Cancer Immunotherapy:

A rapidly advancing new dawn in the treatment of cancer

Kayleigh Walker is a PhD student in the Department of Molecular and Cellular Biology funded by Cancer Research UK. Her research focuses on the identification of small molecule drugs that target PD-L1 that can mimic the action of antibodies. This involves using NMR Spectroscopy, amongst other techniques, to explore protein-small molecule and protein-protein interactions with the aim of informing drug development. Before arriving in Leicester, Kayleigh completed her undergraduate degree in Biochemistry at The University of Manchester. In her spare time Kayleigh enjoys watching true crime documentaries, electronic music and sampling vegan junk food.

Cancer cells are sneaky. They have developed ways to evade the immune system, and hide in plain sight from our natural defences against tumour progression. However, developments in cancer immunotherapy promise to be the most encouraging treatment approach for cancer since chemotherapy. What makes this even more exciting is that the immune system functions all over the body meaning immunotherapy has the potential to treat many different types of cancer.

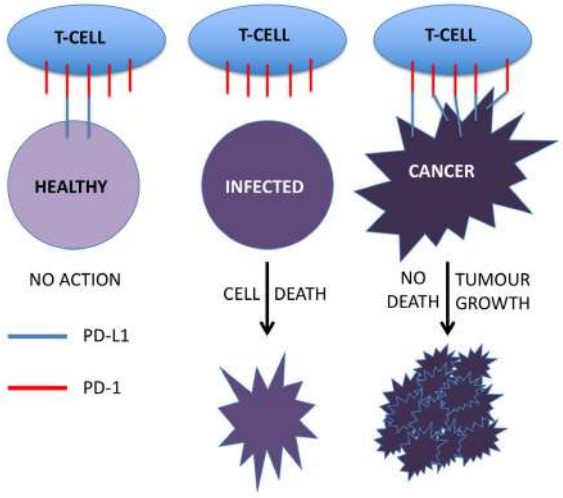
What is Cancer Immunotherapy?

Following numerous promising clinical trial results, cancer immunotherapy was selected as the "Breakthrough of the Year" in 2013 by *Science* magazine. Cancer immunotherapy is any treatment that modulates the immune system to attack and kill tumour cells through small-molecule drugs or antibodies that bind to proteins on the cancer or immune cells. Vaccines or T-cell therapy can also be used, whereby the patient's immune cells are extracted and reprogrammed, then transferred back to the patients to attack tumours. Cancer immunotherapy combats the ability of cancerous cells to avoid immune system detection. One of the ways in which cancer cells avoid detection is via the PD-1/PD-L1 interaction.

PD-1/PD-L1: Friend or Foe

T-cells are immune cells which recognise infected or damaged cells, cytotoxic T-cells can then release toxins to kill these cells. Programmed cell death-1 (PD-1) is a protein expressed on the surface of cytotoxic T-cells. The binding partner of PD-1 is programmed cell death-ligand 1 (PD-L1). This is expressed on the surface of many cell types throughout the body. The T-cell is like an airport security guard. When you set off the scanner you are patted down. If you have nothing illegal or dangerous with you, you can continue on your trip, but if you do, you are taken away and possibly arrested. Similarly, the T-cell "pats down" the cells in your body and it does this by binding to molecules on the surface of the cell being "inspected". The number and type of interactions between the "suspect" cell and the T-cell informs the T-cell whether the cell is healthy or whether it is infected or damaged. Cytotoxic T-cells, unlike airport security guards, take drastic action if a cell is found to be unhealthy and release toxins to kill the cell. Expressing PD-L1 on the surface of the cell sends signals to the T-cell to not take any action. "You have made it through airport security without any problems."

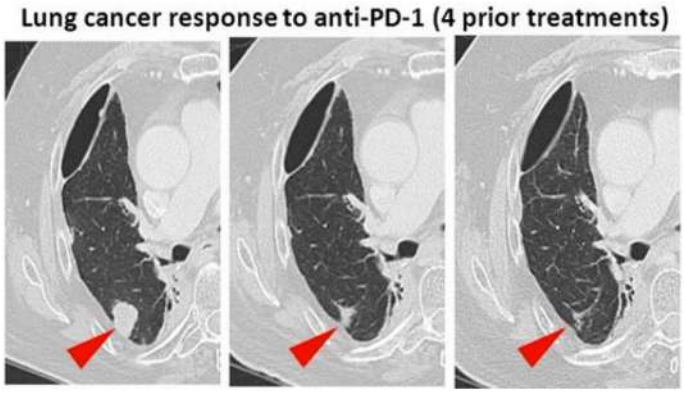
The natural role of this PD-1/PD-L1 interaction is to promote tolerance and prevent autoimmune responses. Some tumours hijack this mechanism, over-expressing the PD-L1 protein on their surface. T-cells that come into contact with a tumour cell expressing



Cancer cells can evade detection by the immune system by over-expressing PD-L1.

PD-1 on its surface binds to the PD-L1 on the tumour. This sends a signal to the T-cell to override an immune response, aiding the cancer cell in escaping detection and proliferating.

Blocking the interaction of PD-1 and PD-L1, with either an antibody or a small-molecule drug, would prevent the inhibiting signal from reaching the T-cells, which may then recognise the cancer cell and initiate an immune response.



https://cancerresearch.org/immunotherapy/timeline-of-progress
Bristol-Myers Squibb BMS-936558 Clinical trial - CA209-003.

Treatment of tumours with anti-PD-1 therapeutics dramatically reduces the size of tumours.

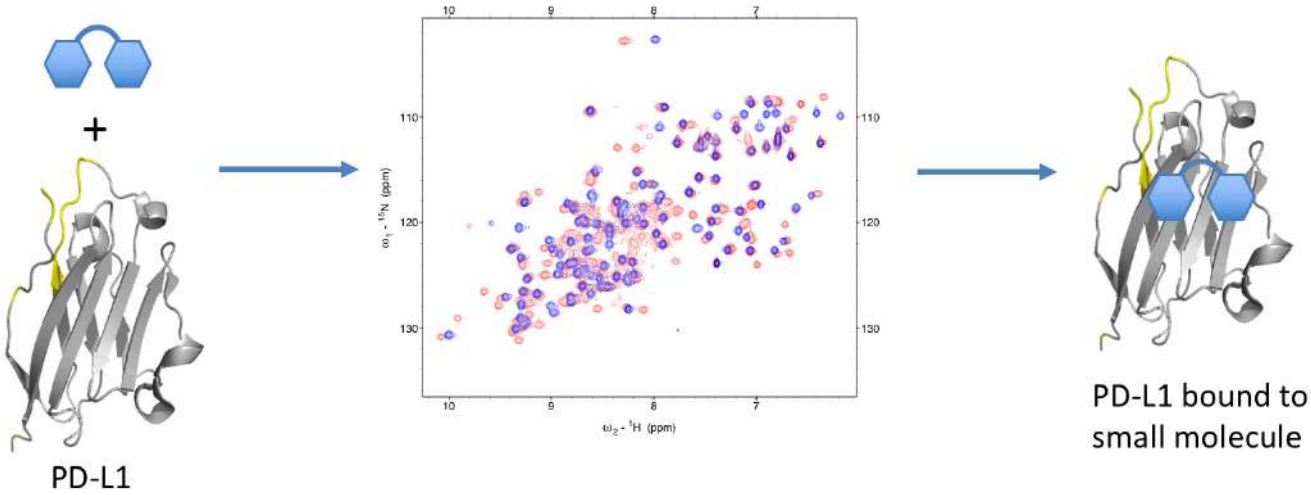
Antibody Therapeutics Targeting PD-1/PD-L1

There are currently five antibody drugs targeting the PD-1/PD-L1 interaction that are licensed by the US Food and Drug Administration (FDA). Two of them bind to PD-1, and the remaining three to PD-L1. These drugs are licensed to treat cancers including melanoma, lung cancer, kidney cancer, lymphoma, and many more. The list keeps growing, as more and more clinical trials are proving successful against many different cancer types. The most well-known of these drugs is Pembrolizumab, also known by the trade name Keytruda, which was first approved in October 2015 by the FDA. Pembrolizumab treats non-small cell lung cancer (NSCLC) in patients no longer responding to chemotherapy. In May 2017, Pembrolizumab was approved as a first-line treatment along with chemotherapy, for NSCLC. In May 2017, the FDA approved Pembrolizumab as a second-line treatment for all metastatic solid tumour types with a specific biomarker. This was the first time the FDA approved a treatment based on biomarkers instead of a specific tumour type. The widespread use of Pembrolizumab in the UK did lag behind that of the US due to concerns about the price of the drug. However, after NICE and the pharmaceutical company Merck came to a confidential agreement for a reduced price for Pembrolizumab, NICE confirmed on the 6th of June this year that it is now routinely available on the NHS for treatment of lung cancer. Pembrolizumab is also available on the NHS for treatment of melanoma and bladder cancer, amongst others, for patients whose cancer has spread or other treatments have not worked. Another drug called Avelumab (Bavencio) has also been FDA-approved for the treatment of Merkel cell carcinoma, and is the first treatment of any kind to be approved for this cancer type, delivering a real boost to patients of this rare type of skin cancer. Thus, antibody therapeutics against PD-1/PD-L1 provide a clear example of how cancer immunotherapy is changing cancer treatments and improving outcomes for patients.

Small Molecules Targeting PD-1/PD-L1

The development of small-molecule drugs that block PD-1/PD-L1 interaction lags behind that of antibodies. Whilst antibodies are performing well showing dramatic life-extending effects for patients, there are a number of benefits, for both pharmaceutical companies and for patients, on focusing development on small-molecule therapies. Firstly the cost of manufacturing a small-molecule drug would be much reduced for the pharmaceutical companies and this could help make the treatment more widely accessible globally. Secondly, since antibodies are proteins they cannot be digested and must be injected, this can be uncomfortable for patients as many would prefer to take a pill instead. Finally, in the case of solid tumours, a small-molecule could penetrate deeper into the tumour and improve outcomes further.

My research has focused on characterising the binding of small-molecules shown to block the interaction *in vitro* to PD-L1. Using NMR spectroscopy I have determined the binding site of these small-molecules and which amino acids of PD-L1 are involved in binding. This research will shed light on how these non-drug-like small-molecules can be chemically modified and improved to produce a drug that has *in vivo* affect.



Comparison of the NMR spectra of PD-L1 with and without the small molecule present allows the mapping of the binding site of the small molecule.