



Current Challenges for the Anti-Devil Facial Tumor Disease (DFTD) Vaccine Development

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The devil facial tumor disease (DFTD) is a naturally occurring contagious cancer in Tasmanian devils (*Sarcophilus harrisii*) which has wiped out more than two thirds of the wild population of devils within just two decades [1] (Figure 1). Because of the alarming rate of the devils decline, it has been listed as an endangered species on the Red List of the International Union for the Conservation of Nature and Natural Resources (IUCN) [2]. While several different programs have been established in order to preserve small healthy devil populations, including the successful translocation of healthy devils to several offshore islands, there is major concern that in the absence of an efficient anti-DFTD vaccine, the disease may lead to the extinction of the world's largest remaining marsupial carnivore.

DFTD is a non-viral transmissible parasitic cancer which spreads from one individual to another through aggressive biting, particularly in fights over mates and food [1,3]. The tumors initially appear around the mouth as small lesions or lumps, which rapidly develop into large tumors around the face, neck, and jaws, and in the oral cavity, interfering with feeding and leading to metabolic starvation [3]. The tumors can also spread to internal organs, such as the lungs, spleen, and kidneys, causing progressive physiological malfunction of the body's most important organs [3].

Genetic analyses and deep sequencing of the DFTD transcriptome provided support for the clonal nature of the tumor [4,5], and together with immunohistochemical studies demonstrated that the DFT originated from a Schwann cell in a female devil [4-6]. More recently, a second independent lineage of transmissible cancer, termed DFT2, was identified in a small number of devils [7]. This tumor, which arose from a male devil, causes facial tumors that are grossly indistinguishable but histologically distinct from those caused by the original DFT cell lineage, also referred to as DFT1.

The reasons for the progression of the DFT cells across allogeneic barriers are only partially clear, and a better understanding of the causes for its modality should contribute to the design of an efficient vaccine that will save the devil from extinction.

Transmissible cancer in nature has been reported thus far only in two mammalian species, which include, in addition to the devil's DFTD, the canine transmissible venereal tumor (CTVT) in dogs [8]. Unlike DFTD, which is a fatal disease that initially appeared only two

decades ago, CTVT is rarely fatal, emerged as a cancer about 11000 years ago and is transmitted from one dog to another by the transfer of living cancer cells during coitus [9]. Furthermore, CTVT cells are relatively immunogenic and the tumor can undergo spontaneous regression. It metastasizes only in rare conditions, predominantly in immune compromised hosts [8].

A third type of transmissible cancer was recently reported in shellfish species, including clams and mussels [10], where one type of a leukemia-like disease was found to be transmitted across species as a xenogeneic tissue [11].

The exact reasons for the occurrence of a rare transmissible cancer specifically in devils are unclear. One factor that is suspected to allow the growth of DFT cells across allogeneic barriers is related to the devils evolution and their limited geographical distribution. Since this species is confined to the island of Tasmania, the devils underwent many generations of inbreeding, which resulted in a very limited genetic diversity. Thus, in contrast to allogeneic tumors that are normally rejected by the immune system of healthy individuals, lack of sufficient heterogeneity among devils might result in inability of their immune system to recognize and destroy the allogeneic DFT cells.

The limited genetic diversity in the wild devil population was demonstrated in several independent studies [12-14], and was recently validated in the closely related Tasmanian tiger (*Thylacinus cynocephalus*), the largest carnivorous marsupial until its extinction in 1936, which underwent a similar geographic isolation on the island of Tasmania [15].

The inability of the devils to mount an effective immune response against DFT cells was also suspected to reflect a reduced expression of immunogenic antigens on the surface of the tumor cells and/or the existence of DFT-mediated immunosuppressive mechanisms.

Recent studies provided support for these assumptions demonstrating that the DFT cells acquired immune evasion mechanisms that enabled them grow in histoincompatible hosts.

In a study by Siddle et al. [16], the authors demonstrated that DFT cells lost the ability to express MHC class I antigens, which apparently enable the tumor cells to evade cytotoxic T cell (Tc) recognition. Downregulation of MHC class I molecule expression on tumor cells is one of the major mechanisms by which they escape immune surveillance [17-19].

Siddle and colleagues demonstrated that the loss of expression of MHC class I proteins by DFT cells was not due to structural mutations, but to regulatory modifications involving epigenetic deacetylation of histones, which lead to a reduced expression of transporter associated with antigen processing 1 (TAP1) and TAP2 [16]. These two proteins are required for the transport of cytosolic endogenous peptides to the endoplasmic reticulum where the peptides bind to the assembled MHC class I molecules. The MHC-peptide complexes are then transported to and expressed on the cell surface where they can be recognized by the T cell receptor (TCR) of the cytotoxic T cells. Allrecognition of MHC-peptide complexes results in a strong Tc-mediated response, leading to death of the target cells.

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DFT cells are also devoid of surface β 2-microglobulin (β 2m) [16], which normally associate with MHC class I proteins at the endoplasmic reticulum and serve to stabilize MHC heavy chain expression at the cell surface [20]. In previous studies, a disruption of the β 2m gene was found to impair MHC class I protein expression and disrupt CD8+ Tc cell development [21].

Further analysis of DFT cells revealed that expression of TAP1, TAP2, and β 2m genes could be upregulated by cell treatment with interferon γ (INF γ) [16], suggesting that in vitro manipulation of DFT cells increases their immunogenicity and perhaps can serve in future strategies of devil immunization against DFTD.

A second study by Flies et al. [22] demonstrated that DFT cells apparently exploit the programmed death-1 (PD-1; CD279): PD-1 ligand (PD-L) inhibitory pathway to evade immune surveillance.

PD-1 is an inducible immune modulatory receptor that plays a role in the maintenance of peripheral tolerance and has recently emerged as a promising target for cancer immunotherapy [23,24]. It is expressed on T cells and other types of immunocytes and plays a major role in down regulating inflammatory immune responses during pregnancy and autoimmune diseases [23,24]. Upon engagement of PD-1 with its ligands, PD-L1 (B7-H1; CD274) and PD-L2 (B7-DC; CD273), it delivers a signal that inhibits the phosphorylation of ZAP70, the interaction of ZAP70 with CD3 ζ and its recruitment to the immunological synapse, and the phosphorylation and activation of PKC θ [25,26]. As a result, the PD-1-regulated downstream events lead to downmodulation of TCR-induced activation signals and inhibit T cell responses. High expression of PD-1 was noted on tumor infiltrating lymphocytes [27] while PD-L1/2 expression was noted on the surface of various types of tumor cells [28-30]. The binding of PD-L1 to PD-1 generates a local immunosuppressive effect that allows the tumor to evade immune destruction [31,32].

Flies et al. demonstrated that the expression levels of PD-L1 on resting tumor cells from DFT1 and DFT2 were undetectable or very low, but increased significantly following INF γ treatment [22].

The lack of expression of MHC alloantigens on DFT cells is a major cause for their inability to evoke an efficient anti-tumor alloimmune response. This effect could be inverted by INF γ treatment, which augments MHC protein expression on the surface of the tumor cells. However, since PD-L1 is also induced by INF γ treatment, it is possible that blocking PD-L1 on the tumor microenvironment might be an essential complementary procedure for anti-DFTD vaccine that involves the induction of INF γ production.

Previous studies of the devil's immune system provided no indications for malfunction or impaired responses. Devils can respond to xenogeneic cells by antibody production and by mounting a strong antibody-dependent natural killer (NK) cell-mediated cytotoxic response [33]. While resting NK cells from devil's peripheral blood do not respond in vitro to DFT cells, they do kill cultured DFT cells following their activation by concanavalin A, a Toll-like receptor agonist, or interleukin 2(IL-2) [34]. The results suggest that despite the inability of the devil to mount an efficient NK cell response against DFT cells in vivo, such a response could probably be induced by proper treatment that boosts the devil's NK cell activity.

In addition, the devil's lymphocytes responded by proliferation in a mixed lymphocyte reaction and reject skin allografts within 14 days of transplantation [35]. These results indicate that the devil's lymphocytes can recognize histoincompatible skin cells and mount



Figure 1: Tasmanian devil (*Sarcophilus harrisii*).

a strong cytotoxic response, but are incapable of recognizing and responding against DFT cells. Alternatively, the devil's lymphocytes might possess the capability of recognizing DFT antigens, but in the context of membrane proteins that deliver inhibitory signals and promote the induction of tolerance to the tumor antigens. Inhibition of anti-DFT cell immune responses might also be induced by DFT cell-secreted factors, which inhibit Tc cell responses at the tumor microenvironment. Furthermore, a possibility exists that regulator T cells (Treg) might also play an important role in the inhibition of immune responses against DFT cells.

It is clear that the development of an efficient anti-DFTD vaccine requires further studies and better understanding of the relationships between DFT cells and the host immune system. However, time is running short and saving the world's largest remaining marsupial carnivore from extinction is critically dependent on the availability of such a vaccine.

The discovery of a second, distinct line of devils' transmissible tumor, the DFT2, poses an extra challenge to vaccine development since a vaccine that protects against one DFT line may not provide protection against the other.

The present prognosis for this endemic marsupial carnivore species remains uncertain and we hope that an efficient anti-DFTD vaccine should be available fast enough in order to save the devils from extinction.

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