Adoptive T-Cell Therapy for Cancer in the United Kingdom: A Review of Activity for the British Society of Gene and Cell Therapy Annual Meeting 2015

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Abstract

Adoptive T-cell therapy is delivering objective clinical responses across a number of cancer indications in the early phase clinical setting. Much of this clinical activity is taking place at major clinical academic centers across the United States. This review focuses upon cancer-focused cell therapy activity within the United Kingdom as a contribution to the 2015 British Society of Gene and Cell Therapy annual general meeting. This overview reflects the diversity and expansion of clinical and preclinical studies within the United Kingdom while considering the background context of this work against new infrastructural developments and the requirements of nationalized healthcare delivery within the UK National Health Service.

Introduction

JUST A FEW SHORT YEARS AGO, exploiting the immune system to tackle cancer was generally considered too complicated and highly unlikely to deliver significant impact upon the clinical stage. Fast forward to 2015 and cancer immunotherapy has been elevated to mainstream focus with monoclonal antibody treatments now the standard of care for an increasing range of cancer indications. Similarly, delivering cells as a therapy outside of bone marrow transplantation was considered complex and impractical, giving rise to the concern that future investment by the pharmaceutical industry was unlikely. However, as of 2015, there is a frenzy of activity and startling sums of money involving established and newly emerging biotech entities in the United States fighting to capture and deliver cell therapies in an industrial context. This activity is driven by three factors. First, there have been highly impressive clinical results where objective clinical responses have been observed in patients with advanced cancer, treated with adoptive T-cell therapy. Second,

recent technological advances have streamlined clinical-grade cell manufacture, thereby providing the potential to deliver treatments to large numbers of patients at randomized phase III trial level and beyond. Third, rapid scientific and preclinical progress within the academic sector has led to the generation of significant amounts of exploitable intellectual property.

The nationalized delivery of healthcare in the United Kingdom through the National Health Service (NHS) provides a unique context to deliver complicated new therapies. The positives of the NHS are many, including free at the point of entry healthcare together with a national framework and governance system for the delivery and evaluation of therapies. The negatives are those associated with any large organization and include the potential lack of flexibility to rapidly test new therapies impacting upon the market. As a contribution to this issue of *Human Gene Therapy* focused on the 2015 BSGCT annual scientific meeting, we provide a timely overview of current clinical and translational adoptive T-cell therapy research activity within the United Kingdom.

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Adoptive T-Cell Therapy

T-cells are key effectors of the adaptive immune system playing many roles that include the targeted elimination of virus-infected cells. This basic function is attractive as a cancer therapy where circulating T-cells could target and eradicate primary and metastatic tumor cells as a systemic and long-lived therapy. Indeed, the importance of T-cells to deliver therapeutic responses in the allogeneic hematopoietic stem cell (Allo HSC) transplant field has long been understood, where patients receiving T-cell-containing HSC grafts experienced improved disease remission as compared with patients receiving T-depleted HSC grafts and where infusions of donor lymphocytes can aid the therapeutic response in certain cancer indications.¹

Over the last two to three decades, autologous T-cell therapies have developed to the stage where the body of positive clinical results has catapulted this form of therapy into public and corporate consciousness. This has largely been the result of a number of factors coming together, including an improved understanding of the basic biology of T-cell function, the development of cell processing technology to improve consistency and reproducibility, and the ability and dedication of a relatively limited number of clinical centers across the globe to develop the infrastructure and expertise to allow the clinical delivery of these complicated therapies.

At present, there are two basic autologous T-cell therapy approaches delineated by whether the naturally occurring tumor antigen specificity of the T-cells is exploited or whether the T-cells are engineered to generate tumor specificity.

"Natural" antitumor T-cells

The challenge of exploiting naturally occurring T-cells for therapy is the low frequency of these cells present within peripheral blood or tissue. Thus, methods that enrich for specific cells generally require sophisticated techniques to isolate T-cells that have responded to the antigen challenge while avoiding nonspecific T-cells, which is challenging to deliver at clinical grade. This approach has been most successfully exploited to isolate virus-specific T-cells, where virus targeting may also treat tumors, such as against cytomegalovirus and Epstein-Barr virus (EBV),² while isolating T-cells specific for nonviral tumor-associated or tumor-specific antigens has been less successful. This relates both to the low frequency of tumor-specific T-cells in the peripheral T-cell repertoire and the influence of immunological tolerance mechanisms. An alternative approach is based upon the enrichment of antigen-specific T-cells within a specific tissue from which the T-cells can be liberated. This has formed the basis for tumor infiltrating lymphocyte (TIL) therapy where T-cells resident within a tumor biopsy are harvested *ex vivo* and expanded to large numbers before adoptive transfer. The hypothesis is that although tumor-specific T-cells are recruited to and become resident within the tumor, their function is impaired by the strongly immune-suppressive tumor microenvironment. *Ex vivo* expansion in a cocktail of various cytokines may reverse the functional deficit before reinfusion. Reports of objective clinical responses in 50–70% of patients with advanced chemoresistant melanoma after TIL therapy^{3,4} suggest that this hypothesis has been proven. The long-term goal in this

area now becomes to make TIL therapy practical and the antitumor effect durable.

A variation on stimulating existing antitumor cells includes exploiting other "natural" lymphocytes. The best studied of these are $\gamma\delta$ T-cells, NK cells, and NKT cells. NK cell-based therapies are advancing into United States and eventual global trials, now that expansion of these cells to clinical scale and purity has been achieved.⁵ Like other T-cells, NKT cells are relatively easy to expand to large numbers, both *in vivo* using lipid antigen stimulation^{6–8} and *ex vivo* for adoptive cell therapy (ACT).^{6,7} Several NKT cell-based therapies have already been pioneered around the world.^{6–9} These include in the context of allogeneic HSCT.⁹ Several groups in the United Kingdom as well as colleagues in Europe are considering direct ACT with NKT cells and/or NKT cells modified with viral vectors encoding chimeric antigen receptors (CARs), based upon their greater efficacy than conventional T-cells in a mouse xenotransplant model.¹⁰ ACT based upon the use of $\gamma\delta$ T-cells has been largely pioneered in Japan, where *ex vivo* expansion of $\gamma\delta$ T-cells using zoledronic acid has been investigated.¹¹

Engineered tumor-specific cells

In what is likely to be the majority of situations, natural T-cells may not be available through the lack of suitable target antigens or unavailable tumor biopsy from which to generate TILs. Consequently, engineering T-cells to endow them with antitumor specificity has evolved as a means to overcome the lack of natural T-cells for therapy. Moreover, current gene transfer technology and recent improvements in *ex vivo* cell culture technology ensure that clinically relevant numbers of tumor-specific T-cells can be generated within a relatively short period, which is of key importance in delivering the therapy in a timely manner.

There are two underlying strategies that have been exploited, resulting in clinical testing of engineered T-cells. The first involves the expression of T-cell receptor (TCR) α and β chains that bestow the engineered T-cell with antigen specificity of the transferred TCR.¹² The two major advantages of this strategy are, first, that the approach exploits the "natural" TCR configuration resulting in physiological T-cell activation/signaling, and second, that the TCR can recognize intracellular targets processed and presented in the context of HLA molecules. Such intracellular protein-derived epitopes include products of oncogenes, nuclear transcription factors, and other proteins critical for driving the malignant phenotype. The second approach involves harnessing the power of fusion receptors consisting of protein binding domains fused to T-cell signaling proteins. These receptors termed "chimeric antigen receptors" provide the opportunity to target the engineered T-cell against potentially any cell surface target antigen, thereby avoiding issues such as HLA loss frequently exploited by tumors as a mechanism to avoid T-cell immune surveillance. Moreover, the targeting of tumors in an HLA-independent manner means that any given CAR construct will be accessible to a broader range of patients than TCR-focused technology that is dependent upon HLA compatibility. These key advantages are counterbalanced by the fact that CAR T-cell technology is currently limited to targeting cell surface proteins, while TCR technology enables the T-cell to target

intracellular tumor antigens demonstrating that each strategy has its own advantages and drawbacks. Both approaches use retro- or lentiviral vector delivery to transfer TCR or CAR genes to the target T-cells.

Importantly, both strategies are now delivering impact upon the clinical stage. T-cells armed with TCRs specific for MART-1 in the context of HLA-A*0201¹³ were the first to be tested in a clinical phase I trial and demonstrated limited efficacy in melanoma. More recently, therapy with HLA-A*0201-restricted NY-ESO-1 TCR-specific T-cells resulted in objective clinical responses in 11 of 18 patients with NY-ESO-1⁺ synovial cell sarcoma and 11 of 20 patients with NY-ESO-1⁺ malignant melanoma.^{14,15} In the CAR field, there is frenzied activity within the United States focusing on CAR technology targeting the CD19 antigen. Reports of objective and sustained clinical responses in patients with B-cell acute lymphocytic leukemia (B-ALL) receiving CD19-specific CAR T-cells in several clinical centers^{16–22} have resulted in several pharma and biotech companies developing commercial strategies to exploit CD19 CAR technology. Beyond CD19 and B-cell malignancies, CAR T-cell technology to target solid tumors has proven more challenging with limited examples of clinical responses to date.²³ It is generally true that most of the disappointing solid tumor studies have involved first-generation CARs or minimal lymphodepletion, or have been stopped early because of “off-tumor, on-target” toxicity. However, a recent case study describing the treatment of two patients with T-cells armed with a mesothelin-specific second-generation CAR provides some early suggestions of clinical efficacy supporting the wholesale development of next-generation CARs for solid cancers. Early reports of responses have been observed despite transient CAR expression and lack of lymphodepletion.²⁴

These high-profile early phase clinical studies have, to a large extent, been performed in major U.S. academic medical centers. This has overshadowed the clinical and translational T-cell research occurring outside of the United States and including the United Kingdom. This in part reflects differences in the regulatory pathways and the funding environment between countries. Nonetheless, there is a breadth of T-cell-focused cancer research taking place outside of the United States. This review examines activity currently occurring within the United Kingdom in response to the BSGCT annual scientific meeting, but it should be borne in mind that major programs of work funded by the European Commission are delivering EU-wide translational research agendas, which include cancer-focused adoptive T-cell therapy.

Clinical Trial Activity Using T-Cell Immunotherapy in the United Kingdom by Location

A summary of recent, open, and about-to-open clinical trials is summarized in Table 1.

Christie Hospital and The University of Manchester

A successfully completed clinical trial of the adoptive transfer of CD25-depleted autologous peripheral blood mononuclear cells to six cyclophosphamide and fludarabine preconditioned renal cell carcinoma patients demonstrated that a transient reduction in circulating CD25⁺ putative regulatory T-cells could be achieved. Moreover, there was a corresponding transient increase in T-cell responses against

the tumor-associated antigen 5T4. No objective clinical responses were observed in these patients, while the sixth patient suffered neurological toxicity and death attributed to known but rare toxicity of fludarabine.²⁵ The observations suggested that a direct reduction of CD25⁺ putative regulatory T-cells in this manner provided only a short time window where Treg frequency was reduced and thereby potentially exploitable for immune-based therapies.

Subsequent trials that opened in 2007/8 involved first-generation CARs to target T-cells against carcinoembryonic antigen (CEA) and CD19. Both trials suffered unfortunate and extended delays going through regulatory approval because of the observations of therapy-induced lymphoproliferation in children receiving gene-modified HSCs to treat X-SCID²⁶ followed by the major adverse events in patients dosed with the Tegenora super-agonistic CD28 antibody.²⁷ Both of these high-profile events raised major questions concerning the safety (separately) of retroviral vectors and immune-directed therapies that required extensive review and re-review of the CEA and CD19 CAR T-cell protocols. The regulators accepted arguments that there was no evidence of oncogenic transformation of T-cells after transduction with retroviruses encoding CARs based upon studies that showed no evidence of adverse events in HIV⁺ patient's receiving a CD4ζ CAR.^{28,29} which has now extended past a decade of safety data.³⁰ This was further supported by pre-clinical studies showing that no transformational events were observed after mouse T-cells engrafted with retrovirus encoding CARs were engrafted for extended periods of time in mouse models.³¹ In terms of safety with respect to the immune targeting by CAR T-cells, the regulators were persuaded by the lack of immune toxicity in early trials of CAR T-cell therapy³² and the inclusion of steroid treatment to eliminate circulating autoreactive CAR T-cells if required.

Consequently, the CEA-targeted CAR T-cell trial opened in 2007 and involved a dose escalation of both CAR T-cells and intensity of chemotherapy (fludarabine alone in cohorts 1–3 increasing in intensity by the addition of cyclophosphamide in later cohorts, resulting in a more severe level of patient lymphodepletion). The trial was halted by the trial sponsor after 14 patients had been treated because of several patients suffering a transient shortness of breath. The report of this trial is in the final stages of preparation. However, the good manufacturing practice (GMP) process used to generate the CEA-specific CAR T-cells for this trial has been reported.³³

The CD19-specific first-generation CAR T-cell trial opened a little after the CEA CAR T-cell trial in March 2008. The protocol involved a cohort-based CAR T-cell dose-escalation to non-Hodgkins lymphoma patients preconditioned with a lower level of cyclophosphamide (15 mg/kg day −7 and day −6) and fludarabine (25 mg/m² day −5 to day −1) before adoptive T-cell transfer followed by IL-2 cytokine support. The study was temporarily suspended in 2010 because of the closure of the local cell processing facility and was reopened in November 2012 once a new cell processing facility had been established and approved for GMP manufacture of gene-modified cell products. The trial is currently open and recruiting with a 100% response rate in cohort 2 recently reported in abstract format at the 29th annual meeting of the Society of Immunotherapy of Cancer (SITC), National Harbor, MD, USA, November 2014.

TABLE 1. A SUMMARY OF T-CELL RECEPTOR, CHIMERIC ANTIGEN RECEPTOR, TUMOR INFILTRATING LYMPHOCYTE, AND VIRUS-SPECIFIC ADOPTIVE T-CELL CLINICAL TRIALS WITHIN THE UNITED KINGDOM AS OF FEBRUARY 2015

	<i>Trial identifier</i>	<i>Title</i>	<i>Current status</i> (Feb 2015)	<i>Transgene</i>	<i>Study start date</i>	<i>Study end date</i>	<i>Sponsor</i>	<i>Principle investigator</i>
CAR T-cells	NCT01493453	A Phase I Study of CD19 Specific T-Cells in CD19 Positive Malignancy	Open and recruiting	First generation CD19z	Mar-08		Christie Hospital NHS Trust, Manchester	Prof R Hawkins
	NCT01212887	Treated Blood Cells, Cyclophosphamide, Fludarabine Phosphate, and Aldesleukin in Treating Patients With Cancer	Closed	First generation MFE23z	Aug-07	Apr-10	Cancer Research UK	Prof R Hawkins
	NCT01195480	CD19-CAR Immunotherapy for Childhood Acute Lymphoblastic Leukaemia (ALL) (CD19TPALL)	Open and recruiting	First generation CD19z in EBV CTL	May-12		University College, London	Prof P Amrolia (Chair)
	NCT01818323	Phase I trial: T4 immunotherapy of head and neck cancer	Not yet open	Second generation ErbB family specific CD28.z receptor	Jun-13		Kings College, London	Dr J Maher
TCR T-cells	NCT01621724	WT-1 TCR gene therapy for Leukaemia: A phase I/II safety and toxicity trial	Open and recruiting	WT-1 specific TCR	Apr-12		Cell Therapy Catapult	Dr E Morris
	NCT01795976	NY-ESO-1 T-cells in OG cancer	Open and recruiting	NY-ESO-1 specific TCR (Adaptimmune)	Aril-2014		Christie Hospital NHS Trust, Manchester	Dr F Thistlethwaite
Suicide gene T-cells	GTAC 169	CMV-TCR gene therapy: A phase I/II	ON hold, pending new vector	CMV specific TCR			UCL	Dr E Morris
	EuDRACT 2014-003111-10	WT1-TCR gene therapy for MDS/AML: Single arm Phase I/II	Not yet open	WT1-Specific TCR			Cell Therapy Catapult	Dr E Morris
	EuDRACT 2005-001925-27	Phase I/II Clinical Trial of T-Cell Suicide Gene Therapy Following Haploidentical Stem Cell Transplantation	Complete	iCD34-HSVTK	2011	2013	Great Ormond Street Hospital NHS trust	Dr W Qasim
	EuDRACT 2014-000584-14	Phase I/II study of CaspaCide T-cells from an HLA partially matched family donor after negative selection of TCR $\alpha\beta$ + T-cells in pediatric patients affected by haematological disorders	Opens 2015	iCAS9			Bellicum	Dr W Qasim
TIL	NCT01995344	TIL therapy in Metastatic melanoma and IL-2 dose assessment (METILDA)	not yet open		Oct-13		Christie Hospital NHS Trust, Manchester	Prof R Hawkins
Virus specific natural T-cells	NCT01077908	Cytomegalovirus - Immunoprophylactic Adoptive Cellular Therapy Study (CMV-IMPACT)	completed	CMV specific selected T-cells	Jul-08	Oct-14	Cell Medica	Dr K Peggs (Chair)
	EuDRACT 2011-001-788-36	ASPIRE- Adenovirus-specific T-cells given to high-risk paediatric patients post HSCT	Open and recruiting	ADV specific T-cells	2012	2015	Cellmedica	Dr W Qasim
	MREC09/H0713/6	Virus specific T-cells for treatment of infection following HSCT	Closed	ADV or CMV specific T-cells	2008	2011	Great Ormond Street Hospital Trust	Dr W Qasim

CAR, chimeric antigen receptor; EBV, Epstein-Barr virus; TCR, T-cell receptor; TIL, tumor infiltrating lymphocyte.

A GMP-compliant TIL production process has been established in Manchester in partnership with colleagues at the NKI, Amsterdam; Herlev Hospital, Copenhagen; and the Sheba Medical Centre, Israel, based upon studies carried out at the National Cancer Institute, Bethesda, MD.^{3,4} Currently, seven patients have received melanoma TIL outside of the clinical trial situation under a “specials” protocol where patients are treated on a compassionate-use basis. A phase II study funded by the NIHR Efficacy and Mechanism Evaluation (EME) program comparing high- and low-dose IL-2 cytokine support in malignant melanoma patients receiving TIL (METILDA) is currently on-hold because of NHS budget issues. An EU-wide randomized phase III led from the NKI, Amsterdam, is open and recruiting in Amsterdam and Herlev Hospital, Copenhagen. Further NHS budgetary issues are holding back opening and recruitment at Christie Hospital, although it is hoped that these will be relaxed with subsequent recruitment to this trial possible soon in Manchester.

Finally, the EU Framework 7 program-funded “ATTACK” trial is led from Manchester (PI: Prof. Robert Hawkins) and involves partners across the EU, including University College London and Adaptimmune, who are providing the NY-ESO-1 TCR technology. This project is focused upon testing the NY-ESO-1-specific TCR in HLA-A*0201⁺ patients with upper gastrointestinal cancers to assess safety, and an initial evaluation of the potency is now open and recruiting. The second arm of the ATTACK trial involves a comparison of NY-ESO-1-specific T-cells generated by standard methods based upon polyclonal T-cell expansion supported by IL-2 compared with NY-ESO-1-specific T-cells isolated based upon CD62L expression and expanded in the presence of IL-7 and IL-15 to question whether the potentially increased naivety or reduced levels of differentiation in the latter cells result in equivalent antitumor activity in patients with malignant melanoma.

Royal Free London Hospital, University College London Hospital, and University College London

There is a growing phase I clinical trial portfolio (all “first-in-man” trials) including four trials with TCR-modified autologous or allogeneic T-cells and one trial with gene-modified HSCs. Three of the trials are testing TCR constructs (WT1 and CMV specific) developed by the Stauss and Morris research group^{34–36} and manufactured at the Great Ormond Street clinical production facility. The UK Cell Therapy Catapult has invested in the WT1-TCR gene therapy phase I clinical trial program and this partnership will oversee its subsequent commercialization, pending results of the early phase trials. The phase I trial for adult patients with acute myeloid leukemia opened in 2013 and is recruiting patients. A trial of cytomegalovirus-specific TCR gene therapy, funded by the Medical Research Council, is on hold (having recruited two patients) awaiting a new batch of vector but is expected to open in the near future. This trial is testing the safety and efficacy of TCR gene-modified allogeneic T-cells in the context of sibling Allo HSCT.

*University College London Hospital,
Great Ormond Street Hospital for Children,
and University College London*

A trial of CD19-CAR T-cell therapy for childhood acute lymphoblastic leukemia (CD19TPALL) is currently open

and recruiting at UCL and other European centers funded through the EU framework program. This protocol involves first-generation CD19 CAR-transduced EBV-specific CTL in children in first remission or relapse.

As a result of extensive preclinical activities, a plethora of trial proposals are funded and are currently at various stages of the regulatory process with some at an advanced stage. These include a trial of second-generation CAR T-cells targeting GD2 in children with relapsed neuroblastoma, which is scheduled to open in 2015 based at UCL and Great Ormond Street Hospital and funded by Cancer Research UK. The CAR will involve a novel humanized GD2 scFv and CD28 and CD3 zeta endodomains. The protocol involves a cohort-based CAR T-cell and lymphodepletion dose escalation up to a maximum regime of cyclophosphamide (300 mg/m²/day on days –4 to –1) and fludarabine (30 mg/m²/day on days –5 to –1) before adoptive transfer of up to 10⁸/m² transduced T-cells. There are several CD19 CAR-focused trial protocols in development with the expectation that these will move into the clinical testing arena within the next 18 months.

*Guy's and St. Thomas's Hospital,
Kings College London*

Investigators at Kings College have developed T4 technology in which a CAR targeted against eight of nine possible ErbB homo- and heterodimers³⁷ is coexpressed with an IL-4-responsive chimeric cytokine receptor.³⁸ The latter enables the *ex vivo* enrichment and amplification of gene-modified T-cells by the addition of IL-4 to the culture media. Clinical testing of T4 cells armed with an ErbB family-targeting domain is due to start in the near future involving local administration of the T4 cells to patients with advanced head and neck tumors.³⁹ In this manner, preconditioning of the patient is not required and also provides a solution to the serious toxicity observed after systemic infusion of Her2/*neu*-specific third-generation CAR T-cells to a patient with advanced metastatic colorectal cancer.⁴⁰

Commercial Developments Within the United Kingdom

In general, the United Kingdom lags behind the United States in its speed to commercially exploit its academic research outputs, and this remains true when considering the commercial landscape of adoptive T-cell therapy in 2015. In the United States, through the acquisition of CD19 CAR T-cell technology, the pharmaceutical giant Novartis has prompted a major surge of commercial investment with several companies raising startling levels of venture funding to deliver gene-modified and natural T-cell therapies. The commercial scene in the United Kingdom is currently largely dominated by companies developing intellectual property based upon identifying novel targets and novel receptors. The Oxford-based companies Adaptimmune and Immunocore are excellent examples focusing upon phage-selection technology to refine TCR technology for T-cell targeting and soluble targeting strategies, respectively.

However, the manufacture of clinical-grade T-cells remains an issue with only few specialized units associated with academic clinical centers able to deliver these cellular products. In response to this, Cellular Therapeutics Ltd. was spun out of the University of Manchester to exploit “clean-room free technology” to increase the potential throughput of

patient samples to support larger-scale clinical trials that the company is now doing for TIL and engineered T-cell activities both for local and now for delivery of cells to partners across the EU. In Glasgow, Scotland, TC Biopharm has developed as a site for the production of autologous $\gamma\delta$ T-cells for the treatment of cancer based upon the aforementioned work from Japan showing some promising early results in trials. Furthermore, CellMedica, based in London, has focused upon selection of virus-specific T-cells for ACT for viral infections following bone marrow transplantation. A trial of cytomegalovirus-specific T-cells has been successfully completed run from University College London and the company is treating adenovirus as part of a multicenter pediatric trial (ASPIRE study) and is also developing treatments for EBV infections.

Much more recently, the announcement of a new CAR T-cell company, Autolus, spun out from University College London and supported by venture capital from the Wellcome Trust, together with the imminent emergence of a further T-cell-focused company from Kings College London, indicates that there is a growing desire to support T-cell approaches. Indeed, the level of financing associated with Autolus (£30M) is the biggest series A in the history of European biotech. The potential promise of cell therapies has clearly been identified by the UK government, which has developed the UK Cell Therapy Catapult (CTC) via investment through Innovate UK (formally the Technology Strategy Board). The CTC aims to expedite the progress of cell therapies in the United Kingdom by investing in commercialization ventures and assisting in the development of cell therapies by providing clinical, regulatory, business, and technical expertise.

Preclinical Activity

There is currently a considerable focus upon TCR engineering across the United Kingdom. In London there is a core of GMP manufacturing expertise at Great Ormond Street Hospital led by Dr. Waseem Qasim and Professor Adrian Thrasher, with both gamma retroviral and lentiviral platforms being used to support trials across UCL and beyond. The group is also leading translational application of emerging nuclease reagents including TALENs for the generation of universal T-cells depleted of alloreactive T-cell receptors. Within University College London, Prof. Hans Stauss and Dr. Emma Morris have shown that TCR-redirectioned T-cells can function *in vitro* and *in vivo* targeting tumor or viral antigens supporting clinical trial activity^{34,41,42} and also that modifications to TCR sequences or transfer of additional molecules can enhance TCR expression and *in vivo* T-cell function,^{43,44} that clinical-grade gene-modified cell products can be generated,^{45,46} and that tolerance to tumor-associated antigens may be overcome.⁴⁷ Moreover, the group has also shown that genetic engineering of HSCs can alter the specificity of developing T-cells⁴⁸ and, in collaboration with others, has shown that gene-modified antigen-specific Tregs have suppressive function *in vivo*.⁴⁹

Dr. Gavin Bendle's research group in the School of Cancer Sciences and the Cancer Immunology & Immunotherapy Centre at the University of Birmingham is focused on the engineering of T-cell immunity to hematological malignancies through TCR gene transfer. A key aim of their current research is the selection and validation of TCRs for the clinical application of engineered T-cell therapy of multiple

myeloma.⁵⁰ The identification of target antigens in myeloproliferative neoplasms and the isolation of TCR genes against these targets is also an area of current research. Furthermore, they are utilizing preclinical models to assess the value of additional genetic engineering to tailor T-cell activity as well as specificity.⁵¹ Dr. Steven Lee's group in Birmingham is focusing upon engineering of T-cells to target the immune response directly to the malignant cells or to the tumor stroma upon which the malignant cells depend. Moreover, TCRs specific for EBV antigens are being exploited as therapies for EBV-associated cancers such as nasopharyngeal carcinoma. Using antibodies specific for several tumor endothelial markers, CARs have also been generated that selectively target the tumor vasculature. The safety and efficacy of these approaches are currently being explored both *in vitro* and using mouse tumor models. In further collaborative studies, they have also demonstrated that cord blood is a potential source of less differentiated effectors when engineering T-cells for therapy.⁵²

Efforts in Prof. Andrew Sewell's group at the University of Cardiff have focused around two main collaborations: those with Adaptimmune and sister company Immunocore and those with Inge Marie Svane and Per Thor Straten at the Center for Cancer Immune Therapy (CCIT) at Herlev Hospital in Copenhagen. Immunocore technology is based around phage display and selection of high-affinity TCRs.⁵³ This technology takes natural antitumor TCRs, which bind with low affinity (K_D 20–300 μM) and can increase the interaction by over a 1 million fold ($K_D < 10 pM$). Although modifications that result in enhanced affinity can occur in complementarity determining regions (CDRs) 1, 2, and 3, these enhanced receptors retain an extraordinary degree of specificity and they have never been observed to stain off-target cells in Cardiff. Curiously, recent results show that mutations in CDR2 that enhance affinity but that do not contact the peptide are overridden by subtle changes in TCR-peptide interactions including single-alanine substitutions.⁵⁴ Specificity of enhanced TCRs can be maintained by altered thermodynamics⁵⁵; however, further work will be required to determine the precise order of molecular events during TCR engagement. Nevertheless, these basic biochemical analyses bode well for enhanced (monoclonal) TCRs maintaining focused specificity. Immunocore are using TCRs with dissociation constants in the $< 50 pM$ range to deliver effector function to tumor cells. Their main platform is a bi-specific comprising a high-affinity TCR fused to an anti-CD3 scFv.^{56,57} This effectively redirects tumor lysis by polyclonal T-cells *in vivo*.⁵⁸ A bi-specific that targets an HLA-A2-restricted epitope from gp100 is currently in phase I/II dose-finding clinical trials in patients with late-stage malignant melanoma in the United Kingdom and United States.

Adaptimmune utilize cell-expressed enhanced TCRs. Use of TCRs with super-enhanced affinities ($K_D < 1 nM$) in this way is not recommended as these receptors are usually self-reactive.⁵⁹ Nevertheless, the phage enhancement process allows generation of TCRs with a whole range of affinities that can be optimized via a rigorous preclinical evaluation process.⁵⁹ High-affinity tumor-specific TCR clonotypes are often deleted from the repertoire as most targeted cancer epitopes are derived from self-proteins. Indeed, there is a wide affinity gap between TCRs that target pathogen-derived epitopes and those that target self-epitopes.⁶⁰ TCR

enhancement allows this affinity gap to be bridged to enable improved breaking of self-tolerance. TCR binding affinity governs the functional profile of TCR-transduced cancer-specific CD8⁺ T-cells, with optimal “anti-pathogen-like” TCRs being more sensitive to antigen and generating a wider range of functions in response to tumor cells.⁵⁹ Adaptimmune have also used their affinity enhancement techniques to select out HIV-specific TCRs that can recognize all known escape variants of the virus,⁶¹ and the future of this approach looks bright. Adaptimmune are currently conducting phase I/II trials of T-cells expressing enhanced NY-ESO-1-specific TCRs. Trials with MAGE-A-10 and AFP TCRs are in the developmental pipeline.

The Cardiff group is also dissecting the “Young TIL” trial for stage IV melanoma run in Copenhagen. This trial has a remarkable clinical response rate of up to 40% with many patients scored as “cure” (> 18 months disease-free). Because of resource limitations, studies have only focused on these success stories, and 2–35% of the TIL that were reintroduced into patients respond to autologous tumor although the exact number varies depending on patient and which effector function is examined. These responses appear to be remarkably broad with the TIL from one HLA A2⁺ patient responding to 18 known HLA A2 antigens. Interestingly, responses from complete remission patients can contain both $\alpha\beta$ and $\gamma\delta$ TCR clonotypes that are broadly tumoricidal and HLA-independent. These cells are of particular interest to the group although they have not yet established that tumor killing is mediated via the TCR. Cardiff University has also recently recruited Oliver Ottmann from Frankfurt and it is hoped that adoptive T-cell therapy trials will be conducted in Wales in the near future.

With the recent commercial focus upon CAR technology, the open discussion involving CAR design and technology has somewhat abated with groups globally focusing upon developing and protecting potentially lucrative intellectual property. However, the Gilham/Hawkins group at the University of Manchester is actively investigating CAR T-cell-mediated toxicity and, in particular, attempting to understand chronic toxicity associated with certain CD19-specific second-generation CARs in syngeneic lymphoma model systems⁶² while also working upon improving the *in vitro* culture conditions used to generate engineered T-cells to improve *in vivo* functionality.⁶³ Additionally, the recent arrival of Prof. Mark Exley to Manchester is stimulating work to investigate the efficacy and potency of CARs in iNKT cells.⁶

Within the London area, there is a considerable level of preclinical activity in the TCR/CAR field. Dr. John Maher continues to refine T4 CAR technology at Kings College London. A number of groups based at University College London are developing technologies that are at various levels of clinical development, including engineering lymphocytes with sort-suicide transgenes,⁶⁴ TCRs specific for viral antigens,⁴⁶ selection of adenoviral-specific T-cells for adoptive transfer,⁶⁵ and the examination of T-cell subsets for adoptive T-cell therapy.⁶⁶

Future Perspectives

In comparison to the United States, the absolute number of clinical trials led from the United Kingdom that are currently open and recruiting is small, reflecting factors including differential clinical regulatory processes, more limited funding

opportunities, and the need to deliver clinical trials within the NHS. Most development has been largely driven by academic initiatives with a relative lack of engagement so far with commercial partners. Future development is likely to be heavily influenced by national initiatives to increase availability of cell therapy production. For example, government investment to the level of £55 million for a phase III cell production center based in Stevenage (approximately 30 miles to the north of Central London) has been announced with a proposed opening date of 2017. This center will be developed by the Cell Catapult, a government-funded agency focused upon enhancing industrial-focused cell therapy activity. Exactly how this center will work and engage with the cell therapy community remains unclear.

Currently, UK-based cell manufacturing and gene modification infrastructure is predominantly academic based or lies with fledgling University spin-out companies (e.g., Cellular Therapeutics) that are working at the phase I/II clinical trial scale. Clearly, protecting this academic activity will be important to providing the early phase development of new technologies and delivering therapies in rare or novel disease indications where the economic drivers are lacking to encourage an industrial focus. However, this level of infrastructure is able to support only modest clinical activity. Moreover, there is an acute lack of capacity to produce GMP-grade viral vectors within the United Kingdom and the EU as a whole. Addressing these limitations will undoubtedly enhance the capacity to deliver early phase clinical studies that will eventually require expansion to randomized trial setting that will benefit from the specific development of a phase III cell production center. There will likely be advances in automation of T-cell engineering, and refinement of transduction processes which should alleviate some of the capacity issues.

Regulatory hurdles for new and experimental treatments are generally a limiting factor in the speed of clinical evaluation. The European Clinical Trials Directive 2001/20/EC was transposed into UK law as the Medicines for Human Use (Clinical Trials) Regulations 2004 (SI 2004/1031) that came into effect on the May 1, 2004. One of the key aspects of this legislation is the requirement that all investigation medicinal products must be manufactured to GMP standards and the manufacturer must have a manufacturing license. In short, this means that cell therapies must be GMP compliant for use in earliest phase clinical trials. The development of full GMP compliance for each cell therapy is a major rate-limiting and costly step before a first-into-man testing of a new therapy. Safety is clearly paramount, but providing the opportunity to move therapies more rapidly into the clinic using material generated to Good Laboratory Practice in a very early phase, limited-patient-number trials such as phase 0 trials provide an early opportunity to assess the potential of the specific cell therapy, potentially reducing phase II/III failures and speeding the entire clinical validation process.⁶⁷

Finally, how cell therapies will be funded within the United Kingdom in the future remains open to question. Reducing budgets will continue to impact upon the ability of the NHS to provide centralized funding of expensive treatments including cell-based therapies even should clinical benefit be demonstrated. Consequently, an underlying driver will be to deliver cell therapies in an economically competitive manner.

Overall, the examples cited here of preclinical activities demonstrate that there is a burst of early phase cell therapy

clinical trial activity on the horizon although it is also clear from this review that here is a growing focus of activity within the London and Manchester areas. The provision of phase III cell production capacity will place the United Kingdom in a pivotal position to deliver the randomized trials of cell therapy that will be required to confirm proof of activity over current standard of care.

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