**Title: An oral bait vaccination approach for the Tasmanian devil facial tumor diseases**

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**Structured abstract**

*Introduction:* The Tasmanian devil (*Sarcophilus harrisii*) is the largest extant carnivorous marsupial. Since 1996, its population has declined by 77% primarily due to a clonal transmissible tumor, known as devil facial tumor (DFT1) disease. In 2014, a second transmissible devil facial tumor (DFT2) was discovered. DFT1 and DFT2 are nearly 100% fatal.

*Areas covered:* We review DFT control approaches and propose a rabies-style oral bait vaccine (OBV) platform for DFTs. This approach has an extensive safety record and was a primary tool in large-scale rabies virus elimination from wild carnivores across diverse landscapes. Like rabies virus, DFTs are transmitted by oral contact, so immunizing the oral cavity and stimulating resident memory cells could be advantageous. Additionally, exposing infected devils that already have tumors to OBVs could serve as an oncolytic virus immunotherapy. The primary challenges may be identifying appropriate DFT-specific antigens and optimization of field delivery methods.

*Expert commentary:* DFT2 is currently found on a peninsula in southern Tasmania, so an OBV that could eliminate DFT2 should be the priority for this vaccine approach. Translation of an OBV approach to control DFTs will be challenging, but the approach is feasible for combatting ongoing and future disease threats.

**Keywords:**

Devil, transmissible tumor, wild immunology, allograft, viral vector, conservation immunology, oral bait vaccine, neoantigen

## 1. Introduction

### *1.1. The Tasmanian devil and transmissible cancers*

The Tasmanian devil (*Sarcophilus harrisii*) is the largest extant carnivorous marsupial. The species became extinct on mainland Australia around 3,000 years ago and is presently found only on the island State of Tasmania [1,2] (**Fig. 1**). Since 1996 the devil population has declined by 77% and is now listed as endangered [3,4]. The precipitous population decline is largely due to the emergence of a clonal, transmissible cancer called devil facial tumor (DFT1) that is usually fatal [5–7].

In 2014, a second transmissible devil facial tumor (DFT2) that originated independently of DFT1 was discovered in wild devils [8]. A few cases of natural DFT1 regression have been reported [9–11], but no regressions or survival have been reported to date for DFT2 [8,12]. Like DFT1, DFT2 likely originated from a Schwann cell [13,14]. There are only nine known naturally-transmissible cancers, two of which occur in devils [5,8,15–17]. Independent studies from the San Diego Zoo (1979) and the Tasmanian Department of Primary Industries, Parks, Water and Environment (2019) performed 40 years apart and using different founder stocks reported that 50% and 43% of devils in captivity developed neoplasms [18,19]. A 1990 study from the Taronga Zoo (Sydney, Australia) also stated that "dasyurids, especially Tasmanian devils, are particularly prone to develop proliferative lesions" [20]. Large studies on neoplasms in captive wildlife by the San Diego Zoo (n=10,317) [19,21] and Taipei Zoo (n=2,657) [22], and domestic animals by the USA National Cancer Institute (n=202,277) reported cancer incidence generally less than 10% for zoo animals and domestic animals [23]. In addition to DFTs, habitat changes, road fatalities, dog attacks, and inbreeding, further limit the chance of population recovery [4,24]. The predisposition for cancer coupled with anthropogenic pressures present a clear threat to the long-term persistence of Tasmanian devils in the wild.

A regionally distributed vaccine could be used to prevent DFT2 from spreading across the state (**Fig. 1**) and provide an adaptable platform for ongoing (i.e. DFT1) and future disease threats. This Special Report will provide an overview of DFT vaccine options and their benefits and limitations. The vaccine option that balances safety with the greatest likelihood of success is an oncolytic viral vector that expresses DFT-specific antigens and is packaged inside an **oral bait vaccine (OBV)** capsule attractive to Tasmanian devils (**Fig. 2)**. Our aim is to develop an adaptable OBV platform that builds on nearly five decades of research and field application of the highly successful OBV approach that has been used to control rabies in more than 30 countries [25,26].

## 2. Current and future devil monitoring and management

Early statistical modelling (2007) predicted that DFT1 would spread across the entire range of the devil within 5-10 years, with extinction "a real possibility and an unacceptable risk" [7]. DFT1 has not yet reached the northwest and southwest regions of Tasmanian, so these regions remain DFT-free for the time being. DFT-affected devil subpopulations generally persist at 10-20% of historical levels and no local extinctions have been reported [4]. With DFT1, juvenile devils (< 1 year of age) are generally not affected, vertical transmission has not been reported, and primary transmission is hypothesized to occur during mating [27,28]. Precocial breeding of one-year old females and more pouch young per female has maintained small local populations [4,29]. However, as the devil pouch can accommodate a maximum of four joeys and further reduction of the breeding age to less than one-year of age is not expected, it is unlikely that increased precocial breeding can increase population density or compensate for additional environmental pressures on the wild population.

Genetic analysis demonstrating strong linkage disequilibrium pre- and post-DFT1 arrival have been used to infer positive selection in genomic regions near particular variants and suggest that the population is rapidly evolving in response to DFT1. However, disease prevalence remains > 20% and devils > 3 years old represent less than 10% of the population following the arrival of DFT1 (4,26). Additionally, the study that documents rapid evolution also states that Tasmanian devils have "extremely low levels of genetic diversity"; it is unknown how a species with minimal genetic diversity and is prone to cancer can simultaneously respond to evolutionary pressures from two different transmissible tumors.

One early disease management strategy considered was culling of infected animals, but trials concluded that removing infected devils did not impact the local prevalence of DFT1 [30]. Therefore, managers have focused on devil breeding programs to establish disease-free insurance populations in captive facilities. A DFT-free devil population was also established on Maria Island off the east coast of Tasmania in 2012 [31], which is the primary source of devils translocated to mainland Tasmania to boost population numbers and genetic diversity in diseased areas. The Maria Island population, together with the captive-breeding program, accounts for an extensive insurance population (~700 devils).

Another potential management strategy is to identify DFT-resistant devils and "attempt to spread the resistant alleles into affected populations" [32]. However, the mechanism for resistance and whether the "resistance" phenotype would have similar effects in other outbred populations is unknown. Additionally, translocation among wild populations risks introducing DFT strains with higher virulence[33] due to the long latent period of DFT, which can extend for at least 13 months in some cases (Save the Tasmanian Devil Program, personal communication). For example, DFT strain replacement has been documented to result in a

"rapid increase in disease prevalence, population decline and reduced mean age of the population" [34]. Other management options for reducing or controlling the impact of DFT1 and DFT2 have been considered but are generally limited to specific regions. Our proposal is to develop a vaccine that can suppress or eliminate DFT1 and DFT2 infections to allow wild devil populations to recover.

## 3. DFT vaccine and immunotherapy approaches

### *3.1. Whole-cell killed vaccine*

A whole-DFT cell vaccine approach was a logical starting point because the DFT cells have the potential to express the full suite of tumor antigens [35–37]. However, the clonal DFT cells have been transmitted through many devils and evolution has refined their immune-evading ability, resulting in a non-immunogenic cell (e.g. low MHC-I expression) that can express immunosuppressive checkpoint molecules and cytokines [38–40]. Furthermore, whole cells can also express the full suite of "self" antigens associated with healthy cells. Regulatory T cells and other tolerogenic cells that recognize the normal "self" proteins can create an immunosuppressive environment that impedes anti-tumor immunity in humans and mice [41].

### *3.2. Live-attenuated vaccine*

Live vaccines closely mimic the natural course of infection and are generally the most effective at stimulating lifetime immunity. Live DFT cells could be modified (“attenuated”) to reduce the likelihood of seeding new tumors. For example, we have developed DFT cells that can be induced to express IFNγ and upregulate MHC-I [42]. Coupling upregulation of immunostimulatory genes with mechanisms that control DFT cell proliferation (e.g. "suicide genes") and inhibitory pathways (e.g. PDL1 blocking antibodies, PDL1 gene knockout) could induce a strong anti-tumor response while enhancing the safety profile. Additional attenuation

mechanisms, such as tissue-culture adapted strains and site-directed evolution of the pathogen, can reduce the probability of reversion to virulence [43–45]. However, this live-DFT cell approach would be difficult to implement in a field setting.

### *3.3. Viral vector-based vaccines*

Oncolytic viruses that preferentially infect tumor cells have shown promise in human clinical trials [46,47]. These immunogenic viruses can directly lyse tumor cells, releasing damage-associated molecular patterns (DAMPs) and pathogen-associated molecular patterns (PAMPs), which stimulate antigen presenting cell (APC) and effector cell migration to the tumor microenvironment (**Fig. 3**). Oncolytic viral vectors that are modified to express genes coding for "cargo" (e.g. tumor-specific antigens, cytokines) have prophylactic and immunotherapeutic potential. The viral vectors are usually attenuated to limit replication in the target host and minimize risk of transmission to secondary hosts.

### *3.4. Recombinant protein-based vaccine*

The safest approach to a DFT vaccine combines immunostimulatory adjuvants and purified recombinant proteins. Effective adjuvants that provide immunogenic "danger signals" have already been identified [48,49] and could be used with recombinant protein targets. Non-synonymous DNA mutations that create altered protein sequences in DFTs can yield neoantigens likely to be viewed as foreign proteins by the host immune system. Clonal DFT cells have accumulated thousands of DNA mutations over years of continued transmission. Interestingly, the majority of these mutations are in non-coding regions or are synonymous mutations (i.e. protein sequence and function not altered) [50]. Of the 2,884 single nucleotide variants (SNVs) and 410 insertions/deletions identified in two DFT1 cell lines but not in 46 host devils, only 18 of these variations resulted in non-synonymous mutations. DFT2 has 3,591

SNVs and 572 insertions/deletions, but only 19 non-synonymous mutations. Short peptides that contain the non-synonymous portion of the protein could be used as alternative targets to full-length recombinant proteins [51]. A recent discovery that aberrantly expressed proteins from non-coding regions can function as tumor-specific antigens [52] is another possibility worth exploring.

**4. An oral bait vaccine (OBV) for DFT1 and DFT2 is needed**

Recent modelling (2019) has predicted a 20% chance of devil extinction due to DFT1 in the next 100 years and that "management interventions are unlikely to be necessary to ensure persistence of Tasmanian devil populations" [53]. However, these predictions are based on data from only a single subpopulation representing only a fraction of the data from long-term studies that have collected samples from "over 10,000 individuals and 2,000 tumor biopsies" [54]. Another study that used statewide data predicted long-term coexistence of devils and DFT1, but the devil population would be limited to 9% of pre-DFT1 size that could lead to "dramatic effects on the Tasmanian ecosystem" [55]. Furthermore, the predictions did not consider dynamic impacts of other ecological factors, such as inbreeding, social behavior, and the Allee effect (i.e. reduced fitness in small populations) [24,56–61]. Additionally, further negative consequences of DFT2, which has been co-circulating since 2014 [8], were not acknowledged in the manuscripts [53]. The ongoing spread of DFT1 and lack of population recovery from this infection, the relatively new threat of DFT2 and anthropogenic threats lead to continuing uncertainty for the long-term persistence of wild devils. Here we present a challenging but feasible OBV option to eliminate DFT2, combat the ongoing DFT1 threat, and provide a platform for managing future disease threats.

The ideal vaccine must be potent, innocuous to humans and other animals, and exhibit negligible excretion and low horizontal transmission risk in hosts. It must also be thermostable for several days at ambient temperatures, genetically stable concerning reversion to a virulent phenotype, free of contaminants, and relatively inexpensive to produce [62,63]. Several factors support the reality of a vaccine to block transmission and eliminate DFTs. First, vaccination of translocated devils from insurance populations has demonstrated that strong anti-tumor immune responses can be induced in vaccinated devils [48]. Second, a DFT1 vaccine coupled with subsequent immunotherapy has induced regressions in devils inoculated with DFT1 cells [35]. Advances in biotechnology incorporated with the expanding toolbox for devil immunology will build on these foundations to accelerate vaccine development [13,39,40,42,64–67]. Third, smallpox in humans and rinderpest in wild and domestic animals have been eliminated on a global scale; rabies has been controlled on national scales through vaccination. In comparison, the relatively small (~65000 $km^2$) island of Tasmania presents a more practical challenge.

## 5. Oral bait vaccine (ORV) platform for landscape distribution

### *5.1. Long history of safe and successful rabies OBV*

The first bait-vaccines consisted of chicken heads filled with a capsule containing live-attenuated rabies virus [25,68,69]. Bait-vaccination methods have been continually refined for efficacy and safety [70]; OBVs that express the rabies glycoprotein in replication-competent human adenovirus serotype 5 (ONRAB®) [71] or a thymidine kinase negative Copenhagen strain of vaccinia virus (RABORAL V-RG®) [72] have been extensively used in recent decades. An estimated 665,000,000 oral rabies vaccine baits were distributed across 33,250,000 $km^2$ in Europe between 1978 and 2014 [73,74]. Thirty European countries have

used OBVs as part of rabies control strategies, and 12 currently report they are rabies free according to international standards [75].

*5.2. Rationale for bait-vaccine approach to controlling DFTs*

An OBV platform for DFTs would allow widespread vaccine distribution across the geographic range of the devil including rugged and remote wilderness areas. Orally-delivered vaccines will be most effective if they infect host oropharyngeal tissues [76,77] (**Fig. 3**). Like rabies, DFT is orally transmitted and DFT tumor masses are most commonly found inside the oral cavity [12,27]. The junctional epithelium in gingival crevices and wounds in the oral cavity are the most likely portals of entry for viral vectors and DFT cells because both are "vulnerable point[s] in an otherwise continuous epithelium" [78]. Accordingly, the oral mucosa is an active site for immune surveillance and inflammatory responses [78–80].

Successful viral vector infection in the oral cavity should stimulate resident lymphocytes, APCs (e.g. macrophages, dendritic cells) and innate lymphoid cells (ILCs), and recruit additional immune cell subsets (**Fig 3**). Migration of APCs to the draining lymph nodes (e.g. submandibular lymph nodes) generates systemic memory and effector lymphocytes. Stimulation of T resident memory cells (Trm) in the oral cavity could play an important role in protection against DFT. $CD4^+$ and $CD8^+$ Trm and resident ILC1 cells produce IFNγ in response to non-specific and specific stimulation [81–87]. DFT1 cells upregulate MHC-I in response to IFNγ [38], so this simple inflammatory response could abrogate a major DFT1 immune-evasion mechanism. Rapid elimination of DFT cells by resident memory cells could circumvent potential tolerance-inducing mechanisms associated with upregulation of PDL1, which is delayed on DFT cells compared to MHC-I upregulation [39]. Interestingly, DFT2 cells do express MHC-I, suggesting that other immune-evasion mechanisms are in play [88].

Recent evidence in humans and mice suggests that virus-specific $CD8^+$ T cells can be repurposed for antitumor immunity [83]. This could be beneficial in DFT infection, as specific Trm induced by bait vaccination could be reactivated by subsequent exposure to DFT cells or the viral vector itself [83]. Reactivation of Trm can induce IFNγ and immune recognition of DFT cells by migrating leukocytes attracted to the site of inflammation, such as memory B cells attracted via CCL9 and CCL10 [86]. This raises the exciting possibility that the bait vaccine can serve as both a prophylactic vaccine and an immunotherapy. One hypothesis to explain natural DFT1 immune responses observed [9–11] is that sufficient "danger signals" occur in the tumor microenvironment to activate anti-DFT immunity; there is a high probability that these danger signals are derived from microorganisms entering wounds (**Fig. 3**) or ulcerated tumors. OBVs could also act as “danger signals” to activate and recruit innate cells (e.g. NK cells) that directly kill DFT cells and produce IFNγ to promote Trm responses [37,47,89,90]. Incorporation of immunostimulatory cytokines (e.g. IL15) or recombinant checkpoint blocking antibodies (e.g. PD1, CD200) could provide a powerful immunotherapy approach [39,40]. In summary, the rabies transmission-immunity cycle shares many key elements with DFT transmission-immunity, suggesting the bait vaccine could powerfully prime and/or boost anti-DFT immunity.

## 6. Development of a DFT bait vaccine

### *6.1 Development and testing of viral vectors and bait capsules (**Fig. 2**)*

The most straightforward DFT bait-vaccine approach would build on the successes and failures of oral rabies vaccine development. Many viral vectors were tested for oral rabies vaccines, including baculovirus [91], canine adenovirus type 2 [92–95], and raccoonpox virus [96]. More recently, an adenovirus platform was more effective in inducing seroconversion of baited

raccoons in comparison to the areas baited with the vaccinia platform [97]. Vaccinia and adenoviruses have both been reported to infect marsupials [98–100], and we have confirmed that adenoviruses infect DFT cells (Flies et al., *unpublished*).

Parallel testing of infectivity in DFT cells, devils, and non-target species (e.g. quolls) is required to identify inadvertent targets of OBVs. Infection tests using unmodified viral vectors (i.e. no DFT antigens) in healthy devils could be achieved using injection and instillations into the oral cavity. Alternatively, initial testing of the bait-vaccine approach could be accomplished using commercially available rabies OBVs. This could simultaneously measure infectivity of viral vectors and immune responses to viral-vector proteins and cargo proteins (e.g. rabies glycoprotein). Weak responses to the rabies glycoprotein could indicate low infectivity or immunogenicity of the viral vector [101]. To maximize attractiveness of the bait to devils and to minimize attractiveness to non-target species, placebo bait-preference tests can be used to select scent coatings for the bait capsules [102–104]. Devils are primarily scavengers and routinely feed on roadkill encompassing many common species (e.g. wallabies), making an attractant easily accessible.

Initial testing in captive devils can be accomplished using existing facilities and by modifying previous vaccine and immunotherapy and animal ethics protocols [35,48,49]. Following successful bait testing in captivity and semi-wild enclosures, initial roll out of bait vaccines can be accomplished in areas frequented by wild devils using automated bait dispensers, modified to limit baits consumed in a single visit. Remote cameras and proximity loggers [28] at bait stations can provide information on devil numbers and bait consumption. This will also give insight into non-target species consuming baits.

Retrospective analyses of European rabies control efforts suggested that cross-border differences in vaccination management hampered progress [73]. In contrast, Tasmania is an island state, DFT infects only a single species, movement of infected devils across geopolitical borders is not an issue, and a single management agency (Department of Primary Industries, Parks, Water and Environment) manages devil conservation initiatives. This current infrastructure may simplify some of the challenges faced by wildlife rabies managers (i.e., multi-lateral coordination and collaboration). Vaccine production and distribution is costly, but if vaccination is successful it could reduce costs in the long-term [105]. Working with teams with extensive OBV experience should help avoid pitfalls and maximize efficiency.

## 7. Conclusion

The ongoing spread of DFT1 and the emergence of DFT2, combined with environmental factors such as climate change [60,106], habitat alteration [24], and the uncertainty of host-pathogen co-evolutionary dynamics [107,108], suggest that an OBV platform should be developed in parallel with other Tasmanian devil conservation approaches. An effective OBV is an intervention with the potential to rapidly eliminate DFT1 or DFT2 on a statewide level. The key criteria that need to be satisfied prior to implementation of a DFT OBV strategy are to develop: (1) a safe and potent vaccine; (2) a delivery system; (3) a method for monitoring bait uptake by target and non-target species; (4) a robust surveillance and evaluation program [109]. Identification of stable DFT-specific antigens that can be used for a statewide vaccination campaign is likely to be a primary challenge. However, by building on effective rabies OBV strategies, key information such as: $R_0$ for DFT1 and DFT2; infectivity and immunogenicity of viral vectors; bait preference; and minimum baiting density can be established in parallel. Successful completion of these tasks can begin to lay the foundation of extensive safety testing

prior to using a DFT OBV in the field. Small-scale (e.g. free-range enclosure) efficacy testing can be done iteratively as suitable vaccine antigens are discovered.

## 8. Expert Opinion

The long-term persistence of devils in the wild hinges on the hope that devils will evolve resistance or tolerance to both DFT1 and DFT2, or that the tumors disappear from the landscape due to evolutionary processes [110]. How many threats can devils face while the unpredictable trajectory of host-pathogen coevolution plays out? The bait-vaccine method proposed here could be rapidly adapted to new threats and deployed across large regions. This approach could achieve the coverage levels needed to establish "herd immunity" to break the DFT1 transmission cycle, eliminate DFT2, and stamp out future disease threats before they take hold.

DFT2 was likely identified soon after it originated and to date has only been detected on a 550 $km^2$ peninsula (**Fig. 2**) [8,12,88]. This presented a chance for early action to eliminate DFT2 or set up a firewall to confine it to the peninsula. However, there were no management tools available for a quick response and no action was taken; it seems likely DFT2 will escape the peninsula in coming years [12]. A trap-vaccinate-release or targeted OBV approach could ensure efficient vaccine delivery in key areas [72,111], such as in a buffer zone surrounding DFT2 or in urban areas that have small devil populations. An adaptable OBV for DFTs would fill the major gap in management options and could eliminate DFT2 before it has the chance to follow the path of DFT1 and further reduce the wild devil population. Thus, we propose that DFT2-specific antigen discovery and an OBV platform should be a foremost conservation priority.

DFT-specific antigen discovery has been hindered by a lack of devil-specific reagents and methods. However, the continual refinement of the devil and tumor genomes [112] should increase efficiency of antigen discovery. Furthermore, we have greatly expanded our toolbox in recent years through high-throughput "omics" approaches and now have the foundation necessary to develop the proposed vaccine [10,11,36,38,39,49,65,88,90,112–119]. In human cancer, each cancer originates from a different genetic background, so identification of tumor-specific antigens must start from scratch for each individual. The clonal nature of DFT1 and DFT2 means that most mutations are carried forward with transmission, so two sets of antigens (DFT1 and DFT2) are needed for a vaccine instead of a new set of antigens for each devil. The DFTs present a naturally reproducible metastatic disease model, so engagement with industry groups to improve understanding of tumor metastasis could allow faster progress for devil vaccine development and facilitate translational advances in human cancer and transplant immunology.

Rabies OBVs have a long safety record, but additional safety mechanisms such as a short-infectious period of the virus inside the capsule (i.e. virus degrades within a month) or use of a viral vector with site-directed mutagenesis to enhance species-specificity, can help ensure the DFT vaccine is safe and effective [34]. Rabies virus has only a single glycoprotein on its surface [120] which is the only target in the rabies vaccine; a DFT vaccine with multiple protein targets should be the most effective, as immune escape via mutation of target proteins in DFT cells is likely to occur iteratively, rather than simultaneously. Identification of several DFT-specific antigens that are critical for cell function, such as those involved in cell cycling (e.g. CDK1), will help prevent immune escape by DFT cells; an ineffective vaccine that allows DFT escape could drive the tumor toward a more virulent phenotype [121]. However, this risk is also present with natural infections [122].

The OBV approach can induce prophylactic resident memory T cells at the most likely site of DFT infection, which could prevent DFT cell establishment before the cancer can induce immunological tolerance in the new host. Furthermore, oncolytic viral vectors have the potential to convert immunologically "cold" tumors into "hot" tumors [47], thus functioning as an immunotherapy. Extensive monitoring of devils in the past 20 years has vastly improved our understanding of devil biology and ecology (e.g. home range and daily movement), so effective vaccine distribution plans are manageable. For example, biting injuries, and potentially transmission, are highest during the breeding season [28], and competition-induced stress during the breeding season could cause general immunosuppression. Vaccination campaigns prior to the breeding season could provide peak immunity during this critical period.

We encourage a vigorous discussion on the scientific and ethical rationale of using a viral-vector bait vaccine for controlling DFTs. Tasmania is a pristine state, with large tracks of wilderness and a moratorium on genetically modified plants and animals. Nearly 50% of devils develop neoplasia in captivity [18,19]; devils harbor two of the three known transmissible tumors in mammals, and both have arisen in the past 25 years [5,8,15]. It is possible that other transmissible tumors could emerge; not being prepared for additional transmissible tumors or disease threats would be careless [123]. Thus, the central question in an evidence-based discussion should be: Is the future of the Tasmanian devil more secure with or without an effective OBV platform?

**Article Highlights**

- Tasmanian devils get cancer at higher rates than most other species.

- The wild devil population has been reduced by 77% over the last 23 years due primarily to the emergence of a transmissible cancer, the devil facial tumor (DFT1).
- The emergence of a second transmissible tumor (DFT2) further threatens the long-term survival of this species.
- There are currently no effective interventions for reducing or controlling the impact of DFT1 or DFT2 on a broad scale, and few tools are in place to rapidly combat future disease outbreaks.
- We propose a rabies-style oral bait vaccine (OBV) as a safe and effective method for eliminating DFTs and this option must be explored to support the long-term survival of this iconic, endemic, endangered species.
- Tasmanian devils are the world's largest carnivorous marsupial after the human-driven extinction of the Tasmanian tiger (*Thylocanus cyanochalus)* several decades ago. Fear of failure should not impede exploration of innovative strategies to save this iconic species.

**Figure legends**

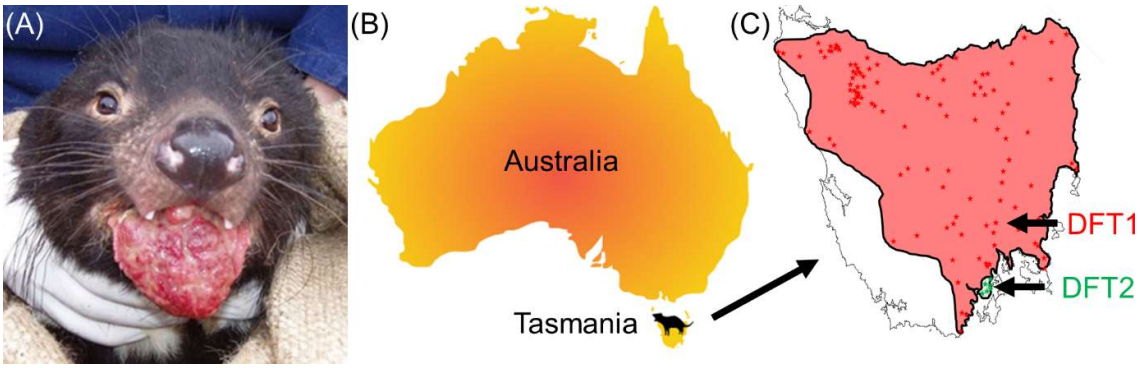
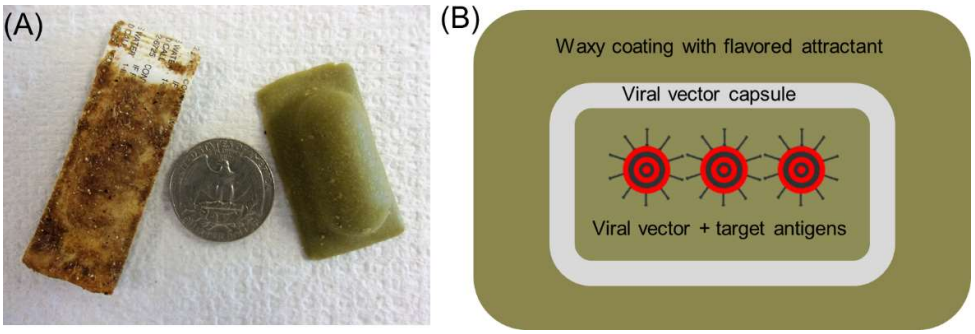



**Figure 1.** (A) Image of devil facial tumor (DFT) courtesy of the Save the Tasmanian Devil Program. (B) Location of Tasmania relative to mainland Australia and (C) distribution of DFT1 (red) and DFT2 (green). DFT2 has only been detected on the peninsula in southern Tasmania to date, which makes it amenable to containment by a vaccine.




420

421 **Fig. 2. Bait vaccines.** (A) Picture of two licensed oral rabies vaccination bait products with 24
422 mm coin for scale. (B) Cross-sectional schematic of an oral bait vaccine (OBV). Viral vectors
423 expressing DFT-antigens are packaged inside a capsule surrounded by a waxy matrix mixed
424 with a flavored attractant. The viral vector contacts and infects the mouth of animals that bite
425 through the outer matrix and inner capsule.

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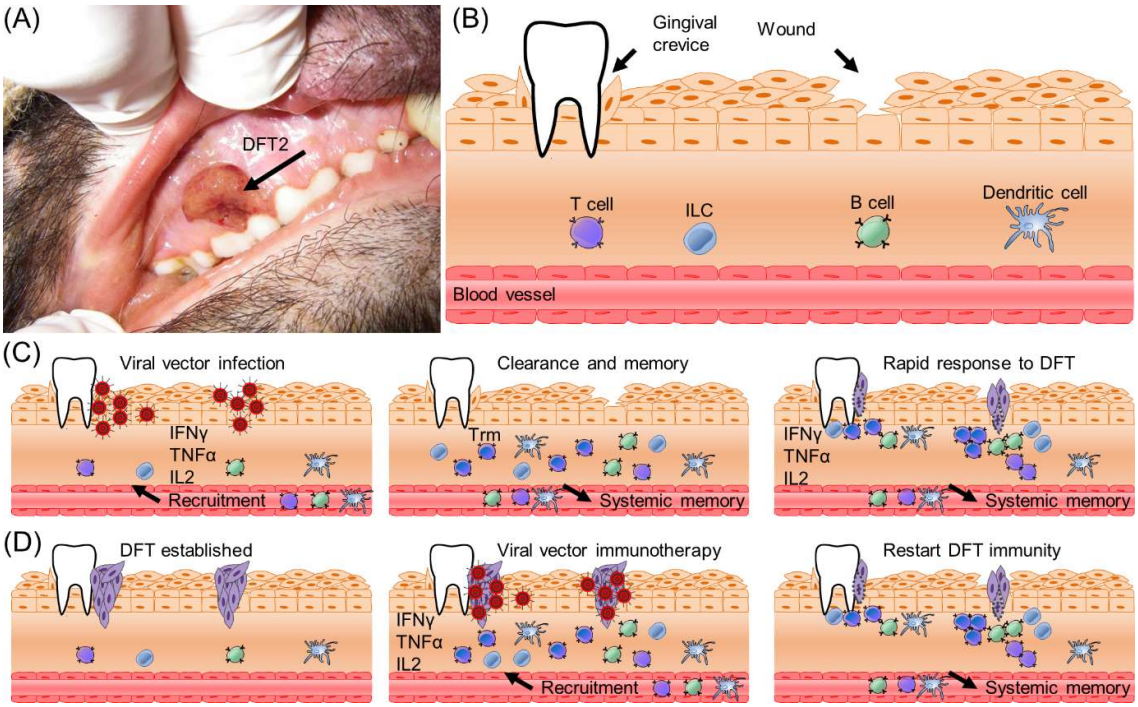


**Fig. 3. Prophylactic and therapeutic potential of DFT vaccine.** (A) DFT2 infection in oral cavity. (B) Diagram of oral epithelium, resident immune cells, and potential portals of entry for DFT cells and viral vectors. (C) In the pre-DFT exposure scenario, immunogenic viral vectors expressing DFT antigens infect normal cells and induce inflammatory cytokines (e.g. IFNγ) and recruit additional immune cells. T resident memory (Trm) cells and other immune cell subsets remain at the site of infection, and T central memory (Tcm) cells circulate through secondary lymphoid tissues. Trm and Tcm rapidly produce cytokines and effector responses when exposed to DFT-antigens. (D) In the post-DFT exposure scenario, DFT invades and establishes immune tolerance. Subsequent exposures to viral vectors expressing DFT-antigens serve as an immunotherapy to induce inflammatory cytokines, such as IFNγ that upregulates MHC-I on DFT cells, and recruitment of additional immune cells.

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