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

August 2023

Risk Assessment and Risk Management Plan (consultation version) for

DIR 196

Commercial supply of Qdenga, a live attenuated GM dengue vaccine

Applicant: Takeda Pharmaceuticals Australia Pty Ltd

This RARMP is open for consultation until 18 October 2023.

Written comments on the risks to human health and safety and the environment posed by this proposed supply of a GM dengue vaccine are invited. You may make your submission

via mail to: The Office of the Gene Technology Regulator
MDP 54, GPO Box 9848, Canberra ACT 2601 or
via email to: ogtr@health.gov.au.

Please note that issues regarding patient safety and the quality and efficacy of the vaccine **do not** fall within the scope of these evaluations as they are the responsibilities of other agencies and authorities.

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Summary of the Risk Assessment and Risk Management Plan (consultation version) for Licence Application No. DIR 196

Introduction

The Gene Technology Regulator (the Regulator) has received a licence application for the import, transport, storage, and disposal of a genetically modified (GM) vaccine, Qdenga, as part of its commercial supply in Australia as a human vaccine against dengue virus. The vaccine would be available under prescription for people travelling to dengue-affected areas.

Before Qdenga can be registered as a human vaccine, its quality, safety, and efficacy must be assessed by the Therapeutic Goods Administration (TGA). If registered as a human vaccine, the TGA may impose conditions relating to the use and labelling of the GM vaccine. As Qdenga is manufactured overseas, a permit from the Department of Agriculture, Fisheries and Forestry will be required for its import into Australia.

The Regulator has prepared a draft Risk Assessment and Risk Management Plan (RARMP) for this application, which concludes that the proposed supply of the GM vaccine poses negligible risks to human health and safety and the environment, and no specific risk treatment measures are proposed. Licence conditions have been drafted for the proposed dealings. The Regulator invites submissions on the RARMP, including draft licence conditions, to inform the decision on whether or not to issue a licence.

The application

Project Title	Commercial supply of Qdenga, a live attenuated GM dengue vaccine
Parent organism	Dengue virus serotype 2 strain PDK-53
Modified trait	Altered antigen expression
Genetic modification	A 'strain' is a genetic variant or subtype of a microorganism. Strains of dengue virus can also be categorised into 4 distinct 'serotypes' based on their surface antigen expression. The genetic backbone for the GMOs in this application is a non-GM dengue virus serotype 2 strain that has been attenuated (weakened) through spontaneous mutations that occurred during a subculturing process in tissue culture. The vaccine contains 4 GM strains of dengue virus, known as TDV-1, TDV-2, TDV-3, and TDV-4, where the serotype 2 backbone has been modified to contain pre-membrane (*prM*) and envelope (*E*) genes from the 4 dengue serotypes. As glycoproteins prM and E are present on the surface of dengue virus particles and are recognised by the human immune system, the GM vaccine is intended to stimulate immune responses against all these serotypes.
Proposed locations	Australia-wide
Principal purpose	Commercial supply of the GM dengue vaccine
Previous approvals	The GM vaccine has not previously been approved in Australia. Internationally, the GM vaccine has been approved by health authorities in Indonesia, the European Union, Great Britain, Brazil, Argentina, and Thailand.
Proposed period of release	From issue of licence

Risk assessment

The risk assessment process considers how the genetic modification and proposed activities conducted with the GM vaccine in the context of import, transport, storage, and disposal might lead to harm to people or the environment. Risks are characterised in relation to both the seriousness and likelihood of harm, taking into account information in the application, relevant previous approvals, current scientific knowledge and advice received from a wide range of experts, agencies and authorities consulted on the preparation of the RARMP. Both the short and long term risks were considered.

Credible pathways to potential harm that were considered include the potential accidental exposure of people to the GMOs during transport and storage, preparation and administration of the vaccine, and during disposal of the GMOs and any associated waste. The potential for the GMOs to be released into the environment and its effects were also considered.

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

The principal reasons for the conclusion of negligible risks associated with the transport, storage and disposal of the GMOs are:

- the GMOs are attenuated in comparison to wildtype (WT) dengue
- the dose received through accidental exposure would be smaller than that administered during vaccination
- the GM vaccine has a favourable safety profile at doses higher than would be expected through accidental exposure
- import, transport, storage, and disposal will follow well established procedures.

Risk management

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 and considers general risk management measures. The risk management plan is given effect through licence conditions. Draft licence conditions are detailed in Chapter 4 of the RARMP.

The risk management plan concludes that risks from the proposed dealings can be managed so that people and the environment are protected by imposing general conditions to ensure that there is ongoing oversight of the vaccine containing the GMOs.

As the level of risk was assessed as negligible, specific risk treatment is not required. However, the Regulator has drafted licence conditions regarding post release review (post-market surveillance) to ensure that there is ongoing oversight of the supply of the GM dengue vaccine and to allow the collection of ongoing information to verify the findings of the RARMP. The draft licence also contains a number of general conditions relating to ongoing licence holder suitability, auditing and monitoring, and reporting requirements, which include an obligation to report any unintended effects.

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Abbreviations

ADE	Antibody-dependent enhancement
AICIS	Australian Industrial Chemicals Introduction Scheme
APVMA	Australian Pesticides and Veterinary Medicines Authority
ARTG	Australian Register of Therapeutic Goods
CDC	Centers for Disease Control and Prevention (United States)
DAFF	Department of Agriculture, Fisheries and Forestry
DENV	Dengue virus serotype
DF	Dengue fever
DHF	Dengue haemorrhagic fever
DIR	Dealings involving intentional release
DNIR	Dealings not involving intentional release
DSS	Dengue shock syndrome
E	Envelope protein
FSANZ	Food Standards Australia New Zealand
GM(O)	Genetically modified (organism)
GTTAC	Gene Technology Technical Advisory Committee
HGT	Horizontal gene transfer
MID_{50}	50% mosquito infectious dose
M	Membrane protein
MVS	Master virus seed
NCR	Non-coding region
NS	Non-structural protein
OGTR	Office of the Gene Technology Regulator
PCR	Polymerase chain reaction
PDK	Primary dog kidney
PFU	Plaque forming units
prM	Pre-membrane protein
PRR	Post release review
RARMP	Risk Assessment and Risk Management Plan
TGA	Therapeutic Goods Administration
the Act	The *Gene Technology Act 2000*
the Regulations	The Gene Technology Regulations 2001
the Regulator	The Gene Technology Regulator
vRNA	Viral RNA
WT	Wildtype
WVS	Working virus seed

Chapter 1 Risk assessment context

Section 1 Background

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

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

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

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

5. Figure 1 shows the information that is considered, within the regulatory framework, in establishing the risk assessment context. This information is specific for each application. Potential risks to the health and safety of people or the environment posed by the proposed release are assessed within this context. Chapter 1 provides the specific information for establishing the risk assessment context for this application.

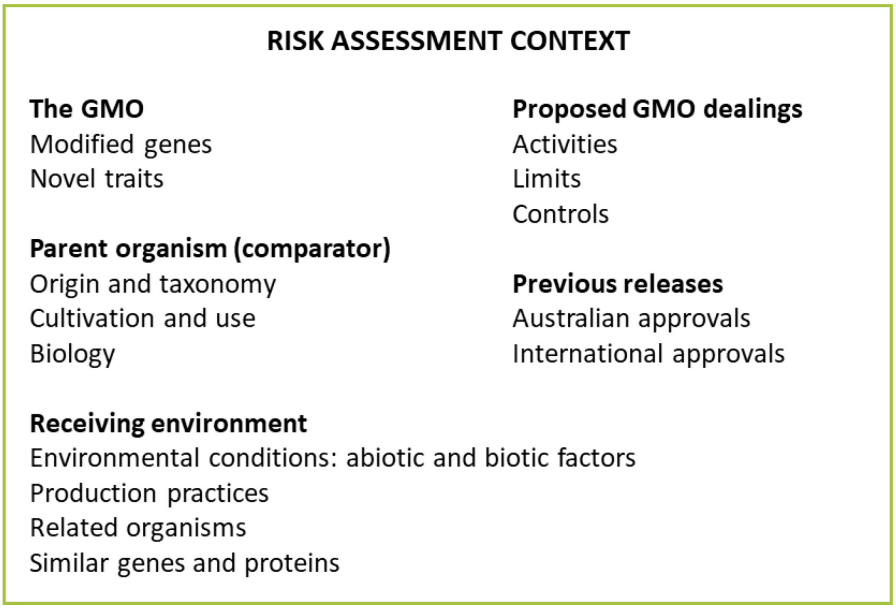

Figure 1. Summary of parameters used to establish the risk assessment context, within the legislative requirements, operational policies and guidelines of the OGTR, and the Risk Analysis Framework

6. Since this application is for commercial purposes, it does not meet the criteria for a limited and controlled release application under section 50A of the Act. Therefore, under section 50(3) of the Act, the Regulator was required to seek advice from prescribed experts, agencies and authorities on

matters relevant to the preparation of the RARMP. This first round of consultation included the Gene Technology Technical Advisory Committee (GTTAC), State and Territory Governments, Australian Government authorities and agencies prescribed in the Regulations and the Minister for the Environment. A summary of issues contained in submissions received is provided in Appendix A.

7. Section 52 of the Act requires the Regulator to seek comment on the RARMP from the experts, agencies and authorities outlined above, as well as the public through a second round of consultation.

1.1 Interface with other regulatory schemes

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

9. To avoid duplication of regulatory oversight, risks that will be considered by other regulatory agencies would not be assessed by the Regulator.

10. For the commercial supply of a live GM vaccine, dealings regulated under the Act include the import, transport, storage, and disposal of GMOs. The Regulator has assessed risks to people as a consequence of these activities and risks from persistence of the GMOs in the environment.

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

12. The TGA provides a national system of controls for therapeutic goods. It administers the provisions of the *Therapeutic Goods Act 1989* which specifies the standard that must be met before a vaccine can be included on the Australian Register of Therapeutic Goods (ARTG). Inclusion in the ARTG is required before a vaccine can be lawfully supplied in Australia. As part of this process, the TGA would assess the quality, safety, and efficacy of the vaccine. Quality aspects could include batch-to-batch consistency in vaccine composition, purity, and potency. Safety aspects could include the toxicological and allergenicity profile of the vaccine, including any excipients, by-products, and impurities from manufacture.

13. The administration/use of GMOs as therapeutics is not regulated under gene technology legislation. The Regulator does not assess vaccine excipients and would not assess manufacturing by-products and impurities unless they are GM products.

14. The labelling, handling, sale and supply of scheduled medicines is regulated through the Scheduling Policy Framework for Medicines and Chemicals (AHMAC, 2018). Guidelines for the safe handling, storage and distribution of Schedule 4 medicines such as vaccines are specified through the Australian Code of good wholesaling practice for medicines in schedules 2, 3, 4 and 8 (NCCTG, 2011). The provisions of this Code, which ensure that quality is maintained during wholesaling, are applied through applicable State and Territory therapeutic goods/drugs and poisons legislation, and/or State or Territory wholesaler licensing arrangements.

Section 2 The proposed dealings

15. Takeda Pharmaceuticals Australia Pty Ltd (Takeda) is seeking authorisation for the commercial supply of a tetravalent live attenuated[1] GM dengue vaccine, Qdenga, in Australia. Qdenga has been developed as a vaccine to prevent against dengue disease in adults, adolescents, and children. The vaccine is intended to be administered by medical professionals to people residing in or travelling to areas where dengue viruses are endemic.

16. For the ongoing commercial supply of Qdenga, the dealings assessed by the Regulator are:

(a) import the GMOs

(b) transport the GMOs

(c) dispose of the GMOs

and the possession (including storage), supply or use of the GMOs for the purposes of, or in the course of, any of the above.

2.1 Details of the proposed dealings

17. Takeda proposes to manufacture Qdenga outside Australia and import the fully packaged product from Germany. The import of the vaccine requires a permit from DAFF.

18. The vaccine would be supplied as a freeze-dried powder in a glass vial with an airtight seal. The glass vial would be contained in outer packaging to minimise breakage and would then be placed in a labelled carton for transport and handling purposes. The vaccine would also be co-packaged with a diluent (sodium chloride solution) either in a glass pre-filled syringe or a glass vial.

19. Once the product has entered Australia, storage, transport, and handling would be conducted in accordance with local regulations, the *World Health Organization Good storage and distribution practices for medical products* (World Health Organization, 2020), and the *Australian Code of Good Wholesaling Practice for Medicines in schedules 2, 3, 4 and 8* (NCCTG, 2011),[2] which includes maintenance of the cold chain and security arrangements to prevent unauthorised access to the medicines.

20. Prior to distribution, the vaccine would be stored in cool rooms (2-8°C) at central storage facilities where access would be limited to authorised personnel. The vaccine would be distributed by a commercial courier that specialises in the handling of temperature sensitive pharmaceuticals and vaccines.

21. If approved by both the Regulator and the TGA, Takeda intends to supply Qdenga Australia-wide for vaccination of travellers. Vaccination sites would be medical or clinical facilities such as specialist travel clinics.

22. Once at the vaccination sites, the vaccine would be stored in a secure location with access limited to medical staff.

23. The vaccination course involves 2 doses, with the second dose administered 3 months after the first. Before administration, the vaccine powder must be reconstituted with the provided diluent, which is introduced into the vaccine vial using a sterile needle and syringe. Once the solution becomes clear, 0.5 mL of the reconstituted Qdenga vaccine is withdrawn from the vial and used for administration by subcutaneous injection. Administration will be conducted by trained medical staff at medical or clinical facilities. Staff administering the vaccine would be expected to follow all relevant

[1] **Attenuated** viruses are weakened strains compared to the WT counterpart and produce no or little disease upon host infection.

[2] In the *Therapeutic Goods - Poisons Standard*, dengue vaccines are listed as Schedule 4: Prescription only medicines (Therapeutic Goods Administration, 2023).

national guidelines for the prevention of transmission of infectious agents in healthcare settings (National Health and Medical Research Council, 2019).

24. Disposal of the GM vaccine and any associated material contaminated with the GMOs would be in accordance with the requirements of the *Work Health and Safety Act 2011* (Commonwealth of Australia, 2011) and related State and Territory legislation.

25. At the central storage facilities, unused or expired vaccine would be disposed of by a clinical waste contractor and all stock destroyed would be recorded.

26. At the medical facilities where vaccination occurs, residual vaccine and waste associated with the vaccination process would be disposed of in the clinical waste stream. Following administration, used vaccine syringes and any waste contaminated with the GMOs would be placed immediately into secured containers or sealed bags and destroyed following institutional procedures for the disposal of biohazardous material. Unused or expired vaccine would be returned to a Takeda nominated central storage facility for destruction or would be disposed of by the pharmacy or healthcare institution following defined procedures in line with Australian regulations.

27. During disposal, waste would be inactivated through a measure such as high temperature incineration.

Section 3 The parent organism

28. The vaccine contains 4 live attenuated strains of dengue virus (see Section 4 for information on the GMOs). The parent organism for the strains is a non-GM attenuated strain of dengue virus known as dengue virus serotype 2 (DENV-2) PDK-53. The characteristics of the parent organism provide a baseline for comparing the potential for harm from dealings with GMOs. As such, the relevant biological properties of dengue virus, and the DENV-2 PDK-53 strain will be discussed here.

29. Dengue is an arbovirus (arthropod borne virus; transmitted by an arthropod to a vertebrate host during a blood meal) that is transmitted to humans by female *Aedes* mosquitoes. Dengue belongs to the genus *Flavivirus* in the Flaviviridae family of RNA viruses, which also includes other genera such as *Hepacivirus* (e.g. Hepatitis C) and *Pestivirus* (e.g. Bovine viral diarrhoea virus). Other viruses in the Flavivirus genus that are human pathogens include yellow fever virus, tick-borne encephalitis virus, Japanese encephalitis virus, and West Nile virus (Simmonds et al., 2017). Dengue is classified as a Risk Group 2 organism (Standards Australia/New Zealand, 2010).[3]

30. There are many different strains (genetic variants) of dengue virus. These strains can be categorised into serotypes, which are distinct 'sub species' that share common surface structures (antigens). There are 4 dengue virus serotypes (DENV-1, DENV-2, DENV-3, and DENV-4), which share approximately 60-79% identity at the amino acid level (Khan et al., 2006).

3.1 Human pathology

31. In humans, the majority of dengue cases are asymptomatic (also called subclinical or inapparent), particularly in children (Endy et al., 2011; Grange et al., 2014; Salje et al., 2018). In those with symptomatic infection, classical dengue fever (DF) presents as an acute flu-like illness, with a sudden onset of a fever accompanied by other symptoms such as headache, fatigue, muscle and joint pains, nausea, diarrhoea, vomiting, or rash (Agrawal et al., 2018). Symptoms appear after an incubation period of 3-10 days, generally last for 2-7 days and are followed by a recovery phase of 3-5 days (Nishiura and Halstead, 2007; World Health Organization, 2009; Chan and Johansson, 2012). While symptoms such as joint and muscle pain may continue for several years after the initial dengue

[3] Definition of a **Risk Group 2** organism (Standards Australia/New Zealand, 2010): "Moderate individual risk, limited community risk – a microorganism that is unlikely to be a significant risk to laboratory workers, the community, livestock, or the environment; laboratory exposures may cause infection, but effective treatment and preventative measures are available, and the risk of spread is limited".

infection (Garcia et al., 2011; Kalimuddin et al., 2022), dengue virus replication in people is considered to be acute and self-limiting. Dengue virus is not known to form latent infection with the potential for reactivation, unlike herpes viruses (Traylen et al., 2011).

32. In a small proportion of people, DF can progress to a potentially lethal form called severe dengue. As shown in Figure 2, there may or may not be warning signs of the progression from DF to severe dengue. If apparent, these warning signs include abdominal pain, persistent vomiting, and fluid accumulation. The severe form of dengue can manifest as severe haemorrhaging (also known as dengue haemorrhagic fever/DHF) or a state of hypovolemic shock from severe plasma leakage (also known as dengue shock syndrome/DSS) (World Health Organization, 2009).

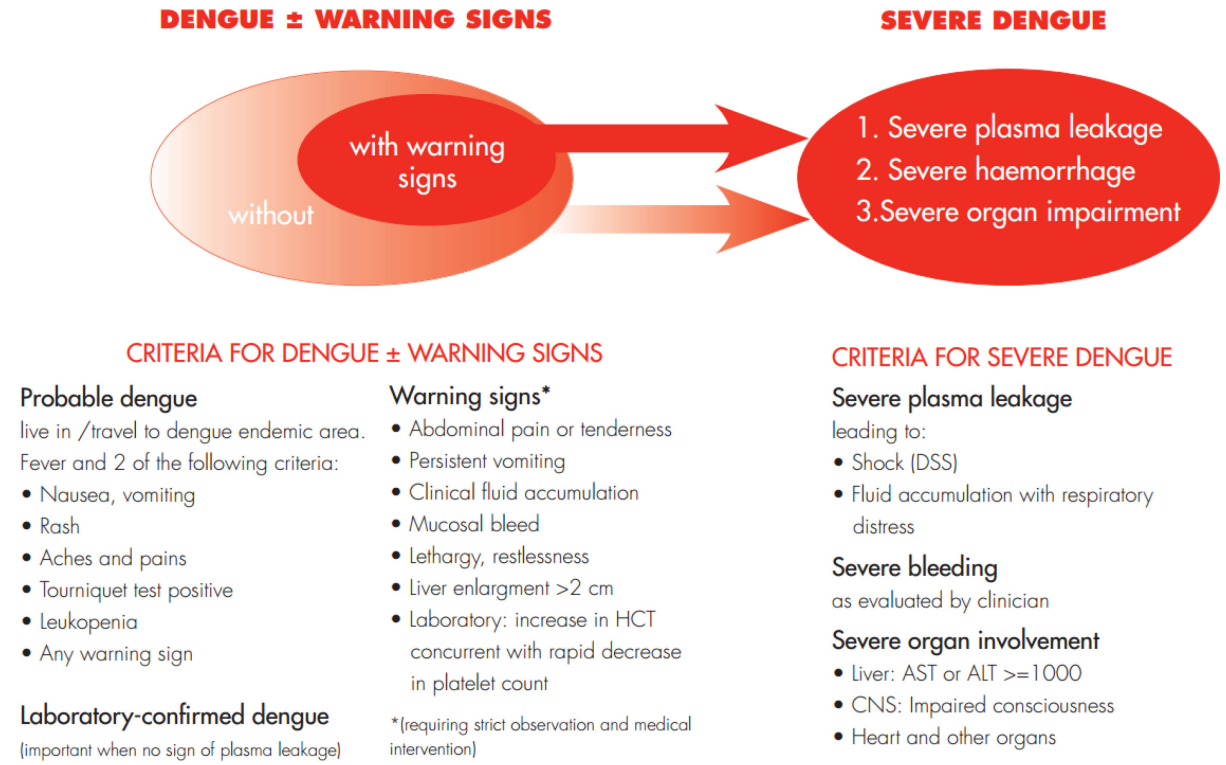

Figure 2. Symptomatic dengue classification and level of severity

Source: World Health Organization (2009). ALT = alanine aminotransferase (in U/L); AST = aspartate aminotransferase (in U/L); CNS = central nervous system; DSS = dengue shock syndrome; HCT = haematocrit.

33. Recovery from primary dengue infection confers lifelong immunity to the same serotype (homologous serotype) and temporary cross-protection against a different serotype (heterologous serotype). However, subsequent infection with a different serotype can result in an increased likelihood of a person developing severe dengue (Sabin, 1952). This phenomenon is largely driven by antibody-dependent enhancement (ADE), where the immune system boosts infection by dengue. On secondary infection with dengue, ineffective cross reactive antibodies do not neutralise the viral particles and instead promote uptake of the particles into monocytes or macrophages where the virus then replicates (Dejnirattisai et al., 2010).

34. As dengue is an immune-mediated disease, particularly in the context of the progression from DF to severe dengue, the possibility of different clinical expression in immunocompromised patients has been suggested. However, no consensus has been reached based on small studies and case reports. Some reports of transplant patients on immunosuppressive therapy suggest more severe clinical sequelae than experienced by the immune-competent population (Prasad et al., 2012; Maia et al., 2015), while others indicate a relatively benign course of disease in the immunocompromised (Renaud et al., 2007; Nasim et al., 2013). A small study of children with dengue infection who were immunocompromised on chemotherapy (cancer in remission) or steroid treatment found higher rates

of headaches, hepatic dysfunction, time to platelet recovery and need for fluid replacement than in immune-competent children with dengue, which the authors suggested shows a trend towards more complicated illness in immunocompromised children (Singh et al., 2017). The contribution of the specific immunocompromising therapies to these findings was not discussed.

35. No specific antivirals exist to treat dengue infection. Mild symptoms can be managed with analgesics (Jasamai et al., 2019). Judicious supportive medical care and fluid replacement is important in cases of severe dengue (World Health Organization, 2009).

3.2 Structure and genomic organisation

36. The dengue genome is approximately 10.7 kb long, including a coding region encoding viral proteins, as well as non-coding functional regions involved in regulating the different stages of the viral life cycle. The coding region comprises a single open reading frame which is flanked at either end by short but highly structured non-coding regions which play important roles in immune modulation and viral replication (Vasilakis et al., 2011; Ng et al., 2017; Ochsenreiter et al., 2019).

37. The open reading frame is translated as a single precursor polyprotein which is then cleaved by viral and host proteases into ten individual proteins; three structural and seven non-structural (NS) proteins. As shown in Figure 3, the three structural proteins capsid (C), pre-membrane (prM), and envelope (E) are grouped at the N terminal end, followed by the NS proteins. The structural proteins are components of the mature virus particle and the NS proteins are involved in viral replication and immune evasion (Vasilakis et al., 2011).

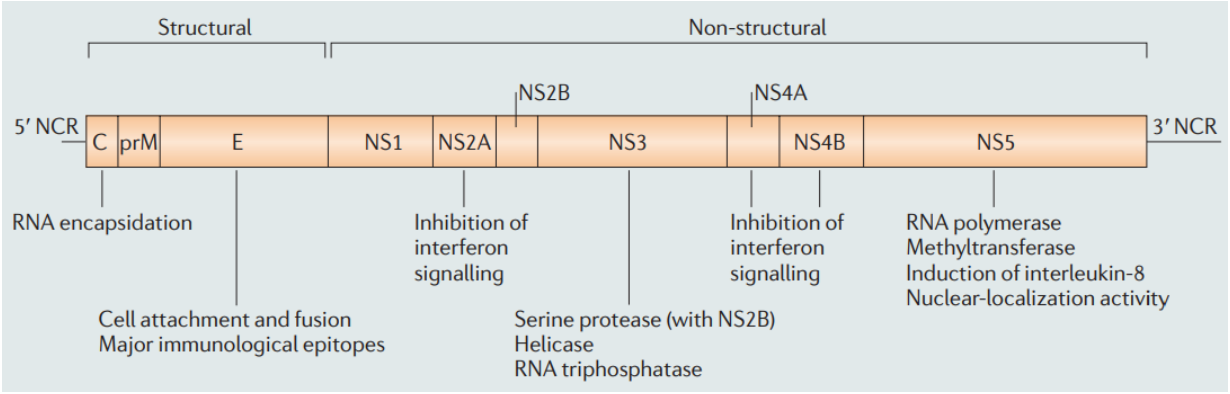

Figure 3. Organisation of the dengue genome

Source: Vasilakis et al. (2011). Encoded proteins and some of their functions are as indicated.

38. Dengue virions are spherical particles about 50 nm in diameter, consisting of a nucleocapsid of capsid (C) proteins and the RNA genome, enclosed within a lipid bilayer envelope (Mebus-Antunes et al., 2022). The lipid envelope is derived from host cell membranes and displays the virally-encoded membrane (prM/M) and E glycoproteins (Diamond and Pierson, 2015).

3.3 Pre-membrane, membrane, and envelope proteins

39. The prM/M and E glycoproteins are major dengue antigenic determinants (Dejnirattisai et al., 2010). There are 180 copies each of prM/M (9 kDa) and E (50 kDa) on the surface of the viral particle (Zhang et al., 2004; Lin et al., 2012; Wong et al., 2012; Thomas et al., 2020).

40. The transition of a dengue virion from the immature to mature form is driven by significant conformational changes in the prM/M and E glycoproteins. The M glycoprotein is first expressed as a precursor (prM), forming a ‘spiky’ heterodimeric structure with the E glycoprotein in immature dengue virions. During the virion maturation process through the host cell secretory pathway, low pH conditions lead to a conformational change in prM and E from an immature ‘spiky’ to mature ‘smooth’ morphology. Prior to viral exit from the cell, the majority of prM is cleaved by a furin-like cellular protease to produce the mature M. In the mature virion, M forms a transmembrane protein under the

E glycoprotein shell. The pr peptide forms a kind of 'cap' on the E protein, preventing premature fusion, until the mature dengue virion is released from the cell. The role of the E protein is to bind to the host receptor and facilitate fusion of the viral and host membranes, with these interactions determining cellular and species tropism (Stiasny and Heinz, 2006).

3.4 Viral infection and replication

41. Infection begins with viral attachment to the host cell surface via attachment molecules and receptors, with the first point of contact between the virus and host cell being glycoprotein E. Virions are internalised by endocytosis, and in the low pH environment of the endosome, viral glycoproteins mediate fusion between viral and cellular membranes and release of viral RNA (vRNA) into the cytoplasm. Genome replication takes place in the cytoplasm and does not involve a DNA intermediate - the positive-strand vRNA genome serves both as mRNA for translation of the polypeptide and as a template for RNA synthesis. A negative-strand RNA intermediate is synthesised, which then directs production of new positive-strand vRNA which can be used for new rounds of translation or as a substrate for encapsidation. Multiple rounds of translation and RNA synthesis produce high levels of viral proteins and vRNA (Gebhard et al., 2011; Back and Lundkvist, 2013). For virion production, vRNA is enclosed by the capsid which then acquires the viral envelope from the rough endoplasmic reticulum (Mebus-Antunes et al., 2022). Following a maturation process in the trans-Golgi network, mature infectious virions exit the cell via the secretory pathway (Mukhopadhyay et al., 2005; Stiasny and Heinz, 2006).

3.5 Viral load and shedding in humans

42. There are a number of methods for measuring the amounts of dengue virus in a sample. Polymerase chain reaction (PCR) can be used to detect vRNA and count the number of viral genomes in a sample, with concentration expressed as genomes/mL, copies/mL or cDNA/mL. In this RARMP, the term genomes/mL will be used for quantitative PCR testing. While PCR is a sensitive assay, it does not differentiate live infectious viral particles from non-infectious particles. Two infectivity assays that will be discussed in this RARMP are plaque assays and endpoint dilution assays. A plaque assay calculates the number of infectious viral particles in a sample as plaque forming units (PFU)/mL. The endpoint dilution assay measures the infectivity of the virus and is reported as 50% infectious dose (ID_{50}) (Payne, 2017). Across the literature, dengue virus quantitation findings are reported in a mixture of logarithmic and non-logarithmic values. To allow simpler comparison between literature findings, values in this RARMP have been converted to non-logarithmic values.

43. Viraemia (the presence of the virus in the blood) typifies dengue infection and occurs in both asymptomatic and symptomatic individuals. In symptomatic cases, viraemia develops several days before symptoms appears and peaks close to onset of symptoms. Peak plasma viral titres of over 10^9 genomes/mL have been observed (Vaughn et al., 2000; Duong et al., 2015). Increased viral titres have been associated with increased disease severity, with a 10-fold increase in peak titre reported for patients with severe dengue compared to DF (Vaughn et al., 2000). In symptomatic cases, viraemia typically clears within 5-7 days after onset of symptoms (Tricou et al., 2011). Several case reports have shown persistent viraemia (> 15 days after initial symptoms) in immunocompromised dengue patients (de Souza Pereira et al., 2017). The kinetics of viraemia in asymptomatic individuals has not been as well defined, however in a study of a small number of asymptomatic household members of symptomatic dengue cases, the mean maximum viral titre was lower in the asymptomatic cases (3.89×10^6 genomes/mL) than in symptomatic cases (7.24×10^9 genomes/mL) and the asymptomatic viraemia took longer to clear (Matangkasombut et al., 2020). A similar trend was seen in another small cohort of asymptomatic cases, with viral titres 100-fold lower in asymptomatic cases compared to pre-symptomatic or symptomatic (Duong et al., 2015).

44. Viral genomes have been detected by PCR in saliva, urine, semen, vaginal secretions, and breast milk. However, viral loads are orders of magnitude lower than those in plasma and it has not been clearly demonstrated that infectious virus particles have been isolated from these fluids:

- **Saliva**: Dengue genomes are shed into saliva with similar kinetics to viraemia. At the time of disease onset, vRNA levels in saliva are highest and decline thereafter, with no virus in the saliva detected after day 10 of onset of fever. In a study of saliva samples from 132 dengue patients, the peak viral load was 10^5 genomes/mL at days 1-3. Viable dengue particles could not be isolated from the 15 dengue-positive saliva samples that were tested (Andries et al., 2015). More recently, viable dengue particles were isolated in 73% of PCR-positive saliva samples tested (n = 22), however these findings are viewed with caution as limited information was provided on the methods, and infectivity of the samples was not reported (Humaidi et al., 2021).
- **Urine**: Dengue genomes are detectable in urine (viruria) with delayed excretion kinetics in comparison with blood, appearing later in the course of infection and remaining for some days after viraemia has disappeared. In a study involving multiple samples from 53 confirmed dengue patients, viruria was first detected from the day following onset of symptoms and were found in ≥ 50% of samples from days 6-16. Dengue virus was detected in serum only until day 11 (Hirayama et al., 2012). Similar results were obtained from a larger study involving 267 dengue patients, with the peak viral load reported as 6.3 x 10^3 genomes/mL. Viable dengue particles could not be isolated from the 15 dengue-positive urine samples that were tested (Andries et al., 2015).
- **Semen**: There has been a single case report describing dengue RNA in the semen of a male DF patient who was hospitalised at day 9 post-symptom onset. Viral genomes were detected in serum and urine at day 9 post-symptom onset only, but persisted in semen to day 37. Fractionation showed vRNA in both cellular and cell-free fractions. Attempts to isolate live virus from semen were unsuccessful, however negative strand vRNA was detected in whole semen and the cellular fraction to day 37. As an indirect marker of viral replication, its persistence well past the periods of viraemia and viruria suggested active replication in the genital tract rather than spill-over from plasma or urine (Lalle et al., 2018). In a study of 5 male dengue patients, no vRNA was detected in semen samples collected between 3-6 days after onset of symptoms (Molton et al., 2018).
- **Vaginal secretions**: A case report described persistent dengue RNA in the vaginal secretions of a female DF patient. Viral genomes were not quantified but were detected in plasma and saliva to day 10 post-disease onset, and in urine and vaginal secretions to day 18. They segregated with the pellet fraction of the vaginal secretion after centrifugation, possibly reflecting an association with vaginal epithelial cells. The authors were unable to isolate replication-competent virus, possibly due to low viral load (Iannetta et al., 2017).
- **Breast milk**: In breast milk samples from 12 women with dengue infection, samples from 9 of the women contained dengue virus. While the peak serum vRNA load ranged from 10^4 to 2.5 x 10^8 genomes/mL, the viral load in breast milk ranged from 20 to 2.5 x 10^4 genomes/mL. Dengue RNA was detectable in breast milk until 14 days after onset of symptoms. Infectious viral particles were not able to be isolated from the breast milk samples (Arragain et al., 2017).

45. No reports could be found of dengue RNA being detected in human faeces.

3.6 Spontaneous mutations, coinfection, and the potential for recombination

46. Dengue virus exhibits a relatively high mutation rate, which is a characteristic of many RNA viruses due to the intrinsically high error rate of their RNA polymerase and lack of the proof-reading enzymes used by DNA-based organisms to enhance the fidelity of genome replication (Peck and Lauring, 2018). In a study of single nucleotide variations in dengue virus serially passaged *in vitro*, genomic mutation rates of approximately 0.7 and 0.6 substitutions per genome per replication were estimated in mosquito and human cell lines, respectively (Dolan et al., 2021). On a population level, viral evolutionary rates for each serotype have been assessed in a number of studies, yielding a mean rate of 7.6 x 10^{-4} substitutions per nucleotide per year (Pollett et al., 2018). These are lower than the evolutionary rates for other RNA viruses such as influenza and human immunodeficiency virus, which

have substitution rates exceeding 1 x 10^{-3} substitutions per nucleotide per year (Jenkins et al., 2002). This has been postulated, although not proven, to reflect a fitness cost due to the alternating human-mosquito lifecycle of the dengue virus (Pollett et al., 2018).

47. While the dengue virus has a relatively high mutation rate, mutations that improve dengue virus fitness in humans and mosquitoes are rare events (Dolan et al., 2021).

48. Humans have been observed to be co-infected with two or more different dengue serotypes (Figueiredo et al., 2011; Senaratne et al., 2020), multiple genotypes of a single dengue serotype (Aaskov et al., 2007), multiple flaviviruses (Dupont-Rouzeyrol et al., 2015) and multiple arboviruses (Caron et al., 2012).

49. *Aedes* mosquitoes have been observed to be co-infected with at least three dengue serotypes (Thavara et al., 2006) and at least two different arboviruses (Caron et al., 2012), and mosquito cell lines can be co-infected with at least three different types of arboviruses (Kanthong et al., 2010).

50. Viral recombination is a process of genetic exchange between two separate viral genomes that are co-infecting and replicating in the same host cell. Even in conditions that favour recombination, the frequency of recombination within the same flavivirus species is very low (Taucher et al., 2010). Sequencing and phylogenetic analysis of dengue genomes has indicated that at an evolutionary level, recombination events have occurred and that they play an important role in the genetic diversity of dengue (Worobey et al., 1999; Tolou et al., 2001; Craig et al., 2003; Twiddy and Holmes, 2003; Aaskov et al., 2007). Recombination has not been observed between different flaviviruses (Twiddy and Holmes, 2003). Based on experimental results, if recombination were to occur, it is highly unlikely that this would result in a more virulent virus. Artificial recombinants of a WT yellow fever virus and a chimeric yellow fever virus/dengue virus vaccine (McGee et al., 2008), and a WT Japanese encephalitis virus and a chimeric yellow fever/Japanese encephalitis vaccine virus (Pugachev et al., 2007) were highly attenuated compared to the WT parent virus strains.

3.7 Epidemiology

3.7.1 Ecology and reservoirs

51. Dengue can be maintained in a sylvatic cycle or an urban cycle. In the sylvatic cycle, mosquitoes transmit the viruses between non-human primates (NHPs), while in the urban cycle mosquitoes transmit the viruses between humans located in rural and urban environments. Dengue can switch from the sylvatic to the urban cycle when infected mosquitoes opportunistically feed on humans who enter the jungle (Vasilakis et al., 2011).

52. Humans are the main vertebrate hosts of dengue and it is hyperendemic in many densely populated countries. In the urban cycle, dengue is transmitted to humans by female mosquitoes, mainly *Aedes aegypti* and, to a lesser extent, *Ae. albopictus* (Whitehorn et al., 2015). Dengue infection is self-sustaining in many urban areas as *Ae. aegypti* thrives in urban environments.

53. The dengue sylvatic cycle generally involves the smaller monkeys in Asia and Africa (Vasilakis et al., 2011; Althouse et al., 2012). Viral strains isolated from NHPs are genetically distinct from those affecting humans. In addition, NHPs develop only a mild infection which does not progress to the more severe forms disease which are seen in humans. When experimentally infected with human-derived strains, the NHP response is similar to that of humans in that there is a viraemia lasting several days, however it is generally asymptomatic with no abnormalities in haematocrit, and reduced platelet count is observed in only a minority of animals. NHPs are nonetheless capable of developing neutralising antibodies in response to dengue infection and are widely used in vaccine testing (Men et al., 1996; Back and Lundkvist, 2013).

54. Non-primate vertebrates have also been investigated as reservoir hosts for dengue. Dengue antibodies have been detected in a limited number of South American species of rodents, marsupials, and bats (de Thoisy et al., 2004), and DENV-2 virus has been found in tissues of 2 Central American

bats (Calderon et al., 2021). It is currently uncertain whether these animals may be dead end hosts, accidental hosts, or potential reservoir hosts for dengue. It is also unclear if these animals are capable of transmitting the virus and a literature search has not revealed any known cases of transmission of dengue from any of these species to humans. These species are not found naturally in Australia.

3.7.2 Transmission by mosquitoes

55. Female vector mosquitoes become infected with dengue virus when taking a blood meal from a viraemic host and transmit it while taking a blood meal from a subsequent host. The blood meal is required for the production and development of eggs (Harrison et al., 2021). Female mosquitoes can also transmit dengue to offspring through transovarial transmission (Thavara et al., 2006; Kurnia et al., 2022).

56. The level of viraemia in the host and the duration of infectiveness are important determinants of transmission of dengue from humans to mosquitoes. The probability of transmission to mosquitoes mirrors the kinetics of human viraemia, and can occur from 2 days before the onset of symptoms until 3 – 5 days after onset of symptoms (Nishiura and Halstead, 2007; Nguyen et al., 2013; Duong et al., 2015). A study documenting transmission to *Ae. aegypti* feeding directly on clinically ill dengue patients supports a sigmoidal relationship between virus dose ingested and proportion of mosquitoes infected, showing little or no transmission at viral plasma titres below 10^3 genomes/mL and close to 100% transmission above 10^9 genomes/mL. Transmission likelihood varied between dengue serotypes with the 50% mosquito infectious dose (MID_{50}) being approximately 10-fold higher for DENV-3 and DENV-4 (~ 3.2×10^7 genomes/mL), compared to DENV-1 and DENV-2 (~2.5×10^6 genomes/mL) (Nguyen et al., 2013). Another study examined infection of mosquitoes with an artificial blood meal containing human DENV-2 isolates and reported blood meal concentrations in PFU rather than vRNA concentration. The dose response also followed a sigmoidal relationship, with 50% infectious dose values ranging from 4×10^4 to 10^6 PFU/mL in the blood meal (Pongsiri et al., 2014). Intriguingly, a study examining transmission through direct feeding of mosquitoes from asymptomatic, pre-symptomatic, and symptomatic subjects indicated that transmission to mosquitoes from asymptomatic and pre-symptomatic hosts was more efficient than from symptomatic hosts reporting MID_{50s} of 2×10^5, 4.8×10^5, and 1.6×10^7 genomes, respectively. A similar trend was observed through indirect feeding. Asymptomatic and pre-symptomatic infections also produced a higher viral load in mosquitoes than symptomatic patients experiencing peak viraemia. It should be noted that there were only 13 asymptomatic cases, compared to 42 pre-symptomatic and 126 symptomatic (Duong et al., 2015).

57. Once ingested, dengue virus must establish a productive infection in the epithelium of the mosquito midgut (Ramesh et al., 2019). If successful, progeny virus is shed into the haemocoel (part of the open circulatory system of invertebrates), from which it disseminates and infects other tissues through the hemolymph (blood-like fluid in the haemocoel). For the mosquito to become infectious to humans (and NHPs), dengue must infect the salivary glands and be shed into saliva. Once this infection of the salivary glands has occurred and sufficient replication taken place, dengue may be transmitted to a new vertebrate host at the insect's next feeding event (Back and Lundkvist, 2013; Carrington and Simmons, 2014). *Ae. albopictus* has been shown to a less effective vector than *Ae. aegypti* because even though *Ae. albopictus* is more easily infected with dengue, its saliva is less infectious (Whitehorn et al., 2015). Female mosquitoes inject their saliva into their prey during feeding. The saliva contains a mild anaesthetic and anti-coagulants to facilitate feeding (Foster and Walker, 2009). Proteins in mosquito saliva have also be shown to promote infection in the host, including through immunomodulatory effects (reviewed in Schneider and Higgs, 2008).

58. There are approximately 6 days (at 30°C) or 15 days (at 25°C) between ingestion and the mosquito becoming infectious (Chan and Johansson, 2012). Once infected with dengue virus, a mosquito can transmit the virus for the rest of its life (World Health Organization, 2009). The lifespan of an *Aedes* mosquito is about 3 – 4 weeks (Goindin et al., 2015). Female *Ae. aegypti* and *Ae. albopictus* have been shown to take multiple blood meals as part of the egg maturation process

(Ponlawat and Harrington, 2005; Harrington et al., 2014), and so have the potential to expose multiple people to dengue in a short period of time.

3.7.3 Vector control

59. Conventional strategies to control the spread of dengue virus are aimed at controlling mosquito vectors through the following approaches:

- chemical (e.g. suppressing the mosquito population with insecticides)
- environmental (e.g. reducing or covering water storage containers)
- biological (e.g. larvae-eating fish, *Wolbachia* infection) (Ogunlade et al., 2023).

60. *Wolbachia* is a maternally-inherited endosymbiotic bacteria that infects a number of insect species, including *Ae. aegypti*. *Wolbachia* infection has two main effects on *Ae. aegypti*. *Wolbachia* infection leads to cytoplasmic incompatibility and early embryonic death when an infected male breeds with an uninfected female (Walker et al., 2011). *Wolbachia* infection has also been shown to block transmission of the virus in mosquitoes (Walker et al., 2011; Ye et al., 2015). While this biological approach has the benefit of potentially being self-sustaining, high temperatures (> 29°C) have been shown to result in low levels or complete loss of *Wolbachia* infection (Ross et al., 2019).

3.7.4 Non-vectored transmission in humans

61. Direct human-to-human transmission can occur vertically (from mother to foetus), by blood transfusion, organ transplant or other parenteral exposure such as needle stick injury involving infected blood.

62. There have been several reported cases of dengue infection passing from the mother to foetus during pregnancy, believed to be passed through the placenta, or during birth (Phongsamart et al., 2008; Arragain et al., 2017). The reported transmissions rates vary. In a cohort of 64 pregnant women with dengue infection, the transmission rate during pregnancy was reported as 1.6% (1/64) (Tan et al., 2008). Another study of 10 paired mothers and newborns reported a vertical transmission rate of 90% (Arragain et al., 2017). While dengue virus has been detected in breast milk, transmission by this route is still uncertain (Barthel et al., 2013; Arragain et al., 2017).

63. Blood-borne transmission to others is possible during the viraemic phase of the disease that develops in both asymptomatic and symptomatic individuals, although is a less common route compared with mosquito-vectored transmission. Successful non-vector transmission increases with viral concentration in the inoculum and the volume transferred:

- **Blood transfusion**: At least ten incidences of blood transfusion transmission, leading to symptomatic illness including DHF, have been documented. Studies of blood donor samples from Brazil have demonstrated approximately 5% of blood donors have asymptomatic infection during dengue outbreaks (Busch et al., 2016; Slavov et al., 2019), and one donor-recipient study found a 37.5% transmission rate after transfusion of viraemic blood (Sabino et al., 2016).
- **Organ and bone marrow transplant**: Dengue infection has also followed organ transplantation from both living and deceased viraemic donors, with potentially severe consequences for recipients, including fever, haemorrhage, shock, and death (Rosso et al., 2019; Shaji Mathew et al., 2019). Transmission through bone marrow transplant has also been reported (Rigau-Pérez et al., 2001).
- **Needle stick injury**: In the occupational setting, symptomatic infection of healthcare workers has been documented on a number of occasions following needle stick injury during blood collection from dengue patients. The exposed workers became ill 4-8 days after the incident (Chen and Wilson, 2004; Wagner et al., 2004). A case report of laboratory worker contracting

dengue after a needlestick injury from filtering dengue solution has also been observed. The concentration of dengue in the solution was not reported (Lee et al., 2016).

- **Other occupational exposure**: A single case report suggests mucous membrane exposure as a viable exposure pathway. A healthcare worker in the United States developed symptomatic dengue after a splash to the face with confirmed viraemic blood, through contact with the eye, nose, and mouth. Infection due to blood contact with mucous membranes was considered plausible (Chen and Wilson, 2004). This transmission pathway is supported by experimental human studies conducted by the United States Army during World War II that demonstrated the capacity for viral infection following ocular or intranasal exposure, but at a 10,000 or 1 million-fold higher dose respectively, compared to the intracutaneous route (Sabin, 1952; reviewed in Snow et al., 2014).

64. While dengue is not considered to be a sexually transmitted disease, two cases of likely sexual transmission have been reported in recent years. In both cases, one female-to-male (Lee and Lee, 2019) and one male-to-male sexual (case summarised in Grobusch et al., 2020), transmission involved a person returning from a dengue endemic region and a partner testing positive for dengue a few days after sexual exposure. Although alternative routes of transmission (e.g. blood-mucosa contact) were not able to be entirely excluded, in both cases sexual transmission was considered most likely.

65. There is currently insufficient evidence to confirm transmission of dengue through aerosols. Dengue was detected in a small percentage of routinely collected respiratory samples collected from patients with respiratory symptoms. The authors postulated that the presence of the virus in the nose and throat could indicate a possible respiratory route of transmission, but highlighted that there is currently insufficient evidence to confirm transmission via this route (Cheng et al., 2017).

3.7.5 Global pattern of distribution

66. The global number of dengue infections is estimated at approximately 50 to100 million per year in more than 100 countries, with approximately 40,000 deaths per year. The regions with the greatest incidence of dengue are South Asia and Southeast Asia, East Asia, West Sub-Saharan Africa, central Latin America, and tropical Latin America (Yang et al., 2021; Zeng et al., 2021)(Figure 4).

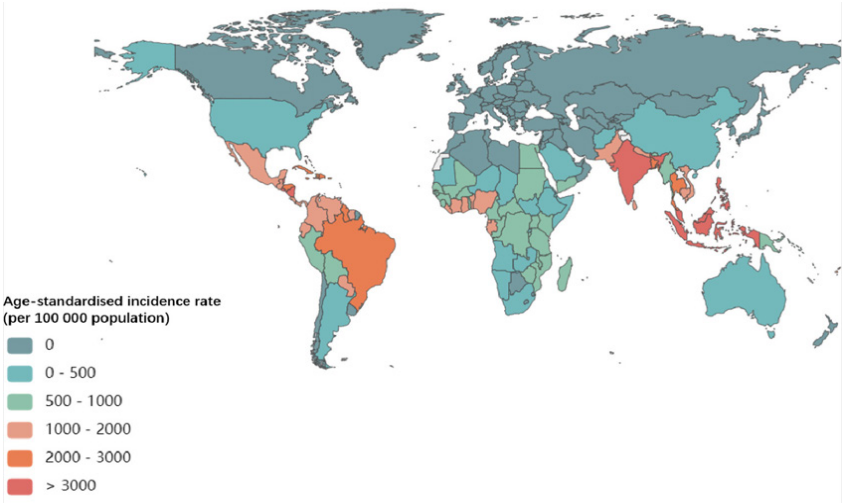

Figure 4. Global age-standardised incidence of dengue fever in 2017

Source: Zeng et al. (2021).

67. The distribution of dengue infections globally is largely dependent on the distribution of the mosquito vectors. The key factors that influence the distribution of *Aedes* mosquitoes have been reported as accessibility of hosts, humidity, and annual minimum temperatures. *Aedes* mosquitoes thrive in densely populated areas with warmer temperatures and higher humidity. There are some

interspecies variations for each of these factors, with *Ae. aegypti* preferring slightly warmer (8°C) minimum temperatures than *Ae. albopictus* (2°C) (Dickens et al., 2018).

68. It is predicted that over 50% of the global population live in areas that are suitable for the transmission of dengue (Messina et al., 2019). Global shifts in climate, such as heatwaves and floods, as well as increasing urbanisation are predicted to expand the areas that are suitable, resulting in increased dengue outbreaks (Nava et al., 2017; Messina et al., 2019). Figure 5 shows predicted changes to suitable environments for dengue occurrence over the next 60 years.

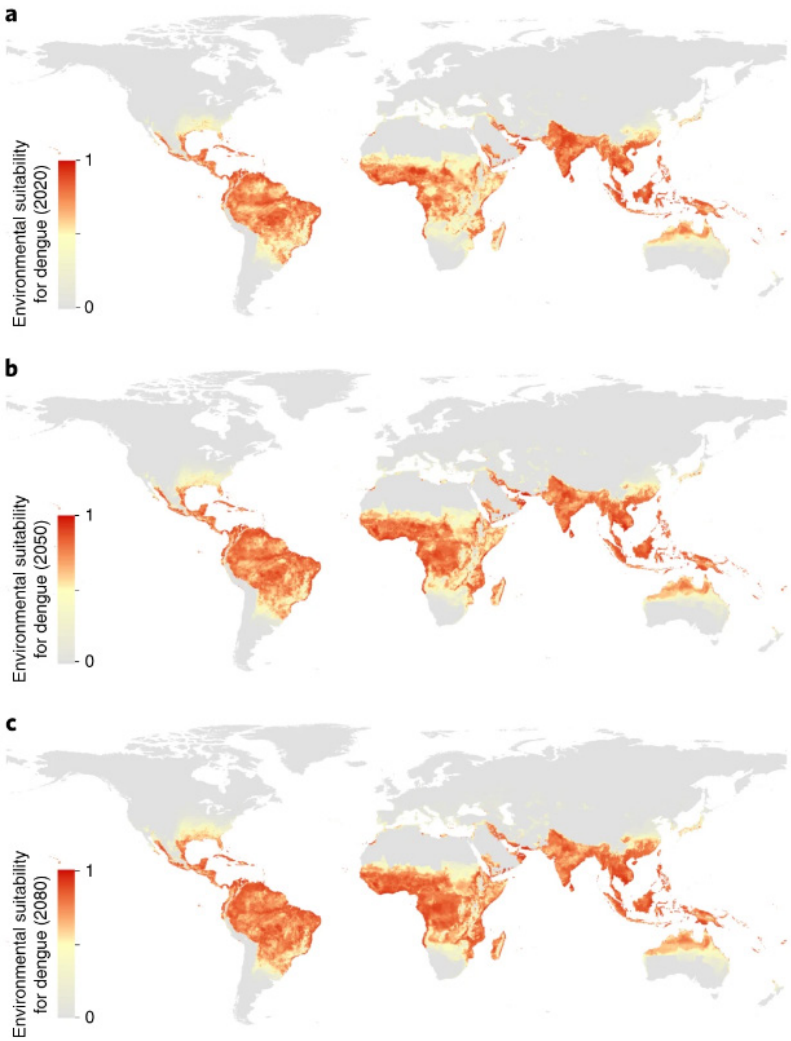

Figure 5. Predicted environmental suitability for dengue occurrence

Projected data shown for 2020 (a), 2050 (b) and 2080 (c). Source: Messina et al. (2019).

69. The prevalence of the different serotypes varies both between geographical regions and over time. A meta-analysis of 174 dengue outbreaks that occurred between 1990 and 2015 showed that DENV-2 was the cause of the most monovalent outbreaks, followed by DENV-1, DENV-3, then DENV-4. DENV-2 was the predominant serotype in monovalent outbreaks in the late 1990's and 2000's, while in 2010 it was DENV-1. From 2010 onwards, DENV-1 and DENV-2 were the more common serotypes in the African and American regions, DENV-1 in Europe and the Western Pacific Region, and DENV-2 and DENV-3 in the Eastern Mediterranean region. Almost 50% of the outbreaks were caused by 2 or more serotypes (Guo et al., 2017).

3.7.6 Outbreaks in Australia

70. In Australia, *Ae. aegypti* was previously found in New South Wales, the Northern Territory, Western Australia, and Queensland (Beebe et al., 2009; Trewin et al., 2017), however following successful elimination measures, including reducing breeding sites for the mosquitoes, the current distribution is limited to parts of northern Queensland (Trewin et al., 2017; Trewin et al., 2022). In 2005, *Ae. albopictus* was found on some islands in the Torres Strait and has since been the subject of successful containment efforts focussed on protecting Thursday and Horn Islands, which are the transport hubs connecting the Torres Strait to the mainland (van den Hurk et al., 2016). *Ae. albopictus* is thought to be capable of colonising mainland Australia but it is not currently present (Atlas of Living Australia, https://www.ala.org.au/, accessed 20 June 2023). *Ae. notoscriptus* (also known as the Australian backyard mosquito) is native to the Southwestern Pacific and is widespread throughout the tropical to temperate regions of Australia (Paris et al., 2023). While it is thought to be involved in transmission of some arboviruses, it is considered an unlikely and ineffective vector for dengue (Kramer et al., 2011; Skelton et al., 2016).

71. Dengue is a notifiable disease in Australia, which means that state and territory health authorities provide the National Notifiable Diseases Surveillance System with deidentified data about new cases of infection.[4]

72. The number of dengue cases in Australia is relatively low compared to countries where dengue is endemic, with 406 cases of dengue reported in Australia from January 2022 – January 2023 (Department of Health and Aged Care, 2023). Although dengue is not endemic in Australia, it can be acquired in dengue-affected areas overseas and brought into the country. Overseas acquisition, particularly from countries in Southeast Asia and the Pacific that are in close proximity to Australia and where dengue is endemic, accounts for the majority of the total dengue cases in Australia (Department of Health, 2021).

73. Transmission within Australia is restricted to areas where the key mosquito vector, *Ae. aegypti* is present in sufficient numbers and human populations are of sufficient density, i.e. some areas of northern Queensland. Introduction of dengue into these areas by a viraemic tourist or returning resident can lead to sporadic outbreaks, the largest recent one occurring in Cairns between November 2008 and May 2009. This outbreak of a DENV-3 virus produced 915 confirmed cases, including six cases of severe dengue and one death. (Ritchie et al., 2013). In 2019, there was a small outbreak of 21 locally acquired dengue cases in Central Queensland when a DENV-2 viraemic traveller returned from Southeast Asia (Walker et al., 2021).

74. As discussed in Section 3.7.4, *Wolbachia* is used as a biological agent to control mosquito populations and reduce transmission of DENV. *Wolbachia*-infected *Ae. aegypti* mosquitoes were first released in Cairns in 2011 and post-release monitoring has indicated that the *Wolbachia* genome has remained stable 7 years post-release (Dainty et al., 2021). As of 2023, there have been releases covering 300 km^2 of northern Queensland with a population of over 300,000 people (for further information see https://www.worldmosquitoprogram.org/, accessed 20 June 2023). A reduction in the local transmission of dengue has been reported in the areas of *Wolbachia* release, based on the number of case notifications of locally-acquired dengue (Ryan et al., 2019).

75. As discussed in Section 3.7.1, the dengue sylvatic cycle involves transmission between mosquitoes and a number of non-human primates, with some other South and Central American vertebrates being debated as potential reservoirs. The sylvatic cycle has not been reported as occurring in Australia, likely due to the lack of suitable hosts outside captivity.

[4] See the Department of Health and Aged Care website for further information about notifiable diseases and the NNDSS.

3.7.7 Environmental stability and decontamination methods

76. Enveloped RNA viruses such as dengue virus are not expected to survive for extended periods outside a host due to their sensitivity to environmental and chemical factors.

77. Flaviviruses in general can be physically inactivated by ultraviolet light, desiccation, gamma irradiation and heat. Due to the sensitivity of their outer lipid envelope, flaviviruses can be chemically inactivated by low pH, detergents, disinfectants and chaotropic agents (Public Health Agency of Canada, 2014).

78. Dengue virus is sensitive to moist heat (121°C for at least 15 min), dry heat (160-170°C for at least 1 hour), and low temperature sterilisation (ethylene oxide or plasma sterilisation). Dengue virus is also susceptible to chemical agents such as 1% sodium hypochlorite, 2% glutaraldehyde, 2% peracetic acid, 70 % ethanol, iodophors, phenolic compounds and 3-6% hydrogen peroxide (Public Health Agency of Canada, 2014).

79. Dengue virus is inactivated by nucleic acid extraction reagents phenol-guanidine isothiocyanate and chaotropic salt (Blow et al., 2004).

80. In an experiment testing methods of storing dengue virus samples in the laboratory for later PCR analysis, dengue RNA has been shown to be stable in dried human blood on filter paper for up to nine weeks at room temperature sealed in a plastic bag. In the same study, DENV-2 in a dried blood spot on filter paper was shown to be capable of infecting a mosquito cell line *in vitro* after being stored up to 48 hours at room temperature sealed in a plastic bag (Prado et al., 2005).

3.8 DENV-2 PDK-53, the attenuated parent strain

81. Culturing viruses in cells from non-host species to encourage spontaneous viral mutations has been an established procedure for viral attenuation for many decades (Rodrigues et al., 2015).

82. DENV-2 PDK-53 (hereafter abbreviated to PDK-53) is a non-GM laboratory-derived strain. It was attenuated by culturing WT strain DENV-2 16681, originally isolated from a dengue patient in Thailand in 1964, for 53 serial passages in primary dog kidney (PDK) cells.[5] Compared to the 16681 WT parent, the PDK-53 strain showed decreased plaque size in monkey kidney cells, increased temperature sensitivity, loss of neurovirulence in suckling mice, and reduced incidence of viraemia in infected rhesus monkeys (Yoksan et al., 1986). In a small clinical trial of 10 participants given an equal volume of a PDK-53 vaccine candidate (consisting of 1.9-2.7 x 10^4 PFU as assessed by subsequent back titration of virus concentration), neutralising antibodies to DENV-2 were detected at 30 days post-vaccination and no significant safety risks were identified. Dengue virus-specific memory T cell responses were also observed in the participants (Dharakul et al., 1994). Viraemia was noted in 1 of the 10 participants. Following amplification in tissue culture, the isolate from the viraemic participant was shown to maintain the small plaque and temperature sensitivity characteristics of PDK-53 (Bhamarapravati et al., 1987) and showed a similar reduction in infection and dissemination in *Ae. aegypti* mosquitos, compared to the 16681 WT strain (Khin et al., 1994).

83. PDK-53 contains 9 mutations which occurred spontaneously during the serial passaging of the 16681 virus in PDK cells. These mutations are:

- **C to T at nt 57 of the 5'NCR**
- 3 silent mutations
- substitutions at prM-29 Asp to Val, **NS1-53 Gly to Asp**, NS2A-181 Leu to Phe, **NS3-250 Glu to Val**, and NS4A-75 Gly to Ala (Kinney et al., 1997).

84. Of these 9 mutations, whole genome sequencing and phenotypic studies have demonstrated that 3 of these mutations (in bold above) are necessary and sufficient for the attenuation: one in the

[5] **Passaging** is a process of subculturing cells in tissue culture by harvesting and reseeding the cells into a new culture flask/dish.

5’ non-coding region (5’NCR), one in non-structural protein 1 (NS1), and one in non-structural protein 3 (NS3). The PDK-53 vaccine candidate discussed in paragraph 82 had a mixed genotype at genome nucleotide 5270 (NS3-250); approximately 29% Glu (matching the parent 16681 strain) and 71% Val. (Butrapet et al., 2000).

85. Phenotypic analysis was conducted on 18 different recombinant dengue viruses to analyse the contribution of the 5’NCR, NS1, NS3, and prM mutations to the attenuated phenotype of PDK-53. Complete reversion of the PDK-53 virus to the virulent phenotypic characteristics of the parental 16681 virus required reversion mutations in at least the 5’NCR and NS1 loci. Reversion at the NS3 locus was also required to reconstitute the large plaque phenotype, and also potentially plays a role in the mouse virulence phenotype of the parent 16681 strain. It was found that the prM locus does not play a role in the attenuated phenotype (Butrapet et al., 2000).

Section 4 The GM vaccine - nature and effect of the genetic modification

4.1 The genetic modifications

86. The vaccine contains 4 live attenuated GM strains of dengue virus; TDV-1, TDV-2, TDV-3, and TDV-4 (Figure 6).

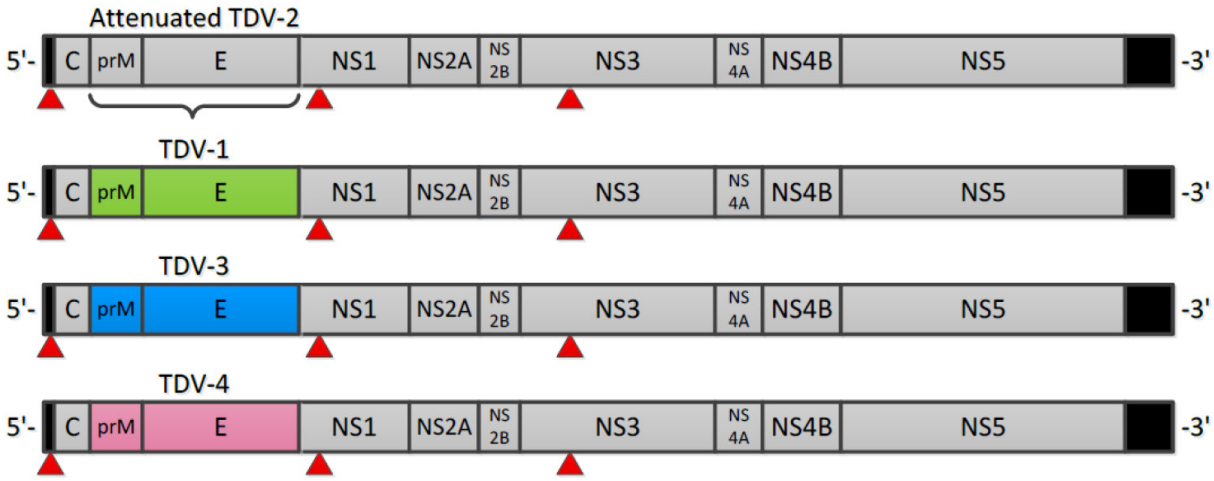

Figure 6. Structure of the four strains in Qdenga

The solid red triangles indicate the 3 attenuation loci present in the 5’NCR, NS1 and NS3 proteins. Figure supplied by applicant, adapted from Patel et al. (2023). Abbreviations: C = capsid; E = envelope; NS = non-structural protein; prM = pre-membrane.

87. TDV-2 was generated by cDNA cloning of the attenuated non-GM strain DENV-2 PDK-53 (see Section 3.8, above) and contains all 3 of the key attenuation mutations, which are located outside the structural genes *prM* and *E*. While no genes have been replaced in TDV-2, it is considered to be a GMO as it was created through gene technology.

88. The other 3 strains were generated by replacing the *prM* and the *E* genes in TDV-2 with the *prM* and *E* genes from dengue serotypes 1, 3, or 4 (to create strains TDV-1, TDV-3, and TDV-4, respectively). As these 3 strains have the TDV-2 backbone, they also contain the 3 attenuating mutations from the PDK-53 parent strain (Figure 6).

89. Table 1 lists the WT virus origins of the *prM* and *E* genes in the GM TDV viruses.

Table 1. Origins of the *prM* and *E* gene regions in the GM Qdenga viruses

Serotype	WT strain	Origin of WT strain	Source	Reference
DENV-1	16007	Thailand, 1964	DHF/DSS patient	Halstead et al. (1970)
DENV-2	16681	Thailand, 1964	DHF/DSS patient	Halstead et al. (1970)
DENV-3	16562	Philippines, 1964	DHF patient	Halstead et al. (1970)
DENV-4	1036	Indonesia, 1976	DF patient	(Gubler et al., 1979)

Abbreviations; DF = dengue fever; DHF = dengue haemorrhagic fever; DSS = dengue shock syndrome.

90. The final product contains the following mixture of the TDVs:

- TDV-1: ≥2 x 10^3 PFU/dose
- TDV-2: ≥5 x 10^2 PFU/dose
- TDV-3: ≥1 x 10^4 PFU/dose
- TDV-4: ≥3 x 10^4 PFU/dose[6]

4.2 Effect of the genetic modification

91. As discussed in Section 3.3, the *prM* and *E* genes encode membrane glycoproteins that are present on the surface of dengue viral particles and are recognised by the human immune system. As the GM strains contain *prM* and *E* genes from the 4 dengue serotypes, the intended effect of the genetic modification is to induce immune responses against all 4 serotypes. The aim is to achieve a reasonably balanced immune response against all 4 serotypes in order to reduce the risk of severe dengue if a vaccinee is exposed to circulating WT dengue viruses.

4.3 Characterisation of the GMOs

92. The original research grade recombinant vaccine viruses were developed at the US Centers for Disease Control and Prevention (CDC) (Huang et al., 2003). The research-grade precursors to commercial strains TDV-1, TDV-2, TDV-3, and TDV-4 were originally designated as D2/1-V, PDK-53-VV45R, D2/3-V, and D2/4-V (Huang et al., 2013). Alternative names in the literature for the GM vaccine are TAK-003 and DENVax, and the TDV strains have also been referred to as DENVax-1 to DENVax-4 (Osorio et al., 2011; Huang et al., 2013; Patel et al., 2023).

4.3.1 Attenuation phenotype

93. The TDV strains are attenuated because they retain the non-GM alterations responsible for the attenuation of the parent PDK-53 strain (see Section 3.8).

94. Studies of the four TDV master virus seeds (MVS; the progenitor of the vaccine batches) showed decreased plaque size and increased temperature sensitivity in monkey kidney cell culture, and decreased neurovirulence in newborn mice compared to the WT control (Huang et al., 2013).

[6] Numbers supplied by the applicant were in logarithmic form and were TDV-1: ≥3.3 $\log_{10}$ PFU/dose, TDV-2: ≥2.7 $\log_{10}$ PFU/dose, TDV-3: ≥4.0 $\log_{10}$ PFU/dose, and TDV-4: ≥4.5 $\log_{10}$ PFU/dose.

These phenotypic characteristics are similar to the attenuated PDK-53 parent (Section 3.8) and to the research-grade vaccine viruses (Huang et al., 2003; Huang et al., 2013).

4.3.2 Genotype stability and reversion

95. It is important that live vaccine strains are genetically stable throughout production and after vaccination to ensure a consistent, safe phenotype.

96. Full length genome sequencing of the four TDV MVS showed that the *prM* and *E* gene modifications, and the attenuating mutations were maintained (Huang et al., 2013).

97. As per international guidelines (European Medicines Agency, 2010; United States Food and Drug Administration, 2010), the genetic stability of the three loci associated with viral attenuation (5'NCR, NS1, and NS3) was evaluated through vaccine production. These attenuation loci remained unchanged upon generation of working virus seed (WVS) and bulk drug substance batches from the MVS stocks.

98. Genetic stability studies of the three attenuating mutations were also performed during serial passage of each of the 4 TDV viruses in monkey kidney cells. Sequence analysis showed no evidence of reversion at the NS1 or NS3 loci after 10 passages, however the 5'NCR mutation did show a propensity to revert to WT in all 4 of the TDVs (Butrapet et al., 2006). As discussed in Section 3.8, a recombinant PDK-53 virus with the WT nucleotide at 5'NCR-57 retained the attenuation characteristics of small plaque size, temperature sensitivity, decreased mouse neurovirulence and reduced replication in mosquito cells when compared to the parent 16681 strain (Butrapet et al., 2000). Therefore, a reversion of only the 5'NCR locus in the TDVs is not expected to result in a pathogenic phenotype as the presence of mutations in NS1 and NS3 are sufficient to maintain the attenuated phenotype.

99. Genetic stability of the TDVs following vaccination was assessed in 6 clinical trials (Phase I and Phase II). In the Phase I trials, reversion was assessed in participants with replication competent virus (129/424 participants). A reversion at a single attenuation loci was detected in 44 participants (10.4% of 424 total in the trials). The majority of these were in the 5'NCR locus (43), with only 1 partial reversion (presence of both attenuated and WT nucleotide) in NS1 detected. In a Phase II trial, all 164 participants with vRNA in the blood were assessed for reversions, with 23/164 (14%) having a reversion at a single attenuation locus. Consistent with the Phase I trials, the majority of the mutations were in the 5'NCR locus (22) and one was in the NS1 locus. For the participants with reversions, the adverse events (AEs) occurring around the time of viraemia were mostly mild to moderate and self-limited, and no important safety risk was identified.

100. In a Phase III trial, reversions were assessed in participants who had a fever within 30 days of vaccination and who had replication competent virus. Single reversions in the 5'NCR locus were detected in 4/15 participants. While one participant was hospitalised due to dehydration, none of the participants developed symptoms similar to severe dengue (bleeding, low platelet counts, plasma leakage) and no important safety risk was identified.

4.3.3 Transmission by mosquitoes

101. The replication potential of the TDV viruses was examined in an *Ae. albopictus* C6/36 cell culture. At 6 days post-infection, the titres of TDV-1, -2, and -4 in the mosquito cells were approximately 10^3 to 10^5 PFU/mL lower, and TDV-3 was approximately 10-1000 PFU/mL lower, than the titre observed in cells infected with homologous WT dengue strains of the same serotype (Huang et al., 2013).

102. The TDV viruses were also assessed for their ability to infect *Ae. aegypti* mosquitoes in a laboratory setting. Groups of 25-50 mosquitoes were given an infectious blood meal containing a single TDV or the equivalent WT strain control, each ranging in concentration from 1.6×10^5 to 7.9×10^6 PFU/mL as assessed by back-titration of the blood meals. The mosquitoes were then tested for infection in the body 14 days later. None of the mosquitoes exposed to TDV-1 or TDV-2 showed evidence of infection, compared to approximately 40% with the WT strains. 3.6% (2/55) of mosquitoes

were infected with TDV-4, compared to 16% (8/50) for the WT control. No mosquitoes were infected with TDV-3 in 2 experiments, however the infection rates with the WT DENV-3 control were also low (8%). In a third TDV-3 experiment with a higher viral blood titre, 3% (1/30) of mosquitoes in both the TDV-3 and WT DENV-3 groups became infected (Huang et al., 2013).

103. Due to the low rates of infection with the TDVs from oral feeding, dissemination into the salivary glands was assessed 7 days following intrathoracic inoculation of the mosquitoes with the TDVs and equivalent WT controls. Despite near 100% body infection rates, the TDVs exhibited very low or not detectable (0-10%) dissemination into the salivary glands compared to the WT strains (43-87%) (Huang et al., 2013).

4.3.4 Viremia in vaccinees

104. In the clinical trials, a number of different tetravalent formulations were tested, including various concentrations and ratios of the GM strains. In a Phase II study examining a high dose formulation and the final Qdenga formulation (Tricou et al., 2020), 175 participants received the final formulation. Of these, 15.8% of participants with pre-existing dengue antibodies developed viraemia (15/95), compared to 48.8% of those without pre-existing antibodies (39/80). Viraemia was first detected at day 7 post-vaccination. The peak of viraemia occurred at approximately 11 days post-vaccination and most participants had negative viraemia after 21 days, regardless of pre-existing dengue antibodies. TDV-2 was the most frequently detected strain (29.1%; 51/175) with blood titres ranging from 10^3 to 2.5 x 10^5 genomes/mL. TDV-3 and TDV-4 were infrequently detected (1.7% and 0.6%, respectively), with blood titres ranging from 5 x 10^2 to 6.3 x 10^3 genomes/mL. TDV-1 was not detected.

4.3.5 Shedding

105. Shedding of the TDVs was assessed in immunocompromised AG129 mice at 1, 5, 13, and 41 days after a single subcutaneous injection. No dengue RNA was detected in the faeces. At 5 days post-injection, one urine sample contained TDV-3 (1/8 mice), and 3 saliva samples contained TDVs (1/10 mice in TDV-2 group, 2/10 mice in TDV-3 group) (information provided by applicant).

106. Shedding of the TDVs has not been assessed in humans.

4.3.6 Safety and immunogenicity

107. More than 38,000 doses of the different tetravalent formulations of the TDVs (ranging from 5 x 10^2 to 1.6 x 10^6 PFU of the different TDVs per dose) have been administered to more than 28,000 people, including adults, adolescents, and children as young as 18 months old. Trials have been conducted in both endemic and non-endemic areas.

108. The GM vaccine was generally well tolerated in the clinical trials. The most common adverse events were injection site pain and headache. No safety concern was identified based on the dengue serostatus (previous exposure to dengue). There were no vaccine related deaths (Patel et al., 2023). There have been no reports of toxic or allergic reactions to the protein encoded by the TDVs. This is consistent with the findings of the non-clinical studies. In single dose, repeat dose, and developmental and reproductive toxicity studies in mice and rabbits at TDV concentrations exceeding the human clinical dose, no safety concerns with respect to toxicity were identified (information provided by the applicant).

109. In the clinical trials, antibody responses to all four serotypes were seen at 1-month post-vaccination. The highest antibody levels were to DENV-2, and generally the lowest were to DENV-4. Antibodies to at least 3 serotypes were detected in nearly 100% of individuals at 1 month, and antibodies to all 4 serotypes in approximately 80% (Saez-Llorens et al., 2018; Rivera et al., 2022). It does not appear that the potential for the development of ADE was directly assessed in the clinical trials. However, it is noted that in the largest clinical trial (>20,000 participants in 8 dengue endemic

countries), participants who received the vaccine had lower rates of severe dengue at 3 years post-vaccination than those who received the placebo (Rivera et al., 2022).

4.3.7 Stability in the environment and decontamination

110. Liquid formulations of the GM vaccine lose effectiveness if left at room temperature for extended periods of time, with approximately 10-fold infectious titre of each serotype lost after 7 days at 25°C and all infectious titres lost by 21 days at 25°C (information provided by applicant).

111. As the powder formulation is to be kept between 2 to 8°C (Section 2.1), degradation of the GMOs would be expected to occur if the vials were not stored in the refrigerator/cool room, however no specific data was supplied by the applicant.

112. Methods of decontamination effective against WT dengue viruses (Section 3.7.7) are expected to be equally effective against the GMOs.

Section 5 The receiving environment

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

5.1 Site of release

114. The GM vaccine is intended to be administered as 2 individual subcutaneous injections 3 months apart, performed by a trained healthcare professional at a medical or clinical facility.

115. Most clinical facilities would be equipped to deal with scheduled drugs and infectious agents and they typically comply with AS/NZS 2243.3:2010 Safety in laboratories – Microbiological Safety and Containment (Standards Australia/New Zealand, 2010).

116. During vaccination of the patients, aerosols of the GMOs could be released into the clinical facility, but the amount would be expected to be very small and there is currently insufficient evidence for dengue transmission through aerosols (Section 3.7.4).

117. The GMOs may also enter the wider environment through blood transmission from a viraemic vaccinee to other humans or mosquitoes. As discussed in Section 4.3, the GMOs are not expected to be shed in urine or faeces. Another route by which the GMOs may enter the wider environment is via accidental spills of the vaccine during transport or storage, or a sharps injury occurring following disposal of vaccine vials or syringes contaminated with the vaccine.

5.2 Related viral species in the receiving environment

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

119. Common mosquito-borne viruses in the Australian environment, such as Ross River virus, Barmah Forest virus and Chikungunya virus (Queensland Health, 2018), are alphaviruses in the *Togaviridae* family and are not closely related to dengue virus (Forrester et al., 2012).

120. The majority of mosquito-vectored flaviviruses in the Australian environment are sufficiently distantly related to the parent organisms that they are not transmitted by *Aedes* mosquitoes but by *Culex* mosquitoes. These include Murray Valley encephalitis virus, the Kunjin strain of West Nile virus and Japanese encephalitis virus (Queensland Health, 2018; Vial et al., 2021).

121. Yellow fever virus and Zika virus are flaviviruses that are closely related virus to dengue and also transmitted by *Aedes* mosquitoes (Lataillade et al., 2020; Obadia et al., 2022). There have been no reports of yellow fever in Australia. There have been no locally acquired cases of Zika in Australia, however a limited number of infections (<30 per year) have been acquired from overseas (Queensland

Health, 2018). As discussed in Section 3.6, recombination has not been observed between different flaviviruses.

5.3 Similar genetic material in the environment

122. The balance of an ecosystem could be perturbed by the introduction of new genetic material through horizontal gene transfer or through release of a GMOs into the environment. However, the effect of perturbation would be relatively small if the genetic material was already present in the system and did not confer any selective advantage to an organism that gained this genetic material.

123. All of the genes in the GM vaccine strains are functionally similar to those present in naturally occurring dengue virus. The genes introduced into the GM vaccine strains were derived from naturally occurring strains representing the four serotypes, and so similar genetic material would already be present in the environment.

124. As discussed in Section 3.7.6, dengue is not endemic to Australia, but occasional outbreaks occur when dengue viraemic travellers return from overseas.

125. Dengue vaccine clinical trials are occasionally conducted in Australia. A search of the Australian New Zealand Clinical Trials Registry (https://www.anzctr.org.au/TrialSearch.aspx, accessed 23 June 2023) listed 5 Australian clinical trials using a live attenuated tetravalent dengue vaccine, however only one is currently active (but not yet recruiting). Therefore, similar live attenuated tetravalent dengue vaccines could be present in people or the environment in Australia, but the scope is expected to be limited.

126. As discussed in Section 4.3, the GM vaccine strains are attenuated compared to WT strains and therefore do not confer a selective advantage over WT dengue strains.

5.4 Alternate hosts

127. Viruses are obligate parasites, which cannot replicate outside a host as they depend on the host's proteins for many replicative processes.

128. As discussed in Section 3.7.1, dengue can be maintained in a sylvatic cycle (non-human primates) or an urban cycle (humans), and while dengue antibodies or virus have been isolated from some non-primate vertebrates, there is currently no evidence of transmission from these species. None of the vertebrate species that are confirmed or suspected to be involved in the dengue sylvatic cycle are present in Australia outside of captivity.

Section 6 Relevant Australian and international approvals

6.1 Australian approvals

6.1.1 Approvals by the Regulator

129. The Regulator has not previously approved any licences in relation to this GM vaccine.

130. Previous approvals of other GM dengue vaccines by the Regulator are shown in Table 2. Dengvaxia (assessed as DIR-148) is currently the only dengue vaccine approved for commercial use in Australia.

Table 2. Previous licences issued by the Regulator for GM dengue vaccine clinical trials or commercial supply

Application reference	Title	Organisation
DNIR-386	Clinical trials of ChimeriVax Tetravalent Dengue Vaccine	Sanofi-Aventis Australia Pty Ltd
DNIR-598	A Phase 1, double blind, randomized, placebo-controlled study to evaluate the safety and immunogenicity of Dengusiil in healthy adults	PPD Australia Pty Ltd
DNIR-650	Clinical trial of a live attenuated tetravalent Dengue vaccine (V181) in adults	Merck Sharp & Dohme (Australia) Pty Ltd
DIR-148	Commercial supply of Dengvaxia, a live attenuated GM dengue vaccine	Sanofi-Aventis Australia Pty Ltd

6.1.2 Approvals by other government agencies

131. As Qdenga is manufactured overseas, a permit from the Department of Agriculture, Fisheries and Forestry will be required for its import into Australia.

132. Assessment by the TGA and inclusion on the ARTG is required before a vaccine can be lawfully supplied in Australia.

6.2 International approvals

133. As of 14 July 2023, the GM vaccine has been approved by health authorities in Indonesia, the European Union, Great Britain, Brazil, Argentina, and Thailand.

Chapter 2 Risk assessment

Section 1 Introduction

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

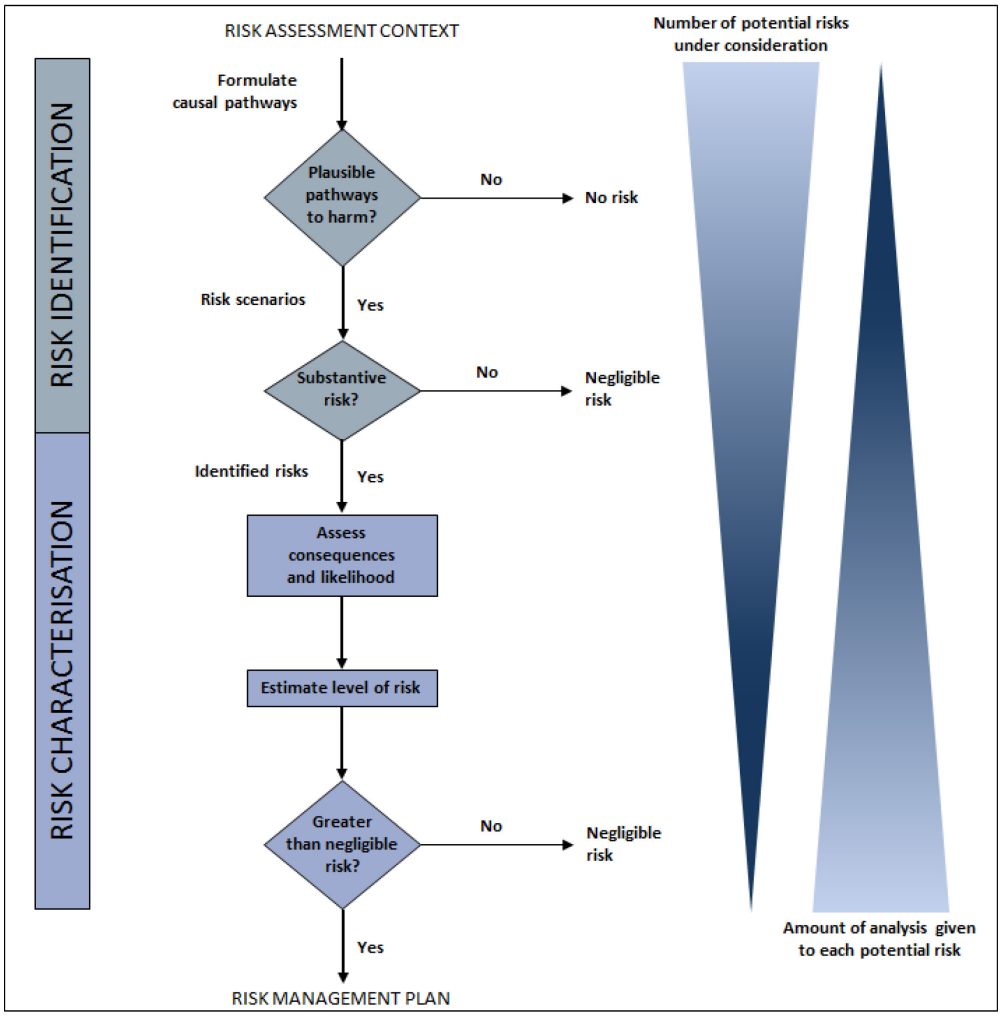

Figure 7. The risk assessment process

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

136. Risk identification first considers a wide range of circumstances in which the GMO, or the introduced genetic material, could come into contact with people or the environment. This leads to postulating plausible causal pathways that may give rise to harm for people or the environment from dealings with a GMO. These are risk scenarios. These risk scenarios are screened to identify those that are considered to have a reasonable chance of causing harm in the short or long term. Pathways that do not lead to harm, or those that could not plausibly occur, do not advance in the risk assessment process (Figure 7), that is, the risk is considered to be no greater than negligible.

137. Risks identified as being potentially greater than negligible are characterised in terms of the potential seriousness of harm (Consequence assessment) and the likelihood of harm (Likelihood

assessment). Risk evaluation then combines the Consequence and Likelihood assessments to estimate the level of risk and determine whether risk treatment measures are required. The potential for interactions between risks is also considered.

Section 2 Risk identification

138. Postulated risk scenarios are comprised of three components (Figure 8):

I. the source of potential harm (risk source)

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

III. potential harm to people or the environment.

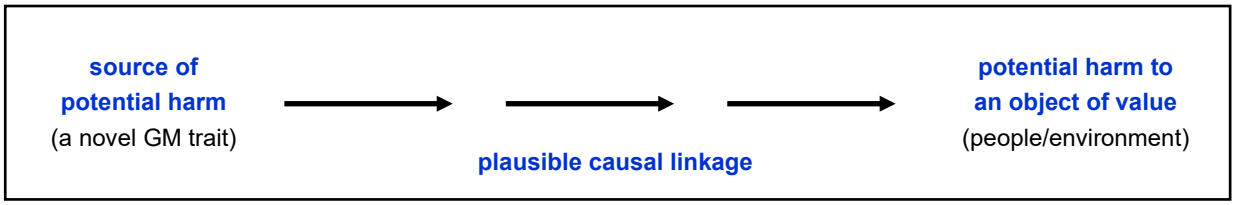

Figure 8. Risk scenario

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

- the proposed dealings
- the proposed limits including the extent and scale of the proposed dealings
- the proposed controls to limit the spread and persistence of the GMOs and
- the characteristics of the parent organism(s).

2.1 Risk source

140. The non-GM parent organism is dengue virus serotype 2 strain PDK-53, which was attenuated through serial passage in tissue culture (Chapter 1, Section 3.8). Details of pathogenicity and transmissibility of WT dengue virus is also discussed in Chapter 1.

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

142. The specific risk source in this application are the introduced genes in the dengue strains in the GM vaccine. As discussed in Chapter 1, Section 4.1, the 4 GM strains all contain an attenuated dengue serotype 2 backbone, and 3 of the strains have been modified to contain *prM* and *E* genes from dengue serotypes 1, 3, and 4, respectively. These introduced genes and their encoded proteins are considered further as a potential source of risk.

143. The GM vaccine formulation also contains a number of excipients. These excipients are not GMOs and will not be considered in the risk assessment.

2.2 Causal pathway

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

- the proposed dealings, which are the import, transport and disposal of the GMOs and possession (including storage) in the course of any of these dealings
- regulations in place for the transport or disposal of the GMOs by other regulatory agencies, the States and Territories
- characteristics of the parent organism
- routes of exposure to the GMOs, the introduced gene(s) and gene product(s)

- potential for transmission
- potential effects of the modified gene on the properties of the organism
- potential exposure of other organisms to the GMOs in the environment
- the release environment
- spread and persistence of the GMOs (e.g. dispersal pathways and establishment potential)
- environmental stability of the organism (e.g. tolerance to temperature, UV irradiation and humidity)
- potential risk of revertant/novel strains due to mutations or horizontal gene transfer (HGT)
- practices before and after administration of the GM vaccine
- potential for unauthorised activities.

145. The current assessment focuses on risks posed to people or the environment, including long term persistence of the GMOs, which may arise from the import, transport, storage, or disposal of the GM vaccine, and the possession, supply, or use of the GMOs for the purposes of, or in the course of, any of these dealings.

146. The TGA regulate quality, safety, and efficacy of vaccines under the *Therapeutic Goods Act 1989*, as mentioned in Chapter 1, Section 1.1. This includes:

- assessment of patient safety, vaccine quality and efficacy prior to inclusion on the ARTG
- recommended practices for the transport, storage, and disposal of the GM vaccine under the *Australian code of good wholesaling practice for medicines in schedules 2, 3, 4 & 8* (NCCTG, 2011)
- requirements for the scheduling, labelling, and packaging under the *Poisons Standard* (Therapeutic Goods Administration, 2023).

147. Use of GMOs for therapeutic purposes is not regulated under the Act, and the Regulator does not assess risks from their use, such as risks to the intended vaccine recipients from the GM vaccine. Therefore, this assessment focuses primarily on risks posed by accidental exposure of people and other organisms and to the environment from the GMO, and not the intended vaccine recipients.

148. As discussed in Chapter 1, dengue is a mosquito-borne illness that results in viraemia in humans and can be spread through blood-blood contact. Dengue is not known to be transmitted by shedding. In natural human infection (WT strains), dengue genomes have been detected in body fluids such as saliva, urine, semen, vaginal secretions, and breast milk. However, vRNA load in urine, saliva, and breast milk is 3-5 orders of magnitude lower than in plasma and attempts to isolate viable virus particles from urine, semen, vaginal secretions, and breast milk were unsuccessful. There are conflicting reports about the presence of viable virus particles in human saliva. The GM strains are attenuated compared to WT and have limited shedding into urine and saliva in mice. Therefore, shedding of the GM strains in human body fluids such saliva, urine, semen, vaginal secretions, and breast milk is expected to be minimal and insufficient to cause infection, and will not be considered further as potential dispersal pathways into the environment.

149. For urban transmission of dengue virus between mosquitoes and humans to occur, sufficient populations of vector mosquitoes and human hosts would need to be within close proximity and:

(a) a viraemic human must be bitten by a vector mosquito

(b) the mosquito must ingest enough viral particles to infect the midgut

(c) the virus must be able to replicate in the mosquito midgut and then disseminate into the salivary gland

(d) the virus must replicate in the mosquito salivary glands to a sufficient level to ensure transmission to a human when taking a blood meal.

150. The GMOs are attenuated compared to WT dengue and have reduced replication potential in humans and mosquitoes. The peak viraemia reported in clinical trial data was 2.5×10^5 genomes/mL for TDV-2 (Chapter 1, Section 4.3.4), which is considerably lower than the 10^9 peak titres seen in natural

dengue infection (Chapter 1, Section 3.5), thus reducing the likelihood for transmission. As discussed in Chapter 1, Section 4.3.3, when *Ae. aegypti* mosquitoes were fed a blood meal with 2.5 x 10^6 PFU/mL TDV-2, none became infected. This lack of, or impaired, infectivity to mosquitoes was also seen for the other TDVs (Huang et al., 2013). In addition, populations of *Ae. aegypti* are limited to northern Queensland (Chapter 1, Section 3.7.6), reducing opportunities for vector exposure. If the GMOs remain attenuated, sustained transmission in the environment is considered highly unlikely, and will not be considered further.

151. The GMOs are live attenuated dengue strains and therefore have the potential to acquire mutations that may cause reversion to a more virulent phenotype. As discussed in Chapter 1, Section 3.6, dengue is an RNA virus with a relatively high mutation rate, although beneficial mutations happen rarely (Dolan et al., 2021). The genetic modifications in the GMOs are the replacement of genes which encode structural prM and E proteins from WT dengue strains and are not expected to increase the likelihood of reversion occurring.

152. Genetic stability of the GMOs has been studied *in vitro* and in clinical trials (Chapter 1, Section 4.3.2). Of the 3 attenuation loci, 5'NCR has shown a propensity to revert, and occasionally NS1, but not both together. *In vitro* phenotypic studies have demonstrated that reversions in 2 or more attenuation loci are required to restore the virulent phenotype (Butrapet et al., 2000).

153. For reversion to occur, the virus must be actively replicating in a host. In genetic stability testing of the research grade GM strains *in vitro* in monkey kidney cells, the NS1 and NS3 loci remained stable after 10 passages. The 5'NCR loci has low levels of reversion (<10%) before passage 8 (Butrapet et al., 2006). The GMOs have impaired replication in both people and mosquitoes compared to WT strains, which reduces the opportunity for reversion. Dengue is also not known to form a latent infection and the viraemia from the GM vaccine is transient, with a lower maximum titre than in natural dengue infection. This also reduces the opportunity for reversion to a virulent phenotype.

154. Recombination of dengue is a rare event. It requires a mosquito or a human to be co-infected with more than one strain of dengue. This is unlikely given dengue is not endemic in Australia. In addition, Australian vaccination guidelines recommend deferring vaccination in those who are unwell, which would exclude those with symptomatic dengue (Australian Technical Advisory Group on Immunisation, 2022). As the vaccine is aimed at stimulating an immune response, the GMOs would be expected to be rapidly cleared by the immune system, which limits the opportunity for recombination. If recombination were to occur between the GMOs and a WT strain, it is highly unlikely that all three attenuation loci would be lost. The introduced genes are the *prM* and *E* genes from WT dengue viruses. Therefore, these genes are not expected to produce proteins with novel properties compared to WT dengue which could result in a more virulent WT virus, should recombination occur. Recombination between the four GM strains would not be expected to result in a virulent virus, as the backbones of all strains are attenuated.

155. Recombination of the GMOs with a different flavivirus is unlikely since there is no reported evidence of recombination of different flaviviruses. Flaviviruses do not circulate widely in Australia and the most common flaviviruses in Australia are not vectored by the *Aedes* mosquito. In addition, artificial recombinants of WT flaviviruses with chimeric flavivirus vaccines showed high levels of attenuation compared to the WT parent virus (Pugachev et al., 2007; McGee et al., 2008).

156. Considering the above factors, reversion of the GMOs to a virulent phenotype and recombination resulting in a virulent strain of the virus in a person leading to sustained transmission in the environment are considered to be highly unlikely and will not be considered further.

157. As a positive strand RNA virus, the dengue virus genome is not capable of integration into the host cell genome. Therefore, the consequences of integration of viral DNA into a host cell genome will not be considered further.

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

2.3 Potential harm

159. Potential harms from the GM vaccine include:

- harms to the health of people or other organisms following accidental exposure to the GMOs, including disease or an adverse immune response to the GMOs
- the potential for establishment of the GM dengue strains in the environment (discussed in Section 2.2).

160. As discussed in Chapter 1, Section 3.7.1, none of the non-human vertebrate species that are confirmed or suspected to be involved in the dengue sylvatic cycle are present in Australia outside of captivity. In addition, mosquitoes are the only invertebrate vectors. Therefore, the potential for harm to occur to organisms other than humans will not be considered further.

161. The proteins associated with the introduced genes in the GM vaccine (*prM* and *E*) are dengue proteins and are not expected to differ structurally or functionally from those in WT dengue viruses present in the environment, or to be allergens or toxins. In addition, no allergic or toxic responses to the vaccine have been observed in the more than 28,000 clinical trial participants who have received at least one dose of the various formulations of the GM vaccine. Therefore, allergenicity or toxicity from the proteins expressed by the introduced genes in the GMOs will not be considered further.

2.4 Postulated risk scenarios

162. One risk scenario was postulated and screened to identify any substantive risks. This scenario is summarised in Table 3 and examined in detail in Section 2.4.1.

163. In the context of the activities proposed by the applicant and considering both the short and long term, the risk scenario did not give rise to any substantive risks.

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

Risk scenario	Risk source	Causal pathway	Potential harm	Substantive risk?	Reasons
1	The introduced genes in the GM vaccine (*prM* and *E* genes from WT dengue serotypes)	Accidental exposure of people to the GMOs through: (a) Unintentional release of GMOs during transport or storage (b) Preparation and administration of the GMOs (c) Disposal of vials or syringes contaminated with GMOs in medical waste ⬇ GMOs enter broken skin ⬇ Person is infected with GMOs	Adverse reaction to the GMOs	No	• The GMOs are attenuated in comparison to WT dengue • The dose received through accidental exposure would be far smaller than that administered during vaccination • The GM vaccine has a favourable safety profile at doses higher than would be expected through accidental exposure • Import, transport, storage, and disposal will follow well established procedures

2.4.1 Risk Scenario 1

Risk source	The introduced genes in the GM vaccine (*prM* and *E* genes from WT dengue serotypes)
Causal pathway	⬇ Accidental exposure of people to the GMOs through: (a) Unintentional release of GMOs during transport or storage (b) Preparation and administration of the GMOs (c) Disposal of vials or syringes contaminated with GMOs in medical waste ⬇ GMOs enter broken skin ⬇ Person is infected with GMOs ⬇
Potential harm	Adverse reaction to the GMOs

2.4.1.1 Risk source

164. The source of potential harm for this postulated risk scenario is the introduced genes in the GM vaccine; *prM* and *E* genes from WT dengue serotypes.

2.4.1.2 Causal pathway

165. Individuals may be inadvertently exposed to the GMOs during transport or storage, or during disposal of vials or syringes contaminated with the GMOs. The two locations where this is most likely to occur are:

- the central storage facilities where stocks of the GM vaccine are being held
- locations where the GM vaccine is being administered.

166. Given the transmission profile of dengue (Chapter 1, Section 3.7.4), the only transmission pathway that is considered to be plausible is exposure to the GMOs through broken skin (including a needlestick injury).

167. As discussed in Chapter 1, Section 4.3.7, the GMOs are expected to lose viability over time above the optimal storage temperature of 2-8 C and to be sensitive to common decontamination methods.

Exposure during transport and storage of the GM vaccine

168. The GM vaccine must be stored between 2-8°C and requires a cold chain, which is a well-controlled and uninterrupted sequence of transport and storage designed to maintain the vaccine in this narrow temperature range, in order to preserve the potency of the vaccine.

169. Takeda proposes to import the vaccine as a freeze-dried powder in a sealed glass vial, which minimises the likelihood of leakage. In addition, the type of glass vial that is commonly used for packaging injectable drug products such as this vaccine, does not shatter easily. The vials would be packaged in cartons and the cartons packed in corrugated cardboard shipping cartons for distribution. Transport of the GM vaccine between the port of entry and the central storage facility would continue in this packaging.

170. Dengue vaccines are classified as Schedule 4 (Prescription only) medicines. The *Australian code of good wholesaling practice for medicines in schedules 2, 3, 4 & 8* (NCCTG, 2011) recommends that:

- upon arrival at the wholesaler, packaging should be removed, and stock should be examined for the absence of damage or evidence of tampering. Damaged stock should be quarantined
- packaging of cold chain medicines should alert the receiver of its contents and that the receiver should place the medicines in appropriate storage facilities as soon as possible
- wholesalers should ensure that persons supplied with medicines are authorised appropriately under State or Territory legislation to be supplied with those medicines.

171. Additionally, storage, handling and transport would be in accordance with both the *Australian code of good wholesaling practice for medicines in schedules 2, 3, 4 & 8* (NCCTG, 2011) and the *World Health Organization's Good storage and distribution practices for medical products* (World Health Organization, 2020). These guidelines require that:

- written procedures for dealing with spillage of items of special hazard are available and training is provided to responsible staff
- in the event of a spill, the spill should be cleaned up promptly and rendered safe as quickly as practicable in accordance with the material safety data sheet (MSDS)
- spills kits should be conveniently located within the storage area
- access to the medical product is restricted to individuals with the appropriate training.

172. These practices would minimise the likelihood of damaged and leaking stock going unnoticed and ensure the GM vaccine is being handled by individuals who are trained in procedures required to decontaminate a spill, thus minimising the likelihood of unintended dispersal of the GMOs.

173. Should the GMOs be unintentionally released, they are highly unlikely to infect people as they cannot replicate outside a host and are readily decontaminated. Exposure leading to infection requires entry of GMOs by broken skin. Should a vial break during transport or storage, it is unlikely that a sufficient amount of the GMOs would enter through broken skin to result in an adverse reaction.

Exposure during preparation and administration of the GM vaccine

174. As discussed in Chapter 1, Section 5.1, the GM vaccine is intended to be administered through subcutaneous injection performed by trained healthcare professionals at medical or clinical facilities. There is potential for exposure of people involved in the preparation or administration of the GM vaccine to the GMOs via needle stick/sharps injury or due to spillage of GM vaccine onto surfaces. Aerosols of the GMOs could be released into the clinical facility during preparation or administration of the GM vaccine, but the amount would be very small and aerosols are not known to be a route of dengue transmission (Chapter 1 Section 3.7.4).

175. The GMOs would be prepared and administered by authorised, experienced and trained health professionals. All personnel working in settings where healthcare is provided, including vaccination services, are required to comply with the standard precautions for working with potentially infectious material, as described in the Australian Guidelines for the Prevention and Control of Infection in Healthcare (National Health and Medical Research Council, 2019). This includes hand hygiene, sharps safety, wearing of appropriate personal protective equipment, and covering cuts and abrasions on exposed skin with water-proof dressings. Compliance with these behavioural practices at vaccination centres will limit unintended exposure of people to the GMOs.

Exposure during disposal of the GM vaccine and any contaminated waste

176. At the central storage facilities, there may be vials of the GM vaccine for disposal which are either excess stock or past their expiry date, but which may still contain viable GMOs. At vaccination facilities, GM waste for disposal will include used vials, syringes, or other material contaminated with the GMOs, such as gloves.

177. The *Australian code of good wholesaling practice for medicines in schedules 2, 3, 4 & 8* (NCCTG, 2011) requires:

- specific training for personnel handling medicines that pose a high risk to personnel if package integrity is breached or spillage occurs
- waste medicines be collected and destroyed by a person who is licensed or permitted to do so under relevant State or Territory legislation
- medicines for destruction be enclosed in sealed packaging or in a container.

178. At the central storage facilities, sealed vials of unused GM vaccine would be decontaminated by a waste contractor and all stock destroyed would be recorded. Given that during waste disposal the GMOs

would still be a dry powder in a sealed vial that does not shatter easily, the people handling the waste are highly unlikely to be exposed to the GM vaccine in a manner that would lead to productive infection. In addition, if the unused vials are kept at room temperature for a period before disposal the viability of the GMOs would be expected to decrease.

179. At the sites of administration, used vials of the GMOs, syringes, or other waste contaminated with the GMOs would be treated as clinical waste and disposed of in accordance with the waste disposal methods approved by the States and Territories. Adherence with these procedures would reduce the likelihood of accidental exposure of people to the GMOs.

180. For productive infection, individuals must be exposed to an infectious dose. Residual liquid in used vials or syringes would be unlikely to contain a sufficient dose to cause productive infection. The same would apply to secondary waste such as gloves that may be contaminated with the vaccine. The GMOs cannot replicate outside a host cell, so viral particles in the used vials or other waste material could not multiply to reach an infective dose. In addition, the viability of the reconstituted GMOs would be expected to rapidly decrease outside of the cold chain. Testing conducted by the applicant indicated that liquid formulations of the GM vaccine lost approximately 10-fold infectious titre of each serotype after 7 days at 25°C and all infectious titre by 21 days at 25°C.

2.4.1.3 Potential harm

181. Laboratory and human studies have shown that the GMOs are attenuated compared to WT dengue and the vaccine has a favourable safety profile in vaccinees (Chapter 1, Section 4.3.6). When administered through subcutaneous injection in humans, the most common systemic and local adverse events are headache and injection site pain, respectively.

182. The amount of the GMOs received through accidental exposure to broken skin would likely be far less than a single intentional dose and would be unlikely to result in symptoms. If the accidental exposure were to produce symptoms, this would likely be limited to mild symptoms such as a headache or a localised skin reaction at the exposure site for a short period of time while the virus is cleared by the immune system.

183. Clinical data regarding the effect of the GMOs on immunosuppressed individuals are lacking as this group has been excluded from the clinical trials. In natural dengue infection there is no well-established link between a more severe disease outcome in immunosuppressed people, although some studies suggest a slight trend towards persistent viraemia and prolonged dengue symptoms in immunosuppressed individuals (Chapter 1, Sections 3.1 and 3.4). The GMOs are attenuated compared to WT dengue and produce mild symptoms in immune-competent people. Therefore, if an immunosuppressed individual was to be accidentally exposed to the GMOs it is unlikely that they would develop severe symptoms, although this is an area of some uncertainty.

184. As discussed in Section 3.1, in natural dengue infection a secondary infection with a different dengue serotype can predispose a person to severe dengue. It does not appear that the potential for the development of ADE on subsequent exposure to WT dengue following vaccination with the GM vaccine was directly assessed in the clinical trials. However, it is noted that not all clinical trial participants developed antibodies to all four serotypes, and in the largest clinical trial (>20,000 participants in 8 dengue-endemic countries), participants who received the vaccine had lower rates of severe dengue at 3 years post-vaccination than those who received the placebo (Rivera et al., 2022). In the case of an accidental exposure to the GMOs, a person would likely get a subclinical dose and therefore may be more likely to not develop antibodies to all four serotypes (compared to people receiving the full vaccine dose). Alternatively, the dose from an accidental exposure may be so low that no antibodies to any of the four serotypes develop. Based on the limited evidence that is available, it seems unlikely that the potential for ADE is a concern for accidental exposure, however this is an area of uncertainty.

2.4.1.4 Conclusion

185. The potential for an unintentional exposure of people to the GMOs during transport, storage, and disposal resulting in an adverse reaction is not identified as a risk that could be greater than negligible. Therefore, it does not warrant further detailed assessment.

Section 3 Uncertainty

186. Uncertainty is an intrinsic part of risk analysis.[7] There can be uncertainty in identifying the risk source, the causal linkage to harm, the type and degree of harm, the likelihood of harm or the level of risk. In relation to risk management, there can be uncertainty about the effectiveness, efficiency, and practicality of controls.

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

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

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

189. Uncertainty can also arise from a lack of experience with the GMOs themselves. While the potential for development of ADE following accidental exposure has been noted as an area of uncertainty, there is extensive clinical trial experience with the GM vaccine in more than 28,000 participants in endemic and non-endemic areas, and marketing authorisation has been granted in several regions internationally following comprehensive independent scientific assessments by regulators (Chapter 1, Section 6.2).

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

191. Post release review (PRR; Chapter 3, Section 4) will be used to address uncertainty regarding future changes to knowledge about the GMOs. This is typically used for commercial releases of GMOs, which generally do not have fixed duration.

Section 4 Risk evaluation

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

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

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

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

194. One risk scenario was identified whereby the proposed dealings might give rise to harm to people or the environment. This included consideration of whether people can be accidentally exposed to the GMOs while conducting the dealings.

195. A risk is substantive only when the risk scenario may, because of gene technology, have some chance of causing harm. Risk scenarios that do not lead to harm, or could not reasonably occur, do not represent an identified risk and do not advance in the risk assessment process.

196. In the context of the range of measures already in place, including the operating guidelines and requirements of the other regulatory agencies, and considering both the short and long term, the risk scenario was not identified as representing a substantive risk requiring further assessment. The principal reasons for this include:

- exposure to the GM vaccine would be minimised by well-established clinical, import, transport, storage, and disposal procedures
- the GM vaccine strains are attenuated
- the dose received through accidental exposure would be far smaller than that administered during vaccination
- the GM vaccine has a favourable safety profile at doses higher than would be expected through accidental exposure.

197. Therefore, any risks to the health and safety of people, or the environment, from the proposed commercial supply of the GM vaccine are considered to be negligible. The Risk Analysis Framework (OGTR 2013), which guides the risk assessment and risk management process, defines negligible risks as insubstantial with no present need to invoke actions for their mitigation. No controls are required to treat these negligible risks. Hence, the Regulator considers that the dealings involved in this proposed release do not pose a significant risk to either people or the environment.[8]

[8] 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.

Chapter 3 Risk management plan

Section 1 Background

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

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

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

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

Section 2 Risk treatment measures for substantive risks

202. The risk assessment of the risk scenario listed in Chapter 2 concluded that there are negligible risks to people and the environment from the proposed supply of the GM vaccine. This risk scenario was considered in the context of the proposed receiving environment and the Australia-wide release. The risk evaluation concluded that no specific risk treatment measures are required to treat these negligible risks. General risk management measures are discussed below.

Section 3 General risk management

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

- applicant suitability
- testing methodology
- identification of the persons or classes of persons covered by the licence
- reporting structures
- access for the purpose of monitoring for compliance.

3.1 Applicant suitability

204. In making a decision whether or not to issue a licence, the Regulator must have regard to the suitability of the applicant to hold a licence. Under Section 58 of the Act, matters that the Regulator must take into account include:

- any relevant convictions of the applicant

- any revocation or suspension of a relevant licence or permit held by the applicant under a law of the Commonwealth, a State or a foreign country
- the capacity of the applicant to meet the conditions of the licence.

205. If a licence were issued, the conditions would include a requirement for the licence holder to inform the Regulator of any circumstances that would affect their suitability.

206. In addition, any applicant organisation must have access to a properly constituted Institutional Biosafety Committee and be an accredited organisation under the Act.

3.2 Testing methodology

207. If a licence were issued, Takeda would be required to provide a method to the Regulator for the reliable detection of the GMO, and the presence of the introduced genetic materials in a recipient organism. This methodology would be required prior to conducting any dealings with the GMO.

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

208. If a licence were issued, any person, including the licence holder, could conduct any permitted dealing with the GMO.

3.4 Reporting requirements

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

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

210. The licence holder is also obliged to submit an Annual Report containing any information required by the licence.

211. There are also provisions that enable the Regulator to obtain information from the licence holder relating to the progress of the commercial release (see Section 4, below).

3.5 Monitoring for compliance

212. 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 the Regulator, inspectors or other person authorised by the Regulator, to enter premises where a dealing is being undertaken for the purpose of monitoring or auditing the dealing.

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

Section 4 Post release review

214. Regulation 10 requires the Regulator to consider the short and the long term when assessing risks. The Regulator takes account of the likelihood and impact of an adverse outcome over the foreseeable future, and does not disregard a risk on the basis that an adverse outcome might only occur in the longer term. However, as with any predictive process, accuracy is often greater in the shorter rather than longer term.

215. For the current application for a DIR licence, the Regulator is including conditions that require ongoing oversight in order to provide feedback on the findings of the RARMP and ensure the outcomes remain valid for future findings or changes in circumstances. If a licence was issued, this ongoing oversight would be achieved through post release review (PRR) activities. The three components of PRR are:

- adverse effects reporting system (Section 4.1)
- requirement to monitor specific indicators of harm (Section 4.2)
- review of the RARMP (Section 4.3).

216. The outcomes of these PRR activities may result in no change to the licence or could result in the variation, cancellation or suspension of the licence.

4.1 Adverse effects reporting systems

217. Any member of the public can report adverse experiences/effects resulting from a GMO to the OGTR through the Free-call number (1800 181 030), mail (MDP 54 – GPO Box 9848, Canberra ACT 2601) or via email to the OGTR inbox (ogtr@health.gov.au). Reports can be made at any time on any DIR licence. Credible information would form the basis of further investigation and may be used to inform a review of a RARMP (see Section 4.3 below) as well as the risk assessment of future applications involving similar GMOs.

4.2 Requirement to monitor specific indicators of harm

218. Collection of additional specific information on an intentional release provides a mechanism for 'closing the loop' in the risk analysis process and for verifying findings of the RARMP, by monitoring the specific indicators of harm that have been identified in the risk assessment.

219. The term 'specific indicators of harm' does not mean that it is expected that harm would necessarily occur if a licence was issued. Instead, it refers to measurement endpoints which are expected to change should the authorised dealings result in harm. Should a licence be issued, the licence holder would be required to monitor these specific indicators of harm as mandated by the licence.

220. The triggers for this component of PRR may include risk estimates greater than negligible or significant uncertainty in the risk assessment.

221. The characterisation of the risk scenario discussed in Chapter 2 did not identify any risks greater than negligible. Therefore, they were not considered substantive risks that warranted further detailed assessment. Uncertainty is considered to be low. No specific indicators of harm have been identified in this RARMP for application DIR 196. However, specific indicators of harm may also be identified during later stages, e.g. following the consideration of comments received on the consultation version of the RARMP, or if a licence were issued, through either of the other components of PRR.

222. Conditions have been included in the licence to allow the Regulator to request further information from the licence holder about any matter to do with the progress of the release, including research to verify predictions of the risk assessment.

4.3 Review of the RARMP

223. The third component of PRR is the review of RARMPs after a commercial/general release licence is issued. Such a review would take into account any relevant new information, including any changes in the context of the release, to determine if the findings of the RARMP remained current. The timing of the review would be determined on a case-by-case basis and may be triggered by findings from either of the other components of PRR or be undertaken after the authorised dealings have been conducted for some time. If the review findings justified either an increase or decrease in

the initial risk estimate(s), or identified new risks to people or to the environment that require management, this could lead to changes to the risk management plan and licence conditions.

Section 5 Conclusions of the consultation RARMP

224. The risk assessment concludes that the proposed commercial release of this GM vaccine poses negligible risks to the health and safety of people or the environment as a result of gene technology.

225. The risk management plan concludes that these negligible risks do not require specific risk treatment measures. However, general conditions are imposed to ensure that there is ongoing oversight of the release.

Chapter 4 Proposed licence conditions

Section 1 Interpretations and Definitions

1. In this licence:

   (a) unless defined otherwise in this licence, words and phrases used in this licence have the same meaning as they do in the Act and the Gene Technology Regulations 2001;

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

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

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

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

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

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

2. In this licence:

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

‘**Annual Report**’ means a written report provided to the Regulator by the end of September each year containing all the information required by this licence to be provided in the Annual Report.

‘**ARTG**’ means the Australian Register of Therapeutic Goods maintained in accordance with the *Therapeutic Goods Act 1989*.

‘**GM**’ means genetically modified.

‘**GMOs**’ means the genetically modified organisms that are the subject of the dealings authorised by this licence.

‘NLRD’ is a Notifiable low risk dealing. Dealings conducted as an NLRD must be assessed by an institutional biosafety committee (IBC) before commencement and must comply with the requirements of the Gene Technology Regulations 2001.

‘**OGTR**’ means the Office of the Gene Technology Regulator.

‘**Regulator**’ means the Gene Technology Regulator.

Section 2 Licence conditions and obligations

3. This licence remains in force until it is suspended, cancelled or surrendered. No dealings with the GMO are authorised during any period of suspension.

4. The licence holder is Takeda Pharmaceuticals Australia Pty Ltd.

5. Any person, including the licence holder, may conduct any permitted dealing(s) with the GMOs.

6. The dealings authorised by this licence are:

(a) import of the GMOs;

(b) transport of the GMOs;

(c) disposal of the GMOs;

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

Note: Use of the GMOs for therapeutic purposes is not covered by the Gene Technology Act 2000 and therefore this licence is not required to authorise such use. The GMOs are also subject to regulation by other federal and state departments and agencies, including the Therapeutic Goods Administration and the Department of Agriculture, Fisheries and Forestry. These other departments and agencies may impose further requirements for, or limitations on, the use of the GMOs or these dealings.

7. This licence does not apply to dealings with the GMOs conducted as a Notifiable Low Risk Dealing (NLRD) or pursuant to another authorisation issued under the Act.

Note: Dealings conducted as an NLRD must be assessed by an Institutional Biosafety Committee (IBC) before commencement and must comply with the requirements of the Regulations.

8. Dealings with the GMOs authorised by this licence may be conducted in all areas of Australia.

9. The licence authorises dealings with the GMOs described in **Attachment A**.

2.1 General obligations of the licence holder

10. The licence holder must immediately notify the Regulator if any of its contact details change.

Note: Please address correspondence to OGTR.M&C@health.gov.au.

Prior to issuing a licence, the Regulator considers suitability of the applicant to hold a licence. The following conditions address ongoing suitability of the licence holder.

11. The licence holder must, at all times, remain an accredited organisation in accordance with the Act and must comply with its instrument of accreditation.

12. The licence holder must:

(a) inform the Regulator immediately, in writing, of:

i. any relevant conviction of the licence holder; or

ii. any revocation or suspension of a licence or permit held by the licence holder under a law of the Australian Government, a State or a foreign country, being a law relating to the health and safety of people or the environment; or

iii. any event or circumstances that would affect the capacity of the licence holder to meet the conditions in it; and

(b) provide any information related to the licence holder's ongoing suitability to hold a licence, if requested by the Regulator, within the timeframe stipulated by the Regulator.

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

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

(b) the cancellation or suspension of the licence; and

(c) the surrender of the licence.

2.2 Provision of new information to the Regulator

Licence conditions are based on the risk assessment and risk management plan developed in relation to the application using information available at the time of assessment. The following condition

requires that any new information that may affect the risk assessment is communicated to the Regulator.

14. The licence holder must inform the Regulator if the licence holder becomes aware of:

    (a) additional information as to any risks to the health and safety of people, or to the environment, associated with the dealings authorised by the licence; or

    (b) any contraventions of the licence by a person covered by the licence; or

    (c) any unintended effects of the dealings authorised by the licence.

Note: The Act requires, for the purposes of the above condition, that:

(a) *the licence holder will be taken to have become aware of additional information of a kind mentioned in condition 14 if he or she was reckless as to whether such information existed; and*

(b) *the licence holder will be taken to have become aware of contraventions, or unintended effects, of a kind mentioned in condition 14, if he or she was reckless as to whether such contraventions had occurred, or such unintended effects existed.*

Note: Contraventions of the licence may occur through the action or inaction of a person.

15. If the licence holder is required to inform the Regulator under condition 14, the Regulator must be informed immediately.

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

16. If at any time the Regulator requests the licence holder to collect and provide information about any matter to do with the progress of the dealings authorised by this licence, including but not confined to:

    (a) additional information as to any risks to the health and safety of people, or to the environment, associated with the dealings authorised by the licence, whether or not the licence holder has provided information to the Regulator under condition 14(a);

    (b) any contraventions of the licence by a person covered by the licence, whether or not the licence holder has provided information to the Regulator under condition 14(b);

    (c) any unintended effects of the dealings authorised by the licence, whether or not the licence holder has provided information to the Regulator under condition 14(c);

    (d) research, including by way of survey, to verify predictions of the risk assessment, or for any purpose related to risks to the health and safety of people, or to the environment;

    (e) scientific literature and reports in respect of the GMOs authorised by this licence, for a nominated period; and

    (f) details of any refusals of applications for licences or permits (however described) to deal with the GMO made pursuant to the regulatory laws of a foreign country;

and the request is reasonable, having regard to consistency with the Act and relevance to its purpose, then the licence holder must collect the information and provide it to the Regulator at a time and in the manner requested by the Regulator.

Note: The Regulator may invite the licence holder to make a submission on the reasonability of a request by the Regulator to collect and provide information relevant to the progress of the dealings with the GMOs.

2.3 Obligations of persons covered by the licence

17. If a person is authorised by this licence to deal with the GMOs and a particular condition of this licence applies to the dealing by that person, the person must allow the Regulator, or a person authorised by the Regulator, to enter premises where the dealing is being undertaken, for the purposes of auditing or monitoring the dealing.

Section 3 Reporting and documentation requirements

3.1 Notification of authorisation by the Therapeutic Goods Administration

18. If the GMOs are included on the ARTG, the licence holder must notify the Regulator in writing within 14 days of registration.

19. The licence holder must notify the Regulator in writing of any subsequent amendments to the conditions of the ARTG registration involving the pattern of usage, handling, storage, transport, or disposal of the GMOs, within 14 days of the change occurring.

3.2 Annual report

20. The licence holder must provide an Annual Report to the Regulator by the end of September each year covering the previous financial year. An Annual Report must include:

    (a) information about any adverse impacts, unintended effects, or new information relating to risks, to human health and safety or the environment caused by the GMOs or material from the GMOs; and

    (b) information about the numbers of the GM vaccine doses imported and distributed to each State and Territory.

3.3 Testing methodology

21. At least 14 days prior to conducting any dealings with the GMOs, the licence holder must provide to the Regulator a written methodology to reliably detect the GMOs, or the presence of the genetic modifications described in **Attachment A** in a recipient organism or environmental sample. The detection method(s) must be capable of identifying, to the satisfaction of the Regulator, the GMOs described in **Attachment A**.

Note: Please address correspondence to OGTR.M&C@health.gov.au.

ATTACHMENT A

DIR No: 196

Full Title: Commercial supply of Qdenga, a live attenuated GM dengue vaccine

Organisation Details

Postal address: Takeda Pharmaceuticals Australia Pty Ltd

Level 39, Grosvenor Place, 225 George Street

Sydney NSW 2000

Accreditation No: Accr 282

GMO Description

GMOs covered by this licence

The GM vaccine contains 4 live attenuated dengue strains (TDV-1, TDV-2, TDV-3, and TDV-4) which have been modified to contain *prM* (pre-membrane) and *E* (envelope) genes from dengue serotype 1 (strain 16007), dengue serotype 2 (strain 16681), dengue serotype 3 (strain 16562), and dengue serotype 4 (strain 1036).

Parent Organism

Common Name: Dengue virus

Scientific Name: Dengue virus serotype 2 strain PDK-53

Modified traits

Category: Vaccine – altered antigen expression

Description: The dengue viruses have been modified to elicit an immune response to targeted dengue serotypes, for use as a live attenuated vaccine.

Purpose of the dealings with the GMO

The purpose of the dealings is the commercial supply of the GM dengue vaccine for use as a human therapeutic Australia-wide. The permitted dealings under this licence are the import, transport, storage, and disposal of the GM vaccine.

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Appendix A: Summary of submissions

The Regulator received several submissions from prescribed experts, agencies, and authorities[9] on matters relevant to preparation of the RARMP. All issues raised in submissions relating to risks to the health and safety of people and the environment were considered. These issues, and where they are addressed in the consultation RARMP, are summarised below.

Submission	Summary of issues raised	Comment
1	Agrees that the following key issues identified by the office should be considered when preparing the RARMP: • Potential for accidental exposure of humans and animals to the GMOs leading to harm • Potential for recombination, reversion, or mutation events which change the viral characteristics and lead to a pathogenic phenotype • Potential for the GMOs to be harmful to the environment. Did not identify additional relevant information that should be considered.	The potential risks to animals are discussed in Chapter 2, Section 2.3. The potential for accidental exposure to people is discussed in Chapter 2, Section 2.4.1 (Risk scenario 1). The potential for reversion and recombination is discussed in Chapter 2, Section 2.2. The potential for sustained transmission in the environment is discussed in Chapter 2, Section 2.2.
2	Has no comment or feedback at this time in relation to the application.	Noted.
3	Does not have any comment at this stage.	Noted.
4	Have the following commentary before being able to provide valuable feedback on this matter: • Further clarification is required in relation to the proposed recipients for this vaccine (such as gender, age etc.). • It is currently unclear if this vaccine is intended for persons who have previously been infected with one of four dengue serotypes or to all persons with no prior infection. • The vaccination uses live strains of dengue virus, as dengue mosquito capable of transmitting the virus is endemic in parts of Australia, would there be a requirement for the recipient of this vaccine to isolate? • What is the estimated timeline for the release of this vaccine? • What is the estimated cost to the proposed recipient for this vaccine?	The use of the vaccine in people, including conditions of use, falls within the regulatory responsibility of the Therapeutic Goods Administration (TGA). The potential for transmission of the GMOs in the environment is discussed in Chapter 2 Section 2.2. Requirements on vaccinees post-vaccination fall within the regulatory responsibility of the TGA. Marketing and trade issues are outside the scope of the *Gene Technology Act 2000*.

[9] Prescribed experts, agencies and authorities include GTTAC, State and Territory Governments, relevant local governments, Australian government agencies and the Minister for the Environment.

Submission	Summary of issues raised	Comment
	• Is this vaccine going to be available in our region? • It is recommended that extensive community consultation be undertaken to adequately communicate the risk and benefits of the GM vaccine.	Public consultation will be conducted for input on the draft RARMP. Consideration of benefits is outside the scope of the *Gene Technology Act 2000*, which considers risks to the health and safety of people and the environment.
5	Lacks specialist scientific knowledge in this field but appreciates being informed about the application.	Noted.
6	Recommends that the following information be including in the RARMP:	
	It is expected the environmental risk of exposure of non-target animals to the GM virus from the vaccinees or mosquito vector will be negligible. However, the following factors that impact the likelihood of transmission should be included in the RARMP: • Shedding of the GMOs by humans • Survival and persistence of the GMOs • Presence of vectors and animal hosts in Australia.	Shedding of the GMOs is discussed in Chapter 1, Section 4.3.5. Environment stability and decontamination of the GMOs is discussed in Chapter 1, Section 4.3.7. The presence of animal hosts in Australia is discussed in Chapter 1, Section 3.7.1 and Chapter 2, Section 2.3, and the presence of vectors in Chapter 1, Section 3.7.6.
	It is expected that the risk of harm to non-target species will be negligible due to the narrow host range and the attenuated nature of the virus. However, the following factors should be included in the RARMP when considering potential consequences: • The risk of reversion to virulence due to the genetic instability of attenuation mutations • The risk of recombination with wild-type viruses (in the vector or host), other vaccine viruses in vaccinees, or other flaviviruses carried by the mosquito to produce recombinants with altered or increased replication ability, shedding, pathogenicity, and host range.	The potential for reversion and recombination has been discussed in Chapter 2, Section 2.2.
7	At this stage, does not have specific advice on risks to the health and safety of people and the environment to be considered in the development of the consultation RARMP.	Noted.

Submission	Summary of issues raised	Comment
8	The evaluation should consider: • Risks to animals, including livestock • Potential for the vaccine to be shed by people • Risks of exposure to people, including priority groups, people handling or administering the vaccine, transmission to mosquito populations and animal reservoirs, potential environmental contamination such as from household shedding and reversion of the genetically modified components • Long term monitoring and re-evaluation based on best available emerging evidence. Dengue is a significant public health concern and notes that Qdenga has been approved in several regions internationally.	Potential harms to animals are discussed in Chapter 2, Section 2.3. Shedding of the GMOs is discussed in Chapter 1, Section 4.3.5 and Chapter 2, Section 2.2. Potential routes of accidental exposure to the GMOs and the potential for reversion are discussed in Chapter 2, Section 2.2. It is noted that the genetic modifications do not relate to the attenuated phenotype of the GMOs. Licence conditions drafted in the consultation RARMP ensure that there is ongoing oversight of the release.