

## What is happening to our Devils?

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### Background

Fears were first raised in June, 2001 when a possible link between the presence of facial tumours in the Tasmanian Devil (*Sarcophilus harrisii*) and a decline in the population was proposed. The tumours present as large, solid soft tissue masses usually with flattened, centrally ulcerated, exudative surfaces. They are typically multi-centric, appearing first on the mouth, head and/or neck regions. The tumours tend to be locally aggressive and infrequently metastasise (see figure 1). There is minimal cytological differentiation amongst the tumour cell population and so the syndrome has been named Devil Facial Tumour (DFT). The distribution of diseased animals are shown in Figure 2.

### Aims of the DFTD project

- To characterise the neoplasms and to identify their cytological origin.
- To develop a diagnostic test that can pick up early disease to help with management of the population.
- To prepare a diagnostic plan for identifying the cause of the tumours.
- To develop a management program to minimise losses in the devil population from the Devil Facial Tumour.

### Materials & Methods

Over 220 normal and affected tissue samples were collected from healthy and sick devils both alive and deceased in the field and in the laboratory. Tissue samples were processed for routine light microscopy, immunohistochemistry and transmission electron microscopy. As a preliminary definition we consider that any Devil with round to spindle neoplasms involving the subcutis and/or other organs has DFT. Those neoplasms that do not fit this criteria and are identifiable, are classified according to Meuten (2002).<sup>1</sup>

### Preliminary Results & Discussion

For the period between June 2001 and October 2004, more than 100 DFT cases have been examined grossly, histologically, immunohistochemically and by transmission electron microscopy. Histology remains the most specific test and gross observation of lesions being the most sensitive test for DFT. Histological examination of the tumours found the neoplastic cells were located predominantly within the sub-epithelial connective tissue of the skin or oral cavity (see figure 3). The neoplastic cells formed expansile to minimally infiltrative, large nodular aggregates. Delicate fibro-vascular septae partially divided the tumours into lobules which were disrupted by scattered areas of necrosis. The neoplastic cells ranged from round to polyhedral to elongate with abundant eosinophilic cytoplasm and often indistinct cell borders. Nuclei were round to ovoid with a stippled chromatin pattern. Mitoses range from 0-12, with an average of 4 field of 400x magnification. The entire DFT cells stained red with Massons trichrome and the cytoplasm was negative for toluidine blue positive granules.

The diagnostic value of a number of immunohistochemical stains were employed to characterise up to 50 representative cases (see figure 3). They were negative for cytokeratin (48/48), smooth muscle actin (15/16), CD-3 (10/18), CD-16 (13/13), CD-57 (43/43), epithelial membrane antigen (42/42), vWF (11/11), desmin (42/47), glial fibrillary acid protein (13/13). DFT was positive for melan A (34/44), vimentin (50/50), S-100 (39/47). The results are still inconclusive, however, these staining characteristics are consistent with cells derived from the neuroectoderm.

Transmission electron microscopy was conducted on 8 cases (see figure 4). There was a lack of differentiating characteristics. Nuclei were round, measuring 6-8µm in diameter and contained scattered condensed chromatin. Recognisable organelles include: rough endoplasmic reticulum, free ribosomes and polyribosomes, cytoskeletal filaments (were observed, but not dominant), mitochondria (large vesicular), secretory granules, endocytotic vesicles, golgi apparatus, centrioles, some ribosome-lamella complexes, some myelin bodies and the occasional desmosome-like elements. Desmosome-like elements are suggestive of epithelial-type cells, however, these were too infrequent to be conclusive. Ribosome-lamella complexes have been associated with leukaemia, but they also occur in other tumour cells. Myelin bodies are associated with cells of neural origin but are also seen in degenerating cells. No definitive conclusions can be drawn at this stage, but the DFT could be a form of neuroendocrine tumour (NET).

### Results from light microscopy

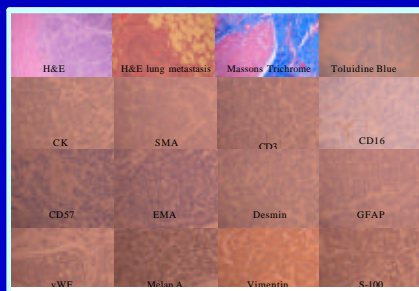


Figure 3. H&E, Masson Trichrome and Toluidine blue were photographed at 4x magnification. Immunostained slides photographed at 10x.

### Ultrastructural characteristics of DFT

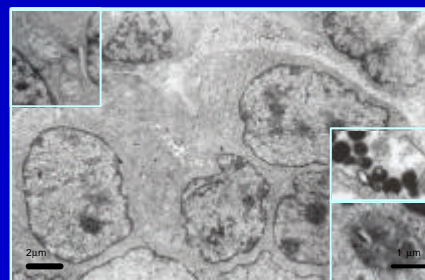


Figure 4. TEM images of the DFT cells. Top left inset shows vesicular mitochondria. Inset on the right are some granules and bottom right shows myelin figures.

### Gross presentation of DFT

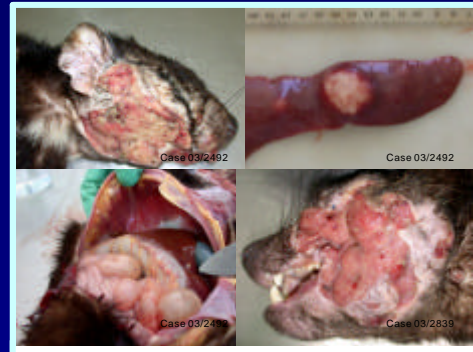


Figure 1. Two representative cases of DFT. Metastases are infrequent, but can sometimes be found in the liver, spleen and lung.

### Geographical distribution of DFT

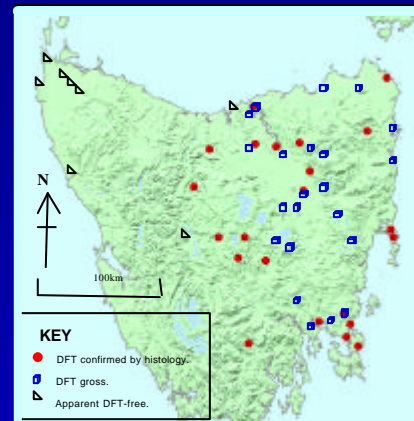


Figure 2. Map produced by Nature Conservation Branch, DPIWE, showing the extent of distribution of DFT in Tasmania.

### Future Directions

Characterisation of the gross, histopathological and ultrastructural features of the Devil Facial Tumour constitutes only one component of the investigation into DFT disease. By identifying the cytological origin of the cells involved, we may be able to draw information from comparative studies in other species and hope to understand what could be initiating and promoting these neoplasms.

Other studies running in parallel with this study include field surveillance to establish and monitor the progress of disease, transmission electron microscopy to look for possible oncogenic viruses and tumour cell culturing and cytogenetics to determine whether there is a genetic basis to DFT. Studies of their immune status is also underway to determine the overall health of the devils and comparing affected with unaffected populations.

### Acknowledgments

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### References

Meuten, DJM. 2002. Tumors in Domestic Animals, 4<sup>th</sup> Edition, Iowa State Press.