World Journal of Immunology

Submit a Manuscript: http://www.wjgnet.com/esps/
Help Desk: http://www.wjgnet.com/esps/helpdesk.aspx
DOI: 10.5411/wji.v5.i3.86

World J Immunol 2015 November 27; 5(3): 86-94
ISSN 2219-2824 (online)

EDITORIAL

Chimeric antigen receptors: On the road to realising their full potential

May CI van Schalkwyk, John Maher

May CI van Schalkwyk, Department of HIV Medicine, Royal Free Hospital, Royal Free London NHS Foundation Trust, London NW3 2QG, United Kingdom

May CI van Schalkwyk, John Maher, CAR Mechanics Group, Department of Research Oncology, Guy's Hospital Campus, King's Health Partners Integrated Cancer Centre, King's College London, London SE1 9RT, United Kingdom

John Maher, Department of Immunology, Barnet Hospital, Royal Free London NHS Foundation Trust, Hertfordshire EN5 3DJ, United Kingdom

John Maher, Department of Clinical Immunology and Allergy, King's College Hospital NHS Foundation Trust, London SE5 9RS, United Kingdom

Author contributions: Both authors made an equal contribution to writing of the manuscript.

Supported by The Wellcome Trust, Cancer Research UK, Bayer, the Medical Research Council, Breast Cancer Now, Leukaemia and Lymphoma Research, Worldwide Cancer Research, June Hancock Mesothelioma Foundation, Jon Moulton Charitable Foundation, Pancreatic Cancer United Kingdom, the Experimental Cancer Medicine Centre at King's College London; and by the National Institute for Health Research (NIHR) Biomedical Research Centre based at Guy's and St Thomas' NHS Foundation Trust and King's College London.

Conflict-of-interest statement: Dr. John Maher is a founder and shareholder in Leucid Bio. There are no other conflicts of interest.

Correspondence to: Dr. John Maher, CAR Mechanics Group, Department of Research Oncology, Guy's Hospital Campus, King's Health Partners Integrated Cancer Centre, King's College London, Third floor Bermondsey Wing, Great Maze Pond, London SE1 9RT, United Kingdom. john.maher@kcl.ac.uk
Telephone: +44-207-1881468
Fax: +44-207-1880919

Received: June 24, 2015
Peer-review started: June 24, 2015
First decision: August 5, 2015
Revised: September 1, 2015
Accepted: October 12, 2015
Article in press: October 13, 2015
Published online: November 27, 2015

Abstract

Chimeric antigen receptors (CARs) are fusion molecules that may be genetically delivered *ex-vivo* to T-cells and other immune cell populations, thereby conferring specificity for native target antigens found on the surface of tumour and other target cell types. Antigen recognition by CARs is neither restricted by nor dependent upon human leukocyte antigen antigen expression, favouring widespread use of this technology across transplantation barriers. Signalling is delivered by a designer endodomain that provides a tailored and target-dependent activation signal to polyclonal circulating T-cells. Recent clinical data emphasise the enormous promise of this emerging immunotherapeutic strategy for B-cell malignancy, notably acute lymphoblastic leukaemia. In that context, CARs are generally targeted against the ubiquitous B-cell antigen, CD19. However, CAR T-cell immunotherapy is limited by potential for severe on-target toxicity, notably due to cytokine release syndrome. Furthermore, efficacy in the context of solid tumours remains unproven, owing in part to lack of availability of safe tumour-specific targets, inadequate CAR T-cell homing and hostility of the tumour microenvironment to immune effector deployment. Manufacture and commercial development of this strategy also impose new challenges not

encountered with more traditional drug products. Finally, there is increasing interest in the application of this technology to the treatment of non-malignant disease states, such as autoimmunity, chronic infection and in the suppression of allograft rejection. Here, we consider the background and direction of travel of this emerging and highly promising treatment for malignant and other disease types.

Key words: Adoptive T-cell immunotherapy; Chimeric antigen receptor; Genetic engineering; Leukaemia; Cancer

Core tip: Adoptive immunotherapy using chimeric antigen receptor-engineered T-cells has been in development for 25 years and, recently, has achieved striking impact in the management of B-cell malignancies. However, the therapy is often accompanied by significant toxicity, in particular cytokine release syndrome. While efficacy in B-cell acute leukaemia provides important clinical proof of concept, this therapy remains unproven in the arena of solid tumours and other disease types. Furthermore, manufacture of cell products is complex and difficult to scale out for widespread clinical use. Significant effort on all of these fronts will be required to enable this promising immunotherapy to enter the therapeutic mainstream.

van Schalkwyk MCI, Maher J. Chimeric antigen receptors: On the road to realising their full potential. *World J Immunol* 2015; 5(3): 86-94 Available from: URL: http://www.wjgnet.com/2219-2824/full/v5/i3/86.htm DOI: http://dx.doi.org/10.5411/wji.v5.i3.86

CHIMERIC ANTIGEN RECEPTORS: THE ROAD TO CREATION

During the latter half of the 20th century, several key advances in our understanding of human immunology have set the stage for the recent emergence of effective immunotherapies for cancer[1]. The pace of this advance is illustrated by the fact that, prior to 1960, the immunological role of the thymus was completely unknown. Thereafter, a cascade of research led to the discovery of two distinct lymphoid cell types of Thymic and Bone marrow origin - namely T- and B-cells - and established their unique roles in the respective establishment of cellular and humoral adaptive immunity. T- and B-lymphocytes proved to have an extraordinarily diverse capacity for antigen recognition, owing to recombination events that generated distinct clonotypic receptors in individual cells. In the case of the B-cell receptor, it emerged that native antigen was engaged directly and often with high affinity. By contrast, a very different system of antigen recognition was uncovered that applies to the predominant circulating T-cell population. These cells were found to interact using a T-cell receptor (TCR) with antigen-derived processed peptide fragments, presented within the groove of a human leukocyte antigen (HLA) on the target or antigen presenting cell surface. Investigation of the HLA gene system demonstrated that it is encoded within a super-locus in which classical transplantation genes are distributed into two major regions, known as HLA class Ⅰ and class Ⅱ. These genes proved to be the most polymorphic ever identified - hindering the clinical development of allogeneic transplantation and cell therapy - and were shown to restrict antigen presentation to two mutually exclusive T-cell subsets that express CD8 or CD4 co-receptors respectively. The discovery of cytokines such as interleukin-2 (IL-2) subsequently proved pivotal in enabling the *ex-vivo* culture and *in-vivo* support of such T-cells for adoptive immunotherapy[2]. As molecular immunology evolved, it became apparent that the ability of antigen to elicit T-cell activation was a complex and titratable phenomenon in which signalling was integrated *via* a multi-molecular synapse containing the TCR/CD3 complex (signal 1) and one or more co-stimulatory receptors, such as CD28 or 4-1BB (signal 2)[3]. More recently, the existence of a counterbalancing family of co-inhibitory receptors has been established, best exemplified by CTLA-4 and programmed death (PD)-1[4].

In parallel with these basic advances, a deepened appreciation has emerged of the dynamic inter-relationship between human cancer and the host immune system. In the 1980s, it was shown that tumour-infiltrating lymphocytes could elicit cytolytic activity against autologous melanoma[5]. Proof of concept for therapeutic activity of *ex-vivo* expanded TIL cells in patients with melanoma followed shortly thereafter[6], in a manner that was potentiated by preparatory lymphodepletion[7]. The increasing appreciation of how transformed cells are subject to such immune surveillance has recently been acknowledged in the proposal that evasion of this process represents a fundamental hallmark of cancer[8]. Two ground-breaking translational outputs of this growth in understanding have emerged more recently, rendering cancer immunotherapy the "Breakthrough of the Year" in 2013. The first of these involves immune checkpoint blockade - a form of immunotherapy that aims to release anti-tumour T-cells from the suppressive effects of co-inhibitory receptor ligation. This rapidly developing therapeutic approach has achieved striking and durable responses in patients with an ever-increasing number of solid tumour types[9]. The second approach aims to target precisely the cytotoxic ability of T-cells through the introduction of an ectopic TCR or chimeric antigen receptor (CAR). Use of TCR engineered T-cells has achieved clinical efficacy in small numbers of patients. However, the approach is limited by the fact that T-cells remain HLA restricted, rendering universal utility problematic and compromising activity against transformed cells that have downregulated HLA antigen

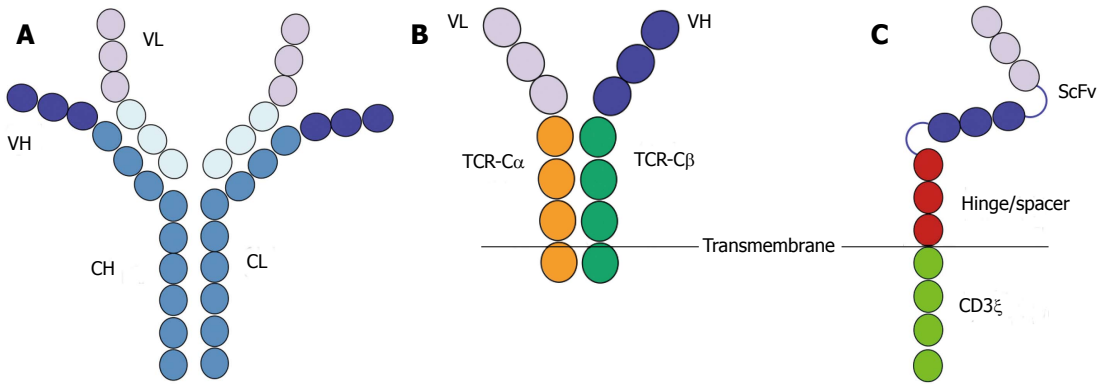

Figure 1 Chimeric antigen receptor structure. A: Most commonly CARs are targeted using the VH and VL domains of a monoclonal antibody; B: In prototypic CAR designs, these were individually fused to the constant domains of TCR-Cα and TCR-Cβ; C: However, more recent iterations entail the initial fusion of VH and VL, thereby creating a scFv moiety which is then fused to a hinge/spacer and a T-cell activating module, such as the endodomain of the ζ subunit of the TCR/CD3 complex. CH: Constant heavy domains; CL: Constant light domains; CARs: Chimeric antigen receptors; VH: Variable heavy; VL: Variable light; scFv: Single chain variable fragment.

expression. Use of CAR-engineered T-cells addresses these obstacles although additional issues continue to hinder this approach, most notably limited efficacy against solid tumours and toxicity due to cytokine release syndrome (CRS). The focus of this editorial is to assess prospects for CAR-based immunotherapy in this emerging new era of cellular therapy.

EVOLUTION OF CAR MODELS THROUGH THE GENERATIONS

CARs are synthetically engineered, membrane-spanning fusion proteins that can engage target molecules in their native form. First described by Eshhar in 1989[10], CARs comprise a targeting moiety, which is coupled to a signalling domain *via* hinge/spacer and transmembrane elements. This was originally achieved by individually combining the variable region of the heavy (VH) or light (VL) chains of a monoclonal antibody (Figure 1A) to the constant regions of the α or β subunits of the TCR (Figure 1B). Since this required the co-expression of two subunits, the initial structure was later simplified such that a single-chain variable fragment comprising a fusion of the VH and VL domains were joined to γ or ζ chains that respectively provide signalling from Fc receptors or the TCR/CD3 complex (Figure 1C)[11]. Alternatively, ligands may be used to engage single or multiple target species, broadening specificity[12]. Expression of CARs may be achieved using integrating viral vectors, notably retrovirus or lentivirus, or through transient non-viral systems (*e.g.*, mRNA electroporation). Identifying the most appropriate host cell population (or combination) for CAR T-cell immunotherapy, such as $CD8^+$, $CD4^+$, naïve, central memory, memory stem T-cell or natural killer cell is an area of intense investigation at present. Using a sequence of *ex-vivo* cell culture techniques, individualised autologous cell-based therapies can be engineered for use in patients (Figure 2).

CARs have evolved through a series of iterative modifications, designed to enhance potency and clinical benefit (Figure 3). First generation CARs possess only one intracellular domain and had limited T-cell activation capacity, leading to little clinical activity in early trials. Mindful of the key role of co-stimulation in the optimal activation and survival of T-cells, fusions were next produced in which modules from CD28 or 4-1BB were placed upstream of CD3ζ. Such second generation CARs mediate enhanced proliferation, cytokine release, *in-vivo* persistence and therapeutic efficacy in comparison to their predecessors[13-15]. Next, third generation CARs were engineered that contain two co-stimulatory modules[16] and which are now being evaluated in clinical trials, although the jury is still out with regard to the magnitude of this advance. Much effort has also been directed at optimising the ectodomain, hinge/spacer region and transmembrane element used in CAR construction as well as the vectors and methods used for T-cell transduction (Figure 2)[1,17]. In parallel, CARs have been co-expressed with accessory molecules to enhance safety (*e.g.*, drug-inducible suicide genes, such as inducible caspase 9[18]) or to improve further their anti-tumour activity. Examples of the latter include the use of cytokine receptors and their derivatives that enable *ex-vivo* expansion of CAR T-cells and which confer responsiveness to tumour-associated cytokines, such as IL-4 or colony-stimulating factor-1[19-21]. Alternatively, CAR T-cells may be engineered to home to the tumour microenvironment and to acquire resistance to immuno-suppressive mechanisms that operate there, for example through constitutive or inducible production of IL-12[22] or engineered resistance to transforming growth factor-β (*e.g.* NCT00889954; https://clinicaltrials.gov/ accessed 22-6-2015).

CLINICAL EVALUATION: WHAT'S ALL THE FUSS ABOUT?

Proof of concept for the game-changing potential of CAR-based immunotherapy has been exemplified by several recent clinical trials in patients with B-cell

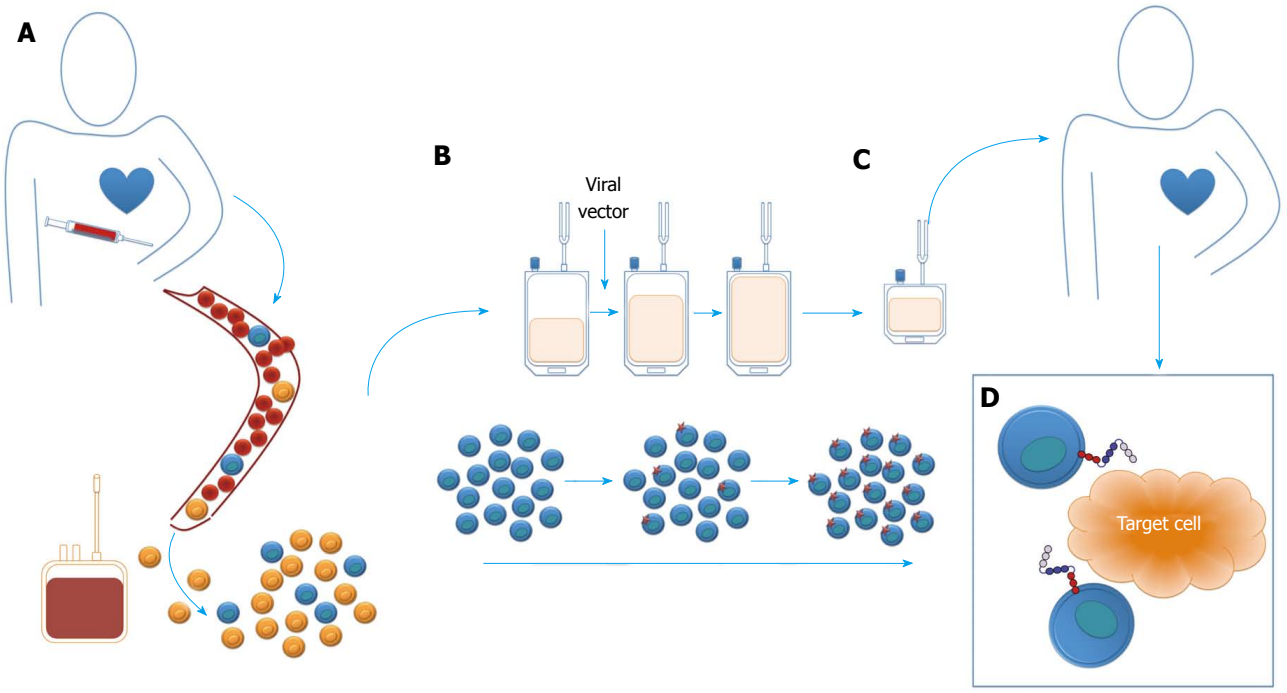

Figure 2 The overview of manufacture of chimeric antigen receptor T-cell products. A: Starting material is most commonly a leukapheresis, although some systems employ whole blood at this stage; B: Peripheral blood mononuclear cells are isolated and T-cells then activated and genetically engineered (in this case with a viral vector); C: After *ex-vivo* expansion, the cell product is formulated and administered; D: After which engineered cells can engage target cells *via* the CAR. CAR: Chimeric antigen receptor.

malignancies. In all cases, patients have received autologous CAR T-cells targeted against the ubiquitous B-cell antigen, CD19, and administered intravenously after lymphodepleting immunosuppression. In patients with acute lymphoblastic leukaemia (ALL), complete remission rates of 80% or above have been reported in several independent United States centres, employing second generation CARs that contain either CD28 or 4-1BB motifs in addition to CD3[23-27]. It is important to emphasise the unprecedented magnitude of these responses in the context of first in man evaluation in patients with otherwise untreatable malignancy. Furthermore, some of these patients had failed treatment with potent antibody derivatives such as blinatumomab, highlighting the greater potency of the CAR-based approach[25]. Highly impressive clinical outcomes have also been observed using CD19-targeted CAR T-cells in the setting of chronic lymphocytic leukaemia, and non-Hodgkin's lymphoma[23,28].

THE CLINICAL JOURNEY THUS FAR - ACHIEVING BALANCE AND CONTROL

The first comprehensive report of clinical testing of CAR T-cell immunotherapy is less than 10 years old[29]. Over that period, we have witnessed both the enormous clinical promise of this technology, together with its potential to cause extreme toxicity (summarized in Table 1). Many patients who have responded to CD19-targeted CAR T-cell immunotherapy have experienced CRS, accompanied in some cases by failure of one or more vital organs and/or macrophage activation syndrome. Pre-clinical studies indicate that such macrophage activation and release of monokines such as IL-6 represents a double-edged event, since this is required for maximal clinical efficacy[30], but contributes substantially to clinical manifestations of CRS[31]. As might be expected, anti-cytokine therapies such as the anti-IL-6 receptor antibody, tocilizumab and the anti-tumour necrosis factor-α antibody, infliximab have been used in patient management, as have traditional agents such as corticosteroids[32]. The inducible caspase 9 suicide system may also find particular utility in this setting since it has been shown to eliminate 90% of engineered cells within 30 min after administration of the triggering dimeriser drug[33].

By contrast to B-cell malignancy, efficacy of CAR T-cell immunotherapy in patients with solid tumours has been much more modest. Nonetheless, some responses have been achieved in paediatric patients, without unacceptable toxicity[34]. Solid tumours impose several key challenges to the successful development of CAR based immunotherapy that do not apply in B-cell malignancy. The first of these is the paucity of "CD19-like" targets - in other words, targets that are exclusively expressed on tumour cells alone, or in addition to tissue(s) that are dispensable or which perform a function that can be bypassed using a pharmacological solution. Consequently, self-antigens that are over-expressed in tumour cells and found at low levels in healthy tissues are generally selected for CAR T-cell

Table 1 Summary of toxicities that may be attributed to immunotherapy using chimeric antigen receptor engineered T-cells

On target on tumour toxicity	
Cytokine release syndrome[32]	Exaggerated activation of multiple leukocyte subtypes Marked elevation in circulating levels of multiple cytokines Pyrexia and acute phase response Vascular leakage Failure of one or more major organs May be related to tumour burden Occurs days to (exceptionally) weeks after T-cell infusion
Macrophage activation syndrome[25]	Haemophagocytosis Organomegaly Elevation of ferritin, aminotransferases, lactate dehydrogenase and triglycerides Hypofibrinogenemia
Tumour lysis syndrome	Rapid tumour cell destruction leading to profound metabolic disturbances, including hyperphosphatemia, hyperuricaemia, hyperkalaemia, hypocalcaemia and/or renal failure
On target off tumour toxicity	
CAR T-cell mediated immune attack of healthy tissue that express cognate target	Exemplified by B-cell aplasia induced by CD19-targeted CAR T-cells[23-28] hepatotoxicity induced by carbonic anhydrase IX-targeted CAR T-cells[37] pulmonary toxicity induced by HER2-targeted CAR T-cells[38]
Antibody mediated toxicity	Exemplified by anaphylaxis induced by mesothelin-targeted CAR T-cells[42]
Off target off tumour toxicity	
Insertional mutagenesis	Not seen with gene-modified T-cells as yet, unlike haemopoietic stem cells
Replication competent virus	Not seen with modern vector systems

CAR: Chimeric antigen receptor.

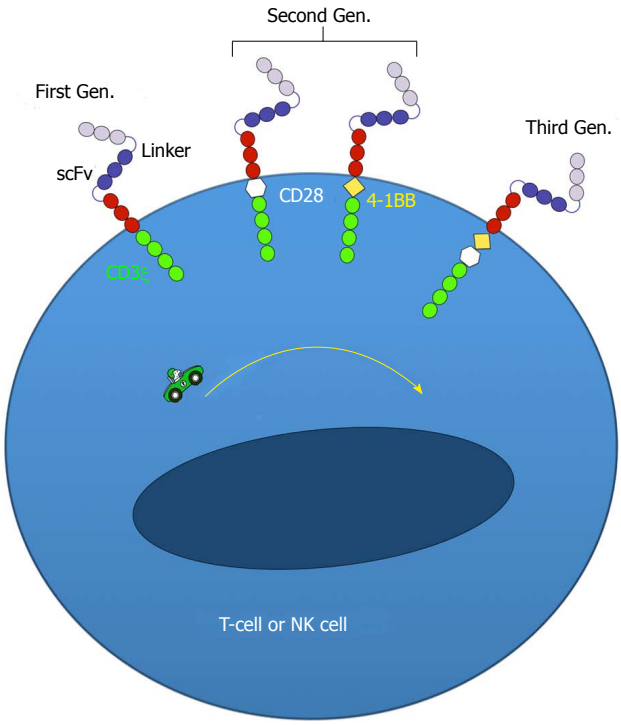

Figure 3 Evolution of chimeric antigen receptors. First Gen. CARs contain a source of "signal 1" alone, exemplified by CD3ζ. This is augmented in a second generation CAR by the inclusion of one co-stimulatory module, commonly from either CD28 or 4-1BB. The inclusion of two such co-stimulatory modules defines third generation CARs. CARs: Chimeric antigen receptors; Gen.:Generation.

immunotherapy. Indeed, targets that are expressed on T-cells, such as CD5, have been successfully targeted in pre-clinical models of T-cell lymphoma[35]. The use of lower affinity targeting moieties may enhance the safety of this approach, enabling better discrimination by CARs between tumour cells (target high) and healthy tissues (target low)[36]. Nonetheless, previous clinical experience has highlighted the difficulty inherent in negotiating this fine line[37]. One particularly unfortunate fatal adverse reaction occurred in a patient treated intravenously with 10 billion T-cells engineered to express a third generation HER2-targeted CAR, administered following lymphodepletion[38]. On-target toxicity ensued rapidly following engagement of HER2 in the pulmonary microvasculature, perhaps owing to the propensity of infused CAR T-cells to impact for several hours at that site[39]. More recently, a more cautiously implemented trial targeting HER2 has been completed and has achieved an efficacy signal without significant toxicity. Moreover, a study targeting mesothelin has also recently been reported in abstract form and, once again, has demonstrated safety, but with limited efficacy[40]. A further key challenge is the immunogenicity of CARs when administered to (non B-cell depleted) recipients[41], owing to the presence of xenogenic or other foreign sequences. The resultant antibody response favours the rapid clearance of these cells and has even resulted in life-threatening anaphylaxis[42]. An additional important obstacle is the need to enable T-cells to home more efficiently into tumour deposits and, once there, to operate effectively within the hostile tumour microenvironment. Pre-clinical approaches designed to address these obstacles are alluded to briefly above. One clinical strategy which may address some of these issues is direct delivery of CAR T-cells to the site of disease, a strategy that has recently been evaluated in glioblastoma[43] and is undergoing evaluation in head and neck cancer[20]. A summary of clinical trials in

Table 2 Clinical trials of chimeric antigen receptor T-cell immunotherapy of haematological malignancy

Target	No. of trials	Diseases
CD19	40	Acute and chronic B-cell malignancies Hodgkin's lymphoma
CD30	4	Hodgkin's lymphoma; CD30+ non-Hodgkin's lymphoma
CD20	2	B-cell malignancy
CD22	1	B-cell malignancy
Kappa light chain	1	B-cell malignancy Multiple myeloma
CD33	1	Acute myeloid leukaemia
CD138	1	Multiple myeloma
CD123	1	Acute myeloid leukaemia
Lewis Y antigen		Acute myeloid leukaemia Myelodysplastic syndrome; Multiple myeloma
NKG2D stress ligands	1	Acute myeloid leukaemia; Myelodysplastic syndrome; Multiple myeloma
Combinatorial	1	CD16-containing "universal CAR" targeted with rituximab for B-cell malignancy

CAR: Chimeric antigen receptor.

haematological malignancy (Table 2) and solid tumours/other diseases (Table 3) that are currently registered on the clinicaltrials.gov website is provided. Trials were based in United States (65), China (15), United Kingdom (5) and 1 each in Japan, the Netherlands, Australia, Sweden, Singapore, Switzerland and Israel.

THE NEED FOR AUTO-MANUFACTURING OF CAR T-CELLS

Manufacture, formulation and certification of autologous CAR-based cell therapy products continues to pose obstacles to robust large-scale production and widespread clinical use[44]. Products must be made to Good Manufacturing Practice standards, meaning that manufacturing protocols are often time-consuming, expensive, labour-intensive and cumbersome. Autologous cell products constitute the ultimate "personalised medicine" since each batch generally provides one or more treatments for a single patient. As a result, there is a need to "scale-out" to maximise the numbers of batches produced, rather than "scale-up" in order to generate large volume batches for multi-patient use. Manufacturing techniques in current use often introduce inter-operator variability, entail the use of open processing systems and require intensive training of personnel. The facilities, equipment, staffing and documentation involved in the engineering and delivery of a single product renders widespread implementation challenging, even in the setting of resource-rich countries. Regulations used to guide production and release were originally developed for traditional pharmaceutical agents with linear supply chains and well established business and supply models. These impose significant challenges for autologous CAR T-cell therapies, which involve circular supply chains in which the first step entails a blood draw or leukapheresis. Adding to complexity, there is a lack of harmonisation in the application of regulations pertaining to manufacture across different geographical sectors. In Europe for example, products require qualified person certification prior to release whereas this requirement does not operate in the United States.

In light of these considerations, efforts need to focus upon standardisation and automation of production, employing existing infrastructure such as exists in blood banks or stem cell facilities[45] and increased reliance on closed manufacturing processes wherever possible[46]. A key dilemma in this regard is the decision to opt for centralised *vs* multi-site manufacture. While the latter can minimise distance between the patient and site of production, it requires the establishment and maintenance of comparability of cell product quality across sites. This in turn emphasises the need for careful characterisation of cellular source material and raw materials. Product stability is also a key factor, which may require development of validated cryopreservation techniques, raising issues about whether products should be thawed immediately prior to shipping or subject to manipulation upon receipt at the site of administration.

An alternative manufacturing solution that is attracting increasing interest entails the development of allogeneic and potentially universally applicable CAR T-cell products. In light of HLA polymorphism and the cross-reactive nature of the TCR (leading to alloreactivity), this requires consideration of strategies to eliminate risk of graft *vs* host disease and rejection of infused cells. Although at an early stage, interest has been raised in the use of genomic editing tools to address such limitations[46]. Alternatively, use of lymphoid precursors that complete thymic education in the recipient, or engineered T-cells derived from induced pluripotent stem cells may warrant consideration as potential solutions to the histocompatibility problem[47].

DRIVING CARS INTO DIVERSE APPLICATIONS

As experience with CAR T-cell immunotherapy of malig-

Table 3 Clinical trials of chimeric antigen receptor T-cell immunotherapy of solid tumours and other diseases

Target	No. of trials	Diseases
HER2	7	Glioblastoma
		HER2 expressing solid tumours[1]
GD2	7	Neuroblastoma[34]
		GD2-expressing malignancy
		Osteosarcoma
		Melanoma
Mesothelin	4	Pancreatic cancer[40]
		Ovarian cancer
		Mesothelioma[2]
CEA	4	Breast cancer
		CEA expressing malignancy[1]
Folate receptor-α	1	Epithelial ovarian cancer[29]
EGFr	2	$EGFr^+$ malignancy
		Glioblastoma
EGFr variant Ⅲ	2	Glioblastoma
Carbonic anhydrase Ⅸ	1	Renal cell carcinoma[37]
Prostate-specific membrane antigen	2	Castrate-resistant prostate cancer
Interleukin-13 receptor α2	1	Glioma[43]
CD171 (L1 cell adhesion molecule)	1	Neuroblastoma
Extended ErbB family	1	Head and neck cancer3[20]
Fibroblast-activation protein	1	Mesothelioma
Glypican-3	1	Hepatocellular carcinoma
Pathogenic T-cell receptors	1	Type 1 diabetes[4]

(https://clinicaltrials.gov/accessed August 26th, 2015 search terms: T cell gene cancer; chimeric and cancer; T-cell cancer and gene). [1]One Trial terminated due to toxicity; [2]Trial includes the co-administration of CD19-targeted CAR T-cells to minimize anti-CAR antibody production; [3]Intra-tumoural route in use to minimize toxicity; [4]CAR targeted using a peptide + HLA-CD3ξ fusion; CAR: Chimeric antigen receptor; CEA: Carcinoembryonic antigen; EGFr: Epidermal growth factor receptor.

nant disease has grown, increasing consideration has been given to the development of CAR-based therapies for diverse non-malignant disease states. Several preclinical studies have demonstrated the potential benefit of the adoptive transfer of purified regulatory T-cells (Tregs) in the treatment of autoimmune disease and donor graft rejection. Currently, many clinical trials are investigating the safety and efficacy of adoptive transfer of *ex-vivo* expanded Tregs in these disease settings, although cells are not targeted in many cases, which may compromise efficacy. It has therefore been suggested that CAR-targeted Tregs may have a potential role achieving more potent and targeted therapeutic immunosuppression[48].

The potential benefit of T-cell based therapies in the treatment of infectious diseases, for example human immunodeficiency virus (HIV), has also been the focus of recent research and discussions. This infection is known to elicit a cytotoxic $CD8^+$ T-cell response, the potency of which is believed to correlate with viral load and disease control. This phenomenon is exemplified by a unique cohort of patients known as "HIV controllers", individuals who appear to have an inherent ability to keep the virus at bay. This phenotype is believed to be due in part to a potent $CD8^+$ HIV-specific T-cell response as opposed to an inherent resistance to initial viral infection. Based on these findings the delivery of T-cell therapies including HIV-targeted engineered CAR-based therapies was postulated to be of potential benefit in the fight against HIV and in the quest to develop a cure. Studies of first generation CAR T-cells in patients with HIV infection have demonstrated the remarkable ability of these cells to persist and to home to sites of disease, calling into question the need to evaluate more modern CAR T-cell strategies in this setting[49].

THE FUTURE OF CARS: ENSURING A SMOOTH RIDE

Based upon 25 years of pre-clinical and clinical experience with CAR-based therapies, we are potentially entering into an era of rapid advancement in design and more widespread use of CAR-based therapies within a broad range of clinical settings. However, there is still much work to be done to ensure efficient and effective progress. Major challenges remain in the cancer setting surrounding issues such as target selection, maintenance of *in-vivo* survival of CAR T-cells, and the achievement of sustained but not excessive function. Future questions need to address the combinatorial use of these cells in conjunction with conventional (*e.g.*, chemotherapy, radiotherapy, tumour-targeted monoclonal antibodies) and emerging therapies, such as immune checkpoint blockade. Development of standardised and robust manufacturing solutions also presents a new challenge to commercial development. Nonetheless, the stunning and unparalleled activity of this technology in B-cell malignancy coupled with its amenability to precise and highly refined engineering emphasises the fact that top

gear for CAR T-cell immunotherapy remains tantalisingly around the corner.

ACKNOWLEDGMENTS

The authors acknowledge the help of all collaborators in our CAR T-cell and immunotherapy programmes.

REFERENCES

1 **Miller JF**, Sadelain M. The journey from discoveries in fundamental immunology to cancer immunotherapy. *Cancer Cell* 2015; **27**: 439-449 [PMID: 25858803 DOI: 10.1016/j.ccell.2015.03.007]

2 **Morgan DA**, Ruscetti FW, Gallo R. Selective in vitro growth of T lymphocytes from normal human bone marrows. *Science* 1976; **193**: 1007-1008 [PMID: 181845]

3 **Bretscher P**, Cohn M. A theory of self-nonself discrimination. *Science* 1970; **169**: 1042-1049 [PMID: 4194660]

4 **Chen L**, Flies DB. Molecular mechanisms of T cell co-stimulation and co-inhibition. *Nat Rev Immunol* 2013; **13**: 227-242 [PMID: 23470321 DOI: 10.1038/nri3405]

5 **Muul LM**, Spiess PJ, Director EP, Rosenberg SA. Identification of specific cytolytic immune responses against autologous tumor in humans bearing malignant melanoma. *J Immunol* 1987; **138**: 989-995 [PMID: 3100623]

6 **Rosenberg SA**, Packard BS, Aebersold PM, Solomon D, Topalian SL, Toy ST, Simon P, Lotze MT, Yang JC, Seipp CA. Use of tumor-infiltrating lymphocytes and interleukin-2 in the immunotherapy of patients with metastatic melanoma. A preliminary report. *N Engl J Med* 1988; **319**: 1676-1680 [PMID: 3264384 DOI: 10.1056/NEJM198812223192527]

7 **Dudley ME**, Wunderlich JR, Yang JC, Sherry RM, Topalian SL, Restifo NP, Royal RE, Kammula U, White DE, Mavroukakis SA, Rogers LJ, Gracia GJ, Jones SA, Mangiameli DP, Pelletier MM, Gea-Banacloche J, Robinson MR, Berman DM, Filie AC, Abati A, Rosenberg SA. Adoptive cell transfer therapy following non-myeloablative but lymphodepleting chemotherapy for the treatment of patients with refractory metastatic melanoma. *J Clin Oncol* 2005; **23**: 2346-2357 [PMID: 15800326 DOI: 10.1200/JCO.2005.00.240]

8 **Hanahan D**, Weinberg RA. Hallmarks of cancer: the next generation. *Cell* 2011; **144**: 646-674 [PMID: 21376230 DOI: 10.1016/j.cell.2011.02.013]

9 **Pico de Coaña Y**, Choudhury A, Kiessling R. Checkpoint blockade for cancer therapy: revitalizing a suppressed immune system. *Trends Mol Med* 2015; **21**: 482-491 [PMID: 26091825 DOI: 10.1016/j.molmed.2015.05.005]

10 **Gross G**, Waks T, Eshhar Z. Expression of immunoglobulin-T-cell receptor chimeric molecules as functional receptors with antibody-type specificity. *Proc Natl Acad Sci USA* 1989; **86**: 10024-10028 [PMID: 2513569]

11 **Eshhar Z**, Waks T, Gross G, Schindler DG. Specific activation and targeting of cytotoxic lymphocytes through chimeric single chains consisting of antibody-binding domains and the gamma or zeta subunits of the immunoglobulin and T-cell receptors. *Proc Natl Acad Sci USA* 1993; **90**: 720-724 [PMID: 8421711]

12 **Davies DM**, Foster J, Van Der Stegen SJ, Parente-Pereira AC, Chiapero-Stanke L, Delinassios GJ, Burbridge SE, Kao V, Liu Z, Bosshard-Carter L, Van Schalkwyk MC, Box C, Eccles SA, Mather SJ, Wilkie S, Maher J. Flexible targeting of ErbB dimers that drive tumorigenesis by using genetically engineered T cells. *Mol Med* 2012; **18**: 565-576 [PMID: 22354215]

13 **Finney HM**, Lawson AD, Bebbington CR, Weir AN. Chimeric receptors providing both primary and costimulatory signaling in T cells from a single gene product. *J Immunol* 1998; **161**: 2791-2797 [PMID: 9743337]

14 **Maher J**, Brentjens RJ, Gunset G, Rivière I, Sadelain M. Human T-lymphocyte cytotoxicity and proliferation directed by a single chimeric TCRzeta/CD28 receptor. *Nat Biotechnol* 2002; **20**: 70-75 [PMID: 11753365 DOI: 10.1038/nbt0102-70]

15 **Imai C**, Mihara K, Andreansky M, Nicholson IC, Pui CH, Geiger TL, Campana D. Chimeric receptors with 4-1BB signaling capacity provoke potent cytotoxicity against acute lymphoblastic leukemia. *Leukemia* 2004; **18**: 676-684 [PMID: 14961035 DOI: 10.1038/sj.leu.2403302]

16 **Pulè MA**, Straathof KC, Dotti G, Heslop HE, Rooney CM, Brenner MK. A chimeric T cell antigen receptor that augments cytokine release and supports clonal expansion of primary human T cells. *Mol Ther* 2005; **12**: 933-941 [PMID: 15979412 DOI: 10.1016/j.ymthe.2005.04.016]

17 **Maher J**. Immunotherapy of malignant disease using chimeric antigen receptor engrafted T cells. *ISRN Oncol* 2012; **2012**: 278093 [PMID: 23304553 DOI: 10.5402/2012/278093]

18 **Straathof KC**, Pulè MA, Yotnda P, Dotti G, Vanin EF, Brenner MK, Heslop HE, Spencer DM, Rooney CM. An inducible caspase 9 safety switch for T-cell therapy. *Blood* 2005; **105**: 4247-4254 [PMID: 15728125 DOI: 10.1182/blood-2004-11-4564]

19 **Wilkie S**, Burbridge SE, Chiapero-Stanke L, Pereira AC, Cleary S, van der Stegen SJ, Spicer JF, Davies DM, Maher J. Selective expansion of chimeric antigen receptor-targeted T-cells with potent effector function using interleukin-4. *J Biol Chem* 2010; **285**: 25538-25544 [PMID: 20562098 DOI: 10.1074/jbc.M110.127951]

20 **van Schalkwyk MC**, Papa SE, Jeannon JP, Guerrero Urbano T, Spicer JF, Maher J. Design of a phase I clinical trial to evaluate intratumoral delivery of ErbB-targeted chimeric antigen receptor T-cells in locally advanced or recurrent head and neck cancer. *Hum Gene Ther Clin Dev* 2013; **24**: 134-142 [PMID: 24099518 DOI: 10.1089/humc.2013.144]

21 **Lo AS**, Taylor JR, Farzaneh F, Kemeny DM, Dibb NJ, Maher J. Harnessing the tumour-derived cytokine, CSF-1, to co-stimulate T-cell growth and activation. *Mol Immunol* 2008; **45**: 1276-1287 [PMID: 17950877 DOI: 10.1016/j.molimm.2007.09.010]

22 **Curran KJ**, Brentjens RJ. Chimeric antigen receptor T cells for cancer immunotherapy. *J Clin Oncol* 2015; **33**: 1703-1706 [PMID: 25897155 DOI: 10.1200/JCO.2014.60.3449]

23 **Maher J**. Clinical immunotherapy of B-cell malignancy using CD19-targeted CAR T-cells. *Curr Gene Ther* 2014; **14**: 35-43 [PMID: 24365143]

24 **Maude SL**, Teachey DT, Porter DL, Grupp SA. CD19-targeted chimeric antigen receptor T-cell therapy for acute lymphoblastic leukemia. *Blood* 2015; **125**: 4017-4023 [PMID: 25999455 DOI: 10.1182/blood-2014-12-580068]

25 **Maude SL**, Frey N, Shaw PA, Aplenc R, Barrett DM, Bunin NJ, Chew A, Gonzalez VE, Zheng Z, Lacey SF, Mahnke YD, Melenhorst JJ, Rheingold SR, Shen A, Teachey DT, Levine BL, June CH, Porter DL, Grupp SA. Chimeric antigen receptor T cells for sustained remissions in leukemia. *N Engl J Med* 2014; **371**: 1507-1517 [PMID: 25317870 DOI: 10.1056/NEJMoa1407222]

26 **Lee DW**, Kochenderfer JN, Stetler-Stevenson M, Cui YK, Delbrook C, Feldman SA, Fry TJ, Orentas R, Sabatino M, Shah NN, Steinberg SM, Stroncek D, Tschernia N, Yuan C, Zhang H, Zhang L, Rosenberg SA, Wayne AS, Mackall CL. T cells expressing CD19 chimeric antigen receptors for acute lymphoblastic leukaemia in children and young adults: a phase 1 dose-escalation trial. *Lancet* 2015; **385**: 517-528 [PMID: 25319501 DOI: 10.1016/S0140-6736(14)61403-3]

27 **Davila ML**, Riviere I, Wang X, Bartido S, Park J, Curran K, Chung SS, Stefanski J, Borquez-Ojeda O, Olszewska M, Qu J, Wasielewska T, He Q, Fink M, Shinglot H, Youssif M, Satter M, Wang Y, Hosey J, Quintanilla H, Halton E, Bernal Y, Bouhassira DC, Arcila ME, Gonen M, Roboz GJ, Maslak P, Douer D, Frattini MG, Giralt S, Sadelain M, Brentjens R. Efficacy and toxicity management of 19-28z CAR T cell therapy in B cell acute lymphoblastic leukemia. *Sci Transl Med* 2014; **6**: 224ra25 [PMID: 24553386 DOI: 10.1126/scitranslmed.3008226]

28 **Kochenderfer JN**, Dudley ME, Kassim SH, Somerville RP, Carpenter RO, Stetler-Stevenson M, Yang JC, Phan GQ, Hughes MS, Sherry RM, Raffeld M, Feldman S, Lu L, Li YF, Ngo LT, Goy A, Feldman T, Spaner DE, Wang ML, Chen CC, Kranick

SM, Nath A, Nathan DA, Morton KE, Toomey MA, Rosenberg SA. Chemotherapy-refractory diffuse large B-cell lymphoma and indolent B-cell malignancies can be effectively treated with autologous T cells expressing an anti-CD19 chimeric antigen receptor. *J Clin Oncol* 2015; **33**: 540-549 [PMID: 25154820 DOI: 10.1200/JCO.2014.56.2025]

29 **Kershaw MH**, Westwood JA, Parker LL, Wang G, Eshhar Z, Mavroukakis SA, White DE, Wunderlich JR, Canevari S, Rogers-Freezer L, Chen CC, Yang JC, Rosenberg SA, Hwu P. A phase I study on adoptive immunotherapy using gene-modified T cells for ovarian cancer. *Clin Cancer Res* 2006; **12**: 6106-6115 [PMID: 17062687 DOI: 10.1158/1078-0432.CCR-06-1183]

30 **Parente-Pereira AC**, Whilding LM, Brewig N, van der Stegen SJ, Davies DM, Wilkie S, van Schalkwyk MC, Ghaem-Maghami S, Maher J. Synergistic Chemoimmunotherapy of Epithelial Ovarian Cancer Using ErbB-Retargeted T Cells Combined with Carboplatin. *J Immunol* 2013; **191**: 2437-2445 [PMID: 23898037 DOI: 10.4049/jimmunol.1301119]

31 **van der Stegen SJ**, Davies DM, Wilkie S, Foster J, Sosabowski JK, Burnet J, Whilding LM, Petrovic RM, Ghaem-Maghami S, Mather S, Jeannon JP, Parente-Pereira AC, Maher J. Preclinical in vivo modeling of cytokine release syndrome induced by ErbB-retargeted human T cells: identifying a window of therapeutic opportunity? *J Immunol* 2013; **191**: 4589-4598 [PMID: 24062490 DOI: 10.4049/jimmunol.1301523]

32 **Maude SL**, Barrett D, Teachey DT, Grupp SA. Managing cytokine release syndrome associated with novel T cell-engaging therapies. *Cancer J* 2014; **20**: 119-122 [PMID: 24667956 DOI: 10.1097/PPO.0000000000000035]

33 **Di Stasi A**, Tey SK, Dotti G, Fujita Y, Kennedy-Nasser A, Martinez C, Straathof K, Liu E, Durett AG, Grilley B, Liu H, Cruz CR, Savoldo B, Gee AP, Schindler J, Krance RA, Heslop HE, Spencer DM, Rooney CM, Brenner MK. Inducible apoptosis as a safety switch for adoptive cell therapy. *N Engl J Med* 2011; **365**: 1673-1683 [PMID: 22047558 DOI: 10.1056/NEJMoa1106152]

34 **Pule MA**, Savoldo B, Myers GD, Rossig C, Russell HV, Dotti G, Huls MH, Liu E, Gee AP, Mei Z, Yvon E, Weiss HL, Liu H, Rooney CM, Heslop HE, Brenner MK. Virus-specific T cells engineered to coexpress tumor-specific receptors: persistence and antitumor activity in individuals with neuroblastoma. *Nat Med* 2008; **14**: 1264-1270 [PMID: 18978797 DOI: 10.1038/nm.1882]

35 **Mamonkin M**, Rouce RH, Tashiro H, Brenner MK. A T-cell-directed chimeric antigen receptor for the selective treatment of T-cell malignancies. *Blood* 2015; **126**: 983-992 [PMID: 26056165 DOI: 10.1182/blood-2015-02-629527]

36 **Song DG**, Ye Q, Poussin M, Liu L, Figini M, Powell DJ. A fully human chimeric antigen receptor with potent activity against cancer cells but reduced risk for off-tumor toxicity. *Oncotarget* 2015; **6**: 21533-21546 [PMID: 26101914]

37 **Lamers CH**, Sleijfer S, Vulto AG, Kruit WH, Kliffen M, Debets R, Gratama JW, Stoter G, Oosterwijk E. Treatment of metastatic renal cell carcinoma with autologous T-lymphocytes genetically retargeted against carbonic anhydrase IX: first clinical experience. *J Clin Oncol* 2006; **24**: e20-e22 [PMID: 16648493]

38 **Morgan RA**, Yang JC, Kitano M, Dudley ME, Laurencot CM, Rosenberg SA. Case report of a serious adverse event following the administration of T cells transduced with a chimeric antigen receptor recognizing ERBB2. *Mol Ther* 2010; **18**: 843-851 [PMID: 20179677 DOI: 10.1038/mt.2010.24]

39 **Parente-Pereira AC**, Burnet J, Ellison D, Foster J, Davies DM, van der Stegen S, Burbridge S, Chiapero-Stanke L, Wilkie S, Mather S, Maher J. Trafficking of CAR-engineered human T cells following regional or systemic adoptive transfer in SCID beige mice. *J Clin Immunol* 2011; **31**: 710-718 [PMID: 21505816 DOI: 10.1007/s10875-011-9532-8]

40 **Beatty GL**, O'Hara MH, Nelson AM, McGarvey M, Torigian DA, Lacey SF, Melenhorst JJ, Levine B, Plesa G, June CH. Safety and antitumor activity of chimeric antigen receptor modified T cells in patients with chemotherapy refractory metastatic pancreatic cancer. *J Clin Oncol* 2015; **33**: 3007

41 **Lamers CH**, Willemsen R, van Elzakker P, van Steenbergen-Langeveld S, Broertjes M, Oosterwijk-Wakka J, Oosterwijk E, Sleijfer S, Debets R, Gratama JW. Immune responses to transgene and retroviral vector in patients treated with ex vivo-engineered T cells. *Blood* 2011; **117**: 72-82 [PMID: 20889925 DOI: 10.1182/blood-2010-07-294520]

42 **Maus MV**, Haas AR, Beatty GL, Albelda SM, Levine BL, Liu X, Zhao Y, Kalos M, June CH. T cells expressing chimeric antigen receptors can cause anaphylaxis in humans. *Cancer Immunol Res* 2013; **1**: 26-31 [PMID: 24777247 DOI: 10.1158/2326-6066.CIR-13-0006]

43 **Brown CE**, Badie B, Barish ME, Weng L, Ostberg JR, Chang WC, Naranjo A, Starr R, Wagner J, Wright C, Zhai Y, Bading JR, Ressler JA, Portnow J, D'Apuzzo M, Forman SJ, Jensen MC. Bioactivity and Safety of IL13Rα2-Redirected Chimeric Antigen Receptor CD8+ T Cells in Patients with Recurrent Glioblastoma. *Clin Cancer Res* 2015; **21**: 4062-4072 [PMID: 26059190 DOI: 10.1158/1078-0432.CCR-15-0428]

44 **Hourd P**, Ginty P, Chandra A, Williams DJ. Manufacturing models permitting roll out/scale out of clinically led autologous cell therapies: regulatory and scientific challenges for comparability. *Cytotherapy* 2014; **16**: 1033-1047 [PMID: 24856894 DOI: 10.1016/j.jcyt.2014.03.005]

45 **Levine BL**, June CH. Perspective: assembly line immunotherapy. *Nature* 2013; **498**: S17 [PMID: 23803946]

46 **Kaiser AD**, Assenmacher M, Schröder B, Meyer M, Orentas R, Bethke U, Dropulic B. Towards a commercial process for the manufacture of genetically modified T cells for therapy. *Cancer Gene Ther* 2015; **22**: 72-78 [PMID: 25613483 DOI: 10.1038/cgt.20 14.78]

47 **Themeli M**, Rivière I, Sadelain M. New cell sources for T cell engineering and adoptive immunotherapy. *Cell Stem Cell* 2015; **16**: 357-366 [PMID: 25842976 DOI: 10.1016/j.stem.2015.03.011]

48 **Jethwa H**, Adami AA, Maher J. Use of gene-modified regulatory T-cells to control autoimmune and alloimmune pathology: is now the right time? *Clin Immunol* 2014; **150**: 51-63 [PMID: 24333533 DOI: 10.1016/j.clim.2013.11.004]

49 **Lam S**, Bollard C. T-cell therapies for HIV. *Immunotherapy* 2013; **5**: 407-414 [PMID: 23557423 DOI: 10.2217/imt.13.23]

P- Reviewer: Corthay A, Ferrante A, Lichtor T, Yves R
S- Editor: Qiu S **L- Editor**: A **E- Editor**: Jiao XK

Published by **Baishideng Publishing Group Inc**
8226 Regency Drive, Pleasanton, CA 94588, USA
Telephone: +1-925-223-8242
Fax: +1-925-223-8243
E-mail: bpgoffice@wjgnet.com
Help Desk: http://www.wjgnet.com/esps/helpdesk.aspx
http://www.wjgnet.com