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Research priorities in the Tasmanian devil facial tumour debate

Priorità della ricerca nel dibattito sul tumore facciale dei diavoli della Tasmania

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Summary

Against a résumé of current understanding of the nature, incidence and spread of the Tasmanian devil facial tumour (DFT), this script is presented with the aim of drawing attention to two particular research priorities; both are currently under-rated and under-funded. This wildlife disease was first recognised over ten years ago in the extreme north-east region of Tasmania. The invariably fatal, infectious neoplasm has affected devil populations covering two-thirds of the island of Tasmania and official estimates recognise that over half of Tasmania's devil population, initially estimated at ~150,000 animals, have died as a direct result of the neoplasm's transmission between wild devils. A significant turning point in defining the pathobiology of this disease was the identification of the near identical determinants of the key *histocompatibility* genes amongst the DFT-affected eastern Tasmanian devil populations and of the clone of neoplastic cells that is the transmissible agent of this disease. Genotyping now represents the best diagnostic tool for detecting individual devils with dissimilar immunogenetic determinants. Currently the transmissible tumour is entering the geographically separate western devil popu-

Riassunto

Sullo sfondo di una sintesi delle attuali conoscenze sulla natura, sull'incidenza e sulla diffusione del tumore facciale dei diavoli di Tasmania (DFT), viene prodotto questo lavoro al fine di porre l'attenzione su due particolari priorità della ricerca; entrambe sono attualmente sottostimate e scarsamente finanziate. Questa malattia dei selvatici è stata riconosciuta per la prima volta più di dieci anni fa nell'area all'estremo nord-est della Tasmania. Il tumore infettivo, sempre letale, ha colpito la popolazione dei diavoli sui due terzi dell'isola di Tasmania, e le stime ufficiali riconoscono che più di metà della popolazione di diavoli della Tasmania, inizialmente valutata in circa 150.000 esemplari, è morta come diretta conseguenza della trasmissione del tumore fra i diavoli. Una svolta significativa per definire la patobiologia di questa malattia è stata l'identificazione di determinanti quasi identici dei geni chiave di istocompatibilità tra la popolazione di diavoli nella Tasmania orientale affetti da DFT e del clone di cellule neoplastiche che è l'agente trasmissibile di questa malattia. L'identificazione del genotipo rappresenta ora il mezzo diagnostico più attendibile per individuare i singoli diavoli con determinanti immunogenici dissimili. At-

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lation; the population offering the greatest prospect of individuals with innate immunogenic resistance to DFT. This research warrants the very highest priority so that its potential to save the species is fully explored. A second research area, all but neglected, is a truthful investigation of the local environmental conditions that preceded the index outbreak within the high density devil population in north-east Tasmania. Based on current knowledge of this unusual new neoplasm, it is becoming increasingly likely that the genesis and effective transmission of this disease was the fateful culmination in a cascade of anthropogenic land-use activities and can more specifically be linked to a toxin-related aetiology occurring in a wild, carrion-feeding marsupial that had reached unprecedented numbers by the 1990s. The spontaneous genesis for this transmissible cell clone commenced in a devil population that already had considerable loss of genetic diversity. Understanding the sudden emergence of a transmissible cancer within a species surely warrants greater attention. Eur. J. Oncol., 13 (4), 229-238, 2008

Key words: **infectious malignancy, marsupials, Tasmanian devil facial tumour (DFT)**

tualmente il tumore trasmissibile sta entrando nella popolazione occidentale di diavoli geograficamente separata, popolazione che offre la maggiore prospettiva di individui con resistenza immunogenica congenita al DFT. Questo studio richiede la più alta priorità in modo che la possibilità di salvare la specie sia pienamente esplorata. Una seconda area di ricerca, quasi trascurata, è un attento studio delle condizioni ambientali locali che hanno preceduto la comparsa dell'evento tra la popolazione di diavoli nella Tasmania nord-orientale. Sulla base delle attuali conoscenze di questo nuovo tumore raro, diviene sempre più verosimile che la genesi e l'effettiva trasmissione di questa malattia sia stata la conseguenza inevitabile di una cascata di attività umane di trattamento dei terreni e più specificatamente abbia un'eziologia legata ad una tossina presente in un marsupiale selvatico portatore del gene che ha raggiunto numeri senza precedenti negli anni '90. La genesi spontanea di questo clone cellulare trasmissibile è insorta in una popolazione di diavoli che già aveva una notevole perdita di diversità genetica. La comprensione dell'improvvisa comparsa di un tumore trasmissibile in una specie richiede certamente la massima attenzione. Eur. J. Oncol., 13 (4), 229-238, 2008

Parole chiave: **tumore infettivo, marsupiali, tumore facciale dei diavoli di Tasmania (DFT)**

Introduction

Devils *(Sarcophilus laniarius syn. harrisii)* are now entirely restricted to the 68,330 km^2 island of Tasmania, although they were once more widespread on the Australian continent. A population estimate range of between 130,000 and 170,000 devils was made just prior to the discovery of the devil facial tumour disease (DFT) at its index location in north-east Tasmania in 1996[1]. Several government reports referred to devil numbers during the 1990s being at unprecedentedly high numbers. The invariably fatal facial tumour disease acted as a contagion within the devil population but it did not become the subject of intensive investigation until late 2003, by which time devils at several locations in eastern and central Tasmania were grossly affected with facial tumours[2]. By 2004 the field studies had demonstrated a pattern of apparent spread of the facial tumour disease from the eastern and central regions of Tasmania into other devil populations in south-eastern, central highlands and central northern regions[3]. By the end of 2006 infected devils were present in over half the island and the prevalence of these tumours in some populations had reached over 80%[4]. Absence of DFT in the western devil populations has been variously attributed to (a) distance decay and slow rate of spread of infection from diseased core areas or (b) insufficient sampling amongst the western population or (c) the possibility of western devils having an

immune response capable of recognising and altering their response to the tumour. The critical impact of the tumour on devil demographics was that of removing sexually mature and the older age cohorts – both males and females – from the population. In the early years of the monitoring it was uncommon to identify the disease in juvenile or sub-adult devils, however in recent years sub-adults and devils less than 2 years of age are affected. After at least 6 years in which DFT has been present in some well-studied populations (Mt William and Freycinet National Park), female devils are now breeding for the first time in their second rather than in their third year of life[5].

In the period since the Senior Scientist's forum on the devil facial tumour in February 2007, research has tended to follow closely on the ideas then espoused[6]. Two avenues have since become ever more obviously crucial and yet undervalued for the objective of saving the Tasmanian devil from extinction in the wild.

In addition to the state-sponsored DFT activities, independent research from noteworthy DFT incidents or opportunistic sampling of affected devils destined for euthanasia has greatly increased knowledge, both conceptually and factually, of the *case definition* and *pathobiology* of this wildlife disease.

Learning from an outbreak of facial tumours at a Tasmanian wildlife park

In mid-March 2006 a captive female devil escaped from a commercially operated wildlife park situated in bushland at Mole Creek in an area previously known to contain DFT-affected wild devils. This female devil was recaptured outside the park boundary fence within a few days and returned to an enclosure she had shared with several other devils. At the time of her recapture it was noted that this devil had sustained obvious bite wounds to her head and face. In May a captive male devil sharing the enclosure with the female was noticed to have obvious skin wounds on his right cheek following intra-specific fighting. By mid-June his wounds had begun to discharge and the soft tissue of the cheek began to swell; the open wounds were repeatedly treated as septic discharging bite wounds. By October both devils were confirmed to have advanced facial tumours and an epidemiological review of the disease outbreak was undertaken. During the devil-mating months two wild devils with DFT were trapped in close proximity to the wildlife park perimeter fence; both had advanced ulcerated tumours on their lips and rostrum.

This escape incident undoubtedly led to the transfer of the DFT infection into a previously DFT-free population of captive devils and whilst it was an unfortunate accident, it represented the first actual opportunity to assess the plausibility of the so-called tumour cell *allograft* theory of DFT transmission[7] and to collect timeline data on the incubation or latent period between exposure incidents and the development of obvious tumours. The initial captive DFT-infected devil and the subsequent transfer of DFT to other captive devils provided important data on the tumour growth rate and the effect of developing tumours on affected devils. From the review of all four cases in this outbreak the following conclusions could now be made[8]:

- an 'infectious' wild devil was the only plausible source of the facial tumour infection in the first captive female devil;
- significant intra-specific facial biting was the most likely means of infectious transfer firstly from the wild devil to the index captive devil and secondly between all captive devils that developed DFT lesions; and
- within approximately two months the original captive devil, in contact with other captive devils, had itself become capable of infectious transfer and then had successfully infected another captive devil inmate. When DFT was discovered in the index captive devil she had an open ulcerous tumour located on the inner lips.

Observations on the grossly ulcerated appearance and the actual anatomical location of the tumour masses (on the inner lips and appositional to the *canine* teeth) reinforced a transmission mechanism relying on penetrating bite wounds inflicted by one or more of the four canine teeth. Previous observations on the gross anatomical pathology of facial tumour disease demonstrated that these teeth were the *only* effective means for transferring viable facial tumour cells dislodged from such adventitiously located open tumour masses from an "infectious"

devil (the donor) to another devil (recipient). This infection scenario required that "infectious" tumours had exposed or ulcerated surfaces and were lying in close proximity to the agents of transmission – namely the long, conical canine teeth, such that these teeth became continuously contaminated with viable tumour cells dislodged from the tumour[9]. In addition such infectious individuals, by virtue of the frequency of their fighting/biting interaction with other devils, could be super-infectors for DFT transmission.

Cytogenetic and immunogenetic studies

By mid-2004 the pattern of the disease in affected populations suggested that natural transmission was almost certainly through biting transmission with the likelihood that the actual tumour cell itself was the 'infectious agent of disease'; in other words, the disease was a transmissible neoplastic cell-line[10]. Pearse and Swift[7] had confirmed that facial tumour cells from a number of devils all had an identical chromosomal rearrangement and that this aneuploid karyotype was remarkably stable, appearing identical both within the same tumour mass and between tumours derived from several DFT-affected devils. These researchers also presented the G-banded karyotype of a particular devil comparing the chromosomes from its normal cells and that of its tumour cells. They concluded that the banding differed to the extent that this DFT-affected devil was clearly not the source of its own tumour growth. The discovery gave support for a devil-to-devil *allograft* cell transfer mechanism for the facial tumour disease. Subsequently the first experimental transmissions of DFT cells were successfully carried out by inserting either tissue-cultured tumour cells or pieces of tumour tissue from wild affected devils into captive devils[10].

The term *syngeneic* tumour has been offered to describe more precisely a neoplasm derived from a transformed cell clone and capable of successful entry and proliferation amongst a group of genetically-related individuals (Kast WM, personal communication, 2007). Immunological work by Woods *et al*[11] demonstrated that devils have competent immune responses capable of mounting innate and humoral immune protection to foreign bodies and pathogens as well as effective cell-mediated immune responsiveness to T and B-cell mitogens. As a simple test of the effectiveness of devil-devil immunological recognition, peripheral lymphocytes from a large number of devils were subjected to *in vitro* mixed lymphocyte cell reactions; the responses were generally weak amongst several eastern devil cohorts (Woods GM, Kreiss A, personal communication, 2008). In 2007 preliminary research into the major histocompatibility (MHC) genes in the devil had commenced. The sequencing of these genes in the devil and comparing those sequences with those from tumours isolated from affected devils indicated that the tumour cells had identical MHC determinants and that all affected devils in eastern populations were also expressing identical MHC genotypes[12]. The only devils that showed a greater diversity in their MHC genes were those from geographically remote north western and far western parts of Tasmania. Such immunological and histocompatibility studies provide further evidence that devils from eastern populations are not capable of recognising facial tumour cells as 'non-self' in that the neoplastic cells originated from within a genotype cohort with extremely limited MHC gene diversity. Thus the pre-existing immunogenetics account for the successful transfer of facial tumour cells between devils within the whole eastern devil population.

How does infectious transmission of facial tumour cells occur?

Whilst pre-existing immunogenetics within a host species might successfully complement direct devil-to-devil disease transmission, the mechanism whereby DFT-infected devils actually become infectious required further assessment. Observations on the biting behaviour of devils involved in intraspecific fighting and anatomical examinations of devil skulls demonstrated that the four canine teeth were the *only teeth* anatomically capable of inflicting deep penetrating wounds into the soft tissue of another devil. To assess the potential *infectiousness* of devils affected with facial tumours Obendorf *et al* [13] categorised a number of free-ranging devils as to whether tumours were within the

oral cavity or on the facial skin and also as to whether the tumours were totally enclosed by intact skin or oral mucosa, or were open tumours with discharging sinuses or exposed ulcerated surfaces. Using cytological brushes (Endoscan + Plus™ cytology brushes, Medico, Melbourne, Australia) to collect free cells, Haem-quik stained smears of the surfaces of naturally moistened canine teeth and their tooth-gum junctions were prepared from anaesthetised devils with facial tumours located in various facial/oral sites. Each devil was also grossly assessed according to whether tumour cells could dislodge from a tumour mass and survive as free cells in the environment of the oral cavity. Smears from each canine tooth were examined microscopically for the presence or absence of the distinctive tumour cells and the results were compared to the photographic appearance of their facial tumours.

Several DFT-affected devils with ulcerated tumours in close proximity to one or more canine teeth showed that the teeth surfaces opposed to the exposed surfaces of tumour masses had caused the tumour to develop a distinct concavity into which the appositional canine tooth rested when the jaw was closed (fig. 1). The number of devils available for oral cytology was small (n=20), however, the cytological screening repeatedly showed that numerous identifiable tumour cells – as single cells or aggregates – could be recovered from those canine teeth in direct contact with ulcerated facial tumours (fig. 2) whereas smears from other canine teeth not in direct contact with these open DFT lesions were free of tumour cells. Smears from all devils with (a) un-ulcerated oral tumours, (b) skin-enclosed facial tumours or (c) tumours only discharging onto the facial skin had no tumour cells in the smears prepared from their canine teeth.

By applying such knowledge, the relative infectiousness of specific wild devils can be gauged based on these tumour-tooth associations. These cytological observations also demonstrate an actual mechanism for DFT cell transfer from infectious devils through the inoculation of viable cells passively transferred onto the surface of its canine teeth and thence into a newly created deep soft tissue wound in a recipient devil through biting. This study of a natural transmission mechanism supports the developing case definition of facial tumour disease and

Fig. 1. *Post mortem* resection of the muzzle depicting an ulcerated DFT lesion with a concavity (*) produced through contact with lower canine tooth
Scale: maxillary canine tooth from gum to tip length ~ 2 cm

was important new knowledge in redrafting the DFT-risk categorisation and biosecurity measures for management of DFT-free captive devils[14].

East-west devils: immunology and genetics

Prior to the emergence of DFT a level of genetic variability amongst micro-satellite loci had been noted in devils from western parts of Tasmania differentiating them to some degree from the eastern populations[15, 16]. Over the whole island the allelic diversity of Tasmanian devils is still quite low with a heterozygosity index of H_E = 0.39-0.47. However, there were sufficient unique alleles at these micro-satellite loci within the western devil subpopulation

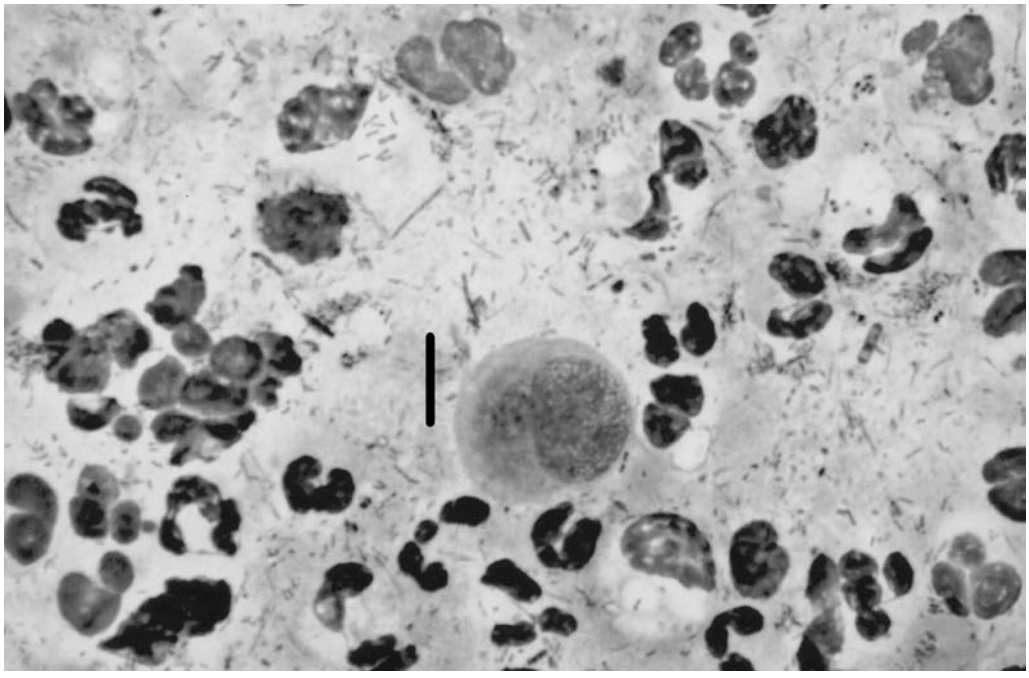

Fig. 2. High magnification of an intact DFT cell taken from the surface of a canine tooth adjacent to an ulcerated tumour
Haem-quik stain: scale bar 10 μm

to offer optimism that the MHC genes might also show greater genetic variability. Recent work by Siddle *et al* [17] indicates that there is indeed greater variability in these gene sequences amongst western devils.

These genetic findings combined with the immunological findings of Woods *et al* [11] sustained "a proof of concept" hypothesis that some devils in the western population – by virtue of their non-matching histocompatibility – might show a different response to DFT cells. Earlier field work[3] monitoring the spread of DFT across Tasmania had demonstrated that vehicular roads by permitting easy movement and abundant roadkill carrion for wild scavengers also allowed diseased devils to migrate into new locations to the south and west. Field observations of Coupland and Anthony[18] have given the first indication that DFT-affected devils moving into new populations in the western parts of Tasmania do not necessarily lead to a dramatic emergence of overt facial tumours amongst the local devils, nor to the decline in the population as seen in eastern Tasmania[3]. Their study involved monitoring the devils within the Cradle Valley area of north western Tasmania using infra-red sensors and cameras to identify individual devils and using images of their heads to determine facial tumour presence over time. After one and a half years of monitoring only four devils in a population of 77 showed visual signs of facial tumours. A longitudinal study at West Pencil Pine (approximately 10 km to the west of Cradle Valley) has also shown a low prevalence of DFT-affected devils within that population two years after the first evidence that a single DFT-infected devil had entered the population (Hamede R, personal communication, 2008).

In the second half of 2007 two devils with western Tasmanian parentage were used in a facial tumour vaccination study; both devils were inoculated subcutaneously with irradiated DFT cells plus adjuvant followed by booster inoculations to increase their antibody response. One devil [*Cedric*] developed high titres of tumour-specific antibody whilst the other devil [*Clinky*] failed show any tumour-specific seroconversion (Woods GM, Kreiss A, personal communication, 2008). In late 2007 both devils were challenged with 25,000 viable tumour cells injected into two sites; one under the oral mucosa beside the mandible and a second intradermally in the side of the cheek. Twelve weeks later *Clinky,* the devil that failed to develop tumour-specific antibodies, had developing swellings at each challenge site, whereas *Cedric,* the devil that developed antibodies, has remained lesion-free ever since. The period that *Cedric* has remained tumour-free has

now exceeded six months[a]: the longest time period that a wild-caught devil has remained lesion-free in captivity before facial tumours developed. Regular biopsies in *Clinky* have confirmed tumours typical of the histopathology of DFT at both injection sites.

Coincidentally the sero-negative devil that developed facial tumours after challenge had MHC genes that were identical to all eastern devils that are succumbing to devil facial tumour in the wild. The devil that developed antibodies to the tumour and has remained tumour-free for twelve months post-challenge has MHC gene sequences that are different to the inoculated tumour and representative of other wild devils found in western population. The question now being asked is whether this immunogenetically competent devil, irrespective of vaccination, would have innately resisted the establishment of the tumour cells based solely on the genetic difference in its MHC genotype.

Future research priorities

In the last 12 months very significant advances have been made in defining the complex pathobiology and natural transmission mechanisms for DFT. In terms of future research there are two areas capable of making a substantial contribution to the continued survival of the Tasmanian devil as a free-living wild marsupial; neither has been given sufficient consideration in future research planning.

Detection of innate immunogenetic resistance to DFT

Through collaborative efforts amongst several research teams, the immunogenetic characteristics of different populations of devils are now being studied following the breakthrough discovery that some western provenance devils like *Cedric* may have natural or innate resistance to the establishment of the transmissible facial tumour cells that originated in an eastern devil population. Encouraging field research supported by immunogenetic studies suggest that at least some western devils have sufficient MHC variations to be capable of recognising these cells as foreign or non-self[17]. The western devil populations at locations where the migration of DFT-affected eastern devils has been detected offer important sites for screening wild devils for the presence of tumour-specific antibodies. Capture-mark-recapture studies to identify the MHC type of all individuals followed by ongoing population surveillance would ensure the detection of: 1) sero-positive devils, and 2) devils that develop overt disease. These devil populations at the east-west interface offer the greatest opportunity to determine whether there is a correlation between the MHC type and any innate (immunogenetic) resistance to DFT. Indeed if this host resistance mechanism were naturally occurring on a wide scale, survivorship of resistant devils may limit the transmission of the current DFT cell strain with its MHC isotype. The present priority is to allow this diagnostic surveillance to occur within these western populations. Based on the results and its application for further free-range reproduction of devils, the current DFT isotype might cease transmission simply by running out of devils with the same histocompatible genotype to infect.

Encouraging as this might seem there are new discoveries that need to be considered. After a long period of apparent stability in the chromosome rearrangement of the original DFT strain, in the last twelve months or so, several new chromosomal rearrangements – predominantly expressed as polyploidy – are being recovered from the wild devil populations where the disease has been endemic for many years[19]. The effect of these new facial tumour strains on the epidemiology of DFT is unclear. In our view, determining the MHC type diversity of wild devil populations is an essential prerequisite in the future management of the species in free-living populations.

Anthropogenic factors and the significance of poison exposure to DFT genesis

The second under-recognised but worthwhile objective should be research into the environmental triggers and potential anthropogenic factors associ-

[a] Following a second challenge using another isolate of viable DFT cells, Cedric developed small subcutaneous tumours in mid-December 2008 at the sites of inoculation. This occurred 9 months after this devil was last vaccinated with irradiated-DFT cells

ated with the index event of DFT genesis, with particular attention given to the situation that prevailed in north-eastern Tasmania where the earliest cases of DFT were identified. The importance of knowing the how and why of the initial emergence of a transmissible cancer has obvious relevance to mankind's stewardship not only of the devils' environment but also of his own. Only when the aetiology of the index incident is understood can there be greater confidence that identical pre-conditions do not recur. In order to justify this line of research the following brief summary is provided.

In Tasmania there has been a long history[20] of the deliberate use of poisons including organophosphates (OPs), specifically to target wildlife considered to be "nuisance" animals or "vermin". This goes back to government sanctioned vertebrate research in the early 1960s using mevinphos (2-methoxycarbonyl-1-methylvinyl dimethyl phosphate) and HETP (hexaethyl tetraphosphate)[20]. Subsequently agricultural OPs have been detected in malicious chemical poisoning incidents involving Tasmanian avian and mammalian species. Some OPs have been shown to cause sub-lethal toxicity by their interaction with biologically-active esterases other than acetylcholinesterase. Human case studies demonstrate that no symptoms are seen immediately post-exposure but neuropathy with muscle paralysis may set in long after residues of the chemical have disappeared from the body. OP-induced delayed neuropathy symptoms are recognised as starting with the ageing of phosphorylated enzymes targeted by OPs[21]. In human subjects organophosphates are known to cause neurotoxic, immunotoxic and genotoxic effects including a variety of significant chromosomal alterations[21, 22]. There are limited demographic studies assessing the impact of sub-lethal exposure to OPs in mammals, however, chromosomal alterations have been readily detected in peripheral lymphocytes collected from humans following sub-lethal exposure to OP pesticides[22].

Predatory species, such as devils, are especially at risk from the environmental use of poisons. They have been directly targeted through exposure to purposely poisoned carcasses or meat baits, and secondarily targeted through eating the remains of other poisoned animals, such as unwanted herbivores. Depending on the amount and potency of toxic residues consumed, secondary poisoning can lead either to mortality or to sub-lethal exposure. There are reasonable grounds for concern about the consequences to wild vertebrate targets of exposure to OPs in the natural environment and the priming of genotoxic effects.

During the twentieth century three distinct periods occurred when Tasmanians used poison to target nuisance wildlife or introduced pest species with exposures especially significant to carrion-feeding carnivores, principally in those areas used for agriculture. Firstly, strychnine was widely used from the late 1860s to 1950 associated with a dramatic and well recorded decline in devil numbers. It is our contention that the impact of the wide scale use of this poison for so many decades was arguably the basis for the loss of genetic diversity within the devils of eastern and central Tasmania. In addition it is well acknowledged that Tasmanian fur-trappers specifically targeted devils with strychnine-laced baits because of the destruction they caused to their snared game. By the 1930s the devil was considered a cryptic and rare animal in the settled regions of Tasmania and by 1941 it was listed as a wholly-protected wild animal.

Secondly, after 1952, Compound 1080 (sodium monofluoroacetate) replaced strychnine and other non-specific poisons as the common agricultural poison to kill vermin or pest animals in Tasmania. The abundance of 1080-poisoned carrion and the tolerance of devils to the lethal effects from secondary 1080-poisoning[23] are sufficient to explain the dramatic resurgence in devil numbers over the next three decades. By the 1970s and '80s, the increase in devil densities in particular regions and their characteristic communal feeding behaviour at carcases was being recognised[24]; competitive and overt aggressiveness towards each other, facial biting and cannibalism were being identified within devil populations[24, 25].

Thirdly, in the period between 1991 and 1998 the OP Mevinphos (Phosdrin™) with its known mutagenic and chromosomal altering properties was unlawfully used to target native wildlife in a location with very high devil densities[26, 27]. This episode had the potential to expose the devil genome sub-lethally to chromosomal damage sufficient to generate a

transformed cell and initiate a unique 'neoplasm': the DFT. This cascade of sequential intrusions on the devil populations by the use of poison also provides explanation for the historic fluctuations in devil numbers remarked upon by several authors[28, 29].

As discussed in the early section of this paper, the vast majority of the eastern genotype devils exposed to this transformed DFT cell do not recognise it as a foreign entity. These cells evade the devil's immunological surveillance, somewhat like "Trojan" cell grafts, entering, establishing and locally spreading in their new host devil just as they did in the donor devil. Had these devil populations possessed a greater degree of genetic diversity perhaps, in some devils, these introduced cells would have been recognised and removed by natural immunogenetic mechanisms.

Conclusion

As a result of the Tasmanian government's concern for the long-term survival of the Tasmanian devil in the wild, the species is now listed as "endangered" under the Tasmanian threatened species legislation. The senior scientist responsible for the "Save the Devil" Program has reported that the disease is likely to cause local extinctions within 15 years after its arrival and, based on current rates of spread, the disease will cover the geographic range of the devil within the next five years. He has warned that devils could become extinct in the wild within 20-30 years through the agency of this single highly lethal disease[30]. Current research suggest that this aneuploid devil cell is able to act as the actual agent of disease amongst other genetically-related devils with all the pathology observed in affected devils attributable to direct animal-to-animal transmission of this neoplastic cell clone.

In this paper, we have discussed two areas of research each so central to the pathobiology of DFT as to warrant major expansion of skilled endeavour. Firstly, to know whether MHC genotype variations can be used as an indicator of natural immunity to DFT among some devils in the wild population; this has obvious and widespread implications for saving the species. Secondly, to investigate the plausibility of a toxigenic trigger for the index cases of DFT in north-east Tasmania thus safeguarding any further harmful exposure of Tasmania's wildlife through neglect of the ecohealth consequences.

Tasmanian natural conservation managers have a responsibility truthfully to understand the harmful impacts that intervention with powerful toxins can cause to the life-sustaining ecosystem of the world's largest marsupial carnivores. They also have a duty to ensure that this unique transmissible neoplasm is properly investigated to ensure that all plausible aetiologies are thoroughly assessed.

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