ADOPTIVE T-CELL THERAPIES
unlocking the potential of engineered antigen receptors

T-cells can and do kill tumour cells, in most instances keeping cancer in check. The concept that the immune system could be unleashed to fight cancer was born in the late 1800s with Coley’s toxins, essentially using what we now know to be inflammatory mediators to provoke an immune response to an established tumour. Adoptive T-cell Therapy (ACT) was first proposed in the 1950s, and typically involves isolating tumour-infiltrating lymphocytes (TILs), expanding them using growth factors (of which IL-2 is the prime candidate), and introducing them back to the cancer patient. These T-cell specific treatment modalities have been bolstered by recent evidence for cancer immunoediting1, and has further encouraged development of experimental approaches for engineering specific mechanisms to achieve cancer targeting of T-cells.

Notwithstanding these approaches, cancer remains a mighty foe. Donning what can be best described as ‘invisibility cloaks’, cancer cells are cleverly able to shield themselves from recognition and attack by the immune system. Multiple strategies are utilised by tumours to escape detection, including decreasing the expression of specific antigen-presenting proteins at the cell surface, inhibiting effector T-cell responses and promoting the production of regulatory T-cells to suppress the immune response.

The scientific community has spent decades studying the ways in which tumours evade immune detection with the goal of devising effective strategies to counteract these methods. Adoptive T-cell transfer, specifically chimeric antigen receptor (CAR) technology, is one such strategy that is generating great excitement largely due to the impressive treatment responses that have been demonstrated in small trials of patients with advanced cancer, in particular with CD19 CAR T-cells targeting hematological malignancies. An equally valid strategy is adoptive T-cell therapy utilising enhanced affinity T-cell receptors (TCRs) for which treatment responses are now being observed with New York esophageal squamous cell carcinoma-1 (NY-ESO-1) expressing solid tumours. Engineered TCRs take advantage of the full complexity of endogenous TCR signalling.

By Dr Cenk Sumen, Dr Dan Williams and Dr Gwendolyn Binder-Scholl
machinery, whereas CARs function independently of the natural T-cell receptor. By harnessing the patient’s own immune system to combat cancer, these approaches hold promise for eliminating or reducing the need for chemotherapy, radiation and surgery. Adoptive T-cell therapies using enhanced receptors such as TCRs or CARs requires harvesting T lymphocytes, or T-cells, from a patient or matched donor’s blood. These cells are then genetically engineered \textit{ex vivo} allowing expression of the receptor on their cell surface that can recognise tumour specific antigens and activate the T-cell to attack the patient’s cancer. These engineered cells are expanded in culture and then infused back into the patient to treat their disease.

Over the last few years, CAR T-cells directed against the CD19 antigen have achieved clinical proof-of-concept. Researchers at multiple academic centres have reported success in a number of small clinical trials of patients with advanced B-cell malignancies who had exhausted all other lines of therapy. Renewed excitement in T-cell immunotherapy arose in 2011, when investigators at the University of Pennsylvania (Penn) announced that three patients with advanced chronic lymphocytic leukaemia (CLL) had long-lasting remissions after a single dose of CD19-directed CAR T-cells\textsuperscript{2}. Since these initial data, the National Cancer Institute (NCI)\textsuperscript{3}, Memorial Sloan Kettering Cancer Center\textsuperscript{4} and Penn\textsuperscript{5} have also presented encouraging results in patients with lymphoma and acute lymphoblastic leukaemia (ALL). It appears that ALL has the highest response rate to date (~80%) among the leukaemias and lymphomas. As a result of these promising clinical data, new academic-industry partnerships as well as new companies have been formed to advance the development of CAR T-cell therapies.

\textbf{Figure 1}

\textbf{A)} T-cell receptor; \textbf{B)} Chimeric antigen receptor
Adaptive transfer of T-cells expressing enhanced receptors holds great promise for cancer patients as these cells can theoretically be engineered to target virtually any tumour-associated antigen. They also overcome the limitation of a weakened immune system in very sick patients by generating an army of reprogrammed T-cells outside of the patient’s body. This article will provide an overview of CAR and TCR transduced T-cell therapies, their early clinical success and how the many challenges facing the field are actively being addressed by companies deeply involved in this space, such as Adaptimmune and PCT.

Redirecting specificity by design

T-cell specificity can be redirected using synthetic T-cell receptors (Figure 1A) or native TCRs (Figure 1B). CARs were originally generated by Zelig Eshhar and colleagues to study TCR signalling. Each CAR is designed to enable the gene modified T-cell to recognise a specific ligand on the surface of a patient’s cancer cells. In other words, target specificity of T-cells can be redirected towards essentially any antigen as long as a proven binding motif is available, although spacer sequences to allow functionality for each CAR must be empirically determined for optimal activity. CARs are engineered to combine the extracellular antigen-binding domain of an antibody, scFv region, with the intracellular signalling domain of either a T-cell receptor or co-stimulatory molecule (ie CD28 and 41BB), or both. They redirect T-cell specificity to target antigens in a human leukocyte antigen (HLA)-independent manner. First-generation CARs only contained the CD3zeta domain and the T-cells expressing these CARs could activate but were deficient in survival (Figure 2, 1G). Second-generation CARs incorporated additional co-stimulatory receptor domains such as the CD28 and 4-1BB domain, which allowed enhanced proliferation and cytokine secretion and in some cases long-term survival and memory T-cell generation (Figure 2, 2G). Third-generation CARs combine two or more domains from co-stimulatory molecules, such as 4-1BB and OX40 (Figure 2, 3G), and data to date suggest that this results in a combination of qualities from the various domains included.

Therapeutic CARs can only recognise proteins expressed on the surface of a cell. This comprises approximately 10% of proteins. The majority of tumour-specific targets are intracellular. This suggests the potential for TCR-based adaptive T-cell therapy could actually be more far reaching than CARs can currently offer. Antibodies to HLA-peptide complexes are another area of active investigation, but these have to date lacked sufficient peptide specificity and will likely fail safety criteria for clinical use.

Most T-cells express the αβ TCR, but a small subset expresses γδ T-cell receptors which have the ability to target lipids (when presented within CD1d on the antigen presenting cells – specificity is maintained through the TCR α chain CDR3 loop). These are used by NK T-cells and have Th1 responses in autoimmune diseases and Th2 responses in cancer. Additionally, this interaction could potentially be enhanced by affinity engineering and used as a therapy. Thus, TCR-based T-cell therapy also has the potential to target other molecules.

To date, there are relatively few engineered CARs that specifically direct T-cells to antigens other than CD19 and CD22 that have been entered into the clinic. At Adaptimmune, we have a pipeline of specific engineered TCRs targeting a number of antigens and the potential to produce TCRs to virtually any target, many of which we hope to enter into the clinic in the near future. The technology platform features antigen target validation and the generation of TCRs of appropriate specificity and affinity. These competencies encapsulate years of TCR engineering expertise and represent a significant barrier to entry.

We believe that the potential for the range of antigens that enhanced TCRs can target is similar to, if not greater than, that potentially targetable by CARs (although the antigens may be different to those targetable by CARs).

A variety of non-viral and viral-based methods are being used to deliver CAR and TCR constructs into T-cells. Transposon-based systems offer more efficient gene transfer than plasmids, although culture time remains a limitation. The Sleeping Beauty transposon system is actively being used in clinical trials to deliver CAR T-cells. Retro and lentiviral vectors efficiently and permanently transduce T-cells and remain the most commonly-used method for gene transfer. To date, these viral methods are proving safe with respect to insertional mutagenesis, and T-cells are known to be far more resistant to transformation by integration than stem cells where such events have been detected. Targeted recombination into safe sites within the genome is under investigation, but this will most likely be used primarily in stem cell applications. RNA transfer for transient expression of the engineered receptor is sometimes used in cases where the target antigen is considered high risk.

With respect to the T-cell, it has not yet been determined if there is a subtype (or population distribution of T-cell subtypes) which is best for
adoptive therapy. Initial data suggest that less mature or central memory T-cells are more likely to proliferate and persist longer in patients compared to their more differentiated counterparts and clinical studies are under way using some of these subsets. It is possible that a mixture of phenotypes may be required for optimal effect.

**Manufacturing T-cell therapies**

Figure 3 provides an overview of a typical autologous adoptive T-cell manufacturing process. In brief, the process begins with harvesting T-cells from a patient or matched donor’s blood and then shipping the apheresis product to the manufacturing facility. The T-cells are isolated, activated and transduced with the enhancing receptor construct. The manipulated cells are expanded in culture, a process that takes approximately 10 days, and then shipped back to the clinical site for infusion back into the patient.

There are a number of manufacturing constraints that must be taken into consideration when developing autologous cell therapies. The most critical of these constraints impact the patient, including failure of the product lot and delivery of the final product to the wrong patient. Product lot failure results in failure to treat the patient but, more importantly, delivering the final product to the wrong patient can be life threatening to the patient.

Also, there can be severe ‘fresh product’ scheduling constraints when trying to collect enough blood from patients who are very ill because apheresis should only be collected when the patient is not experiencing any severe symptoms. Additionally, it is very challenging to isolate T-cells from a blood sample that is full of cancer cells.

With autologous products, a separate good manufacturing practice (GMP)-compliant batch record and quality assurance (QA) release is required for each patient, and the production schedule is often constrained. There is typically a very short shelf life of starting material or final product – as short as 24 hours – in addition to hospital scheduling needs.

There are additional challenges associated with downstream processing. Terminal sterilisation of the final product is not an option for T-cell therapies; aseptic processing is required throughout the process to maintain sterility assurance. There is also limited ability to clear or eliminate impurities or contaminants except by dilution. Therefore, reliance must be placed on very high quality or inherently safe raw materials. Additionally, with limited capability to test the final product, increased reliance must be placed on validated, robust manufacturing processes as well as in-process controls.

**Figure 2**

The design of CAR T-cells has evolved considerably over time. Figure reused with permission from the Journal of Cancer (Casucci, M, Bondanza, A. Suicide Gene Therapy to Increase the Safety of Chimeric Antigen Receptor-Redirected T Lymphocytes. J Cancer 2011; 2:378-382)
Along with these challenges, we must consider how we evaluate the quality of the engineered T-cells generated for these therapies and establish how to test these qualities through well designed potency assays. However, before being able to do this, we need to understand what T-cell qualities (persistence, dose, cell phenotype and/or gene expression profile, cytotoxic profile, etc) are associated with strong clinical responses, and there remains a lot of learning in the field to establish this information. The tumour type and target antigen may also determine the cell product qualities necessary for deep and durable clinical responses.

As T-cell therapies become added to the treatment armamentarium for hematologic and solid cancers, efficiencies and scalability will need to be engineered into the manufacturing process. With the development of more efficient, streamlined processes, the production of high-quality engineered T-cells should become more economical. Realising economies of scale for patient-specific T-cell therapy will prove a greater challenge. Unlike conventional drugs and biologics, which are scaled up to increase lot size, autologous engineered T-cells must be scaled out to increase lot number. As effective and durable T-cell therapies are brought towards commercial development, current manufacturing constraints on dose (the number of viable, potent T-cells given to the patient) may be ameliorated through efficiencies of scale and automation, reducing culture times and treatment cost per patient.

**Challenges and opportunities**

We are in a very promising and exciting stage for realising the full potential of cancer immunotherapy, encompassing targeted T-cell therapies. Clinical trials have revealed several key challenges that must be overcome, including identifying optimal T-cell dosage and managing toxicities. Reprogrammed T-cells proliferate rapidly once they are infused back into the patient. It is therefore important to determine how many infused T-cells are needed to elicit a safe and strong response in patients. Early clinical data do not appear to demonstrate a clear correlation between dose and efficacy, although hematologic malignancies may behave differently than solid tumours where the T-cells need to get into the tissue and find the tumour and antigen in order to elicit a response.

The biggest restriction on the use of CARs may be finding an antigen to target that is expressed on solid tumours and not elsewhere in the body. Even CD19 is not absolutely specific; it is expressed on B cells and so the therapy also targets a patient’s normal B cell population.

Ironically, safety becomes an issue when CAR T-cells do their job too well, primarily because of the cytokines they unleash. Cytokine release syndrome is a potentially lethal side-effect that must be managed in these patients. Another safety concern that arises when CAR T-cells do their job too well is tumour lysis syndrome. This side-effect occurs when large amounts of dying cancer cells release metabolites that ultimately build up in the kidney. Engineered TCRs may provide an enhanced safety profile in this regard, and reduce bystander killing. Toxicities can also be clinically managed by the administration of approved biologics which block inflammatory cytokine signalling.

Researchers are responding to these safety concerns by embedding a safety gene (or ‘kill switch’) in the gene construct used to express the engineered receptor. The safety gene can be activated in the event of a life-threatening toxicity, and stop the proliferation of a majority of T-cells, or kill the transduced T-cells. The FDA does not require use of a safety switch for first in human products, but they can be useful if there are concerns about the expression of the target antigen on normal tissue, or about the specificity of the receptor used, which cannot be sufficiently tested in preclinical studies.

While cell therapies in general can be expensive and difficult to develop, the ultimate promise of these treatments is the ability to affect long term suppression of the tumour cells without the need for chronic treatment, in effect achieving a cure. Over the long term, the economics are favoured for therapies that can become cures as opposed to long-term drug treatments. Market opportunity for anti-cancer engineered T-cell therapies could exceed $20 billion.

Most clinical trials for CAR T-cell therapies have been in hematological malignancies primarily because these cancers are easily accessible and their cell surface tumour antigens well characterised. New clinical efforts are under way, however, to study CAR T-cells in solid tumours, including mesothelioma, sarcoma, glioblastoma and advanced pancreatic cancer.

There are specific challenges associated with the development of CAR T-cell therapies in solid tumours that are not present in hematological malignancies, including tumour size and microenvironment. Solid tumour masses contain billions of cancer cells and these cells are much harder for T-cells to access compared to individual cancer cells that are floating in the bloodstream. Moreover, the tumour microenvironment is designed to repel an immune response. For these reasons, it remains to be seen whether CAR T-cell therapies will be able to...
to elicit as strong a response in solid tumours as they do in hematological malignancies. CAR T-cells may have a role as an adjunctive therapy in the treatment of solid tumours, in combination with biologics which serve as checkpoint blockade inhibitors such as Yervoy (ipilimumab). Some of the most promising solid tumour data has been in a synovial sarcoma and melanoma study, where patients received cells expressing an affinity enhanced NYESO-1 TCR. As described earlier, use of TCRs to target tumour antigens may be more effective in the solid tumour setting due to their ability to target intracellular antigens and thus avoid normal tissue damage.

The development of engineered T-cell therapies is associated with many logistical and manufacturing constraints primarily because the therapies must be individualised for use in the autologous setting. To overcome the manufacturing and economical limitations of autologous therapies, significant efforts are under way to eliminate the need for patient-specific T-cells. The goal of these efforts is to develop universal, standardised, off-the-shelf, allogeneic T-cells.

One approach to allogeneic CAR T-cell (UCART) development involves inactivating the TCR and CD52 genes from donor T-cells using transcription activator-like effector nucleases (TALEN) gene editing technology. Inactivating TCR prevents the potential of allogeneic cells to induce graft-versus-host disease (GvHD) in recipients; inactivating CD52 enables CAR T-cell administration following lymphodepleting therapy.

Innovation always presents challenges, and both CAR and TCR-transduced T-cell therapies are no exception. For example, these T-cells are currently autologous therapies which pose economical, logistical and manufacturing constraints. They are also considered a living drug, which again poses additional challenges, as these cells proliferate and remain in the body long after initial treatment.

The demonstration of effective and safe allogeneic engineered T-cell therapy will shift the cost curve down and will help make these treatments available to a broader patient population. In particular, allogeneic therapies may prove less taxing to seriously ill patients who have been heavily pretreated. In these patients, the T-cells may be low in

Figure 3
Typical autologous CAR T-cell manufacturing process
number, only partially functional, or unable to expand, which currently limits their ability to be manufactured into autologous engineered T-cells.

In summary, engineered T-cell therapy is a powerful modality capable of achieving deep and durable clinical responses in the treatment of refractory patients. Although adoptive T-cell manufacturing is currently a complex and commercially challenging process, compelling early success with both CAR and engineered TCR T-cells is driving innovation in the field to address these hurdles. Adoptive transfer of T-cells expressing chimeric antigen receptors or engineered T-cell receptors hold great promise for cancer patients as a patient’s cells can theoretically be engineered to target virtually any tumour-associated antigen. As such, one can envision a time in the not too distant future where a patient would come to a hospital, have his/her tumour characterised and then receive a personalised cell therapy targeting antigens known to be specific for his/her tumour type. Cell therapy is becoming an essential part of the immuno-oncology armamentarium, and we believe it will become a fourth pillar of healthcare9.

Dr Cenk Sumen is the Manager of Technology and Business Development at Progenitor Cell Therapy. He received his PhD from Stanford University, where he studied T-cell activation at the immunological synapse. He previously worked at PerkinElmer, STEMCELL Technologies and Life Technologies, and completed postdocs at Harvard Medical School and Memorial Sloan-Kettering Cancer Center.

Dr Dan Williams is the Head of Translational Development at Adaptimmune Ltd. He is responsible for translation of development candidates from research through development and into early phase clinical studies. He also oversees Adaptimmune’s clinical trials in the UK and EU. He received his PhD from the Division of Molecular Physiology at the University of Dundee.

Dr Gwendolyn Binder-Scholl is Executive Vice-President at Adaptimmune Ltd, responsible for driving all aspects of the company, including ongoing clinical trials and strategic development planning. She has extensive industry and academic experience in cellular and gene therapy translational research and development. She received her PhD in cellular and molecular medicine from Johns Hopkins University.

Disclaimer
This article was finalised by the authors for publication in March 2015. The article contains subjective statements made by the individual authors which represent their views as at the time of this finalisation. These statements are not necessarily authorised by or representative of the formal positions taken by the entities employing those individuals.

References