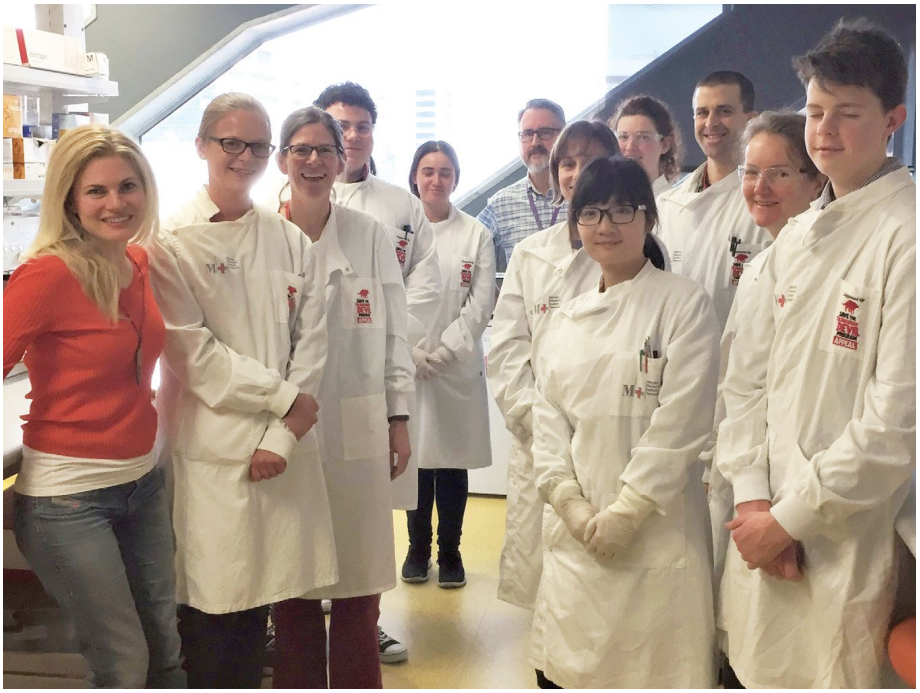
Luciferase comes to the devil's rescue

Wild Tasmanian devils are vulnerable to a facial cancer discovered in 1996 and identified as a transmissible tumor a decade later. The contagious disease originated in northeastern Tasmania and spread throughout the country, decimating the devil population and raising the real possibility of extinction. Scientists at the Menzies Institute for Medical Research, University of Tasmania, have pioneered research into the problem – drawing upon the latest developments in human immunology and bioluminescence cytotoxicity assays – in the hope of developing a vaccine to save the island's iconic marsupial.

The Tasmanian devil facial tumor disease is responsible for as much as a 90 percent decline in the population since 1996, proving fatal in nearly every case of infection. In 2014, a second genetically distinct and independent transmissible tumor was discovered with similar consequences – large lumps appear around the mouth and head that prevent eating and lead to starvation. The recent discovery of some disease-resistant devils suggests that the species may be rapidly evolving, but there is still no devil immune response to the tumor in the majority of the population.

Andy Flies, a postdoctoral research fellow at the University of Tasmania supported by the Morris Animal Foundation and a Commonwealth grant co-funded by Nexvet Biopharma, explained: "An organism's immune system should launch an attack on any foreign cells, which is what makes the transmissible tumors so intriguing. The cancer cells are transferred as an allograft when the devils bite one another, and our team is trying to figure out why there is no immune response. In a recent publication,[1] we showed that the key immune checkpoint molecule PD-L1 is upregulated in devil facial tumor cells in response to interferon-gamma – a cytokine released by activated T cells. The PD-L1 binds to PD-1 receptors on the T cells, inhibiting the antitumor immune response and enabling the tumor to evade detection. In short, the production of PD-L1 acts as an invisibility cloak."



The research team at the Menzies Institute for Medical Research with actress and ambassador Bonnie Sveen (left)

"The big headlines in recent human immunology have covered checkpoint molecule inhibitors. We're tracking what is working in human clinical trials, and trying to reverse-engineer and adapt it for our own use. Our latest publication compared key immune checkpoint molecules in nine different species, ranging from humans to bats to Tasmanian devils.[2] Despite the last common ancestor of marsupial and placental animals occurring 162 million years ago, we found a remarkable level of similarity in key regions for these critical immune molecules, suggesting that some immunotherapy or vaccine approaches for humans might also work in Tasmanian devils. The lack of devil-specific antibodies has been a real obstacle to progress in this area. Using antibodies from other species has yielded only moderate success, so we have developed around 50 monoclonal antibodies of our own against seven or eight different targets. We're starting to gather the necessary resources to carry out more advanced immunology, beginning at a similar point to human and mouse immunology 20 to 30 years ago with regard to the availability of species-specific reagents."

Andy continued: "To date, the chromium-51 release assay has been the gold-standard approach to studying cellular immune responses. Target tumor cells are labeled with the isotope and incubated with effector cells, and the immune response can be determined by measuring radioactivity, as the cancer cells release the isotope when they are killed. Working with radioactive chemicals is a hassle, as there are many regulations and chromium-51 has a really short half-life of only 21 days; after a single month, half of a $500-dollar isotope stock has decayed and, if you encounter a delay, you end up wasting your money."

"There was a need and a desire to move on and find an alternative method, and that's where the Spark® comes in. Our colleague, Nuri Guven, raised the funds to purchase the instrument, and I first became aware of the Spark's great potential after I attended a Tecan Spark workshop organized at the Menzies Institute for Medical Research. Nuri's previous experience with Tecan systems was very helpful in getting us up to speed on the instrument. In the new assay, I transfect the target tumor cells to express luciferase – an enzyme derived from the firefly – making them luminescent. The bioluminescence is proportional to the number of surviving tumor cells, so the cell survival rate can be measured by quantifying the luminescence. Although we have a few other readers in the department, they were not capable of carrying out this particular assay."

"The Spark software is pretty straightforward and the results from the first two tests confirmed that it was suitable for our needs. The cytotoxicity assay is really fast; running a whole plate takes about 90 seconds, and it is much better to run eight plates in 15 minutes than waiting for a chromium counter for two hours. I now use the Spark for all my ELISAs, due to its speed and the option to use different wavelengths. In the future, we plan to purchase the Humidity Cassette and Te-Cool®, which will enable us to incubate cells in the reader, allowing us to run kinetic assays with automatic injection overnight. Our ultimate hope is that these new assays will help us to develop a vaccine but, whatever the outcome, our research has implications for our understanding of human and veterinary cancer," Andy concluded.

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1) Flies Andrew, S *et al*. PD-L1 Is Not Constitutively Expressed on Tasmanian Devil Facial Tumor Cells but Is Strongly Upregulated in Response to IFN-γ and Can Be Expressed in the Tumor Microenvironment. *Frontiers in Immunology*, 2016, **7**, 513.

2) Flies Andrew, S *et al*. Comparative Analysis of Immune Checkpoint Molecules and Their Potential Role in the Transmissible Tasmanian Devil Facial Tumor Disease. *Frontiers in Immunology*, 2017, **8**, 581.

To find out more about the Spark's luminescence optics, visit
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To learn more about Andy Flies' research, visit
utas.edu.au/profiles/staff/menzies/andrew-flies