

## Overview

# Transmissible Cancers and Immune Downregulation in Tasmanian Devil (*Sarcophilus harrisii*) and Canine Populations

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Known as devil facial tumor disease (DFTD) and canine transmissible venereal tumor (CTVT), transmissible cancer occurs in both Tasmanian devil and canine populations, respectively. Both malignancies show remarkable ability to be transmitted as allografts into subsequent hosts. How DFTD and CTVT avoid detection by immunocompetent hosts is of particular interest, given that these malignancies are rarely seen in other species in nature. Both of these transmissible cancers can downregulate the host immune system, enabling proliferation. DFTD is characterized by epigenetic modifications to the DNA promoter regions of  $\beta_2$  microglobulin, transporters associated with antigen processing 1 and 2, MHC I, and MHC II—crucial proteins required in the detection and surveillance of foreign material. Downregulation during DFTD may be achieved by altering the activity of histone deacetylases. DFTD has caused widespread destruction of devil populations, placing the species on the brink of extinction. CTVT demonstrates a proliferative phase, during which the tumor evades immune detection, allowing it to proliferate, and a regressive phase when hosts mount an effective immune response. Alteration of TGF $\beta$  signaling in CTVT likely impedes the antigen-processing capabilities of canine hosts in addition to hindering the ability of natural killer cells to detect immune system downregulation. Immunosuppressive cytokines such as CXCL7 may contribute to a favorable microenvironment that supports the proliferation of CTVT. When viewed from an evolutionary paradigm, both DFTD and CTVT may conform to a model of host–parasite coevolution. Furthermore, various genetic features, such as genetically active transposons in CTVT and chromosomal rearrangements in DFTD, play important roles in promoting the survival of these disease agents. Understanding the mode of transmission for these transmissible cancers may shed light on mechanisms for human malignancies and reveal opportunities for treatment in the future.

**Abbreviations:**  $\beta_2$ m,  $\beta_2$  microglobulin; CTVT, canine transmissible venereal tumor; DFTD, devil facial tumor disease; LINE, long interspersed element; TAP, transporter associated with antigen processing; TLR, toll-like receptor

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Transmissible cancers deviate from the traditional cancer paradigm in that they can be transmitted as allografts and proliferate throughout a host population.<sup>32</sup> Currently, only 2 transmissible cancers are known to exist in vertebrates in nature: devil facial tumor disease (DFTD), affecting Tasmanian devils (*Sarcophilus harrisii*), and canine transmissible venereal tumor (CTVT), inflicting members of the canid family, mostly domesticated dogs.<sup>32</sup> Although both cancers have similar characteristics in terms of transmission, they differ in disease progression as well as severity (Figure 1). Without treatment, CTVT tumors usually regress after 1 to 3 mo.<sup>42</sup> In contrast, DFTD has been shown to cause close to 100% mortality in some devil populations.<sup>30</sup> CTVT is often enters a regressive phase after proliferation.<sup>5,50</sup> CTVT responds well to traditional chemotherapy treatments and host species have shown the ability to mount an effective immune response in the regressive phase.<sup>50</sup> However, CTVT continues to persist in the canid family and in fact has been referred to by some as the oldest malignancy to have ever

been identified.<sup>43</sup> CTVT is transmitted primarily through physical contact, and tumors tend to predominantly affect the genital region. Lesions usually appear as bright red, cauliflower-like circular masses in the genitalia of both female and male dogs.<sup>28</sup> Lesions are known to bleed easily and become ulcerated, giving them their contagious characteristics.<sup>28</sup> Since its divergence, CTVT has been shown to occur in canine species on virtually almost every continent.

Tasmanian devils are carnivorous marsupials native to the island of Tasmania. This species is the largest extant carnivorous marsupial.<sup>34</sup> *S. harrisii* are nocturnal foragers and scavengers that typically feed on dead remains or smaller insects and animals.<sup>34</sup> DFTD was first reported in the mid to late 1990s and has since proliferated throughout the population, bringing *S. harrisii* to the brink of extinction.<sup>25</sup> Devil facial tumor disease, another transmissible cancer, is transmitted clonally as an allograft.<sup>32</sup> The baseline genome has remained remarkably stable throughout the last 20 y; however, some differentiation exists, and subclones have developed.<sup>39</sup> The tumors are usually spread through biting during intercourse or altercations between devils. Tumors appear as rounded, ulcerative, and exudative lesions typically located on the face and oral mucosa.<sup>27</sup> The Tasmanian

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Disease	Methods of escape from immunosurveillance
DFTD	Downregulation of functional MHC molecules on the cell surface and epigenetic transformation through: <ul style="list-style-type: none"> <li>- deacetylation of histone proteins</li> <li>- modification in the antigen-processing pathway itself, particularly downregulation of <math>\beta 2</math> microglobulin, TAP1, TAP2, and MHC I molecules</li> </ul>
CTVT	Proliferative phase—disruption of the TGF $\beta$ signaling pathway leading to: <ul style="list-style-type: none"> <li>- disruption of the MHC antigen-processing pathway</li> <li>- impeding of the ability of natural killer cells to detect downregulation of the various arms of the immune system</li> <li>- CXCL7-immunosuppressive cytokine leading to downregulation.</li> </ul> Regressive phase—inhibition of the effects of TGF $\beta$ and CXCL7 due to IL6 secreted by tumor-infiltrating lymphocytes

**Figure 1.** Disease and pathophysiologic characteristics of DFTD and CTVT.

devil population showed a decline in 1863 and then again, from 1908 to 1920, perhaps suggesting that a population bottleneck had occurred, reducing genetic diversity. However, the population had since rebounded and become numerous prior to the proliferation of DFTD.<sup>14</sup>

### Immunologic Escape Mechanisms in DFTD: Epigenetic Downregulation

DFTD can evade recognition by the host's immune system.<sup>48</sup> Failure to elicit an immune response has been correlated with epigenetic modifications of the DNA promotor regions of  $\beta 2m$ , transporters associated with antigen processing (TAP) 1 and 2, MHC I, and MHC II (Figure 2)<sup>47</sup>. These genes ultimately lead to the expression of functional MHC I and II molecules.<sup>48</sup> Tumor cells do not express functional MHC molecules on the surface.<sup>47,48</sup> Downregulation is not solely due to somatic mutations or structural changes but rather to reversible modifications in promoter regions of important immune genes involved in the antigen-processing pathway ( $\beta 2$  microglobulin, TAP1, TAP2).<sup>48</sup>

One pathway of epigenetic modification involves deacetylation of histone proteins.<sup>4,53</sup> Acetylation, as well as methylation, is a crucial factor in regulating the expression of genes.<sup>19</sup> Histones are a family of positively charged or basic proteins that associate with DNA.<sup>19</sup> Five types of histones have been identified (H1, H2A, H2B, H3, and H4), which wrap DNA into a more condensed form by associating with the negatively charged phosphate backbone.<sup>19</sup> When acetyl groups are added, they bind to lysine residues in the N-terminal portions of histone proteins, effectively negating some of the positive charge used to bind the DNA phosphate backbone.<sup>19</sup> Therefore, chromatin is transformed into a more relaxed structure, enabling DNA binding proteins to initiate gene transcription.

Another method used by DFTD to escape immunologic detection is modification of the antigen processing pathway itself, in particular in  $\beta 2m$ , TAP1, TAP2, and MHC I molecules.<sup>48</sup> Each of these molecules plays an important role in the presentation of foreign peptides to cells of the innate and adaptive immune systems.  $\beta 2m$  is an integral component of the antigen-presenting complex of the MHC I molecules present on the surface of nearly all cells, excluding erythrocytes.<sup>19</sup> MHC I is involved in the endogenous presentation of antigens that is, antigens present in the cytosol of cells.<sup>19</sup>  $\beta 2m$  ensures appropriate antigen binding in the peptide-binding groove of MHC I molecules.<sup>19</sup>

If DFTD can be clonally transmitted successfully as an allograft and avoid immune detection, DFT cells should not express molecules associated with the host immune system, specifically the components of the antigen-processing pathway (that is,  $\beta 2m$ , TAP 1, MHC). To address this issue, one group set out to identify whether MHC I molecules were present in DFTD cells.<sup>48</sup> By using 4 DFTD cell lines as well as a host fibroblast cell

line, a monoclonal against the MHC I molecules and a polyclonal antibody against  $\beta 2m$  were created.<sup>48</sup> Western blotting and flow cytometry revealed no evidence of  $\beta 2m$  and little to no evidence of MHC I molecules.<sup>48</sup> Previous studies have found limited evidence of infiltration of lymphocytes and neutrophils into tumor regions.<sup>41</sup> However, this effect is likely due to the intertwined nature of the neoplasms with host connective tissue.<sup>44</sup>

$\beta 2m$  is a component of the MHC I complex and is required for the appropriate binding of foreign peptides presented on the surface of MHC I molecules.<sup>19,48</sup> The binding groove, which is recognized by CD8<sup>+</sup> T cells, is a heterotetramer consisting of 3  $\alpha$ -subunits as well as the  $\beta 2m$  subunit.<sup>19,48</sup> Thus, in the absence of  $\beta 2m$ , endogenous antigens are unable to be presented. TAP proteins are composed of 2 subunits (TAP1 and TAP2) that together form part of the ATP-binding cassette.<sup>17,51</sup> The protein complex uses ATP hydrolysis to deliver degraded foreign peptides, resulting from proteasome degradation, to the lumen of the endoplasmic reticulum, where these peptides will eventually be loaded on to MHC molecules and displayed for immune detection.<sup>17,51</sup>

Investigators used RT-PCR to identify whether mRNA transcripts for proteins involved in the antigen-processing and peptide-loading pathways were expressed in 4 DFTD cell lines.<sup>48</sup> Specifically, the investigators examined mRNA sequences for  $\beta 2m$ , TAP1, and TAP2 and found that DFTD cells express little to none of these mRNA transcripts, indicating that these proteins are not constitutively expressed.<sup>48</sup> Interestingly, PCR analysis revealed that DNA transcripts for  $\beta 2m$ , TAP1, TAP2, MHC I, and MHC II molecules were present and not deleted.<sup>48</sup> Furthermore, these transcripts showed all the capabilities of undergoing translation into functional proteins with no deletions present when compared with normal devil fibroblast cells.<sup>48</sup> This finding suggests that some mechanism of downregulation is playing a role. Normally, in vivo histone acetyltransferases catalyze the transfer of acetyl groups from acetyl-CoA to lysine residues on the N-terminals of histone proteins, thus relaxing chromatin structure and enabling transcription.<sup>13,24</sup> In addition, factors such as histone deacetylases catalyze the removal of acetyl groups, resulting in a more compact chromatin structure and thereby halting translational activity.<sup>13,24</sup>

Exploitation of either method of acetylation or deacetylation may result in cancer. However, downregulation most likely results from DFTD cells increasing histone deacetylase activity. Several histone deacetylase inhibitors have been widely used to regulate transcriptional activity when histone deacetylases are inhibited.<sup>2,31</sup> Treatment of DFTD cells with histone deacetylase inhibitor trichostatin A increased the transcription of MHC I,  $\beta 2m$ , TAP1, and TAP2 genes.<sup>48</sup> This result indicates that downregulation by DFTD is due at least in part to deacetylation of these transcripts, which are essential to promoting an immune response.

	Genetic aspects	Immunologic aspects	Evolutionary aspects
DFTD	3 strains, each with substrains; multiple substrains be found in the same e subject; possible chromotripsis  Tumor cell line but lacking the random mutational process, leading to genetic instability  Cell Line (DFT2) =	Escape from immunosurveillance through epigenetic transformation	Allograft transmission  Recently emerged, possible malignant pattern potentially due to lack of genetic diversity in MHC genes, perhaps with potential to conform to the coevolutionary model
CTVT		Escape from immunosurveillance through TGFβ and CXCL7 in the proliferative phase  Inhibition of effects of TGFβ and CXCL7 through IL6 in the regressive phase	Allograft transmission  Evolutionary pathways leading to reduction in virulence, suggesting possible host-parasite coevolution

**Figure 2.** Genetic, immunologic, and evolutionary interpretations regarding DFTD and CTVT.

### Escape of Immune Surveillance in CTVT

Similar to DFTD, CTVT has also shown the ability to escape detection by the host immune system but with notable exceptions. Most importantly, CTVT is characterized by a proliferative phase, during which the tumor evades immune detection and the malignancy grows pervasively. The proliferative phase typically continues for 11 to 12 mo.<sup>9</sup> Usually after this proliferative phase is a stage of regression, when the host exhibits immune rejection to the tumor cells.<sup>9</sup>

Immunologic escape by CTVT is due to at least in part by disruption of the TGFβ signaling pathway.<sup>9</sup> TGFβ is a cytokine that is elevated in many forms of human cancers,<sup>29</sup> due to its role in the regulation of the cell cycle, as well as its immunosuppressive functions.<sup>6,29</sup>

Using experimentally induced tumors, one group of researchers has shown that TGFβ disrupts the MHC antigen-processing pathway, allowing CTVT to grow undetected by the innate immune system.<sup>9</sup> In addition, the ability of natural killer cells to detect downregulation of the various arms of the immune system may be impeded by tumor cells secreting TGFβ.<sup>9</sup> Furthermore, chemokines from the CXC family have shown to play an important role in the development and progression of CTVT, as well as various human cancers.<sup>9</sup> CXC chemokines consist of small (8 to 10 kDa) proteins involved in inflammation and immune system activation. This type of chemokine acts by inducing various cells of the adaptive and innate immune systems to migrate toward sites of infection or invasion by foreign particles (chemotaxis).<sup>19</sup> The CXC notation is conventionally used to indicate that the conserved cysteine residues used to characterize these proteins are separated by a single amino acid in this family of chemokines.<sup>9</sup> Scientists have demonstrated that one particular chemokine in the CXC family, CXCL7, is associated with the proliferative phase of CTVT.<sup>9</sup> By perpetuating a state of chronic inflammation, both TGFβ and CXCL7 may contribute to a favorable microenvironment for tumor proliferation.<sup>9</sup> Interestingly, the effects of both TGFβ and CXCL7 are at least in part inhibited by IL6 that is secreted by tumor-infiltrating lymphocytes during the regressive phase.<sup>9</sup> The cytokine IL6 is involved in many aspects of the immune system, including the differentiation of B cells into their immunoglobulin-producing form.<sup>7</sup> IL6 seems to play a pivotal role in the progression of CTVT to the regressive phase.<sup>9</sup> CTVT cells treated with IL6 were shown to significantly downregulate their expression of CXCL7, indicating that IL6 has potential to be used in vaccination.<sup>9</sup>

The exact mechanisms of how these complex interactions can lead to proliferation or regression in CTVT is not yet fully understood. In humans, a combination of both positive and negative signaling is required to ensure a specific and efficient immune response.<sup>19</sup> For example, the activation of CD4<sup>+</sup> T helper cells, which respond to MHC II molecules, requires an additional signal distinct from the MHC II molecule.<sup>19</sup> This signal usually comes in the form of a protein present on the surface of antigen-presenting cells that, along with the MHC II molecule, binds to the T-cell receptor.<sup>19</sup> Once activated, T cells begin to secrete cytokines such as TGF-β and IL6.<sup>19</sup> However, without the correct stimulatory signals, no such activation occurs, and thus the secretion of cytokines may be altered. Whether signaling pathways mediated by cytokines are altered in CTVT has yet to be determined, but further investigation is warranted and may provide insight into the transitions into the progressive and regressive phases of CTVT.

### Evolutionary Considerations

CTVT and DFTD are transmitted as allografts. Therefore, host-parasite dynamics come into play, and the transmissible cancers of these 2 vertebrates can be viewed from an evolutionary paradigm. Given that the sources of transmission for both CTVT and DFTD are clonal cell lines, they can be viewed as analogous to parasites invading a host. Host-parasite coevolution was first described in 1949, in which the host and parasite were envisioned as engaged in an everlasting evolutionary arms race.<sup>15</sup> In essence, how virulent a pathogen or parasite is in relation to its host can be shaped by natural selection; therefore thus adaptations from both sides (host and parasite alike) enable a parasitic relationship to persist.<sup>40</sup> Mathematical relationships can be applied to describe the dynamics in host and parasite evolution. One notable model, proposed in 1981, describes how successful a parasite or pathogen can be in transmission and infection, depending on several factors:<sup>3</sup>

$$R_0 = \frac{\beta N}{\alpha + b + v},$$

where  $R_0$  indicates the basic reproductive rate of the parasite, which is a measure of its fitness;  $\alpha$  represents the disease-induced mortality rate of the host;  $b$  is the disease-free mortality rate (that is, the number of hosts dying due to causes other than parasitic infection);  $v$  indicates the recovery rate of the host; and



$N$  represents the population density of the host.<sup>3</sup> A couple of predictions can be made by using this model. First, the basic reproductive rate of the parasite is maximized when the  $\alpha$  value reaches 0, representing an inverse relationship. Because  $\alpha$  represents the number of hosts dying in the presence of parasitic infection, a 0 value for this parameter indicates that a majority of the infected hosts should not succumb to the disease. Therefore, to ensure successful transmission and success, a parasite or pathogen would evolve to be less virulent over time. Intuitively, a high virulence rate would almost ensure the death of the particular host and impede a parasite or pathogen from reproducing. The model also predicts that success of the pathogen or parasite is directly related to host density. As a population of hosts begin to decline, the basic reproductive rate of the parasite decreases. Considering that CTVT has been in existence for 6000 to 10,000 y, some form of host-parasite coevolution likely could be involved. Given that CTVT is not particularly lethal to canids and that most canines enter the regressive phase, it stands to reason that the reduction in virulence is due at least in part to these evolutionary parameters. In contrast, DFTD is incredibly aggressive and leads to death of almost 90% of its hosts in certain areas.<sup>25</sup> The Tasmanian devil species is currently listed as endangered and has decreased dramatically in size.<sup>14</sup> Whether DFTD adopts a malignant pattern similar to CTVT is an important question to consider. Given the relatively recent emergence of this disease in comparison to CTVT,<sup>8</sup> DFTD still has the potential to conform according to the coevolutionary model. Indeed some immunologic responses to DFTD have been observed.<sup>11</sup> One group has demonstrated that devils are showing signs of evolutionary response particularly in genomic regions related to immunologic detection and surveillance.<sup>11</sup>

The Tasmanian devil population has experienced population size reductions over the past 150 y.<sup>18</sup> A large susceptible population may be another requisite for transmissible cancer or parasite to spread with high virulence, as indicated in the model presented earlier.<sup>3</sup> Because the Tasmanian devil is strictly an island species, the notion of reduced genetic diversity may be invoked to explain such susceptibility to a devastating transmissible cancer in these marsupials. Of particular importance are the allelic frequency and genetic diversity of MHC loci in the population. Because MHC proteins play a crucial role in the immunologic response, a lack of genetic diversity among these genes could explain an inability for the devil population to recognize DFTD as nonself. In humans, 20 different MHC genes have been identified, with each gene showing incredibly high degrees of variability.<sup>19</sup> In fact, more than 6000 alleles for MHC genes have been discovered,<sup>19</sup> owing to the remarkable ability of the MHC to recognize a vast variety of antigens. The MHC alleles in a population are under selective pressures and thus have been shaped by natural selection.<sup>19</sup> If, for example, an individual in a population possesses a particular MHC allele that is better suited to present peptides of a threatening infectious agent, that individual is more likely to survive and pass on its genetic information.<sup>19</sup> This is one reason why population bottlenecks are known to reduce genetic diversity. Even if the population were to rebound, the lack of genetic diversity cannot be overcome without input from an additional source of diversity. Devils have been restricted to the island of Tasmania for a significant time, and thus the island itself has presented a barrier to gene flow. Devils possess moderately low genetic diversity and heterozygosity likely due to a founder effect in combination with population bottlenecks.<sup>18</sup>

Given the aggressive nature of DFTD and its high mortality rate, it is reasonable to assume that devils have altered some

life-history characteristics to ensure their survival. It has long been known that host species can alter life-history characteristics in the presence of parasitic infections to ensure reproduction and survival. This behavioral plasticity not only involves the host, but also research has shown that parasites themselves are capable of remarkable acclimation in the face of changing host behavior—antibiotic resistance is often cited as one such example.<sup>20</sup> Recent studies suggest that Tasmanian devils are indeed altering life-history characteristics to survive a devastating disease.<sup>45</sup> Female devils seem to show a propensity toward semelparity, with precocial breeding also being observed.<sup>25,45</sup> DFTD tends to affect adult devils more than juvenile devils. This effect could be due to aggressive behavior, such as biting, during mating in adults.<sup>25</sup> Given the imminent death that occurs once a devil acquires DFTD, an earlier reproductive bout would ensure that the individual will pass on its genetic information. In addition, female devils seem to reproduce in a facultative manner to favor production of female offspring, thus biasing the sex ratio.<sup>25,45</sup> This effect may help to ensure that fitness is preserved. Interestingly, one aspect of the Tasmanian devils' life history is crucial to the spread of DFTD, namely the aggressive behavior males display toward other males when competing for mates.<sup>25,45</sup> The biting that occurs is one of the primary means by which DFTD is transmitted among the population.<sup>25</sup> Therefore, an evolutionary stable strategy<sup>49</sup> would ensure a balance between the benefits of aggression toward other males (not being infected) and the reduction in reproduction.<sup>45</sup> An evolutionary stable strategy, first proposed in 1972, is a strategy that, when adopted, is 'iron clad,' that is, is impenetrable by any other strategy as it ensures the highest fitness.<sup>49</sup>

## Immune Response to DFTD in Devils

Despite the moderately low genetic diversity among the devil population, research has shown that these marsupials are fully capable of immunologic rejection of foreign agents characterized by a robust immune response.<sup>21,22,23,41</sup> DFTD cells treated with the cytokine IFN $\gamma$  have demonstrated an immune response characterized by T-lymphocyte infiltration of the tumor eventually leading to regression.<sup>41</sup> This response, however, is relatively rare.<sup>41</sup> When skin grafts containing foreign MHC molecules were transplanted onto devils to illicit immunologic rejection, devils showed a robust immune response and rejected the tissue within 14 d, invalidating the claim that low genetic diversity has made the devil population susceptible to DFTD.<sup>22,38</sup> It is more likely that the mechanism employed by the tumor allograft itself allows it to proliferate.

In addition, devils can be induced to show cytotoxic and humoral immunity when treated with irradiated DFTD cells combined with adjuvants—montanide and CpG oligonucleotides.<sup>21</sup> Montanide is a mixture of oil and water combined with a specific antigen designed to enhance the immune system's cytotoxic T-lymphocyte response.<sup>33,21</sup> It is a type of immune modulator currently being studied to enhance the body's response to cancer treatment. The CpG oligonucleotides are short, single-stranded DNA molecules containing a modified phosphate backbone; that is, a phosphorothioate backbone as compared with phosphodiester backbone.<sup>55</sup> CpG molecules have shown to be powerful stimulators of immune cells, and they may work by activating toll-like receptors.<sup>55</sup> Toll-like receptors (TLR) are transmembrane proteins that belong to a family of pattern-recognition receptors.<sup>19</sup> TLR can act extracellularly, by detecting various portions of microbes or bacteria, or even intracellularly, functioning in the endogenous recognition of viral particles, foreign fragments, and the like.<sup>19</sup> When a TLR binds a molecule derived from a

pathogen, a signal cascade is initiated, which leads to the activation of the adaptive immune system, among other immune responses.<sup>19</sup> TLR are known to recognize unmethylated CpG oligonucleotides which are present in bacterial DNA.<sup>19</sup> In addition, the TLR family activates a specific transcription factor, NF $\kappa$ B, which is a crucial element in the immune response in vertebrates.<sup>19</sup> Among devils tested for immunity against DFTD, one animal showed a transient immune response after a second challenge with DFTD cells that were not irradiated, after an initial injection of irradiated DFTD cells and immune system adjuvants.<sup>21</sup> A booster injection was required for this devil to reject the challenge initially, however, a subsequent challenge in this devil resulted in tumor growth, indicating that continuous vaccination would be required for any long-term immunity.<sup>21</sup> The cited study<sup>21</sup> may give evidence to support further vaccine development. Large-scale results are needed, but given the endangered nature of this species, it is difficult to conduct appropriate studies with reliable controls. Nonetheless, the study<sup>21</sup> showed promising results, suggesting that perhaps supplementing an immune response in devils with adjuvants and activation of TLR may lead to successful immune responses to DFTD.

Another promising drug used in the treatment of DFTD is imiquimod (Aldara).<sup>35</sup> Imiquimod is currently being used in human cancers (melanoma)<sup>10</sup> as an immunotherapy agent that activates both the innate and adaptive immune response and is effective in treating certain tumors.<sup>10,16,54</sup> Imiquimod has been shown to activate TLR7, in addition to many apoptotic pathways, most likely by enhancing cytokine release.<sup>10,16,54</sup> Interestingly, imiquimod tends to be effective due to its specificity in certain human cancers. The drug can selectively target and kill malignant cells without affecting normal cells.<sup>10</sup> One team demonstrated that imiquimod-treated DFTD cells underwent apoptosis upon continued treatment, and, after 120 h, total apoptosis had occurred.<sup>36</sup> Continuous treatment was required to maintain the reduction in DFTD tumor cells.<sup>36</sup> Imiquimod is known to regulate the transcription of antiapoptotic genes,<sup>54</sup> which is a TLR-independent mechanism. In normal devil fibroblast cells, both pro- and antiapoptotic proteins likely are differentially regulated to ensure appropriate apoptosis of damaged or foreign cells.<sup>36</sup> This regulation may not occur in DFTD and might be a pathway elicited by imiquimod in triggering apoptosis.

### Genetic Characteristics of DFTD and CTVT

The devil genome is diploid consisting of 6 pairs of autosomes plus an additional pair of sex chromosomes, in which the male is the heterogametic sex (XY), similar to most mammals.<sup>39</sup> The Tasmanian devil belongs to the family of marsupials known as Dasyuridae.<sup>39</sup> This family of marsupials has displayed a highly conserved genome over time, enabling karyotype comparison as well as the ability to map the devil genome.<sup>39</sup> The first DFTD karyotype was matched in 2006 and since then, multiple subclones have arisen. These subclones, however, are characterized by a few rearrangements and little variation, and the baseline tumor genome has remained relatively conserved. Chromosome painting results support the hypothesis that DFTD arose from a single progenitor cell line that likely was from a female devil.<sup>39</sup> Considering that DFTD identifies as a tumor cell line, the random mutational process that is a hallmark of most tumors and characterizes their genetic instability has not been found in DFTD. Tumor cell lines observed in vitro and in vivo have gone through as many as 10 cycles of replication without any significant rearrangements or alterations to the normal DFTD genome.<sup>39</sup> This outcome is unexpected, given the mitotic rate of most neoplasms.

Three strains of DFTD have been identified across the island of Tasmania; subclones have been identified for each of the 3 strains, and tumors have sometimes contained multiple subclones within the same host,<sup>39</sup> suggesting that transmission likely occurs through the passage of multiple cells and not just a single cell. The exact mechanism through which DFTD has developed into subclones but retained its characteristic karyotype is not yet fully understood. The hypothesis of chromothripsis—in which tumor progression is characterized by a burst of rearrangements, breaks, and nonhomologous repair in a very small portion of one or a few chromosomes—might explain the variation and rearrangements seen in DFTD.<sup>44</sup> Chromothripsis deviates from the traditional model of oncogenesis, in which mutations accumulate over time. DFTD chromosome painting revealed high-density regions of breaks and rearrangements in a few chromosomes, 1 and X in particular, and the authors suggested that chromothripsis might be associated with degeneration of the telomeres.<sup>39</sup> Telomere length varies among chromosomes, and various chromosomes are more predisposed to telomeric degeneration than others.<sup>39</sup> Cytogenic differences between tumor cell strains are low, and intrastrain and interstrain differences are of similar magnitudes.<sup>39</sup> Intrastrain and interstrain differences suggest that tumor evolution is occurring while the underlying characteristic karyotype remains stable.<sup>39</sup> For example, chromosome 1 is hypothesized to be the chromosome that underwent the initial rearrangement in the progenitor cell.<sup>39</sup> All DFTD strains seem to maintain the rearrangements in chromosome 1, suggesting its importance in the survival of the tumor.<sup>39</sup> Although chromosome 1 rearrangements are consistent among clones and subclones, chromosomes 4, 5, and X show variable rearrangements.<sup>39</sup> A combination of genetic instability and natural selection may be at play. Genes essential for survival and proliferation may be conserved while other nonessential genes may be free to mutate. Mutations in 4, 5, and 6 appear to be neutral.<sup>39</sup>

Since its origin, CTVT has spread to nearly every continent. Estimates suggest that the original progenitor cell arose anywhere from 6000 to 10,000 y ago.<sup>43</sup> Tumors sampled from different continents have little microsatellite variation when compared with each other,<sup>43</sup> suggesting CTVT is likely due to a single origin that is now spreading clonally, as occurs in DFTD. Genetic characteristics of CTVT suggest that it is transmitted asexually as an allograft.<sup>43</sup> The most recent common ancestor of extant tumors is dated to only a few hundred years ago, well after the emergence of the first progenitor cell.<sup>43</sup> The typical domestic dog has a diploid genome (2n) consisting of 76 autosomes, as well as a pair of sex chromosomes.<sup>43</sup> In contrast, CTVT shows a reduction in chromosome number (2n = 57 to 59) that is observed in tumor samples from different continents.<sup>43</sup> CTVT possesses a characteristic long interspersed element (LINE), which is a transposable element that inserts nearby a known oncogene, such as *myc*.<sup>12,43,46</sup> LINE are retrotransposons capable of inserting and removing themselves from the genome.<sup>12,46</sup> Humans possess these transposable elements, but most of them are inactive and do not undergo transcription.<sup>26,46</sup> The only active LINE in the human genome today is LINE1.<sup>26,12,46</sup> This characteristic transposable element may have been crucial to the early success and divergence of CTVT, given that it has likely been maintained over time.<sup>1,43</sup> CTVT can transfer to a large variety of hosts, including even distantly related canid species, such as foxes.<sup>43</sup>

Recently, a second genetically distinct transmissible cancer (DFT2) was identified in the southern portion of the island.<sup>42</sup> The characteristic genetic mutations present in DFTD are not

seen in this emerging transmissible cancer.<sup>42</sup> DFT2 possesses X and Y chromosomes and does not stain positive for periaxin, the diagnostic marker for DFTD.<sup>42,52</sup> Although this second strain appears to be cytogenetically distinct from the original DFTD, it is identical in gross morphology and phenotype.<sup>27,42</sup> DFT2 demonstrates the same transmission patterns (allograft transmission) as DFTD.<sup>42</sup> The identification of a second distinct form of DFTD challenges the notion that transmissible cancers are rare entities. Whether DFT2 alters the evolutionary landscape and the manner in which DFTD and DFT2 interact in the host population is unknown as yet but will provide new insight into transmissible cancers.

## Conclusion

The 2 transmissible cancers seen in vertebrates provide a unique insight into host-tumor dynamics and a nuanced perspective of immunosurveillance concerning malignancies (Figure 2). Typically cells with neoplastic potential are rejected by the innate and adaptive immune systems as nonself. However, in cases where an individual is immunocompromised, allorecognition sometimes fails to occur. Both canines and Tasmanian devils have been shown to have competent immune systems with the ability to reject foreign grafts. The transmission of these 2 cancers in their respective hosts therefore presents an aberration to the norm. DFTD and CTVT have provided an in-depth perspective of how immune system downregulation might occur in not only these transmissible cancers but also other malignancies as well. To date, no known transmissible cancers have been found in humans, other than cases in which the host is immunocompromised. In such instances, the transmission of cancer can occur during tissue grafting.<sup>37</sup>

Furthermore, many of the same pathways elicited by typical cancers have been used by DFTD and CTVT with modification. Ongoing research is warranted to ascertain how these transmissible cancers continue to affect host populations, especially among Tasmanian devils. Immunologic studies have shown promising results for vaccine development. Tasmanian devils represent a keystone species on the island of Tasmania, and its extinction will likely have major ecologic effects on the ecosystem as a whole. Conservation efforts are therefore warranted, and a prudent approach needs to be considered for a species teetering so closely to extinction.

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