Environmental Protection Authority
Te Mana Rauhī Taiao

Staff Assessment Report

Application APP202854: To import and release a genetically modified conditionally replicative oncolytic adenovirus for use in a Phase 2 clinical trial to examine safety and efficacy in patients with stage IIIa and IV melanoma

December 2017

Application number:	APP202854
Purpose:	To import for release a genetically modified conditionally replicative adenovirus for use in a Phase 2 clinical trial to examine safety and efficacy in patients with Stage IIIa and IV melanoma
Applicant:	Oncolys Biopharma Inc.
Application Lead:	Tim Strabala, PhD

Executive Summary and Recommendation

On 20 December 2017, Clinical Network Services Pty Ltd lodged an application to import and release a genetically modified conditionally replicative oncolytic adenovirus (Telomelysin) for use in a Phase 2 clinical trial to examine safety and efficacy in patients with stage IIIa to IV inoperable melanomas. The application was made on behalf of Oncolys Biopharma Inc (the applicant), pursuant to section 34 of the Hazardous Substances and New Organisms (HSNO) Act 1996 (the "HSNO Act").

Section 38I of the HSNO Act provides for a rapid assessment of applications received under section 34, if the application seeks the release of a qualifying organism. A qualifying organism is, in part, a new organism (including a genetically modified organism) that is a medicine or is contained in a medicine.

Based on the information and references in the application and other readily available sources, we found that it is highly improbable that the dose and route of administration of Telomelysin will have significant adverse effects on the health of the public or any valued species. Moreover, we found that it is highly improbable that Telomelysin could form a self-sustaining population and have significant adverse effects on the health of the public, any valued species, natural habitats, or the environment.

Therefore, we recommend that the application be approved subject to the proposed controls.

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1. Introduction

Purpose of this document

1.1. This document has been prepared by EPA staff in the New Organisms team to advise the decision-maker in considering APP202854, the release of Telomelysin with controls for the purpose of a Phase 2 clinical trial study under s38I of the Hazardous Substances and New Organisms Act (1996) (the Act). This document discusses information provided in the application as well as information from other readily available sources.

Application summary

1.2. On 20 December 2017, Clinical Network Services Pty Ltd lodged an application under section 34 of the HSNO Act (on behalf of Oncolys Biopharma Inc, the applicant) seeking approval to import and release a genetically modified conditionally replicative oncolytic adenovirus (Telomelysin; also known as OBP-301). The applicant intends to use Telomelysin in a Phase 2 clinical trial to examine its safety and efficacy as a therapeutic oncolytic vaccine immunotherapy in patients with unresectable[1] Stage IIIa to IV melanoma (ie, metastasised[2] melanoma).

1.3. The applicant intends to conduct the Phase 2 clinical trial at four to five clinical trial sites in New Zealand. The areas where Telomelysin will be used include public hospitals and associated facilities (such as pharmacies and laboratories) where Telomelysin will be transported, stored and prepared for intratumoural injection into patients with metastasised melanoma tumours. These sites will include laboratories where specimens from treated patients will be analysed to monitor patient safety during the clinical study.

1.4. Telomelysin-treated patients will be allowed to return to their homes after Telomelysin is intratumourally administered.

[1]An “unresectable tumour” is defined as one that cannot be removed through surgery. (https://www.cancer.gov/publications/dictionaries/cancer-terms?cdrid=45936)

[2]“Metastasis” is defined as the spread of cancer cells from the place where they first formed to another part of the body. The metastatic tumour is the same type of cancer as the primary tumour. (https://www.cancer.gov/publications/dictionaries/cancer-terms?cdrid=46710)

2. The organism: Telomelysin

2.1. The organism Telomelysin (also known as OBP-301) is a genetically modified conditionally replicative oncolytic Human adenovirus C, serotype 5 (HAdV-C, serotype 5).

Adenoviruses and the viral vectors derived from them

Taxonomy of the adenovirus family

2.2. Adenoviruses are classified as members of the family Adenoviridae. The Adenoviridae are subclassified into five genera, *Atadenenovirus, Siadenovirus, Aviadenovirus, Ichtadenovirus,* and *Mastadenovirus,* which infect terrestrial vertebrates (ExPASy 2015a, b), birds (ExPASy 2015c), fish (ExPASy 2015d), and mammals, including humans (ExPASy 2015e). The taxonomy of this family was originally operationally based, depending on whether a particular virus infected birds or mammals (ExPASy 2015a). However, researchers found a greater degree of similarity at the DNA sequence level between a frog adenovirus and a turkey adenovirus than the turkey adenovirus had to other bird adenoviruses (Anonymous 2002). Therefore, the taxonomy of the Adenoviridae is now based on DNA sequence (Fig. 1), which reveals that there are distinct differences between each of the genera in their structures and genes they comprise (Harrach 2009a). The *Mastadenovirus* genus includes all human adenoviruses.

2.3. Human adenoviruses are classified into seven species, called Human Adenovirus (HAdV) A, B, C, D, E, F, and G (Fig. 1). Each of these species contains three or more serotypes. Human Adenovirus C is the type species of the genus, which comprises four serotypes, Ad1, 2, 5 and 6 (Fig. 1). Many viral vectors, including Telomelysin, are derived from Ad5.

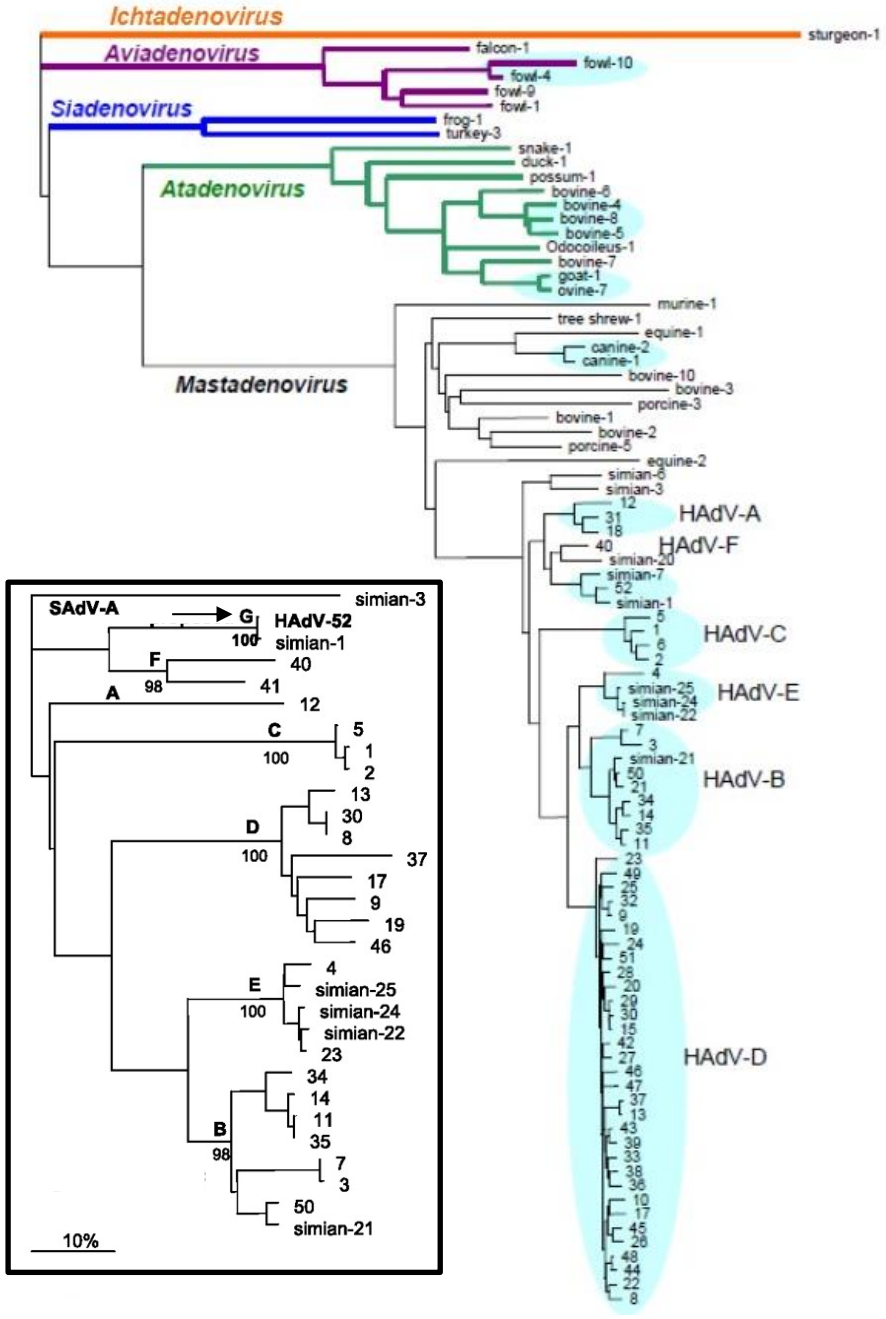


Figure1: Phylogenetic tree of the Adenoviridae family (adapted from Harrach 2009a). The human adenoviruses are denoted as HAdV-A through F. Adenoviruses that belong to the same species are grouped by the *light blue ovals*. Human adenovirus type 5 (from which Telomelysin was derived) is a member of the HAdV-C species. Inset: Phylogenetic tree of the Human Mastadenoviruses (adapted from Harrach 2009b), reflecting the later identification and naming of Human Mastadenovirus G (*arrow*).

Adenovirus structure, function and life cycle

2.4. The adenovirus capsid is an icosahedral (20-sided) particle composed primarily of three major proteins (Douglas 2007; Warnock et al, 2011): the trimeric hexon protein, which makes up the bulk of the viral capsid, the penton protein, found at the base of each vertex of the icosahedron, and the trimeric fibre protein, which extends from each penton protein and terminates in a globular fibre knob domain (Fig. 2).

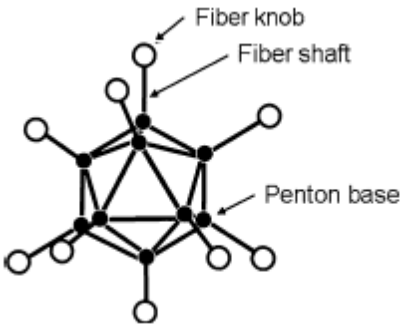


Figure 2: Schematic diagram of an adenovirus capsid. (Excerpted from Douglas 2007).

2.5. The globular fibre knob and penton base domains of the adenoviral particle are responsible for recognising and binding to specific receptors on the host cell, in a two-stage attachment and entry infection process (Zhang & Bergelson 2005, Fig. 3).

2.6. The different HAdV species have different mechanisms of binding to human cells. Focusing on serotype Ad5 (since this is the serotype from which the genetically modified Ad5 Telomelysin is derived), the cellular attachment receptor is the Coxsackievirus-Adenovirus Receptor (CAR), an immunoglobulin superfamily protein. The normal role of the CAR protein is to mediate cell adhesion. Ad5 is also known to bind heparin sulphate glycosaminoglycans, a feature shared with serotype Ad2. Both Ad5 and Ad2 normally cause respiratory infections (Zhang & Bergelson 2005).

2.7. Attachment to the cell brings a specific arginine-glycine-aspartic acid (RGD) domain in the Adenovirus penton base into close proximity to the cell the cell adhesion protein, integrin. The RGD sequence is a specific recognition sequence for the integrin protein. The RGD-integrin interaction triggers a process known as receptor-mediated endocytosis, in which the cell membrane encloses the viral particle in a membranous structure known as a vesicle (Fig. 3).

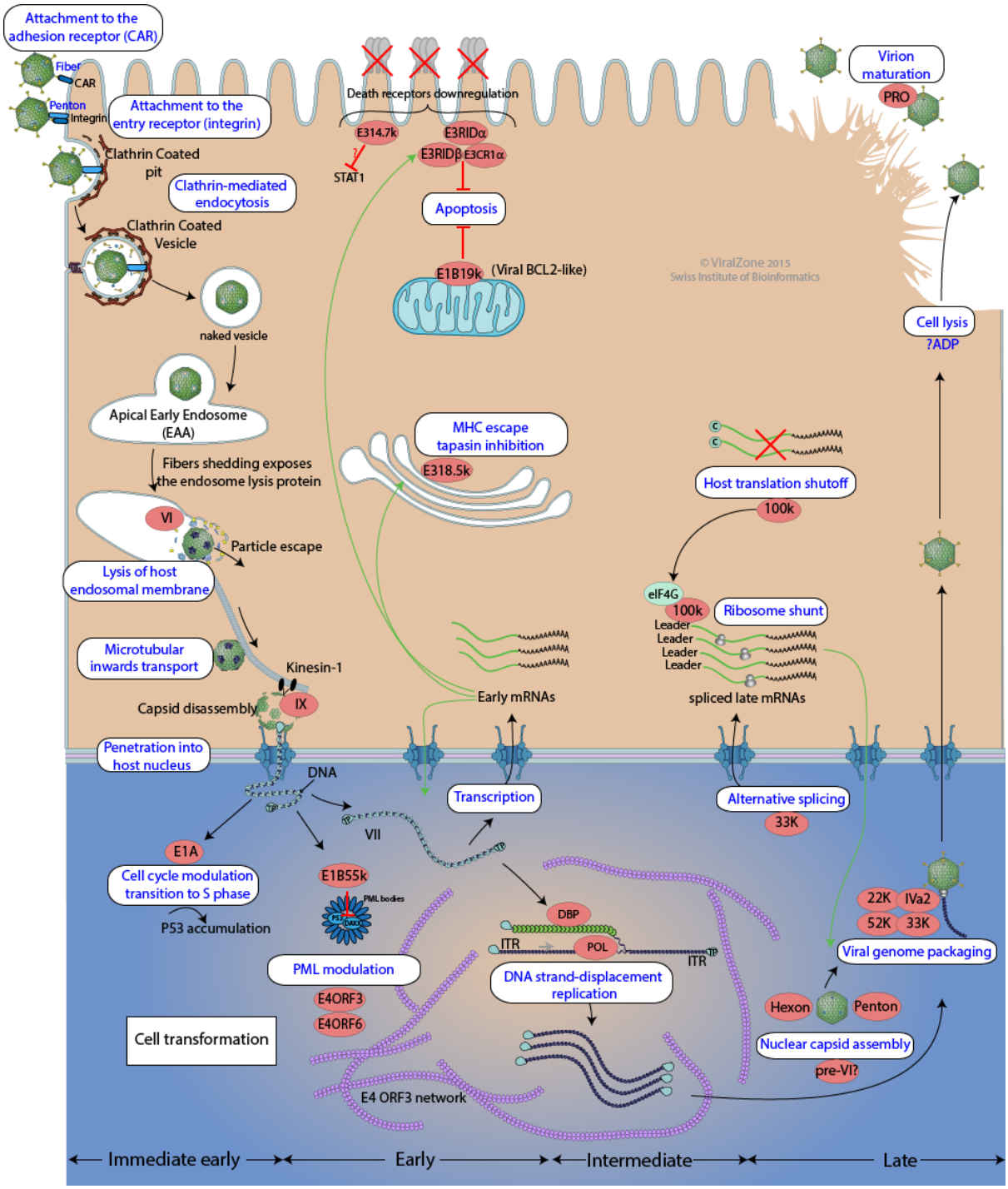


Figure 3: Human Adenovirus C infection cycle (ExPASy 2015f). Infection cycle begins at the top left of the figure with the binding of the CAR receptor and ends at the top right of the figure with cell lysis.

2.8. After completion of endocytosis, the vesicle is broken down, releasing the virus particle into the cytoplasm. The virus then binds to the host cell nuclear membrane, where it releases the viral DNA into the nucleus (Fig. 3).

2.9. Once the adenoviral genome is delivered into the nucleus, a suite of genes, known collectively as the adenoviral “early” genes are activated (Warnock et al, 2011). These are divided into four major transcriptional units, specifically E1, E2, E3, and E4, from which the viral early proteins are derived. Table 1 describes in broad terms the functions of the proteins derived from each of these transcription units.

Table 1. Early transcribed genes in adenoviral infection and their functions (Adapted from Warnock et al, 2011)

Transcription unit	Function
E1A	Activates early-phase transcription and induces the S (nuclear DNA replication) phase of the host cell
E1B	Encodes the E1B 19K and E1B 55K proteins, which inhibit apoptosis (programmed cell death – a common defensive response to viral infection) and thus allow viral DNA replication
E2	Encodes DNA polymerase preterminal protein and DNA-binding protein, required for viral replication
E3	Encodes proteins that block natural cellular responses to viral infection
E4	Encodes a variety of proteins that function in DNA replication, messenger RNA transport and splicing – allowing entry into the intermediate phase of viral DNA replication

2.10. The E1A large protein (one of three proteins produced from alternative splicing of the E1A transcript) is of critical importance in the infection process. E1A large protein is a non-sequence-specific transcriptional activator (Liu & Green 1994) that is responsible for boosting transcriptional activity from all the Adenovirus early gene promoters, including E1B, E2, E3 and E4 (Berk 1986). After E1A transcription initiates, the temporal sequence of gene activation is: E4, E3, E1B and E2 (Nevins 1987). E1A activates its own promoter, further amplifying transcription. Despite being called “early” genes, E1A and E1B transcription continues until very late in the infection process, and E3 and E4 transcription gradually declines through the infection process. Only E4 transcription is limited to the early phase of transcription, when it is shut off by an E2 transcript gene product (Nevins 1987). E2 gene expression allows viral DNA replication to commence, marking the intermediate stage of infection (Fig. 3).

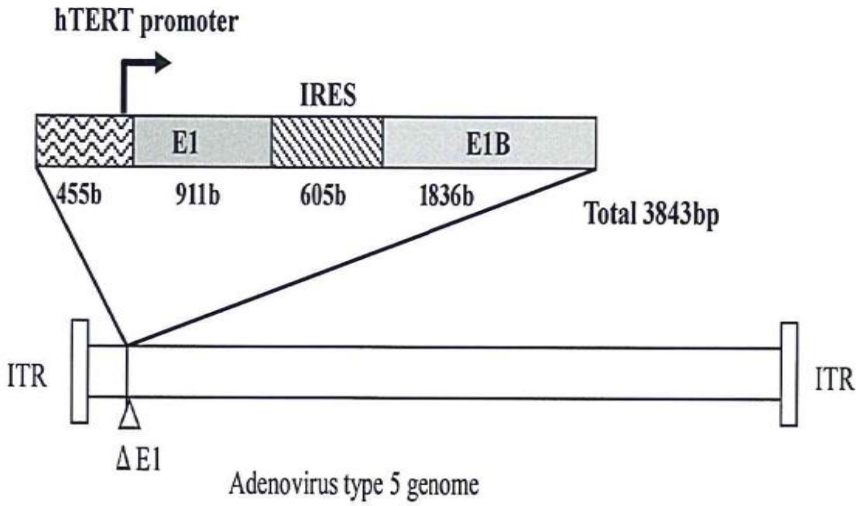
2.11. The amplification of the viral DNA templates allows the transcription of a large number of copies of long (20 kilobase pairs) mRNAs from the major late promoter, which marks the late phase of infection. Translation of the host cell mRNAs is suppressed, and the viral late mRNAs enter into a number of different alternative splicing pathways to allow the translation of five proteins that comprise either viral capsid proteins or are involved in ensuring proper assembly of new virus particles (Warnock et al, 2011). The infection cycle ends with the lysis and death of the host cell and the escape of new virus particles that can infect new cells (Fig. 3).

The genetic modifications used to create Telomelysin

2.12. Telomelysin (also known as OBP-301) is a conditionally replicative oncolytic HAdV-C serotype Ad5 that contains two genetic modifications designed to take advantage of Adenovirus’s cell-killing ability by redirecting it to be specific to cancer cells (Fig. 4). The first is a substitution of the adenoviral E1A promoter with a 467 base pair fragment (Oncolys 2006) containing the promoter of the human telomere reverse transcriptase (hTERT) gene. The second, an insertion of a 656 base pair internal ribosome entry sequence (IRES) from the commercially available

pIRES vector (Oncolys 2006), originally from the encephalomyocarditis virus (EMCV; Bochkov & Palmenberg 2006), between the E1A and E1B genes. An important feature of Telomelysin is that there are no protein-coding genes that have been removed or inserted relative to wild-type Ad5. Instead, its genetic modifications solely affect the regulation of the transcription and translation of the E1A and E1B genes, which make the modified virus more specific for replication in tumour cells, as described in more detail below (See heading: “Capacity of Telomelysin to replicate within cancer cells over normal cells.”)

Figure 4. The genetic modifications of Telomelysin (Excerpted from the APP202854 application form). The Adenovirus Type 5 virus was genetically modified by the deletion of the wild-type E1 transcriptional unit (Table 1) and replacing it with a 3843 base pair insertion comprising a new E1 transcriptional unit in which the native adenovirus E1 transcriptional promoter is replaced by the human telomerase reverse transcriptase (*hTERT*) promoter, and an internal ribosome entry sequence (*IRES*) is inserted between the E1A and E1B genes.



Abbreviation: *ITR,* Inverted Terminal Repeat sequence, required for recognition of the viral DNA for packaging into new virus particles.

Intended use of Telomelysin

2.13. The applicant states that they intend to administer Telomelysin intratumourally to patients with non-resectable Stage IIIa to IV melanoma (inoperable metastasised skin cancer) who are enrolled in a Phase 2 clinical trial. Melanoma patients received single-dose intratumoural injections of Telomelysin in a previous clinical trial, with some evidence of efficacy in reducing the size of metastatic melanoma lesions (Nemunaitis et al, 2010).

2.14. Control 1 is proposed to limit the use of Telomelysin to its intratumoural administration to patients enrolled in a Phase 2 clinical trial for patients with non-resectable Stage IIIa and IV melanoma (see paragraph 6.1). That means that Telomelysin cannot be used for any other purpose without a further HSNO Act approval.

2.15. In accordance with proposed Control 2 (see paragraph 6.1), EPA and MPI will be notified of the location of the clinical trial sites.

Potential benefits from Telomelysin use

2.16. In 2014 (the most recent year for which the Ministry of Health has published data, the incidence of melanoma in New Zealand was 2289 new cases (Torre et al, 2015). The average incidence in the years 2012-2014 was 2326 new cases per year. The disease is approximately 25% more prevalent among men than women; and non-Māori have about seven and one half times the rate of melanoma than Māori overall (Torre et al, 2015). Melanoma is responsible for approximately 300 deaths per year in New Zealand, about 1% of the annual total deaths in the country.

2.17. Data from previous clinical trials support Telomelysin as a promising candidate therapy for metastasised melanoma (Nemunaitis et al, 2010). As such, the potential immediate benefits of Telomelysin to the patients recruited to the applicant's Phase 2 clinical trial include tumour shrinkage, alleviation of cancer symptoms and improved quality of life, cancer remission and prolonged survival (section 5 of the application).

2.18. Telomelysin also has the potential to benefit the wider New Zealand community diagnosed with metastasised melanoma, subject to successful progression through the clinical trial process to full regulatory approval and release.

Potential adverse effects from Telomelysin use

2.19. A wide variety of studies have shown that the administration of human adenoviruses (including Ad5, from which Telomelysin is derived) as either vaccines or therapies is generally safe (reviewed in Lichtenstein & Wold 2004; Gray 2013). For example, live, wild-type adenoviruses Ad1, Ad2, Ad4, Ad5, Ad7, and Ad21 have been administered as vaccines against infections caused by these viruses in the form of enteric-coated tablets, which produce asymptomatic infections in the gut that confer immunity to later infection. Such vaccines are widely considered to be very safe, after the vaccinations of hundreds of thousands of people (Lichtenstein & Wold 2004; Gray 2013). Several studies have found that when wild-type Ad5 is administered to patients, the virus is not transmitted to uninfected people by casual contact (Lichtenstein & Wold 2004).

Potential clinical complications of Telomelysin treatment based on the vaccination history of Ad5

2.20. Experimental infections with wild-type Ad5 are usually either asymptomatic, or can induce fever, conjunctivitis, and pharyngitis, depending on the route of administration (Lichtenstein & Wold 2004). Similar to these symptoms, Telomelysin is known to cause fatigue, chills and fever in patients, as well as injection site reactions such as pain and injection site erythema (Nemunaitis et al, 2010). Importantly, as stated in section 3.1 of the application, and echoed in comments on the application from Medsafe (see Appendix 2), adenovirus can cause significant clinical complications, particularly in immunocompromised people. The applicant has stated that immunocompromised people (as well as children, who have a lower likelihood of previous exposure to Ad5) will be excluded from the trial.

2.21. Human Adenovirus is a Risk Group 2 microorganism[3], meaning it is unlikely to be a serious hazard to laboratory personnel, the community, animals, or the environment, and it presents a limited risk of spreading beyond the patient receiving the treatment. Further, there are preventive measures with respect to any infections that it could potentially cause (outlined below; see paragraph 6.1).

Capacity of Telomelysin to replicate within cancer cells over normal cells

2.22. As immortalised cells, cancer cells have several key differences from the normal cells. One such difference is the collective changes in gene expression that prevent the shortening of chromosomal ends, known as telomeres. In normal cells, telomeres have a certain length, which decreases after each round of cell division. Thus, a primary function of telomeres is to delay chromosome end-shortening induced gene degradation for many rounds of cell division. Telomeres also protect chromosomes from end-to-end fusion and recombination (Ramlee et al, 2016).

2.23. Telomeres are maintained by an enzyme called telomerase, which has two components: a reverse transcriptase subunit (hTERT) and an RNA subunit (hTR). In most slowly or non-dividing somatic cells, hTERT expression is strongly downregulated, and such cells eventually senesce as telomeres gradually shorten through each round of cell division (Ramlee et al, 2016). Cancer cells often avoid this fate by upregulating hTERT gene expression, thus maintaining telomere length, as part of the carcinogenesis process, and as such, 85 to 90 percent of cancers express hTERT to high levels (Hiyama et al, 2001).

2.24. The substitution of the native adenovirus E1 promoter with the hTERT promoter makes Telomelysin more specific for cancer cells by decreasing the ability of Telomelysin to replicate within cells that lack the necessary transcription factors to bind to the hTERT promoter that allow Telomelysin to replicate. Most normal somatic cells do not express these transcription factors, whereas telomerase activity is detected in 85-90% of human cancer specimens (Hiyama et al, 2001). The use of the hTERT promoter further exploits the fact that the expression of the adenoviral late genes in the course of the adenoviral life cycle is completely dependent on the successful expression of the early phase genes and the completion of the early phase of infection. Therefore, even if Telomelysin infects a cell that is not expressing human telomerase (ie, a differentiated normal somatic cell), it generally cannot produce new copies of itself in such a cell. This characteristic, shared by Telomelysin with many other engineered oncolytic viruses, has been described in many peer-reviewed publications as ‘selective replication’ or ‘conditional replication’ within cancer cells (Alemany et al, 2000; Khuri et al, 2000; Suzuki et al, 2002; Kawashima et al, 2004; Taki et al, 2005; Nemunaitis et al, 2010). The ability to replicate is critical to Telomelysin’s cancer cell-killing function because, like

[3]As Risk Group 2 is defined in the Australian/New Zealand Standard *Safety in laboratories. Part 3: Microbiological aspects and containment facilities* (AS/NZS 2243.3.2002).

adenovirus, Telomelysin causes replication-dependent cell lysis, which destroys the cell. Thus, the inability of Telomelysin to make more copies of itself within a non-cancerous somatic cell also means that it cannot kill that cell.

2.25. Although the use of the hTERT promoter renders Telomelysin selectively replicative for most cancer cells, there are also non-cancerous cell types in the human body that express telomerase (and thus have active transcription driven by the hTERT promoter). These cells are usually epithelial stem cells, such as gut crypt epithelial cells or epidermal stem cells, or reproductive stem cells, particularly spermatocytes (Hiyama et al, 2001). In theory, such cells could also be targeted by Telomelysin. Despite this possibility, neither viral replication nor gut toxicity have been reported in normal colorectal tissue or in testes following Telomelysin treatment. Although such cells are proliferative, as stem cells, they only divide occasionally, unlike rapidly dividing cancer cells (Shay et al, 2001). Further decoupling of the amplification of viral replication through the use of an IRES sequence instead of an E1A-responsive promoter (as described in the next paragraph) likely keeps viral replication to a minimum in such cells.

2.26. In addition to the hTERT promoter, the insertion of the EMCV IRES sequence adds further selectivity of replication of Telomelysin to cancer cells. This specificity is brought about because it attenuates the effect of the E1A protein on E1B transcription, by eliminating the E1A-activated E1B promoter. Instead, E1B is co-transcribed with E1A on the same cistron (mRNA) and E1B protein is produced from the bicistronic mRNA by ribosome recruitment to the IRES instead of the usual 7-methylguanosine cap. This modification has been demonstrated to provide up to 100-fold decreased viral yields in non-cancer cells relative to control oncolytic adenovirus vectors that use the native E1B promoter instead of the IRES (Li et al, 2001).

2.27. Epidermal (skin) stem cells are cells that express telomerase, and not surprisingly, based on the biological properties of Telomelysin, some redness and induration has been reported by patients at the site of injection following Telomelysin treatment (Nemunaitis et al, 2010).

Capacity of Telomelysin to spread from treated patients

2.28. Telomelysin transmission from treated patients to untreated people has not been reported (see section 3.1 of the application), meaning Telomelysin transmission is currently only a theoretical concern.

2.29. Adenovirus is very stable in the environment and is resistant to many chemical treatments (refs. and section 3.1 of the application), yet many studies have demonstrated a low rate of transmission of wild-type adenovirus from infected/vaccinated people to uninfected/unvaccinated people (Lichtenstein & Wold 2004; Gray 2013). Telomelysin has been found in the sputum of clinical trial patients, but not urine or saliva (Section 3.1 of the application, Nemunaitis et al, 2010).

2.30. We note that Telomelysin-treated patients will be permitted to leave the clinical setting following Telomelysin administration, and may be required to cover injection sites in non-clinical settings (their homes). Therefore, we propose Control 3 and Control 4.

2.31. Control 3 requires that all patients who are treated with Telomelysin be educated about the potential for Telomelysin transmission, and instructed on avoiding contact with immunocompromised persons.

2.32. Control 4 requires all patients who are treated with Telomelysin to be provided with a biohazard container for disposal of used injection site dressings (if any) at home. These containers must be returned to the clinical trial site for medical waste disposal. This will provide for the appropriate disposal of dressings.

2.33. In light of the data from other unattenuated adenovirus vaccine studies, we consider that Telomelysin transmission from a treated patient to an untreated person is highly unlikely. If transmission did occur, the level of exposure is predicted to be low compared to the doses received by Telomelysin-treated patients. In the highly unlikely event of a Telomelysin infection resulting from the exposure of an untreated person to a Telomelysin-treated patient, the exposed individual would most likely be asymptomatic rather than experience a significant adverse reaction. This is because Telomelysin replication is significantly impaired or non-existent in healthy tissue, and most people have exposure (and thus antibodies) to the Telomelysin "parent" serotype (Ad5) before the age of two (Gray 2013).

2.34. Therefore, we consider that the likelihood of an untreated person being infected with Telomelysin and developing a significant adverse reaction is *highly improbable*.

Ability of Telomelysin to infect and replicate in animal hosts

2.35. In the unlikely event of Telomelysin transmission from a treated patient to a contacted animal, Telomelysin is not expected to replicate within the healthy tissue of the animal because Telomelysin replication primarily exhibits selectivity for tumours. Additionally, adenoviruses are known to be specific for their hosts, and the only other species that human adenoviruses are known to be capable of infecting are simian species (Fig. 3). The HAdV-C species is entirely specific for humans under natural circumstances. The cotton rat (*Sigmodon hispidus*) is used as an animal model for Ad5 studies (Prince et al, 1993), but high viral doses are required for a productive infection and viral replication. Other animals, such as rabbits, pigs and mice have been found to be non-ideal models for adenovirus infection for various reasons, including poor viral replication (Wold & Toth 2012). The Syrian hamster (*Mesocricetus auratus*) is the current preferred HAdV model species (Wold & Toth 2012), but this species is not present in New Zealand.

2.36. Therefore, we consider that the likelihood of any animal species being infected with conditionally replicative Telomelysin and developing a significant adverse reaction is *highly improbable*.

Genetic stability of Telomelysin and its capacity to recombine with other adenoviruses

2.37. Telomelysin could theoretically revert its genome sequence back to that of the unmodified adenovirus (ie, HAdV-C serotype 5) by recombining with a wild-type HAdV-C serotype 5 virus. However, to EPA's knowledge, spontaneous revertants have not been reported.

2.38. Horizontal gene transfer (HGT) is the stable transfer of genetic material from one organism to another without reproduction or human intervention. For viruses, HGT can occur via genetic recombination between replicating viruses within a co-infected cell (Keese 2008) – such events have the potential to generate a novel virus with altered pathogenic properties.

2.39. Intraspecies genetic recombination between HAdV-C serotypes is thought to be prevalent in an evolutionary sense, based on phylogenetic analyses of HAdV-C isolates, but interspecies recombination is thought to be rare (Lukashev et al, 2008). One report of an interspecies recombination with simian adenovirus in the evolution of Ad4 (HAdV-D) was found (Dehghan et al, 2013), which likely explains its close phylogenetic relationship to other simian adenoviruses (Fig. 1). However, EPA did not identify any examples of adenovirus recombinants having arisen under natural conditions (ie, no reports of direct recombination between adenovirus serotypes in the case of a co-infection).

2.40. The likelihood that administered Telomelysin (or shed Telomelysin) will recombine with other adenoviruses within treated persons (or within infected untreated people or animals) and yield recombinant adenoviruses of concern (ie, an undesirable self-sustaining population), is unlikely given that:

- In the 25 year history of vaccination against adenovirus in the US military using unattenuated Ad4 and Ad7, there has never been a verifiable report that such vaccines have caused disease, let alone spread amongst unvaccinated people. On the contrary, the resurgence of Ad4 and Ad7 infections after discontinuation of the vaccination programme has prompted the US military to resume it (Gray 2013).
- If a Telomelysin viral recombinant was generated within a treated patient, the recombined virus would likely remain within the individual, because transmissibility of adenovirus from casual contact is known to be low (Lichtenstein & Wold 2004). Telomelysin transmission from a treated patient has never been reported (and is only a theoretical concern based on the transmissibility of the unmodified host).
- If an untreated person or animal infected with an adenovirus were exposed and subsequently infected with Telomelysin, genetic recombination could only occur in the short period where any adenoviruses were viraemic (approximately 24 hours; Nemunaitis et al, 2010), which would be rendered even more unlikely by the fact that the viruses are encapsidated while viraemic, or when the replicating adenoviruses co-infect a cancerous cell (Telomelysin replication is highly impaired or non-existent in normal cells).

2.41. Therefore, we consider the likelihood of a virulent recombinant Telomelysin virus forming is *highly improbable*.

3. Information from other agencies

3.1. The Department of Conservation (DOC), the Ministry for Primary Industries (MPI) and Medsafe (responsible for the regulation of Medicines under the Medicines Act 1981) were invited to comment on the application. Replies were received from DOC and Medsafe. MPI did not comment.

3.2. DOC noted that they did not consider that Telomelysin would establish an undesirable self-sustaining population, nor did they expect any adverse environmental effects from its release (See Appendix 1).

3.3. Medsafe provided a detailed response to the request for information. Much of the information Medsafe provided was in reference to the effects on the patient, which is not the subject of this assessment, because s38I of the HSNO Act proscribes EPA from considering any effects on the patient. However, much of this information can also be applied to people with whom the patient is likely to come into contact. Thus, any such information is noted in this context in this section.

3.4. Medsafe noted that Ad5 is globally ubiquitous in the environment, and that it causes a mild respiratory infection, to which many people have likely developed antibodies. Thus, exposure to Ad5 is unlikely to result in infection/illness in most healthy people, and the genetic modifications in Telomelysin that make it conditionally replicative in cancer cells make infection in an exposed person highly improbable. However, Medsafe also noted that immunocompromised people generally do not experience mild symptoms when infected with Ad5, and currently available antiviral treatments are limited in their effect on Ad5.

3.5. Medsafe also noted that wild-type Ad5 infection continues asymptomatically after the initial infection, and Telomelysin could be shed by the patient for several months after infection. They further noted that the virus is very stable in the environment. Coupled with the potential for shedding, they recommended that patients should be educated in infection control and avoid contact with immunosuppressed people.

3.6. Finally, Medsafe noted that Telomelysin could also do damage to other cells with active telomerase, ie, germ cells, and somatic stem cells that express the hTERT gene. However, they did not consider that the potential risk was any higher than other current chemical anti-cancer treatments, and that, like other treatments, hyperuricaemia/tumour lysis syndrome[4] was also a possibility. As these side effects are essentially patient-specific due to the intratumoural route of

[4]Tumour lysis syndrome is a potentially life-threatening condition that can be caused by the simultaneous deaths of a large number of tumour cells, thereby releasing their contents into the bloodstream.

injection of Telomelysin, we did not consider these points (particularly tumour lysis syndrome) further.

4. Statutory criteria to be considered

4.1. Section 38I of the HSNO Act provides for the rapid assessment of applications that seek to release qualifying organisms. A qualifying organism is, in part, a new organism that is or is contained in a medicine (as defined in section 3 of the Medicines Act 1981).

4.2. Telomelysin is a medicine as it is "*for administering to 1 or more human beings for a therapeutic purpose*" (in accordance with section 3 of the Medicines Act 1981).

4.3. In order to be approved for release as a qualifying organism, section 38I(3) of the HSNO Act requires that the decision-maker be satisfied that, taking into account all the controls that will be imposed (if any), it is highly improbable that:

(a) the dose and routes of administration of the medicine would have significant adverse effects on-

(i) the health of the public; or

(ii) any valued species; and

(b) the qualifying organism could form an undesirable self-sustaining population and would have significant adverse effects on-

(i) the health and safety of the public; or

(ii) any valued species; or

(iii) natural habitats; or

(iv) the environment.

4.4. In doing so, the effects of the medicine or qualifying organism in the person who is being treated with the medicine are not to be taken into account as per section 38I(4) of the HSNO Act.

4.5. In the first instance, we have assessed the organism against these criteria. This assessment is set out in the following section of this report.

4.6. If the organism does not meet these criteria, the applicant may request that the application be considered under section 38, or section 38A.

5. Assessment of the risk of the conditional release of Telomelysin (OBP-301) against statutory criteria

5.1. It is highly improbable that the dose and intratumoural administration of Telomelysin to patients with non-resectable Stage IIIa to IV melanoma will have significant adverse effects on the health of the public or any valued species, given that:

- Telomelysin transmission from treated patients to untreated people or animals has not been reported, and transmission of unmodified strains of adenovirus from vaccinated patients to unvaccinated people or animals is rare;

- In the highly unlikely event of a Telomelysin infection resulting from the exposure of an untreated person or animal to a Telomelysin-treated patient, an exposed person or animal would most likely clear any conditionally replicative Telomelysin rather than experience an adverse reaction because Telomelysin replication is significantly impaired or non-existent in healthy tissue. Furthermore, Ad5 infection is generally mild or asymptomatic in healthy people.

5.2. Moreover, it is highly improbable that Telomelysin could form an undesirable self-sustaining population that would have significant adverse effects on the health and safety of the public, any valued species, natural habitats or the environment, given that Telomelysin replication is significantly impaired (attenuated) or non-existent in healthy tissue due to the use of the hTERT promoter to drive E1A expression, and further attenuated by the use of the ECMV IRES sequence for E1B gene translation.

5.3. Based on the intrinsic properties of adenovirus and the specific properties of conditionally replicative Telomelysin described and discussed above, we consider that it is highly improbable that Telomelysin will have any significant adverse effects on the health and safety of the public, any valued species, natural habitats or the environment if Telomelysin is used in a Phase 2 clinical trial for patients with non-resectable Stage IIIa to IV melanoma.

5.4. We acknowledge that all clinical trials in New Zealand are expected to be conducted in accordance with the standards set out in the *Note for Guidance on Good Clinical Practice* (Medsafe 2015); and that all New Zealand clinics and hospitals are expected to follow New Zealand *Health and Disability Services (Infection Prevention and Control) Standards* (NZS 8134.3.3:2008)[5].

6. Recommendation

6.1. We recommend that this application to import and release Telomelysin (OBP-301) be approved subject to the following controls:

Control 1 - The organism (Telomelysin; OBP-301) must only be administered:

- by suitably trained medical practitioners
- to patients who are enrolled in a Phase 2 clinical trial approved under the Medicines Act 1981 to examine the safety and efficacy of Telomelysin in patients with non-resectable Stage IIIa to IV melanoma
- intratumourally
- at a Phase 2 clinical trial site

[5] General practices are expected to operate in accordance with Australian/New Zealand Standard (AS/NZS): Office-based health care facilities - Reprocessing of reusable medical and surgical instruments and equipment, and maintenance of the associated environment (AS/NZS 4815:2006).

Laboratories are expected to adhere to various standards, including AS/NZS 2243.3:2010: Safety in laboratories - Microbiology safety and containment.

Control 2 - The New Zealand sponsor of the Phase 2 clinical trial must notify EPA and MPI (in writing, at least one month prior to its first use) before Telomelysin is administered at the site for the first time, of:

- the location of any clinical trial site
- the qualifications of the suitably trained medical practitioner or practitioners, who will administer the organism

Control 3 – Prior to the treatment with the organism of any patient in the trial, the New Zealand sponsor must ensure that a suitably qualified person or persons:

- educates the patient about the potential for Telomelysin to be transmitted to untreated people, particularly immunocompromised persons
- educates the patient about infection control
- advises the patient to avoid contact with immunocompromised persons
- advises the patient to report any adverse effects that they suspect to be related to Telomelysin transmission

Control 4 – Prior to the treatment with the organism of any patient in the trial, the New Zealand sponsor must ensure that a suitably qualified person or persons:

- provides the patient with a biohazard container for any bandages, plasters or other dressings applied to the site of injection of the organism
- instructs the patient to return such containers to the Phase 2 clinical trial site for disposal as medical waste
- disposes of such containers in accordance with medical waste disposal procedures established at the Phase 2 clinical trial site

Control 5 – The New Zealand sponsor must:

- ensure, prior to the commencement of the Phase 2 clinical trial, that protocols and suitably qualified persons are available to investigate reports of adverse effects suspected to be related to Telomelysin transmission from treated patients
- ensure that reports of adverse effects that are reasonably suspected to be related to Telomelysin transmission are investigated to confirm whether or not transmission has occurred, and whether or not adverse effects have resulted from confirmed transmission
- notify EPA and MPI, in writing, of any occurrence of Telomelysin-induced adverse effect resulting from the confirmed transmission of Telomelysin from treated patients to untreated people as soon as is practicable.

Control 6 – The New Zealand sponsor must write a report to EPA and MPI, in which they describe compliance with these controls, initially to be submitted six months after the

commencement of the Phase 2 clinical trial, and then to be submitted on or before 30 June every year thereafter until the conclusion of the Phase 2 clinical trial.

6.2. EPA, Clinical Network Services Pty Ltd, and Oncolys Biopharma Inc recognise that an approval granted under section 38I of the HSNO Act is not an approval to use a qualifying medicine until the medicine has been approved for use under the Medicines Act 1981.

7. References

Alemany R, Balague C, Curiel DT 2000. Replicative adenoviruses for cancer therapy. Nature Biotechnology 18(7): 723-727.

Anonymous 2002. Proposals relating to a third genus in the Adenoviridae. In: ICTV ed., International Committee on Taxonomy of Viruses.

Berk AJ 1986. Adenovirus promoters and E1A transactivation. Annual Review of Genetics 20: 45-79.

Bochkov YA, Palmenberg AC 2006. Translational efficiency of EMCV IRES in bicistronic vectors is dependent upon IRES sequence and gene location. BioTechniques 41(3): 283-292.

Dehghan S, Seto J, Liu EB, Walsh MP, Dyer DW, Chodosh J, Seto D 2013. Computational analysis of four human adenovirus type 4 genomes reveals molecular evolution through two interspecies recombination events. Virology 443(2): 197-207.

Douglas JT 2007. Adenoviral vectors for gene therapy. Molecular Biotechnology 36(1): 71-80.

ExPASy, Swiss Institute of Bioinformatics, 2015a. Atadenovirus. Retrieved 30 November 2015. http://viralzone.expasy.org/all_by_species/531.html

ExPASy, Swiss Institute of Bioinformatics, 2015b. Siadenovirus. Retrieved 30 November 2015. http://viralzone.expasy.org/all_by_species/532.html

ExPASy, Swiss Institute of Bioinformatics, 2015c. Aviadenovirus. Retrieved 30 November 2015. http://viralzone.expasy.org/all_by_species/184.html

ExPASy, Swiss Institute of Bioinformatics, 2015d. Ichtadenovirus. Retrieved 30 November 2015. http://viralzone.expasy.org/all_by_species/768.html

ExPASy, Swiss Institute of Bioinformatics, 2015e. Mastadenovirus. Retrieved 30 November 2015. http://viralzone.expasy.org/all_by_species/183.html

ExPASy, Swiss Institute of Bioinformatics, 2015f. Human adenovirus type C replication cycle. Retrieved 21 November 2016. http://viralzone.expasy.org/all_by_species/4378.html

Gray GC 2013. Adenovirus vaccines. In: Plotkin SA, Orenstein WA, Offit PA ed. Vaccines (Sixth Edition). London, W.B. Saunders. Pp. 113-126.

Harrach B 2009a. New genus Ichtadenovirus containing a new species in the family Adenoviridae; a new species in the genus Siadenovirus. In: ICTV ed. 2008.010-017V, International Committee on Taxonomy of Viruses.

Harrach B 2009b. Create species Human adenovirus G in genus Mastadenovirus and species Falcon adenovirus A in genus Aviadenovirus, family Adenoviridae. In: ICTV ed. 2008.014,016V, International Committee on Taxonomy of Viruses.

Hiyama E, Hiyama K, Yokoyama T, Shay JW 2001. Immunohistochemical detection of telomerase (hTERT) protein in human cancer tissues and a subset of cells in normal tissues. Neoplasia 3(1): 17-26.

Kawashima T, Kagawa S, Kobayashi N, Shirakiya Y, Umeoka T, Teraishi F, Taki M, Kyo S, Tanaka N, Fujiwara T 2004. Telomerase-specific replication-selective virotherapy for human cancer. Clinical Cancer Research 10(1): 285-292.

Keese P 2008. Risks from GMOs due to horizontal gene transfer. Journal of Environmental Biosafety Research 7: 123-149.

Khuri FR, Nemunaitis J, Ganly I, Arseneau J, Tannock IF, Romel L, Gore M, Ironside J, MacDougall RH, Heise C and others 2000. A controlled trial of intratumoral ONYX-015, a selectively-replicating adenovirus, in combination with cisplatin and 5-fluorouracil in patients with recurrent head and neck cancer. Nature Medicine 6(8): 879-885.

Li Y, Yu D-C, Chen Y, Amin P, Zhang H, Nguyen N, Henderson DR 2001. A hepatocellular carcinoma-specific adenovirus variant, CV890, eliminates distant human liver tumors in combination with doxorubicin. Cancer Research 61(17): 6428-6436.

Lichtenstein DL, Wold WSM 2004. Experimental infections of humans with wild-type adenoviruses and with replication-competent adenovirus vectors: replication, safety, and transmission. Cancer Gene Therapy 11(12): 819-829.

Liu F, Green MR 1994. Promoter targeting by adenovirus E1a through interaction with different cellular DNA-binding domains. Nature 368(6471): 520-525.

Lukashev AN, Ivanova OE, Eremeeva TP, Iggo RD 2008. Evidence of frequent recombination among human adenoviruses. Journal of General Virology 89(2): 380-388.

Medsafe 2015. Guideline on the regulation of therapeutic products in New Zealand. Part 11: clinical trials - regulatory approval and good clinical practice requirements.

Nemunaitis J, Tong AW, Nemunaitis M, Senzer N, Phadke AP, Bedell C, Adams N, Zhang Y-A, Maples PB, Chen S and others 2010. A Phase I study of telomerase-specific replication competent oncolytic adenovirus (Telomelysin) for various solid tumors. Molecular Therapy 18(2): 429-434.

Nevins JR 1987. Regulation of early adenovirus gene expression. Microbiological Reviews 51(4): 419-430.

Oncolys 2006. Supporting Information (Telomelysin IND) supplied to the Food and Drug Administration. In: Administration FD ed. Technical information ed.

Prince GA, Porter DD, Jenson AB, Horswood RL, Chanock RM, Ginsberg HS 1993. Pathogenesis of adenovirus type 5 pneumonia in cotton rats (*Sigmodon hispidus*). Journal of Virology 67(1): 101-111.

Ramlee M, Wang J, Toh W, Li S 2016. Transcription regulation of the human telomerase reverse transcriptase (hTERT) gene. Genes 7(8): 50.

Shay JW, Zou Y, Hiyama E, Wright WE 2001. Telomerase and cancer. Human Molecular Genetics 10(7): 677-685.

Suzuki K, Alemany R, Yamamoto M, Curiel DT 2002. The presence of the adenovirus E3 region improves the oncolytic potency of conditionally replicative adenoviruses. Clinical Cancer Research 8(11): 3348-3359.

Taki M, Kagawa S, Nishizaki M, Mizuguchi H, Hayakawa T, Kyo S, Nagai K, Urata Y, Tanaka N, Fujiwara T 2005. Enhanced oncolysis by a tropism-modified telomerase-specific replication-selective adenoviral agent OBP-405 (`Telomelysin-RGD'). Oncogene 24(19): 3130-3140.

Torre L, Bray F, Siegel R, Ferlay J, Lortet-Tieulent J, Jemal A 2015. Global cancer statistics, 2012. CA: A Cancer Journal for Clinicians 65: 87-108.

Warnock JN, Daigre C, Al-Rubeai M 2011. Introduction to viral vectors. In: Merten O-W, Al-Rubeai M ed. Viral Vectors for Gene Therapy: Methods and Protocols, Springer Science+Business Media. Pp. 1-25.

Wold WSM, Toth K 2012. Syrian hamster as an animal model to study oncolytic adenoviruses and to evaluate the efficacy of antiviral compounds. In: Curiel DT, Fisher PB ed. Advances in Cancer Research, Academic Press. Pp. 69-92.

Zhang Y, Bergelson JM 2005. Adenovirus receptors. Journal of Virology 79(19): 12125-12131.

Appendix 1: Text of Comments from DOC (received via e-mail).

Thank you for the opportunity to provide feedback on this application to import for release the genetically modified oncolytic virus Telomelysin, for the purposes of trialling tumour cell treatment.

Please be advised that the Department agrees that this live-attenuated virus (Telomelysin) is unlikely to be able to form a self-sustaining population in the environment, and we don't expect there to be adverse environmental effects from this release.

Kind regards,

Verity Forbes

Technical Advisor - Biosecurity Threats (National) *Kai-mātanga Matua, Koiora Mōrearea*

Department of Conservation *Te Papa Atawhai*

Appendix 2: Text of Comments from Medsafe (received via e-mail).

I have had one of the team look at the application. Our opinion is that the benefits outweigh the risks and it is fine if the trial goes ahead.
The virus has been engineered to target tumour cells and to lyse them and there is evidence that this is what happens in vivo.

Points for noting:
Human adenovirus 5 is ubiquitous in the environment and causes a respiratory illness. This means that patients may already have antibodies to the virus which may reduce efficacy, however since the virus injected directly into the tumour this may be a minimal problem.

The illness caused by Ad5 is generally mild, but not in immunocompromised patients. Although this is a weakened strain this is still a risk (for example there have been deaths in children given varicella vaccine who were immunocompromised). This risk has been noted by the company but we may wish to get more detail given that many cancer treatments are immunosupressive [*sic*]. It was also not clear if this will be an add on or stand alone treatment which is also important in terms of immunosuppression.

There is no approved treatment for Ad5 infection, some antivirals have been tried but generally with low success.

Ad5 infection appears to continue without symptoms after the initial infection phase. It appears that this is a low level infection rather than a latent infection (like chicken pox coming back as shingles) which may be less of an issue for the patient, but will mean that the potential for viral shedding will continue for many months after treatment. I don't think this is a problem but is something to be aware of.

There doesn't seem to be a risk of autoimmune disease with this virus although it has been linked with the development of COPD, I doubt this is a problem for these patients.

The length of infection may increase the risk of reassortment occurring, but again I think this is a very low risk since this is a DNA virus.

There is a potential for transmission and the virus is very stable in the environment. Therefore patients should be educated in infection control and avoid contact with immunosupressed [*sic*] people.

There is a risk of damage to germ cells and stem cells. Although the virus is injected into the tumour the information provided suggests it still has the ability to access other parts of the body. This is an advantage if the patient has metastases but there is a risk that there could be an affect [*sic*] on normal cells that have active telomerase. This potential risk is no worse than the effect of any other anti-cancer medicine on these cell types, but something to keep an eye on.

As with any effective anti-cancer treatment there is also a risk of hyperuricaemia/ tumour lysis syndrome.

I expect most of these points would be considered by GTAC as well.

Please note that Medsafe has no mandate to audit clinical trials. This is a risk that should be noted, however EPA can do a level of audit.

If you have any further questions, please let me know.

Kind regards

Alison Cossar
Acting Manager
Product Regulation Branch
Medsafe
Protection Regulation and Assurance
Ministry of Health