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CAR-T: expanding the horizons

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Immunotherapy in cancer treatment

The human immune system provides a powerful response to invading organisms, destroying bacteria and viruses, as well as aberrant cells that turn cancerous and proliferate uncontrollably. The enquiring mind naturally wonders whether this system could be exploited to treat cancers that have somehow evaded our immune defences. Those cancers that do not respond to chemotherapy or radiotherapy, necessarily harsh and sometimes ineffective treatments, could potentially be thwarted by harnessing the power of our antibodies. Evidence to date suggests that this approach is feasible, although there is still much work to be done before we can say we have finally found a 'cure' via this route.

There have been several attempts to investigate immunotherapy as a course of cancer treatment. In the late 1800s, clinicians noted their patients' tumours sometimes shrank following a bacterial, viral or even fungal infection that had induced a feverish response. From this observation, Dr William Coley formulated a vaccine made of 'killed' bacteria and also noted that the tumours often contained large numbers of lymphocytes (white blood cells) that had infiltrated into the centre of the tumour.

Over 100 years later we are still investigating the role these cells play in cancer and, as is usual in scientific advancement, it has become a lot more complex than previously imagined.

Different approaches to immunotherapy

Whilst there are numerous avenues of research into immunotherapeutic treatment, the basic concept of immunotherapy utilizes the specific molecular characteristics of tumour cells to bring about their destruction. In any form of therapy, there has to be a way to differentiate healthy cells and tissue from diseased, and immunotherapy is no different. This can be achieved by identifying specific unique markers of the tumour cells, usually proteins expressed either on the surface of a cell or within the cell itself. These molecules are sometimes part of the pathway by which cell-to-cell communication takes place, an important process in the development and spread of cancer.

Early on, immunotherapy researchers used a specific kind of immune cell called dendritic cells that play a role in cancer recognition. They mixed these cells, taken from the patient, with tumour cells so that the dendritic cell would

learn to recognize the markers and direct an immune response towards the tumour. However, early promise in this research led to disappointment during clinical trials. It appears that tumours have the ability to hijack and switch off the immune system and create an environment for themselves in which cancerous cells cannot be recognized as something to be destroyed.

Another approach involves the use of antibodies to perturb cell-to-cell communication by masking a protein marker on the tumour cell. This intervention can, for example, deprive the cell of some essential metabolic process or, via a different process, stimulate cell-to-cell communication and 'turn on' immune cells that destroy the cancer cells. The idea is that a 'monoclonal' antibody, raised against a specific antigen, will only work on that target and should, in theory, only destroy cancerous cells and not the healthy ones.

A series of markers called checkpoint inhibitors (Figure 1), work by blocking proteins that stop the immune system attacking cancer cells. Once these proteins are taken out of the process, the immune system can then get on with the job of destroying cancer cells.

The problem researchers have found, however, is that cancerous cells have methods to adapt and evade treatments. By their very nature, cancer cells have accumulated mutations in their DNA and, in a process similar to evolutionary natural selection, those cells that have the necessary mutations required to survive a treatment will form the next generation of tumour cells resistant to that treatment and, all too often, the tumour comes back or relapses.

This brings us to the latest candidate for immunotherapy, chimeric antigen receptor T-cell therapy or CAR-T. This approach involves taking a T cell (so called because it matures in the thymus) from a patient and using

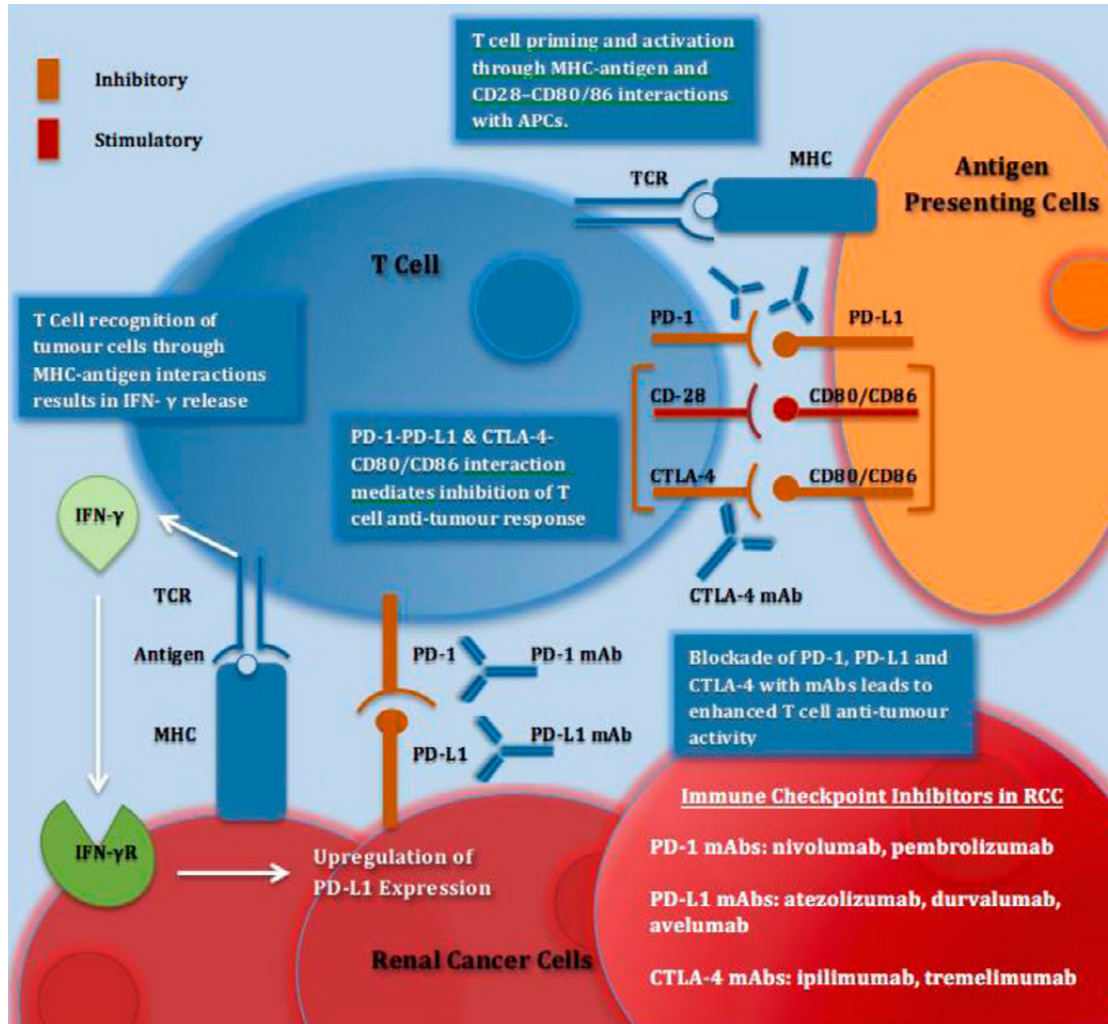


Figure 1. Checkpoint inhibitors, both inhibitory and stimulatory, of the immune system and their interactions with tumour cell markers. PD-1, programmed death-1; PD-L1, programmed death ligand-1; MHC, multi histocompatibility complex; CD, cluster of differentiation, CTLA-4, cytotoxic T lymphocyte associated protein-4; INF- γ , interferon gamma; TCR, T cell receptor (Ross and Jones (2017) Clin Sci. **131** (21), 2627–2642). Image used under the creative commons license.

a viral vector to insert a DNA sequence into the genome of this immune cell. In this way, it is possible to engineer a cell that expresses a receptor on its surface which identifies a tumour-specific surface protein and triggers destruction of the cancerous cell, without harming healthy cells.

This system has proven successful in the treatment of some forms of blood cancer, which express a single, identifiable and cancer-specific target called CD19. However, problems start to appear when we try to translate that approach towards other cancers, such as solid tumours. In these diseases, the tumour is very often heterogeneous (Figure 2) which means that the markers we are looking for are present in some areas of the tumour but not in others. In addition, the markers can come and go during the course of the illness at different times making it almost impossible to pin down with a treatment like CAR-T.

Angiogenesis and immunosuppression

The crucial question that remains is whether CAR-T can be modified to reproduce the success seen with blood

cancer into the treatment of solid tumours, such as brain cancer. The answer may lie in targeting angiogenesis and immunosuppression, two ‘hallmarks of cancer’, which are characteristic of malignant tumours.

Angiogenesis simply means the creation of new blood vessels. One of the defining properties of a malignant cancer is the ability for it to spread to other parts of the affected organ, as well as to other sites in the body. To do this the tumour creates its own network of blood vessels to sustain itself on its travels. The process of angiogenesis is driven by a specific cell-to-cell signalling mechanism, which involves the collision and interaction of specific molecules on the surface of tumour cells, as well as the newly created blood vessels. These molecules could be a potential new target for CAR-T therapy.

Immunosuppression means the dampening down of the immune system, and is a necessary process that is meant to stop our immune system attacking our own cells, as well as those of any offspring we may be carrying. To this end it is a vital function to enable life to continue. However, cancers have a sneaky way of subverting this process to avoid monitoring by the immune system. During the

progression of a cancerous disease, a modification often occurs to generate a tumour-promoting role for the immune system. This dual role of tumour promotion and tumour protection is termed immunoeediting and has three phases: **elimination**, which is the recognition of cancer cells by the immune system, **equilibrium**, in which the expansion of cancer cells is controlled by the immune system and **escape**, which is the development of an immunosuppressive tumour microenvironment.

One of the principal architects of this immunosuppressive microenvironment is a specific regulatory T-cell, or Treg for short. Look inside most solid tumours and you will see that these cells have infiltrated into the middle of the tumour, attracted by cell-signalling proteins called cytokines, which are produced by the tumour itself to create its own 'safe space' within the body. The brain has long been thought of as an immunologically privileged site, with the tight junctions of blood vessel endothelial cells providing a blood–brain barrier that prevented the infiltration of T lymphocytes into the central nervous system. However, under the pathological state of a tumour, the blood–brain barrier is disrupted and lymphocyte trafficking to the brain increases. Immune-competent lymphocytes that can get to the site, however, are immediately switched off by those unwelcome Treg cells that have been brought in by the tumour to do this very job. The question emerges then, can these Tregs also be destroyed by engineered CAR-T cells designed to take them out of the tumour site?

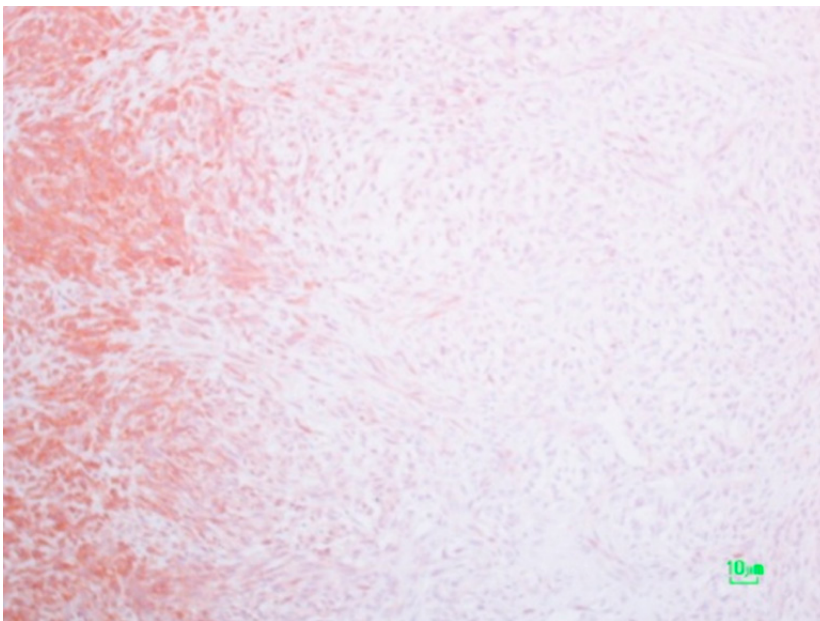


Figure 2. Immunohistochemistry of glioma tissue. The marker labelled (CD25) in red is not present in all parts of the tumour showing its heterogeneous nature. Image produced by the author.

Toxicity and limitations of CAR-T

One of the main limitations of CAR-T therapy is toxicity associated with the treatment. Although you have a specific target in mind for your engineered cells, there can be cross-reactivity with other molecules on healthy cells. In addition, the immune system can be overstimulated, leading to a condition known as cytokine release syndrome (CRS). CRS is caused by a sudden and overpowering release of cytokines from the lymphocytes, stimulated by the CAR-T cells, and this can cause an onset of nausea, rashes and difficulty breathing. On some occasions this can be life-threatening. To avoid this, the CAR-T target must be as specific as possible to the tumour cell. It would appear that targeting cells involved in angiogenesis and immunosuppression would lead to exactly this sort of non-specific reactivity, since both of these are normal processes involved in the healthy functioning of our bodies.

Overcoming the limitations

But what if a specific marker could be found for solid tumour angiogenesis or for Tregs that infiltrate into tumours? The possibility of targeting those tumours would then become feasible. These markers are unlikely to change, because the process of angiogenesis is not normally targeted by the immune system in the same way as cancer cells, and so there has been less time for resistance to the immune system to develop.

Another way of overcoming tumour heterogeneity is by producing CAR-T cells with multiple antigen-binding regions. The fourth generation of CAR-T allows cells to have the ability to bind more than one target, making it possible for the CAR-T producer to refine their target to make sure healthy cells are not attacked (Figure 3). CAR-T cells unresponsive to the immunosuppressive properties of the solid tumour could also be produced, as well as those that have an amplified response to the target. This could be done by engineering a pro-inflammatory response to the CAR-T when it is bound to the specific target you are looking for.

The potential for CAR-T therapy to treat solid tumours as well as blood cancers is very exciting. The search for a good, reliable target that causes negligible side-effects, is resistant to tumour heterogeneity and the immunosuppressive micro-environment will be key to expanding the horizons of this therapy. Finding these markers is the current focus of my research. To this end I am investigating a range of angiogenesis markers called platelet-derived growth factors (PDGF). This molecule and its receptor is strongly implicated in the angiogenesis process and, from my

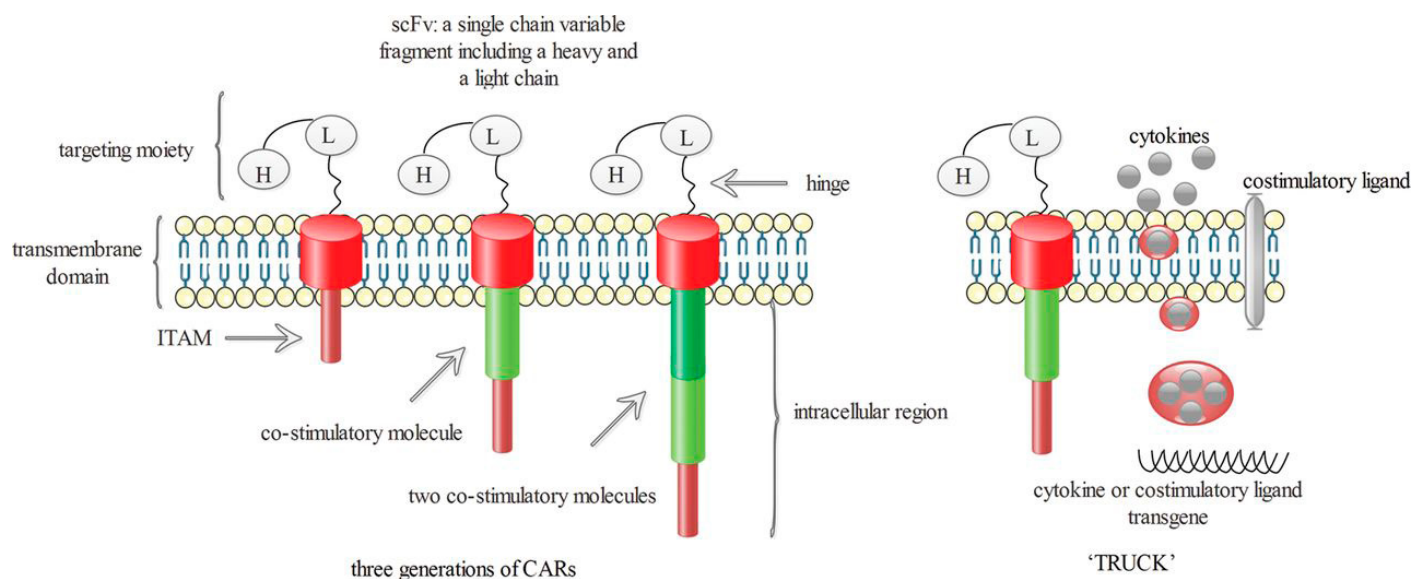


Figure 3. Classes of CAR-T cells showing increased functionality of the cell by the addition of co-stimulatory domains and the addition of genes to produce cytokines or a co-stimulatory ligand. TRUCK, T cells redirected for antigen-unrestricted cytokine-initiated killing; ITAM, immunoreceptor tyrosine-based activation motif (Sha, H-H., Wang, D-D., Yan, D-L., Hu, Y., Yang, S-J., Liu, S-W. and Feng, J-F. (2017) Bioscience Reports, **37** (1), BSR20160332). Image used under the creative commons license.

experiments, is found in increased amounts in the sera of brain cancer patients compared with the sera from healthy control participants. This finding suggests that this may be an appropriate place to start looking for a unique and significant isoform of PDGF from which an effective CAR-T therapy may be formulated. ■



Dr Peter Abel spent 19 years working for the NHS in haematology departments before taking a senior lecturing post at the University of Central Lancashire in the School of Pharmacy and Biomedical Sciences. He is Course Leader for the undergraduate Healthcare Science degree and supervises placement provision within the NHS pathology laboratories allied to his course. In research he is a member of the Brain Tumour North West research alliance and is interested in immunosuppression and the search for a blood-borne biomarker for glioblastoma. He is currently looking for a novel target for potential use in adoptive cell transfer therapy. Email: pabel@uclan.ac.uk

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