. This trial will form part of a worldwide Phase III clinical trial which is intended to be conducted in Argentina, Belgium, Brazil, Canada, Chile, Denmark, Estonia, France, Germany, Iceland, Israel, Netherlands, Poland, Puerto Rico, Russia, Slovakia, Spain, United Kingdom and the USA. The approving agency and any conditions imposed on the trial are detailed in Table 4 below.

Table 4 Overseas applications and approval of trials of the GM vaccine.

Country	Agency	Status of application
ARGENTINA	National Administration of Drugs, Foods and Medical Devices (ANMAT)	Submission planned
AUSTRALIA	Therapeutic Goods Administration	Submission planned
BELGIUM	Federal Agency for Medicines and Health Products	Submitted on 22nd November 2011
BRAZIL	National Health Surveillance Agency (Anvisa)	Submission planned
CANADA	Therapeutic Products Directorate Office of Clinical Trials	Approved on 2nd February 2012
CANADA	Environment Canada	Approved on 10 August 2012
CHILE	Public Health Institute of Chile	Submission planned
DENMARK	Danish Medicines Agency	Submission planned
ENGLAND	Department for Environment, Food and Rural Affairs (DEFRA) GMO deliberate release application	Approved on 8th January 2012
ESTONIA	State Agency of Medicines Department of Human Medicines Bureau of Clinical Assessment and Drug Information	Approved on 19th March 2012
FRANCE	French Agency for the Safety of Health Products Department Evaluation Of Clinical Trials And Drugs With Special Status Unit for Clinical Trials on Medicinal and Non-Medicinal Products	Submitted on 11th January 2012
GERMANY	Paul-Ehrlich-Institut (PEI) German Federal Institute for Vaccines and bio-medical drugs	Submitted on 11th June 2012
ICELAND	Lyfjastofnun (Icelandic Medicines Control Agency)	Approved on 2nd February 2012
ICELAND	Environment Agency Of Iceland	Approved on 6 July 2012
ISRAEL	National Coordinator for Clinical Trials Ministry of Health, Pharmaceutical Department	Approved on 5th February 2012
NETHERLANDS	CCMO (Central Committee on Research Involving Human Subjects)	Submission planned
POLAND	The Office for Registration of Medicinal Products, Medical Devices and Biocidal Products	Submission planned
PUERTO RICO	Food and Drug Administration Center for Drug Evaluation and Research	Approved 2nd November 2011
RUSSIA	Federal State Institution “Scientific Centre for Expert Review of Products for Medical Use” (FGU)	Approved on 9th February 2012
SLOVAKIA	State Institute for Drug Control, Drug Safety and Clinical Trials Section	Submission planned

Country	Agency	Status of application
SPAIN	Spanish Agency for Medicines and Health Products Directorate General for Medicinal Products for Human Use Division of Pharmacology and Clinical Evaluation. Area of ClinicalTrials	Approved on 23rd March 2012
SPAIN	Ministry of Agriculture Food and Environment	Approved on 1st August 2012
UNITED KINGDOM	Medicines and Healthcare products Regulatory Agency (MHRA)	Approved on 29th December 2011
USA	Food and Drug Administration Center for Drug Evaluation and Research	Approved 2nd November 2011
WALES	Welsh Assembly GMO deliberate release application	Approved on 11th January 2012

Chapter 2 Risk assessment

Section 1 Introduction

131. 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 3). Risks are identified within the context established for the risk assessment (see Chapter 1), and takes into account current scientific and technical knowledge. A consideration of uncertainty, in particular knowledge gaps, occurs throughout the risk assessment process.

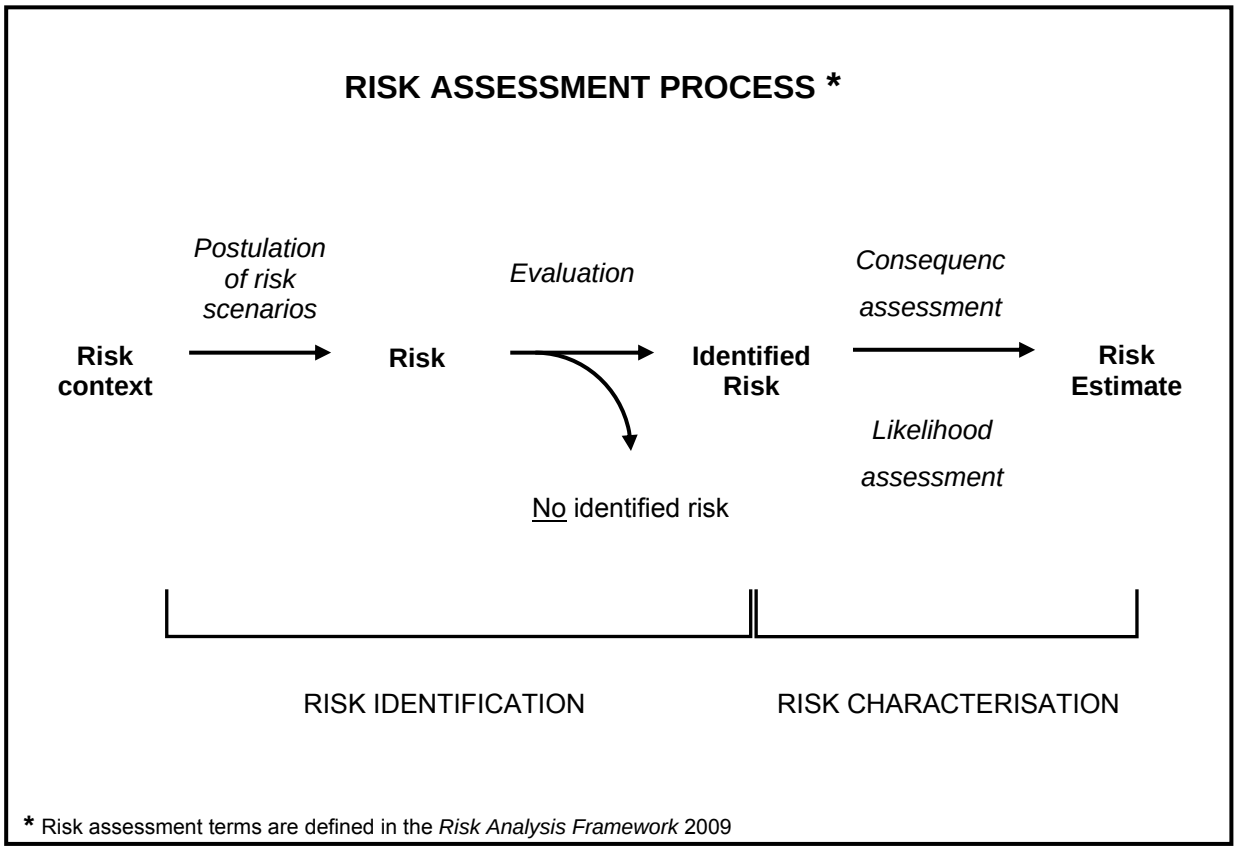

Figure 3. The risk assessment process.

132. Initially, risk identification considers a wide range of circumstances whereby the GMO, or the introduced genetic material and expressed gene products, could come into contact with people or the environment. Consideration of these circumstances leads to postulating plausible causal or exposure pathways that may give rise to harm for people or the environment from dealings with a GMO (risk scenarios).

133. Each risk scenario is evaluated to identify those risks that warrant detailed characterisation. A risk is only identified for further assessment when a risk scenario is considered to have some reasonable chance of causing harm in either the short or long term. Pathways that do not lead to harm, or could not plausibly occur, do not advance in the risk assessment process.

134. A number of risk identification techniques are used by the Regulator and staff of the OGTR, including the use of checklists, brainstorming, common sense, reported international experience and consultation (OGTR 2009). In conjunction with these techniques, risk scenarios postulated in previous RARMPs prepared for licence applications of the same and similar GMOs are also considered.

135. Identified risks (i.e. those identified for further assessment) are characterised in terms of the potential seriousness of harm (Consequence assessment) and the likelihood of harm (Likelihood

assessment). The level of risk is then estimated from a combination of the Consequence and Likelihood assessments.

Section 2 Risk identification

136. The following factors are taken into account when postulating relevant risk scenarios:

- the proposed dealings, which may be to conduct experiments, develop, produce, breed, propagate, grow, import, transport or dispose of the GMOs, use the GMOs in the course of manufacture of a thing that is not the GMO, and the possession, supply and use of the GMOs in the course of any of these dealings
- the proposed limits;
- the proposed controls;
- characteristics of the parent organism(s);
- routes of exposure to the GMOs, the introduced gene(s) and gene product(s);
- potential effects of the introduced gene(s) and gene product(s) expressed in the GMOs;
- potential exposure to the introduced gene(s) and gene product(s) from other sources in the environment;
- the environment at the site(s) of release; and
- clinical management practices for the GMOs.

137. Seven risk scenarios were postulated and evaluated. They are summarised in Table 5 where circumstances that share a number of common features are grouped together in broader risk categories. In the context of the control measures proposed by the applicant, and considering both the short and long term, none of the risk scenarios were identified as a risk that could be greater than negligible. Therefore, they did not warrant further detailed assessment. More detail of the evaluation of these scenarios is provided later in this section.

Table 5 Summary of risk scenarios from dealings with the GM virus.

Risk category	Risk scenario		Identified risk?	Reason
	Pathway that may give rise to harm	Potential harm		
Section 2.1 Production of a substance toxic to people or other organisms	1. Exposure to the GM virus material containing proteins encoded by the introduced genes	Toxicity in people and other organisms	No	• Each of the four encoded proteins is of human origin. They occur naturally in the environment and are unlikely to be toxic to people or other organisms. • The limited scale, and other proposed limits and controls reduce exposure of people and other organisms to products of the introduced genes.

Risk category	Risk scenario		Identified risk?	Reason
	Pathway that may give rise to harm	Potential harm		
Section 2.2 Increased disease burden as a result of the genetic modification	2. Exposure of clinical staff to either of the GM viruses resulting in infection	• Increased disease symptoms; • Inappropriate immune response (including allergy)	No	• Transmission to clinical staff was not observed in previous clinical trials Previous clinical trials have not reported any severe adverse reactions. • Vaccine administered by trained, authorised staff who can meet the medical exclusion criteria (see Section 2.2, below). • Transmission minimised by proposed limits and controls. Previous vaccinia exposure combined with inoculation method means patients are less likely to form pustules compared to people who have not previously received a vaccinia inoculation. Bandaging and injection-site care instructions are designed to contain any shed GM vaccinia. These factors minimise transmission of GM vaccinia. • Fowlpox doesn't replicate in mammals, transmission from patient to clinical staff not expected to occur.
	3. Exposure of people or animals to either of the GM viruses resulting in infection	• Increased disease symptoms; • Inappropriate immune response (including allergy	No	• Previous clinical trials have not reported any severe adverse reactions. • Transmission to non-trial participants was not observed in previous clinical trials. • Transmission minimised by proposed limits and controls. Previous vaccinia exposure combined with inoculation method means patients are less likely to form pustules compared to people who have not previously received a vaccinia inoculation. Bandaging and injection-site care instructions are designed to contain any shed GM vaccinia. These factors minimise transmission of GM vaccinia. • Fowlpox doesn't replicate in mammals, transmission to susceptible species not expected to occur.
	4. Exposure of people or animals to either of the GM viruses due to unintentional release	• Increased disease symptoms; • Inappropriate immune response (including allergy)	No	• Transport of viral stocks will be according to the Regulator's *Guidelines for the Transport, Storage and Disposal of GMOs*. • Storage will be at secure clinical sites. • Disposal of GM vaccinia through clinical waste stream. • Stocks will be accounted for. • Risk Scenarios 1 – 3 were not identified as risks that could be greater than negligible. • The limits and controls on the trial minimise the likelihood of people or animals being exposed due to unintentional release.

Risk category	Risk scenario		Identified risk?	Reason
	Pathway that may give rise to harm	Potential harm		
Section 2.3 Unintended changes in viral characteristics	5. Changes to the structure and function of the GM viruses	• Increased disease symptoms • Inappropriate immune response • Altered host range	No	• Previous clinical trials with the GM viruses have not reported any severe adverse reactions attributable to changes in the virus characteristics. • Pathway to any unintended adverse effects would be minimised by the proposed limits and controls.
Section 2.4 Horizontal transfer of genes or genetic elements to other organisms	6. Infection of people or animals co-infected with another virus, leading to recombination	• Increased disease burden	No	• HGT to another virus unlikely. • Expression of the introduced genes in the GM viruses have not yet shown any increase in disease burden in people. • Expression following HGT not likely to increase disease burden.
Section 2.5 Unauthorised activities	7. Use of the GMOs outside the proposed licence conditions (non-compliance)	Potential adverse outcomes mentioned in Sections 2.1 to 2.4	No	• The Act provides for substantial penalties for non-compliance and unauthorised dealings with GMOs and also requires consideration of the suitability of the applicant to hold a licence prior to the issuing of a licence by the Regulator.

2.1 Production of a substance toxic/allergenic to people or toxic to other organisms

138. Toxicity is the adverse effect(s) of exposure to a dose of a substance as a result of direct cellular or tissue injury, or through the inhibition of normal physiological processes (Arts et al. 2006).

139. A range of organisms may be exposed directly or indirectly to the proteins encoded by the introduced genes or end products of immunological pathways regulated by the introduced proteins. Trial participants will be intentionally exposed to the GM vaccines. Clinical staff administering the vaccines or other staff handling the vaccines during transport, storage and disposal may be exposed through a needlestick injury, a spill or contact with contaminated items. People and other organisms may be exposed to the GM virus shed by trial participants. Transmission and infection as a result of viral shedding is considered in Chapter 2, Section 2.2, Risk scenario 2.

Risk scenario 1. Exposure to GM vaccine material containing proteins encoded by the introduced genes.

140. There is a possibility that exposure of people or other organisms to the proteins encoded by the introduced genes could cause a toxic response.

141. Expression of the introduced genes is not expected to result in the production of novel toxic compounds in the GM vaccine.

142. The insertion of the introduced genes (discussed further in Risk scenario 5) into each of the viruses is not expected to result in the expression of new or novel proteins of viral origin.

143. The proteins encoded by the four introduced genes are of human origin, and not expected to be toxic. The PSA gene has been modified to change one amino acid (at position 155) from isoleucine to leucine, to enhance immunogenicity (induce higher levels of T cell activation) (Terasawa et al. 2002). While this alteration has been shown to make the protein more immunogenic, it is not expected to alter the function of the protein. As such, this protein is not expected to be toxic when expressed.

144. The three other genes, LFA-3, ICAM-1 and B7.1, were all amplified from human cDNAs. There are no introduced nucleotide modifications to these genes, and as such there is no reason to think that the proteins would be toxic when expressed on an infected cell. The proteins are not expected to be expressed on the surface of the viral particles.

145. All components of the GM vaccines have been tested in various combinations in mouse, rabbit and non-human primates, as well as in a number of *in vitro* experiments. Data provided by the applicant shows that no signs of toxicological effects were noted in the rodent or non-human primate studies. Similarly, there have been no reports of toxicity despite the numerous clinical trials that have taken place using these GM vaccines.

146. The proposed limits and controls of the trial (Chapter 1, Sections 4.2 and 4.3) will minimise the likelihood of exposure of people not enrolled in the trial and other organisms in the environment to the GM vaccine. Human contact with the GM vaccines prior to and during inoculation would be limited to trained, authorised staff who can meet the medical exclusion criteria discussed in Chapter 1, Section 4.3. The staff will be wearing appropriate personal protective equipment, including a laboratory coat, gloves and safety glasses. The proposed trial sites are located within hospitals so access to the general public would be minimised.

147. ***Conclusion***: The potential for toxicity in people and other organisms as a result of exposure to GM vaccines containing proteins encoded by the introduced genes is not identified as a risk that could be greater than negligible. Therefore, it does not warrant further assessment.

2.2 Increased disease burden from the GM virus

148. Baseline information on the characteristics of and the factors limiting transmission of *Vaccinia virus* and *Fowlpox virus* are given in Chapter 1.

149. In summary, *Vaccinia virus* is not known to occur naturally in Australia. The virus was previously used as a vaccine for smallpox, with millions of Australians inoculated before smallpox eradication.

150. Vaccinia can be transmitted between people by direct contact with the pustule at the inoculation site, or by secondary contact with items that have been contaminated by contact with the pustule (e.g. towels, sheets, clothes, bandages). The virus can persist for extended periods of time when in contact with organic material.

151. *Fowlpox virus* commonly occurs in Australian chicken flocks, and fowlpox vaccine strains are used to inoculate chickens, often in the face of an outbreak. Fowlpox can be transmitted by close contact between chickens (pecking/fighting etc), or by insect vectors, usually mosquitos. Similarly to vaccinia, fowlpox shows environmental stability when in contact with organic material.

152. Trial participants will be intentionally exposed to the GM viruses. Clinical staff administering the vaccine, or other staff handling the vaccine during transport, storage and disposal, may be exposed through a needlestick injury, a spill or contact with contaminated items. People and animals may be exposed to the GM viruses shed by trial participants.

153. An increased disease burden could be due to an increase in disease symptoms, or inappropriate immune response to the GM virus as a result of expression of the proteins encoded by the introduced genes. An inappropriate immune response would be considered to be an abnormal/unintended increase or suppression of the immune response, or an allergic response. Pathways that could lead to an increased disease burden from the GM viruses include:

- exposure of clinical staff to the GM viruses, leading to viral infection and protein expression;
- exposure of contacts of trial participants (household contacts and animals) to the GM viruses, leading to viral infection and protein expression;

- unintentional release of the GM viruses, leading to viral infection and protein expression in other people or animals;

where expression of the introduced proteins (PSA, B7.1, ICAM-1, LFA-3) leads to an increase in disease symptoms or an inappropriate immune response. These are discussed below.

Risk scenario 2. Exposure of clinical staff to the GM virus resulting in infection

154. Clinical staff administering the vaccine, or other staff handling the vaccines during transport, storage and disposal, may be exposed through a needlestick injury, a splash to the eye or mouth, a spill, or contact with patients or items that have been contaminated with the GM viruses.

155. Previous vaccination with vaccinia is a prerequisite for participation in the trial. This previous immunological experience, combined with the subcutaneous infection route to be used means that the reactions associated with the traditional percutaneous scarification administration of vaccinia vaccines (scarring, pustules, vesicle formation) are less likely to occur than in vaccinia-naïve recipients or non-naïve recipients receiving a second inoculation through the percutaneous method. These symptoms were not observed in two previous clinical studies of the GM vaccines (DiPaola et al. 2006; Kantoff et al. 2010), although some pock formation was noted in another trial (Arlen et al. 2007).

156. A National Cancer Institute clinical trial using the GM vaccinia with the same intended dose and route of administration as that proposed in this application showed that, in people with evidence of prior vaccinia vaccination, while viral DNA persisted in some patient samples, live virus was shed transiently and probably exclusively from the vaccination site (Arlen et al. 2007). The level of shedding of GM vaccinia from trial participants is an important factor in determining exposure to and transmission of GM vaccinia.

157. Unlike trial participants, clinical staff may not have been previously vaccinated with vaccinia. If exposed to GM vaccinia, they may develop a localised vaccinia response (pustule/pock), and may also develop the vaccinia related complications described in Chapter 1, Section 5.5.1. However, the exclusion criterion for clinical staff (described below) limits the likelihood of other vaccinia related complications.

158. The Investigators Brochure and the Study Staff Instructions documents outline precautions to be taken by clinical staff when administering the vaccine. Staff members in the following categories are restricted from working (loading syringes from vials; giving injections) with GM vaccinia: people with an immunodeficiency or taking immunosuppressant drugs; people with chronic eczema or skin conditions that cause skin damage; and women who are pregnant or breast feeding.

159. The Study Staff Instructions document provided by PPD also state that when handling GM vaccinia, staff must wear protective clothing such as a laboratory coat, disposable gloves and protective eyewear. They are also advised to use a biological safety cabinet for syringe loading, if available.

160. Fowlpox replicates abortively in mammalian cells. This means that the virus can enter a cell, express some proteins, and replicate DNA. However, the virus cannot express the proteins necessary for viral assembly, and new infectious virus cannot be formed. As a result, any shedding will be minimal, as only those viral particles from the inoculation will be available to be shed. Any shedding will be restricted in location to the injection site. Transmission of GM fowlpox from the patient to clinical staff is not expected. The medical exclusion criteria that applies for staff members when handling GM vaccinia does not apply for the GM fowlpox injections. However, universal precautions (World Health Organisation 2007) will still be followed, minimising staff contact with the GM fowlpox.

161. The use of personal protective clothing and equipment, as well as the use of a biological safety cabinet, minimises the likelihood of exposure via a splash. The use of gloves will minimise transmission via contact with the injection site.

162. In summary, the proposed limits and controls of the trial (Chapter 1, Sections 4.2 and 4.3) would minimise the likelihood of exposure of clinical staff to the vaccines. Human contact with GM vaccines prior to and during inoculation would be limited to trained, authorised staff who can meet the medical exclusion criteria discussed above. The staff will be wearing appropriate personal protective equipment, including a laboratory coat, gloves and safety glasses. The proposed trial sites are located within clinical facilities, and the vials will be stored in a secure location within these facilities, thereby limiting access to the vaccine stocks.

163. Information provided by the applicant states that comparison of GM vaccinia and GM fowlpox with their parent viruses demonstrate that the two GM viruses do not show any growth advantage, increase in virulence, or increase in stability in the environment.

164. While exposure to GM vaccinia through any of the above exposure routes could result in the formation of pustules and febrile illness, data from previous clinical trials with GM vaccinia, as well as numerous studies on laboratory animals, suggests that the expression of the four human proteins is not expected to increase the symptoms of the disease, affect the virulence and pathogenicity of the virus, or lead to inappropriate immune responses.

165. ***Conclusion*****:** The potential of either GM virus to increase disease burden following infection of clinical staff, resulting in increased disease symptoms or an inappropriate immune response due to the expression of introduced genes, is not identified as a risk that could be greater than negligible. Therefore, it does not warrant further assessment.

Risk scenario 3. Exposure of people or animals to GM virus resulting in increased disease burden

166. As discussed for Risk scenario 2, the previous immunological experience of trial participants, combined with the route of administration of GM vaccinia, means that the likelihood of a pustule forming is reduced. A previous clinical trial with this vaccine suggests that virus may be shed transiently and exclusively from the injection site (Arlen et al. 2007). The level of shedding of the GM vaccinia from trial participants is an important factor in determining exposure to and transmission of the GM vaccinia.

167. Patients are excluded from participation in the trial if they have not previously received a vaccinia vaccine; have eczema or other exfoliative skin conditions; have a heart condition; or an immune suppression. Furthermore, patients are excluded from the trial if they are unable to avoid close contact with the following groups of people, for a period of 3 weeks after the initial GM vaccinia inoculation:

- Persons with weak or suppressed immune systems (through disease or treatment)
- Individuals with eczema or other significant rashes, itching infections, burns, chicken pox or skin injury
- Pregnant and breast-feeding women
- Children under 3 years of age.

168. The applicant has stated that patients will be educated in injection site care. Patients will receive a patient care kit that includes bandages, disposable gloves, absorbent towelling, disinfecting wipes, a digital thermometer, biohazard bags and instructions on caring for the injection site. The instruction materials contain detailed descriptions of bandaging, bathing and reporting any possible side effects. Patients will be instructed to keep the injection site covered with a bandage, and keep the bandage covered with a sleeve (or pants if injection site is on leg). Instructions for changing the bandage explain that all contaminated waste (dressing, gauze, gloves etc) should be placed in a provided biohazard bag, which should be sealed and returned to the hospital or clinic for destruction.

169. Vaccinia viral shedding is not expected to occur at sites other than the injection site (Arlen et al. 2007). The bandaging will prevent shedding from this location. This means that there are very

few opportunities for the patient's household contacts to come into contact with shed virus. Patient instructions for care of the injection site, how to change dressings and how to dispose of dressings, means that the likelihood of transmission of GM vaccinia via shedding is drastically reduced.

170. The GM viruses are designed to produce an immune response against PSA. Previous laboratory and clinical data has shown that multiple exposures to the antigen (e.g. one GM vaccinia vaccination followed by six GM fowlpox "boosts") are necessary to produce the desired immune response against the prostate cancer antigen. This means that a single accidental exposure to/infection by either GM vaccinia or GM fowlpox is unlikely to result in an adverse immune response to PSA.

171. Previous clinical trials with the GM vaccines showed that the placebo (an initial injection with unmodified *Vaccinia virus*, and subsequent injections with unmodified *Fowlpox virus*) and GM virus arms of the trials had similar safety profiles, as discussed in Chapter 1, Section 6.6 (Kantoff et al. 2010). This suggests that expression of the four human proteins does not lead to an increase in replication or virulence of the GM viruses. Further information provided by the applicant states that comparison of GM vaccinia and GM fowlpox with their parent viruses demonstrate that the two GM viruses do not show any growth advantage, increase in virulence, or increase in stability in the environment.

172. Fowlpox replicates abortively in mammalian cells. This means that the virus can enter a cell, express some proteins, and replicate DNA. However, the virus cannot express the proteins necessary for viral assembly, and new infectious virus cannot be formed. As a result, any shedding will be minimal, as only those viral particles from the inoculation will be available to be shed. Any shedding will be restricted in location to the injection site.

173. As clinical studies have shown an acceptable safety profile with no medically significant vaccine-related adverse events for the GM virus it is highly unlikely that the genetic modifications would increase the virulence of the GM virus.

174. The insertion of the four human genes will not change the host ranges of vaccinia and fowlpox, as these genes are not determinants of host range. Vaccinia can replicate in a range of mammalian hosts. Fowlpox causes disease in chickens and turkeys, but cannot replicate in mammals.

175. The likelihood of household animals, or backyard chickens, coming into contact with the injection sites (upper arm or upper outer thigh) is minimal.

176. The patient instructions on how to dispose of dressings following the GM vaccinia injection will minimise the likelihood of a household animal coming into contact with shed GM vaccinia contained in a dressing. The dressings from the GM vaccinia inoculation site will be returned to the clinic for disposal in the clinical waste stream.

177. There is no plausible pathway for GM fowlpox to be transmitted from a patient to a bird in the patient's home, backyard or workplace, as the GM fowlpox cannot replicate in humans, meaning that the only virus available for transmission is from the initial inoculation. A bird would need to come into direct contact with the inoculation site, which will not be possible as the patient will be instructed to wear an adhesive dressing over the site. The adhesive dressing will be permitted to be disposed of in house-hold waste. This is considered appropriate for several reasons:

1) The GM fowlpox will not replicate in the patient, meaning that there is limited potential for GM fowlpox to be present on the adhesive dressing (limited to those virus particles remaining on the skin surface following the injection).
2) Unlike the GM vaccinia inoculation, the GM fowlpox inoculation site will not form a pock, meaning that there will be no organic material (scab) present on the adhesive dressing. When fowlpox is not protected by organic material, it has limited viability, so any residual GM fowlpox that is on the adhesive dressing is not expected to remain viable for long.

3) Fowlpox is considered endemic in Australia, and fowlpox vaccines are used widely by the poultry industry. Fowlpox is only known to cause disease in chickens and turkeys, and can cause asymptomatic infection in pigeons (Barthold et al. 2011; Siddique et al. 2011). While a virus similar to a fowlpox vaccine strain was recently isolated from birds in New Zealand (Ha et al. 2011), it is not known if the virus caused disease in these birds.

4) Finally, the GM fowlpox is based on a vaccine strain of fowlpox, meaning that accidental infection of a bird would result in immunity to fowlpox.

178. The proposed limits and controls of the trial (Chapter 1, Sections 4.2 and 4.3) would minimise the likelihood of transmission of the GM vaccinia. The inability of fowlpox to replicate minimises the likelihood of transmission of GM fowlpox. The applicant proposes a number of control measures, including exclusion of potential trial participants that are more likely to shed virus (see trial participant exclusion criteria, Chapter 1, Section 4.3), and of those expected to be in contact with classes of people more susceptible to vaccinia complications. With these limits and controls the potential for transmission of the GM vaccine to more susceptible hosts is greatly diminished.

179. ***Conclusion*:** The potential of either GM virus to increase disease burden due to transmission of the virus to people or animals that come into contact with patients, resulting in increased disease symptoms or an inappropriate immune response due to the expression of introduced genes is not identified as a risk that could be greater than negligible. Therefore, it does not warrant further assessment.

Risk scenario 4. Exposure of people or animals to the GM virus due to unintentional release

180. An unintentional release would include spills outside of the containment environment. This could occur as a spill during import, storage, transport or disposal.

181. The applicant has stated that the vaccine vials will be transported in sealed primary containers packed within secondary sealed and unbreakable containers marked with a label to indicate that they contain GMOs. The outside of the package will include the address and phone number of the relevant contact person.

182. The study vaccines and placebos will be shipped from the BN ImmunoTherapies storage depot, ALMAC Clinical Services, in the United States to Flinders Clinical Trial Services, Adelaide, South Australia. Distribution to clinical sites will then occur from this site.

183. GM vaccinia and GM fowlpox will be supplied as frozen single-dose preparations, in borosilicate (2R) glass vials sealed with rubber stoppers and aluminium-plastic closures. Each dose of GM vaccinia contains 2×10^8 infectious units in a 0.5 mL volume of PBS/10% glycerol. GM fowlpox will be supplied as a single dose of 1×10^9 infectious units in 0.5 mL PBS/10% glycerol.

184. Disposal of medical waste from the vaccination process will be via the clinical/biohazardous waste stream at the study site. Following administration, used vials and syringes that contained GM vaccine will be immediately placed into sealed infectious waste containers or into sealed bags, and retained for accountability. Upon reconciliation and accountability, this waste will be destroyed by the clinical site following standard clinical waste disposal methods such as steam sterilisation or incineration (Australian Capital Territory 1991a; EPA Victoria 2009; New South Wales 1997; Northern Territory 2009; Queensland 2000; 2011; 2012; South Australia 2009; Victoria 2000; West Australia 2004). 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 Australia and New Zealand (BWI) 2010). The clinical waste stream typically involves destruction of infectious waste by incineration or autoclaving, and is considered appropriate for disposal of the GMOs.

185. All unused study vaccine will either be returned to ALMAC clinical services, USA, for destruction, or disposed of via the clinical waste stream at the site.

186. Any spills occurring in a clinical setting would be disinfected and cleaned according to standard clinical procedures. Spills outside of clinical facilities (i.e. during transport, storage or disposal) would be disinfected and contained according to the requirements of the Regulator's *Guidelines for the Transport, Storage and Disposal of GMOs*. In addition, the GM vaccines are supplied as purified virus particles, which have reduced capacity to survive in the environment compared to virus found in scabs and other biological specimens. Therefore there is very little potential for exposure of humans or other animals to the GM viruses.

187. Risk scenarios 1 – 3, associated with infection of people and animals with the GM viruses, was not identified as a risk that could be greater than negligible. As such, exposure of people and animals from unintentional release of the GM virus is not a risk that could be greater than negligible.

188. ***Conclusion*:** The potential of either GM virus to increase disease burden due to infection of susceptible hosts, resulting in increased disease symptoms or an inappropriate immune response due to the expression of introduced genes, is not identified as a risk that could be greater than negligible. Therefore, it does not warrant further assessment.

2.3 Unintended changes in viral characteristics

189. When genes are inserted into a genome, there is a possibility that the insertion may have unintended consequences on the expression of other genes. This is particularly of concern in small viruses that have a limited number of genes, meaning that the gene products of individual genes may display pleiotropy (the genetic effect of one gene on apparently unrelated, multiple phenotypic traits (Kahl 2001)). The viruses used here, both poxviruses, are large DNA viruses that encode hundreds of genes, making pleiotropic effects less common.

190. It is also important to note that the human gene products will not be expressed on the viral surface. Rather, following viral infection, the infected host cell will express the four human gene products on the host-cell surface. This means that the viral surface won't be altered by the genetic modification.

Risk scenario 5. Changes to the characteristics of the GM virus resulting from expression of the introduced genes

191. Although the molecular properties of the GM viruses are well characterised, there is some possibility that there could be unexpected changes to the characteristics of the GM virus as a result of the introduced genes.

192. Human and animal trials involving the GM viruses, their parent viruses and other viruses with similar genetic modifications have not demonstrated unexpected changes in the characteristics of the GM viruses resulting from the introduced genes.

193. For the GM vaccinia, the four human genes were inserted into the intergenic region between open reading frames F12L and F13L. For the GM fowlpox the four human genes were inserted into the fowlpox FPV426 gene. Therefore no viral genes were altered in the GM vaccinia, and the FPV426 gene can no longer be expressed in the GM fowlpox. The absence of this gene, which has homology to the ankyrin repeat gene family, is not predicted to have an effect on the properties of fowlpox virus. No other plasmid sequences were integrated into the GMOs, only the four human genes, together with the poxviral regulatory sequences, are present in the final GM viruses.

194. As discussed in Chapter 1, Section 6.5, the genomes of the working seed viruses and one production lot have been fully sequenced to confirm the identity of the GMOs. In addition, the presence of the insertion is confirmed by PCR for each production lot. There is no evidence that the insertion is unstable. If the insertion were to be lost, the resulting virus would be equivalent to the parent organism, which has been used as a placebo in previous clinical trials.

195. As discussed above, information submitted by the applicant shows that the expression of the four human genes is not expected to affect viral growth rates, infectivity or pathogenicity.

Unintended changes in viral characteristics have not been seen in clinical and non-clinical experiments.

196. Exposure to the GM viruses is minimised by the limits and controls in place for this trial. This means that an adverse outcome is not expected, as the pathway to harm is not expected to occur.

197. ***Conclusion*:** The potential for an adverse outcome as a result of altered viral structure or function is not identified as a risk that would be greater than negligible. Therefore, it does not warrant further assessment.

2.4 Horizontal transfer of genes or genetic elements to other organisms

198. Horizontal gene transfer (HGT) is the stable transfer of genetic material from one organism to another without reproduction (Keese 2008). All genes within an organism, including those introduced by gene technology, are capable of being transferred to another organism by HGT. HGT itself is not considered an adverse effect, but an event that may or may not lead to harm. A gene transferred through HGT could confer a novel trait to the recipient organism, through expression of the gene itself or the expression or mis-expression of endogenous genes. The novel trait may result in negative, neutral or positive effects.

199. Baseline information on the presence of the introduced gene or similar genetic elements is provided in Chapter 1, Section 7.2. The introduced genetic elements are derived from humans.

Risk scenario 6. Presence of the introduced genetic material in other organisms as a result of horizontal gene transfer

200. Possible risks arising from HGT of the introduced genetic material to other organisms involves consideration of the potential recipient organism and the nature of the introduced genetic material. Risks that might arise from HGT from a GMO to another organism have been reviewed (Keese 2008).

201. Horizontal gene transfer from host to poxviruses is thought to have occurred many times during evolution of their hosts. HGT is considered to have played an important part in poxvirus evolution. Poxviruses do not enter the nucleus of the host. The mechanism of host gene capture is unknown - it may occur through reverse transcription of host mRNAs followed by integration of the cDNA into the virus genome (Bratke & McLysaght 2008).

202. Three genes found in poxviruses that are thought to have been as a result of HGT are known to improve the survival of the virus. Two of these protect the virus from environmental damage, while the third, viral IL-10, is a cytokine that, in humans, inhibits activation and maturation of dendritic cells (Bratke & McLysaght 2008). Viral IL-10 has been shown to delay the development of acquired immunity to the orf virus in humans (a parapox virus that infects sheep and goats and is transmissible to humans) (Chan et al. 2006).

203. Recombination between two viruses occurs during simultaneous infection of the same cell (DeFillipis & Villarreal 2001). Recombination can occur within and between viral types (DeFillipis & Villarreal 2001), meaning that introduced genes could be potentially transferred to other viruses. While recombination between different classes of virus can occur, the frequency of this happening decreases with decreasing relationship between the viruses – meaning that the GM viruses are more likely to recombine with another pox virus than with an unrelated virus.

204. A recent report of recombination between two live viral vaccines used for chickens raises issues about the use of DNA viruses as vaccine vectors (Lee et al. 2012). However, for either of the GM viruses used in this study to undergo recombination, a host cell would need to be concurrently infected with the GM virus and another virus.

205. GM fowlpox is unlikely to come into contact with other fowlpox viruses due to the limits and controls put in place for this trial. Fowlpox recombination will not occur in the human host (the

patient is not expected to be infected with fowlpox, and virus particles aren't formed in humans) or in chickens (there is no plausible route of transmission from the trial participant to a chicken).

206. There is no reservoir of vaccinia in the Australian environment to allow recombination between the GM vaccinia with a non-modified vaccinia.

207. Recombination may occur between a GM virus and another virus, if the patient was infected with another virus at the time of inoculation. The study protocol provided by the applicant states that injections sites (arms or thighs) will be alternated between subsequent vaccinations, which are three weeks apart. The time between GM vaccinia and GM fowlpox inoculations (three weeks), together with the different injection locations means that it is highly unlikely that GM fowlpox and GM vaccinia will infect the same host cell, meaning that a recombination between these two viruses is not expected.

208. The study protocol also states that patients will undergo a brief medical exam before each inoculation. If an acute illness is present at the time of vaccination, the inoculation will be postponed until symptoms subside. While this is a standard measure for any vaccination protocol, it does have the added benefit of reducing likelihood of viral co-infection.

209. Some DNA viruses, such as herpes viruses, sustain a latent infection in humans. Viruses such as herpes simplex viruses (cold sores and genital herpes), *Varicella virus* (chickenpox/shingles) and *Epstein Barr virus* (glandular fever) are herpes viruses. Poxviruses and herpes viruses replicate in different locations within the cell - poxviruses replicate in the cytoplasm, and herpes viruses in the nucleus (Gammon 2009). This difference in replication locations minimises the potential for recombination.

210. Recent reports suggest that poxvirus replication and virion assembly takes place in intracellular structures called virosomes. This intracellular milieu appears to create constraints that limit the fusion of co-infecting viral particles and the mixing of different viral DNAs (Lin & Evans 2010), reducing the ability of poxviruses to recombine.

211. HGT could also result in the presence of the introduced genes in bacteria and in animals or other eukaryotes. However, the introduced sequences were isolated from humans and are already widespread in the environment (See Chapter 1, Section 7.3).

212. A key consideration in the risk assessment process should be the safety of the protein product resulting from the expression of the introduced genes rather than horizontal gene transfer per se (Keese 2008). If the introduced genes or their end products are not associated with harm to people or other organisms then even in the unlikely event of HGT occurring, they should not pose risks to humans, animals or the environment. Conclusions reached for Risk scenarios 1-4 associated with the expression of the introduced genes did not represent an identified risk. Therefore, any rare occurrence of HGT of introduced genetic material to other organisms is expected to be unlikely to persist and/or result in an adverse effect.

213. ***Conclusion*:** The potential for an adverse outcome as a result of horizontal gene transfer is not identified as a risk that could be greater than negligible. Therefore, it does not warrant further assessment.

2.5 Unauthorised activities

Risk scenario 7. Use of the GMOs outside the proposed licence conditions (non-compliance)

214. If a licence were to be issued, non-compliance with the proposed conditions of the licence could lead to exposure to the GM vaccines outside the scope of the proposed trial. The adverse outcomes that may result are discussed in the sections above. The Act provides for substantial penalties for non-compliance and unauthorised dealings with GMOs. The Act also requires that the Regulator has regard for the suitability of the applicant to hold a licence prior to the issuing of a

licence. These legislative provisions are considered sufficient to minimise risks from unauthorised activities.

215. ***Conclusion*****:** The potential for an adverse outcome as a result of unauthorised activities is not identified as a risk that could be greater than negligible. Therefore, it does not warrant further assessment.

Section 3 Risk estimate process and assessment of significant risk

216. The risk assessment begins with postulation of potential pathways that might lead to harm to the health and safety of people or the environment during the proposed release of GMOs due to gene technology, and how it could happen, in comparison to the parent organism and within the context of the receiving environment.

217. Seven risk scenarios were identified whereby the proposed dealings might give rise to harm to people or the environment. This included consideration of whether expression of the introduced genes could: result in products that are toxic to people or other organisms; alter characteristics that may impact on the disease burden of GM virus, or produce unintended changes in viral characteristics. The opportunity for gene transfer to other organisms, and its effects if this occurred were also considered.

218. A risk is only identified when a risk scenario is considered to have some chance of causing harm as a result of the gene technology. 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.

219. The characterisation of the seven risk scenarios in relation to both the seriousness and likelihood of harm, in the context of the control measures proposed by the applicant, did not give rise to any identified risks that required further assessment. The principal reasons for this include:

- Transmission of the two GM viruses via viral shedding during the trial will be minimised through the participant exclusion criteria, the route of inoculation (subcutaneous), bandaging of the injection site and appropriate training of both healthcare workers and patients.
- No increase in disease severity due to the introduction of the four human genes has been observed in previous clinical trials.
- The products of the four introduced genes are not expected to be toxic to humans or other animals, due to their widespread presence in the environment.

220. Therefore, any risks to the health and safety of people, or the environment, from the proposed release of the GM vaccines into the environment are considered to be negligible. Hence, the Regulator considers that the dealings involved in this proposed trial do not pose a significant risk to either people or the environment [6].

Section 4 Uncertainty

221. Uncertainty is an intrinsic property of risk and is present in all aspects of risk analysis, including risk assessment, risk management and risk communication. Both dimensions of risk (consequence and likelihood) are always uncertain to some degree.

[6] As none of the proposed dealings are considered to pose a significant risk to people or the environment, section 52(2)(d)(ii) of the Act mandates a minimum period of 30 days for consultation on the RARMP. However, the Regulator has allowed up to 6 weeks for the receipt of submissions from prescribed experts, agencies and authorities and the public.

222. Uncertainty in risk assessments can arise from incomplete knowledge or inherent biological variability[7]. For clinical trials, because they involve the conduct of research, some knowledge gaps are inevitable. This is one reason they are required to be conducted under specific limits and controls to restrict exposure to the GMOs and their genetic material in the environment, rather than necessarily to treat an identified risk.

223. For DIR 116, the possibility of increased disease burden and unintended change to viral characteristics was considered in individual risk scenarios. Uncertainty is noted particularly in relation to the characterisation of the potential shedding of GM virus from trial subjects.

224. Additional data, including information to address these uncertainties, may be required to assess possible future applications for commercial release of the GM vaccines.

225. Chapter 3, Section 5 discusses information that may be required for future releases.

[7] A more detailed discussion is contained in the Regulator's *Risk Analysis Framework* available at <http://www.ogtr.gov.au/internet/ogtr/publishing.nsf/Content/riskassessments-1> or via Free call 1800 181 030.

Chapter 3 Risk management plan

Section 1 Background

226. 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 evaluates and treats identified risks, evaluates controls and limits proposed by the applicant, and considers general risk management measures. The risk management plan informs the Regulator's decision-making process and is given effect through licence conditions. In addition, the roles and responsibilities of other regulators under Australia's integrated regulatory framework for gene technology are explained.

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

228. All licences are required to be 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 permit OGTR monitors to enter premises where the dealings are being conducted, and section 65 requires the licence holder to report any information about risks or unintended effects of the dealing to the Regulator on becoming aware of them. Matters related to the ongoing suitability of the licence holder are also required to be reported to the Regulator.

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

Section 2 Responsibilities of other Australian regulators

230. Australia's gene technology regulatory system operates as part of an integrated legislative framework. Other agencies that also regulate GMOs or GM products include FSANZ, APVMA, Therapeutic Goods Administration (TGA), National Health and Medical Research Council (NHMRC), National Industrial Chemicals Notification and Assessment Scheme (NICNAS) and DAFF Biosecurity. Dealings conducted under a licence issued by the Regulator may also be subject to regulation by one or more of these agencies[8].

231. The *Gene Technology Act 2000* requires the Regulator to consult these agencies during the assessment of DIR applications. *The Gene Technology Act 2000* requires the agencies to consult the Regulator for the purpose of making certain decisions regarding their assessments of products that are, or contain a product from, a GMO.

232. The applicant will require appropriate authorisation under the *Therapeutics Goods Act 1989* for this proposed clinical trial of the GM vaccines. The applicant has notified the TGA of the trial. Each trial site will also notify the TGA through the Clinical Trial Notification (CTN) Scheme.

233. HREC assessment and approval is an integral part of the governance structure for clinical trials and is also required before the trial can commence.

[8] More information on Australia's integrated regulatory framework for gene technology is contained in the *Risk Analysis Framework (OGTR 2009)* available from the Office of the Gene Technology Regulator. Free call 1800 181 030 or at <http://www.ogtr.gov.au/internet/ogtr/publishing.nsf/Content/riskassessments-1>

Section 3 Risk treatment measures for identified risks

234. The risk assessment of risk scenarios listed in Chapter 2 concluded that there are negligible risks to people and the environment from the proposed trial of a GM vaccine. The risk scenarios were considered in the context of the scale of the proposed trial (up to 1200 adult male trial participants worldwide, across clinical sites in Australia, over a period of up to five years), the proposed containment measures (Chapter 1, Section 4.3), and the receiving environment (Chapter 1, Section 6.6). The *Risk Analysis Framework* (OGTR 2009), which guides the risk assessment and risk management process, defines negligible risks as insubstantial with no present need to invoke actions for their mitigation. Therefore, no conditions are imposed to treat these negligible risks.

Section 4 General risk management

235. Licence conditions have been imposed to prevent dissemination of the GMOs in the environment and limit the trial to the size and locations proposed in the application. These considerations were important in establishing the context for the risk assessment and in reaching the conclusion that the risks posed to people and environment are negligible. The conditions are summarised in this Chapter.

4.1 Licence conditions to limit and control the release

4.1.1 Consideration of limits and controls proposed by PPD Pty. Ltd.

236. Chapter 1, Sections 4.2 and 4.3 provides details of the limits and controls proposed by PPD in their application, which are discussed in the risk scenarios characterised for the trial in Chapter 2. The appropriateness of these limits is considered further below.

237. The proposed trial would be confined to 1200 trial participants worldwide. A proportion of these trial participants will be in Australia, and trial activities will take place at clinical sites in the ACT, NSW, Queensland, South Australia, Victoria and Western Australia. The applicant has proposed that the trial will be completed within five years of trial commencement. These measures would limit the exposure of people and animals to the GM viruses and have been included as licence requirements.

238. Limiting the trial to participants who have previously been inoculated with vaccinia as a smallpox vaccination, combined with the subcutaneous administration method minimises the likelihood of pock formation following inoculation with GM vaccinia, and consequently minimises shedding of GM vaccinia. Education of staff and patients on inoculation site care, bandaging and hygiene should further minimise transmission of GM vaccinia in the environment. Licence conditions require that the Licence Holder provide appropriate medical care to all persons accidentally exposed to GM vaccinia, or showing signs of infection with GM vaccinia.

239. Exclusion of participants from the trial that may come into contact with individuals at risk of complicated disease from exposure to the GM vaccinia will reduce the opportunity for transmission of the GM virus. These include people with immunodeficiency, eczema and other exfoliative skin disorders, women who are pregnant or breastfeeding, and children less than three years of age. These exclusion criteria have been included as licence requirements.

240. Inoculations will be performed by trained nurses and/or physicians at clinical facilities in accordance with the World Health Organisation *Standard Precautions in Health Care* (World Health Organisation 2007) and the International Conference on Harmonisation *Good Clinical Practice Guidelines* (ICH 1996). The WHO standard precautions detail appropriate hygiene, personal protective equipment and decontamination procedures to prevent direct contact with the GM viruses. These practices and procedures will minimise exposure of people undertaking in the dealings to the GM viruses and have been included as licence requirements.

241. The applicant has proposed standard infection control practices and procedures that minimise exposure to the GM viruses. Storage and transport of vaccine stocks containing GM virus will be in

accordance with the Regulator's *Guidelines for the Transport, Storage and Disposal of GMOs* (http://www.ogtr.gov.au/). These practices and procedures will minimise exposure of other people and the environment to the GM viruses and have been included as licence requirements.

242. The applicant has stated that all waste, except patient-generated waste following the GM fowlpox inoculation, will be disposed of in accordance with standard clinical waste disposal practices. The appropriate disposal of clinical waste and unused pharmaceuticals is regulated through relevant state and local government OH&S and environmental protection legislation (Australian Capital Territory 1991a; 1991b; EPA Victoria 2009; New South Wales 1997; Northern Territory 2009; Queensland 2000; 2011; 2012; South Australia 1993; 2009; Victoria 2000; West Australia 2004). Typically clinical waste is destroyed through high temperature incineration or sterilised through an appropriate steam or temperature regime followed by burial in designated land fill sites. These methods are considered appropriate to dispose of the GMOs and therefore no further conditions related to disposal are required. An audit of waste disposal practices in certified facilities that included clinical facilities has been conducted by the Compliance Investigation Unit of OGTR. An acceptable level of compliance with designated practices was found. These practices and procedures will minimise exposure of other people to the GM viruses and have been included as licence requirements.

4.1.2 Summary of measures proposed by the Regulator to limit and control the proposed release

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

- limit the trial to a maximum of 1200 trial participants inoculated with the GM viruses at designated clinical facilities
- restrict exposure of at-risk individuals by specific exclusion criteria
- restrict trial participation to people who have previously received a vaccinia vaccination
- restrict the method of inoculation of GM vaccinia to subcutaneous inoculation
- ensure that inoculations be performed by trained nurses and/or physicians at clinical facilities in accordance with standard universal precautions and ICH-GCP[9], and that appropriate personal protective equipment is worn.
- store and transport all GM vaccines in accordance with relevant regulations and guidelines[10]
- dispose of all clinical waste, and patient waste following GM vaccinia inoculation, in accordance with standard clinical waste disposal practices.

4.1.3 Measures to control other activities associated with the trial

244. The Regulator has issued *Guidelines for the Transport, Storage and Disposal of GMOs* (http://www.ogtr.gov.au/). The licence imposes conditions for transport and storage of the GMOs based on these guidelines.

245. Conditions applying to the collection of samples for experimental analyses are also included in the licence conditions.

[9] The international conference on harmonisation of technical requirements for registration of pharmaceuticals for human use, guidelines for good clinical practice (ICH 1996)

[10] The Gene Technology Regulator's *Guidelines for the Transport, Storage and Disposal of GMOs;* IATA Transportation Regulations

4.2 Other risk management considerations

246. All DIR licences issued by the Regulator contain a number of general conditions that relate to general risk management. These include, for example:

- applicant suitability
- contingency plans
- identification of the persons or classes of persons covered by the licence
- reporting structures, including a requirement to inform the Regulator if the applicant becomes aware of any additional information about risks to the health and safety of people or the environment
- a requirement that the applicant allows access to the trial sites by the Regulator, or persons authorised by the Regulator, for the purpose of monitoring or auditing.

4.2.1 Applicant suitability

247. 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 (both individuals and the body corporate)
- 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.

248. On the basis of information submitted by the applicant and records held by the OGTR, the Regulator considers PPD suitable to hold a licence.

249. The licence includes a requirement that the licence holder inform the Regulator of any circumstances that would affect their suitability.

250. PPD must remain an accredited organisation under the Act and continue to have access to a properly constituted Institutional Biosafety Committee.

4.2.2 Contingency plans

251. PPD is required to submit a contingency plan to the Regulator within 30 days of the issue date of the licence. This plan must detail measures to be undertaken in the event of:

a) the unintended release of the GMOs, including exposure of, or transmission to, persons other than trial participants, or spills

b) a person exposed to PROSTVAC-V developing a severe adverse response, including those resulting from exposure to Vaccinia virus such as eczema vaccinatum, progressive vaccinia, generalised vaccinia and postvaccinal encephalitis.

252. PPD is also required to provide a method to the Regulator for the reliable detection of the presence of the GMOs and the introduced genetic materials in a recipient organism. This instrument would be required within 30 days of the issue date of the licence.

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

253. The persons covered by the licence are 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 commencing the clinical trial, PPD is also required to provide a list of people and organisations who will be covered, or the function or position where names are not known at the time.

254. Trial participants are expected to collect and return waste that has come into contact with the vaccinia inoculation site, such as bandages or gloves, as this waste may contain viable GM vaccinia. Therefore, trial participants are also covered by the licence.

4.2.4 Reporting structures

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

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

256. The licence holder is also required to submit an Annual Report within 90 days of the anniversary of the licence containing any information required by the licence, including the results of inspection activities.

4.2.5 Monitoring for Compliance

257. 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.

258. 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.

259. 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 health and safety of people or the environment could result.

Section 5 Issues to be addressed for future releases

260. Additional information has been identified that may be required to assess an application for a large scale or commercial release of the GM vaccines, or to justify a reduction in containment conditions. This relates to the potential shedding of GM vaccinia from trial subjects.

Section 6 Conclusions of the RARMP

261. The risk assessment concludes that this proposed limited and controlled release of GM vaccines, to be administered in clinical facilities in ACT, NSW, QLD, SA, VIC and WA, involving up to 1200 trial participants and expected to run for up to five years, poses negligible risks to the health and safety of people or the environment as a result of gene technology.

262. The risk management plan concludes that these negligible risks do not require specific risk treatment measures. However, licence conditions have been imposed to limit the trial to the size, locations and duration, and to require controls in line with those proposed by the applicant, as these were important considerations in establishing the context for assessing the risks.

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Appendix A Summary of issues raised in submissions received from prescribed experts, agencies and authorities[11]

The Regulator received several 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.

Issue raised	Consideration in RARMP	Comment
Excluding patients with close association to poultry, pigeons or cage birds from the trial – transmission of GM fowlpox.	Risk Scenario 3	Further consideration has been given to the potential pathway of transmission of GM fowlpox to chickens and possible consequences should such transmission occur. As discussed in Chapter 1, Section 5.2.2 and Risk Scenario 3, *Fowlpox virus* does not replicate in mammals and would not be shed by the trial participants subsequent to inoculation. Any virus present at the vaccination site following inoculation will be contained by the initial bandage. In the absence of stabilising biological material such as scabs or other cell debris, this negligible amount of purified virus would have limited environmental viability on the bandage. *Fowlpox virus* is endemic to Australia. The Risk Assessment did not identify any harm to susceptible avian hosts resulting from exposure to GM fowlpox. Therefore, no additional waste treatment measures are considered necessary for patient waste following GM fowlpox inoculation and there is no need to exclude trial participants with close association to susceptible avian hosts.
Potential for transmission of the GM vaccinia to persons other than trial participants	Risk Scenario 3, Chapter 2, Section 4.	Further consideration has been given to the likelihood of pustule formation and subsequent transmission to persons not involved in the clinical trial. As discussed in Chapter 1, Section 5.2.1, a large body of scientific literature exists which examines the rate of transmission of *Vaccinia virus* following vaccination to protect against smallpox. The genetic modification is not expected to alter the rate of transmission of GM vaccinia. Trial participants will be provided with detailed instructions on injection site care, including the bandaging of pocks and handling of waste, and will be required to return any GM vaccinia waste produced to the clinical site for destruction. This will reduce the possibility of non-trial participants being exposed to GM vaccinia. Licence conditions have been imposed which require that all cases of transmission of GM vaccinia to a person in Australia not involved in the trial be reported to the Regulator. The Licence holder is also required to provide medical treatment to affected individuals and prevent further transmission. Characterisation of the potential shedding of GM virus from trial subjects was identified as an area of uncertainty which should be addressed for future applications. The licence requires that the Regulator be advised of the percentage of trial participants worldwide developing a pustule following exposure to GM vaccinia.

[11] GTTAC, State and Territory Governments, Australian Government agencies, LGAs and the Minister for the Environment.

Issue raised	Consideration in RARMP	Comment
Vaccination of health care workers against vaccinia	-	The *Australian Immunisation Handbook* (2008) recommends vaccination for laboratory workers using live pox virus in recombinant gene research, but does not recommend health care professionals being vaccinated prior to administering the vaccine for smallpox (vaccinia). Health care professionals involved in the trial will be provided with information on both GM viruses, instructions on how to safely handle the viruses and what to do should accidental exposure occur.
Identification and exclusion of potential trial participants with close contact with susceptible individuals	Chapter 1, Section 4.1	Exclusion criteria for trial participants are discussed in Chapter 1, Section 4.1. Licence conditions prohibit the Licence Holder from enrolling persons who they have ascertained are likely to have contact with susceptible persons.
Previous experience with the GMOs	Chapter 1, Section 6.6, Risk Scenarios 2 and 3	The applicant has provided data from previous clinical trials using the GM viruses, including information on the rate of pustule formation and other safety data.
Effect of the GM vaccines on males that do not have prostate cancer.	Chapter 1, Sections 6.6 and 7.3	All males naturally produce the gene products of all four introduced genes. Typically *Vaccinia virus* produces a localised infection and is rapidly cleared by the immune system. Therefore, the introduced genes would be expressed transiently in an infected individual. Results from previous clinical and non-clinical trials with the GM vaccinia proposed to be used in this trial, have shown that a single exposure to either GM virus is not sufficient to induce a lasting immune response against PSA.
Availability of VIG and cidofovir in the advent of an adverse response to Vaccinia	Chapter 3, Section 4.1	Licence conditions have been imposed which require the Licence Holder to provide appropriate medical treatment to persons developing symptoms of a severe adverse response to GM vaccinia.
GMO accounting requirements	-	A Licence condition has been imposed which requires that all vials of GMOs imported into Australia, and their contents, be accounted for from import to disposal.
Suitability of the proposed methods of waste disposal	Risk Scenario 4, Chapter 3, Section 4.1	Licence conditions have been imposed which require that GMOs be appropriately destroyed. They will be placed in two sealed containers, the outermost of which must be labelled, prior to destruction which may include through the clinical waste stream. Standard clinical waste disposal methods have been assessed as suitable for the destruction of the GM viruses covered by this application. The disposal of clinical waste is regulated through relevant state and local government OH&S and environmental protection legislation. An audit of waste disposal practices in certified facilities, including clinical facilities, has been conducted by the Compliance Investigation Unit of OGTR. An acceptable level of compliance with designated practices was found. The proposed method of disposal meets the requirements of Part 3.1 of the Regulator's Guidelines for the Transport, Storage and Disposal of GMOs.

Appendix B Summary of issues raised in submissions received from the public

The Regulator received one submission from the public on the consultation RARMP. The issue raised in this submission is summarised in the table below. 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 currently available scientific evidence in finalising the RARMP that formed the basis of the Regulator's decision to issue the licence.

View (general tone): n = neutral; x = do not support; y = support

Issues raised: RA: Risk analysis;

Type: I: Individual

Sub. No:	Type	View	Issue	Summary of issues raised	Comment
1	I	n	RA	Concerned with the use of the term 'identified risk' to only refer to those risks where the level of risk is assessed as greater than negligible, rather than for all risks that have been considered. Suggests the term 'significant risk' would be more accurate.	The risk assessment terminology used by the Regulator is discussed in the Regulator's *Risk Assessment Framework* (2009) which has been developed in association with the relevant international standards. The term 'significant risk' is used in the *Gene Technology Act 2000* to refer to a risk which would require a longer period of consultation, and would usually require control or mitigation measures to be imposed.