Can you briefly define and introduce your present research focus?

Microbial cancer immunotherapy is simply the use of a microbe, or its parts, to provoke an immune response that can be therapeutic. In the context of cancer, it was recognised hundreds, if not thousands, of years ago that inflammation, often associated with infection, could be beneficial in reducing or eliminating tumours. This was the vague conceptual origin of cancer immunotherapy, but it’s taken a long time to tame microbes into therapies fit for the clinic.

How did you come to study cancer immunotherapy?

My research has always centred on vaccine development, and the immune system’s amazing ability to recognise and control foreign pathogens, such as viruses or intracellular parasites. The mechanisms used by the immune system to seek out and destroy these intracellular pathogens are, in essence, the same mechanisms that the immune system must use to prevent cancer, or to attack it if it has already established a foothold in the body. Unfortunately, as with many infectious pathogens, developing cancers seize control over the immune system and shut down natural immune responses that could otherwise eradicate the tumour. With this shared theme of manipulation and immune trickery, it was quite natural to turn to the field of cancer immunotherapy.

The immunotherapeutic strategy outlined in your latest paper seems to play a permissive role, allowing for an innate immune response to ‘invisible’ tumours. Is this therapy therefore likely to be less deleterious to the patient than traditional interventions?

Achieving high specificity and low toxicity with immunotherapeutics will be challenging, because systemic application targets the whole body. However, in animal studies, immunotherapy with Toxoplasma gondii is strongest at the local tumour site, and we do not see any significant signs of uncontrolled systemic toxicity or inflammation.

Attenuated vaccines often impart so-called ‘heterologous effects’, benefits which cannot be explained by the remediation of the targeted disease alone. Might this anticancer property explain such non-specific benefits?

This is an intriguing question, since indeed one would assume a Toxoplasma vaccine should only confer immunity to Toxoplasma. This vaccine certainly confers a lifelong immunity to Toxoplasma, and this may turn out to be useful in the veterinary sphere. What is remarkable about the vaccine is that we see the full anticancer effect even if there is already immunity to Toxoplasma. Even after repeated treatments, the next treatment still boosts anti-tumour immunity. The innate myeloid cells are clearly responding to the Toxoplasma vaccine every time, and these same cells appear to be co-activated to quickly respond more aggressively to the cancer.

What are your hopes for the future of microbial cancer immunotherapy?

We hope to learn how to cure what are currently considered incurable cancers. In the near future, this could involve the direct use or vaccine engineering of our novel cancer immunotherapy platform. In the long term, this will likely also involve harvesting biological knowledge from immunotherapy to identify novel pathways and targets for developing more effective cancer interventions.
**Immune to cancer**

The Geisel School of Medicine at **Dartmouth College**, USA, is a hub for paradigm-shifting research in cancer therapy. Typifying the institution’s unconventional approach to oncotherapy, the use of parasite **Toxoplasma gondii** to reprogramme the immune system is among the School’s specialties.

**JUST TWO GENES** separate a pandemic parasite capable of infecting almost any warm-blooded creature from a therapy that offers new hope to those with the most refractory forms of cancer. With the use of a proprietary gene-targeting technology, immunologists at the Geisel School of Medicine at Dartmouth College, USA, are repurposing **Toxoplasma gondii**, one of the most prevalent human parasites, as a cancer immunotherapy.

**Toxoplasma** is a protozoan parasite that resides in the feline intestine, and is thus principally transmitted via cat faeces. While potentially fatal to the immunocompromised, **Toxoplasma** contraction typically results in little more than a transient flu-like syndrome in healthy humans. Indeed, with a little genetic manipulation, **Toxoplasma** may be rendered completely benign.

Dr David Bzik and colleagues use this attenuated **Toxoplasma** for the purpose of microbial cancer immunotherapy. This intervention entails the use of a bacterium or virus, or constituents thereof, to elicit an immune response of therapeutic value. Patented genetic technology developed at Dartmouth, by Senior Research Associate Barbara Fox, reawakens the immune system to aggressive solid tumours.

**TRICKS OF THE TUMOUR**

Cancer is a real ‘cloak and dagger’ pathology, duping the immune system so as to go unnoticed as it grows without pause. The mechanisms by which tumours keep below the immune radar are, slowly but surely, being uncovered. One such mechanism involves the inhibition of CD8+ T-cells, immune cells that would otherwise act to eliminate tumours.

Truly efficacious cancer immunotherapies may only be conceived once the mechanisms by which tumours evade immune detection are comprehensively delineated. Fortunately, this is Bzik’s ultimate endeavour: “We think the **Toxoplasma** vaccine can reveal fundamental new aspects of what tumours are actually doing to shut down immunity, and we’re using a genetic approach to track down the key targets”.

The **Toxoplasma** vaccine itself is therefore not necessarily destined for the clinic, it may merely serve as an intermediate tool. If Bzik and his team can tap into the parasite’s genetic library, then they will have at their disposal all the immuno-oncologic knowledge accrued by the protozoan across its evolutionary timeline.

**TOXOPLASMA GONDII MODUS OPERANDI**

A number of microbes share with cancer the aforementioned stratagem of T-cell inhibition, while the contrary **Toxoplasma** does the very opposite, activating the CD8+ T-cells to an anti-tumour effect.

Bzik’s recent work implicates specific myeloid cells, such as dendritic cells or macrophages, as the mediators of **Toxoplasma**’s immunogenic (immune response-inducing) effect. Bzik expands: “**Toxoplasma** appears to directly reprogramme the immunosuppressive myeloid cell types in the tumour microenvironment, effectively waking them up”.

Bzik’s team has demonstrated the anti-tumoural efficacy of a genetically modified parasite in mice with pancreatic or ovarian cancer, notoriously treatment-resistant forms of the disease. In the case of ovarian cancer, **Toxoplasma** seems to preferentially invade the immunosuppressive CD11c+...
antigen-presenting cells of the carcinoma microenvironment. Tumour-associated CD11c+ cells invaded by *Toxoplasma* are transformed into immunostimulatory cells, which express increased levels of the T-cell-stimulating molecules CD80 and CD86. Following *Toxoplasma* treatment of the immunosuppressive ovarian tumour environment, CD11c+ cells regained the ability to efficiently prime CD8+ T-cell responses. Consequently, treatment markedly increased tumour antigen-specific responses by CD8+ T-cells. While T-cells obtained from untreated tumour-bearing mice lacked the ability to control solid ovarian tumours, T-cells obtained from treated mice exhibited a potent ability to suppress ovarian tumour development.

**SAFETY FIRST**

When dealing with attenuated vaccines, there often exists the possibility of said vaccine’s fall from grace and reversion to virulence. By virtue of so-called ‘secondary mutation’, a genetically nullified microbe may reveal itself as a double agent, potentially causing great harm to those who have been given the vaccine.

One must demonstrate the safety, and thus non-reversion, of a vaccine before even the most preliminary of clinical trials. Fortunately, the patented gene-targeting technology employed at Dartmouth does not permit reversion, being fundamentally distinct to the conventional means of microbial genetic manipulation. By deleting two *Toxoplasma* genes, the encoded proteins of which are key to an essential metabolic pathway, the parasite is rendered robustly avirulent, unable to replicate or grow. “This vaccine cannot revert to a virulent form, and our genetic technology can be used to further engineer the *Toxoplasma* vaccine as a more specific and potent cancer vaccine,” adds Bzik, underscoring the further potential of Fox’s genetic technology.

Highlighting the magnitude of attenuation achieved by Bzik’s lab, a single wild-type (genetically unaltered) *Toxoplasma* parasite is sufficient to kill a mouse. Following attenuation, mice were found to tolerate the injection of approximately 10 million *Toxoplasma* cells. Given that *Toxoplasma*’s nearest relative is malaria, this transformation represents a milestone in its own right, and might conceivably be applied to other virulent microbes.

**CLINICAL POTENTIAL**

With some microbe-based cancer therapies currently in phase III clinical trials, and showing promise of clinical approval, medicine is certainly open to the idea of turning pathogens into therapeutic immunogens. The road to the clinic is, however, invariably fraught with obstacles. Chief among these obstacles, translational studies supporting the safety and efficacy of the therapy must be conducted.

While we know the *Toxoplasma* vaccine to be well tolerated and effective in mice, it is not prudent to generalise these findings to cancers seen in the clinic without first having tested the treatment on non-human primates, and other species akin to humans. Translational studies in turn necessitate a considerable cash injection; though Bzik’s lab seems to be covered in the preclinical pioneering studies, additional investments in translational research are needed to bring this innovative technology to the clinic.

As for the vaccine’s ultimate transition into the pharmacopeia, Bzik recognises venture capital as the most promising form of funding. “For the current ideas being developed in this exciting and rapidly expanding therapeutic field, the products approaching the marketplace are primarily developed through business ventures in the form of visionary biotechnology start-ups. This is the obvious avenue to pursue because the cancer vaccine and immunotherapeutic technologies developed at Dartmouth represent an entirely new type of therapeutic vaccine platform, and one that possesses malleable and synergistic features,” he concludes.