CD19 chimeric antigen receptor T cell therapy for haematological malignancies

Sara Ghorashian,[1] Martin Pule[2,3,4] and Persis Amrolia[1,5]

[1]Molecular and Cellular Immunology Unit, Institute of Child Health, University College London, [2]Department of Haematology, Cancer Institute, University College London, [3]NIHR University College London Hospitals Biomedical Research Centre, [4]Department of Haematology, University College Hospitals NHS Trust, and [5]Department of Bone Marrow Transplantation, Great Ormond Street Hospital for Children National Health Service Trust, London, UK

Summary

T cells can be redirected to recognize tumour antigens by genetic modification to express a chimeric antigen receptor (CAR). These consist of antibody-derived antigen-binding regions linked to T cell signalling elements. CD19 is an ideal target because it is expressed on most B cell malignancies as well as normal B cells but not on other cell types, restricting any 'on target, off tumour' toxicity to B cell depletion. Recent clinical studies involving CD19 CAR-directed T cells have shown unprecedented responses in a range of B cell malignancies, even in patients with chemorefractory relapse. Durable responses have been achieved, although the persistence of modified T cells may be limited. This therapy is not without toxicity, however. Cytokine release syndrome and neurotoxicity appear to be frequent but are treatable and reversible. CAR T cell therapy holds the promise of a tailored cellular therapy, which can form memory and be adapted to the tumour microenvironment. This review will provide a perspective on the currently available data, as well as on future developments in the field.

Keywords: T lymphocytes, immunotherapy, gene transfer, cellular therapies.

Haematological malignancies often express tumour-associated antigens that are shared with normal cells. Such tumours evade the host immune system because many T cells directed against self-antigens are deleted during thymic education, and those that persist are suppressed by a regulatory population of T cells (Tregs). Genetic modification of T cells with DNA encoding recombinant receptors (chimeric antigen receptor, CARs) can redirect them to recognize self-antigens on the tumour (Pule *et al*, 2003). CARs consist of an antigen recognition domain derived from an antibody fused to T cell receptor (TCR) signalling domains. CAR T cells can recognize a wide range of cell surface molecules without the constraint of major histocompatibility complex/human leucocyte antigen (HLA) restriction, such as is the case for TCR recognition. Thus, a single construct can be applied to any individual whose tumour expresses the targeted antigen, and there is no risk of reduced efficacy in the face of tumour down-regulation of HLA. T cells are not the only cytolytic cells that can express CARs. Natural Killer (NK) and NKT cells have also been studied and may have a lower propensity to cause graft-*versus*-host disease (GVHD) in an allogeneic setting (Heczey *et al*, 2014; Klingemann, 2014). However, at present, only CAR T cells have been tested clinically.

This review will focus on therapies based on CAR-expressing T cells that target the CD19 antigen. Research on this area from a number of groups has led to a rapid trajectory from pre-clinical work to established efficacy in multiple clinical studies in several disease settings. The potency of CD19 CAR T cells, particularly in acute lymphoblastic leukaemia (ALL) but also in other B cell malignancies, is unprecedented within the field of immunotherapy (see Table I).

CD19 as a tumour-associated antigen

CD19 is a 95 kD transmembrane glycoprotein expressed on the B lineage from the early pro-B to mature B cell stages and is part of the B cell surface signal transduction complex. It is expressed on a range of B cell malignancies, including >95% of cases of ALL, B cell non-Hodgkin lymphoma (NHL) and chronic lymphocytic leukaemia (CLL). It is not expressed on other haematopoietic populations or non-haematopoietic cells. Targeting this antigen therefore should not lead to toxicity to the bone marrow or non-haematopoietic organs, making CD19 an ideal CAR target. However, its expression on normal B cells means effective CD19 CAR T cell therapy will result in B cell aplasia and hence, hypogammaglobulinaemia.

Correspondence: Professor Persis Amrolia, Bone Marrow Transplantation Department, Great Ormond Street Hospital, Great Ormond St, London WC1N 3JH, UK.
E-mail: persis.amrolia@gosh.nhs.uk

First published online 5 March 2015
doi: 10.1111/bjh.13340

Table I. Summary of published clinical studies of CD19 CAR T cell therapy.

Reference	Centre	Patients (*n*)	Disease (median age, years)	Vector (T cell activation method)	CAR design	Adjunctive therapy	Cell dose administered	Persistence of CAR T cells	Adverse events	Outcomes	Best response duration
Jensen *et al* (2010)	City of Hope National Medical Center	2	NHL (ages not given)	DNA electroporation of CAR, plus hygromycin resistance and HSV-Tk genes (anti-CD3 antibody, IL2, stimulation with irradiated LCLs	1st generation	Fludarabine 125 mg/kg, IL2	100–2000 × 10^7/m^2 total T cells	Only detected by PCR 1 d post CAR T cell infusion	Grade 3–4 lymphopenia, eosinophilia	No responses	
Kochenderfer *et al* (2010) Kochenderfer *et al* (2012)	National Institutes of Health	8	4 CLL, 4 NHL (55)	Retrovirus (anti-CD3 antibody, IL2)	2nd generation, CD28 domain	Cyclophosphamide 120 mg/kg + fludarabine 125 mg/kg IL2 iv 8-hourly up to max 10 doses	0·3–3 × 10^7 CAR+ T cells/kg	Up to 6 months	4/8 long term B cell aplasia 5/8 grade 3–4 CRS 1/8 grade 3–4 diarrhoea and fatigue 1 death associated with bacteraemia and viral pneumonitis	6/8 PR and 1/8 CR	>18 months
Savoldo *et al* (2011)	Baylor College of Medicine	6	NHL (55)	Retrovirus (immobilized CD3 antibody, IL2)	1st generation and 2nd generation (CD28 domain) CAR T cells co-infused	None	2–200 × 10^7/m^2 total T cells	2nd generation CAR T cells detectable by PCR up to 6 months	None reported	2 SD	

Table I. (*Continued*)

Reference	Centre	Patients (*n*)	Disease (median age, years)	Vector (T cell activation method)	CAR design	Adjunctive therapy	Cell dose administered	Persistence of CAR T cells	Adverse events	Outcomes	Best response duration
Brentjens *et al* (2011)	Memorial Sloane Kettering Cancer Center	10 of which 9 treated	8 CLL, 2 ALL (60)	Retrovirus (CD3/CD28 beads, IL2)	2nd generation, CD28 domain	None, or cyclophosphamide 1·5–3 g/m^2	0·4–3 × 10^7 CAR+ T cells/kg	Detected by PCR and flow cytometry up to 5 weeks, by IHC up to 6 weeks	1/9 long term B cell aplasia, 3/9 grade 3 neutropaenic sepsis, 3 grade 3–5 hypotension (1 death)	2 SD, 1 PR, 1 durable B cell aplasia	8 months
Porter *et al* (2011) Kalos *et al* (2011)	University of Pennsylvania	3	CLL (65)	Lentivirus (CD3/CD28 beads)	2nd generation, 4-1BB domain	Pentostatin or bendamustine ± cyclophosphamide	1·0–1·6 × 10^7 CAR+ T cells/kg	Detected in blood by flow cytometry and PCR for 6 months	3/3 CRS, 3/3 B cell aplasia	2 CR, 1 PR (2 MRD−)	11+ months
Brentjens *et al* (2013) Davila *et al* (2014)	Memorial Sloane Kettering Cancer Center	16 (4 post allo-SCT)	ALL (50)	Retrovirus (CD3/CD28 beads, IL2)	2nd generation, CD28 domain	Cyclophosphamide 1·5–3 g/m^2	0·14–0·3 × 10^7 CAR+ T cells/kg	Detected by deep sequencing in BM to 4 months	7 severe CRS	14 CR, 12 MRD−,	3 months (then allo-SCT)
Kochenderfer *et al* (2013)	National Institutes of Health	10 (all post allo-SCT)	4 CLL, 6 NHL (50)	Retrovirus (anti-CD3 antibody, IL2)	2nd generation, CD28 domain	None	0·04–0·8 × 10^7 allogeneic CAR+ T cells/kg	Detectable by flow cytometry and PCR. By PCR detectable up to 30 d	3/4 B cell aplasia (of those evaluable), 2 grade 3–4 CRS, 2 grade 3–4 hypotension, 1 grade 3–4 headache, 1 grade 3–4 tumour lysis syndrome	1 PR, 1CR, 6 SD	Up to 9 months

Table I. (*Continued*)

Reference	Centre	Patients (*n*)	Disease (median age, years)	Vector (T cell activation method)	CAR design	Adjunctive therapy	Cell dose administered	Persistence of CAR T cells	Adverse events	Outcomes	Best response duration
Kochenderfer *et al* (2014)	National Institutes of Health	15	9 NHL (56)	Retrovirus (anti-CD3 antibody, IL2)	2nd generation, CD28 domain	Fludarabine 125 mg/kg + cyclophosphamide 60–120 mg/kg	0·1–0·5 × 10^7 CAR+ T cells/kg	Detectable by flow cytometry and PCR, detected by PCR for up to 11 weeks	3/3 B cell aplasia (of those evaluable), 9 grade ≥3 CRS, 6 grade ≥3 infectious complications	8 CR, 4 PR, 1 SD	Up to 22 months
Lee *et al* (2014)	National Institutes of Health	21 (8 post allo-SCT)	20 ALL, 1 NHL (13)	Retrovirus (CD3/CD28 beads, IL2)	2nd generation, CD28 domain	Fludarabine 75 mg/m^2 + cyclophosphamide 0·9 g/m^2	0·003–0·3 × 10^7 CAR+ T cells/kg	Detected by flow cytometry up to 7–8 weeks	4 grade 3–4 CRS, 1 grade 3 dysphasia, 17 B cell aplasia for up to 6 weeks, 1 grade 4 cardiac arrest	14 CR, 13 MRD−	Up to 19 months
Grupp *et al* (2013) Maude *et al* (2014)	University of Pennsylvania	30 (18 post allo-SCT)	ALL (14)	Lentivirus (CD3/CD28 beads)	2nd generation, 4-1BB domain	None (3 patients) or various, most commonly fludarabine 120 mg/m^2 + cyclophosphamide 1 g/m^2	0·076–1·7 3 × 10^7 CAR+ T cells/kg	Detectable by flow cytometry up to 15 months, and by qPCR for up to 2 years	22 mild-moderate CRS, 8 grade 3–4 CRS, with coagulopathy in 3 of these 8	27 CR, 22 MRD−	Up to 24 months

CAR, chimeric antigen receptor; NHL, non-Hodgkin lymphoma; CLL, chronic lymphocytic leukaemia; ALL, acute lymphoblastic leukaemia; HSV-Tk, herpes simplex virus thymidine kinase; IL2, interleukin 2; LCLs, lymphoblastoid cell lines; (q)PCR, (quantitative) polymerase chain reaction; BM, bone marrow; CRS, cytokine release syndrome; IHC, immunohistochemistry; PR, partial response; CR, complete response; SD, stable disease; MRD−, minimal residual disease negative; allo-SCT, allogeneic haematopoietic stem cell transplantation.

Chimeric antigen receptor design

CAR structure

T cell receptors are known to have modular signalling domains (Irving & Weiss, 1991). This led Eshhar *et al* (1993) to generate the first CAR by fusing a single chain variable fragment (scFv) derived from an antibody with a CD3ς signalling domain. In this way, CARs graft the specificity of a monoclonal antibody onto the dynamic and persisting characteristics of an effector T-cell.

The common elements of all CARs (Fig 1) consist of:

1 A targeting domain, typically an scFv. The affinity and the density of the target molecule on the tumour cell are key determinants of the degree of cellular activation and the nature of downstream signalling. A study on CD22-specific CAR T cells suggests the proximity of the CAR binding site to the cell membrane may also influence antitumour efficacy, with CARs binding more proximally giving enhanced cytotoxicity (James *et al*, 2008).
2 An extracellular spacer domain both extends the binding domain away from the T-cell membrane and allows suitable freedom of orientation. The optimal design is crucial – too long a spacer can impair effector responses (Hudecek *et al*, 2013) whilst incorporation of Fc domains may lead to activation-induced cell death following ligation by host Fc receptors (Hudecek *et al*, 2014). This region is usually derived from IgG, CD8α, or CD28 molecules.
3 A transmembrane domain (e.g., from CD28).
4 An intracellular signalling domain, usually the TCRς chain.

Importance of co-stimulation

These initial CAR designs triggered T-cell killing but not full activation, resulting in an absence of proliferation or cytokine secretion in response to antigen (Hombach *et al*, 2001a, b). It has long been known that full T cell activation arises from the combination of antigen-related TCR/CD3 signalling (signal 1) and the interaction of a host of co-stimulatory receptors on the T cell with ligands expressed by antigen presenting cells, including CD28, 4-1BB (CD137, TNFRSF9), inducible T cell co-stimulator (ICOS) and OX40 (CD134, TNFRSF4) (Kershaw *et al*, 2013). In the absence of co-stimulation, TCR stimulation leads to anergy (Schwartz, 2003). This is critical because tumour cells often lack co-stimulatory ligands. CD28 promotes T cell proliferation, survival and cytokine production through enhanced TCR signalling, as well as activation of AKT and BCL-xl (BCL2L1). 4-1BB and OX40 are inducibly expressed on T cells following TCR signalling, and serve to support proliferation at later stages of the immune response.

This deficit in CAR function was remedied by constructing CARs with compound endodomains, incorporating co-stimulatory molecules such as CD28, 4-1BB and OX40 along with CD3ζ (CD247) (Finney *et al*, 1998, 2004; Maher *et al*, 2002). T-cells expressing these 'second generation' CARs (Fig 2) not only kill CD19-expressing targets at lower effector:target ratios (Imai *et al*, 2004), but show greater cytokine and proliferative responses (Haynes *et al*, 2002; Carpenito *et al*, 2009; Milone *et al*, 2009). T cells expressing second generation CARs also mediate more effective regression of ALL in xenograft models (Brentjens *et al*, 2007). As well as improving activation, the incorporation of co-stimulatory domains may improve the persistence of CAR-transduced T cells *in vivo*. 'Third generation' CARs (Fig 2) have also been described (Pule *et al*, 2005).

The importance of co-stimulation has been directly confirmed in a clinical trial, which demonstrated enhanced expansion and persistence of T cells expressing a second generation CAR (Savoldo *et al*, 2011). While there is general agreement that T cells bearing second generation CARs are more effective than first generation CARs, whether there is any advantage of using one co-stimulatory domain over another is unclear. *In vitro* data comparing second generation CD19 CARs with CD28 or 4-1BB co-stimulatory domains suggested that whilst cytotoxicity was equivalent, 4-1BB containing CARs induced improved proliferation. Consistent with this, T cells transduced with this receptor had greater *in vivo* expansion, longer survival and mediated more potent anti-leukaemic effects against established primary human ALL in immunodeficient mice (Milone *et al*, 2009). However, this may be specific to CD19 CARs as conflicting data are reported for other CARs (Carpenito *et al*, 2009). An ongoing clinical trial aims to determine which is the best co-stimulatory domain for CD19 CARs (NCT00466531). Combinations of co-stimulatory domains, such as in third generation CARs, have not clearly shown a benefit. Indeed, in a pre-clinical study comparing CD19 CARs containing a 4-1BB domain or both 4-1BB and CD28 domains, the former outperformed the latter in cytotoxic function (Kochenderfer *et al*, 2009), and a similar observation was made in a study of CD22-reactive CARs (Haso *et al*, 2013). Third generation CARs, e.g., recognizing CD20, are now starting to be tested in humans (Till *et al*, 2012).

Pre-clinical studies

In vitro studies of CD19 CAR T cells demonstrated that these could lyse primary CLL and ALL cells (Brentjens *et al*, 2003, 2007; Cooper *et al*, 2003). *In vivo* anti-tumour efficacy was then demonstrated in immunodeficient mice bearing established human B cell tumours derived from both cell lines and primary human malignancies without apparent toxicity to non-haematopoietic tissues (Brentjens *et al*, 2007; Cheadle *et al*, 2010; Kochenderfer *et al*, 2010; Landmeier *et al*, 2010). However, study of persistence of human T cells in such xenogeneic tumour models is precluded because of a lack of a human cytokine milieu. The development of CARs directed

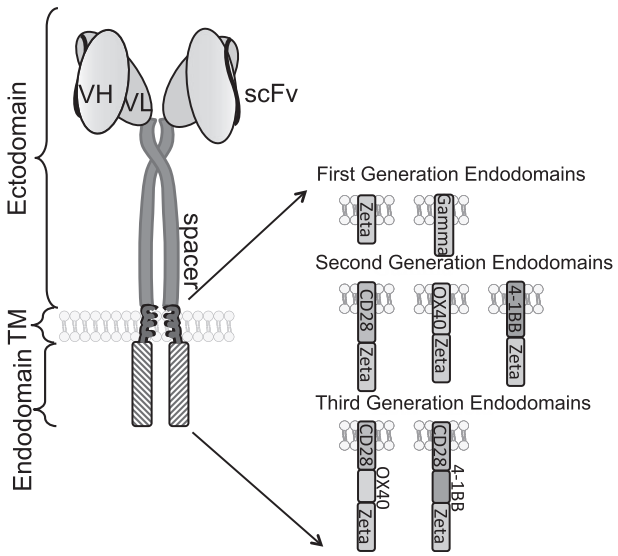

Fig 1. Chimeric antigen receptor (CAR) structure and design. CARs are artificial type I transmembrane proteins: an amino terminal extracellular segment or 'ectodomain' is connected to an intracellular carboxy-terminal segment or 'endodomain' via a transmembrane domain (TM). The ectodomain consists of an antigen-binding domain and a spacer domain. The antigen-binding domain is typically a single-chain variable fragment derived from a monoclonal antibody, but can be any antigen-recognizing domain. The spacer acts to extend the antigen-binding domain out from the T-cell membrane. Typical spacers include the stalk segment of CD8, the Fc portion of immunoglobulin, the IgG1 hinge, the ectodomain of CD28 or the low-affinity nerve-growth factor receptor. The endodomain acts to transmit T-cell signals and typically comprises of one, two or three signalling components. Early first generation CARs had endodomains which contained immunomodulatory activation domains (e.g. CD3zeta), but had no co-stimulatory components. Second generation CARs have endodomains containing a single co-stimulatory component as well as CD3zeta endodomain. Third generation CARs have endodomains which contain CD28, either a 4-1BB or OX40 component, and a CD3zeta endodomain. VH, variable region of immunoglobulin heavy chain; VL, variable region of immunoglobulin light chain; scFv, single-chain variable fragment.

against murine CD19 enabled investigation of the efficacy of CAR-transduced murine lymphocytes against syngeneic $CD19^+$ tumours in immune-competent mice (Cheadle *et al*, 2010; Kochenderfer *et al*, 2010). Whilst these studies established the potential of CD19 CAR T cells to mediate potent anti-tumour effects *in vivo*, they are inherently limited in terms of modelling the toxicity and persistence of the CD19 CAR T cells in humans. Further useful data could therefore only be obtained by clinical trials.

Methods by which CARs can be introduced into T cells

A number of methods have been used to introduce CARs into T cells. Each has its advantages and disadvantages in terms of complexity, stability of transgene expression, safety and cost.

Integrating viral vectors

Both γ-retroviral or lentiviral vectors can permanently insert DNA into the genome. Modern packaging systems provide viral components separately (split packaging) and are safe, eliminating initial concerns of generating replication-competent vectors. These vectors are highly efficient in transducing T cells after *in vitro* activation with various cytokines ±CD3/CD28 stimulation. One concern is the potential for oncogenesis caused by randomly inserting transgenes into the genome, as observed when retrovirally-corrected haematopoietic stem cells were used to treat patients with X-linked severe combined immunodeficiency (Hacein-Bey-Abina *et al*, 2003). However, retroviral gene transduction in T cells has been safe (Scholler *et al*, 2012) with no recorded cases of insertional mutagenesis in over 200 patients treated to date.

Lentiviral vectors are capable of transducing quiescent cells, while γ-retroviral vectors require cells in mitosis. This difference may be moot, given that all CAR T-cell production protocols currently employ powerful mitogenic stimuli. Lentiviral vectors are theoretically safer: their integration preference is less focused on transcriptional start sites compared with γ-retroviruses and they are typically 'self-inactivating', so after insertion the powerful viral promoters are truncated. The advantage of γ-retroviral vectors is that it is possible to generate a stable producer cell line allowing production of indefinite quantities of vector. In contrast, lentiviral vector production depends on transient transfection, limiting broader application.

Transposon-based integration

Transposon-based systems, such as Sleeping Beauty, introduce plasmid DNA (which is considerably cheaper than retroviral vectors) by electroporation along with DNA or RNA which codes for a transposase. Specific terminal repeats flanking the CAR expression cassette are recognized by the transposase and the cassette inserted into the T-cell's genome. However, such protocols result in considerable cell death and typically require prolonged culture for cellular recuperation. These systems are being applied in clinical studies. If equivalent to integrating vector approaches, the cost and complexity of CAR T-cell production will be considerably reduced.

Electroporation of mRNA

Introduction of synthetic mRNA into T-cells without integration into the host genome is not toxic to the T-cell but leads to only transient expression of CAR, which may limit anti-tumour responses. Temporary CAR expression may mean toxicity will be short-lived, however. One proposed application of mRNA CAR T-cells has been to detect unexpected toxicity before administration of permanently engineered T-cells.

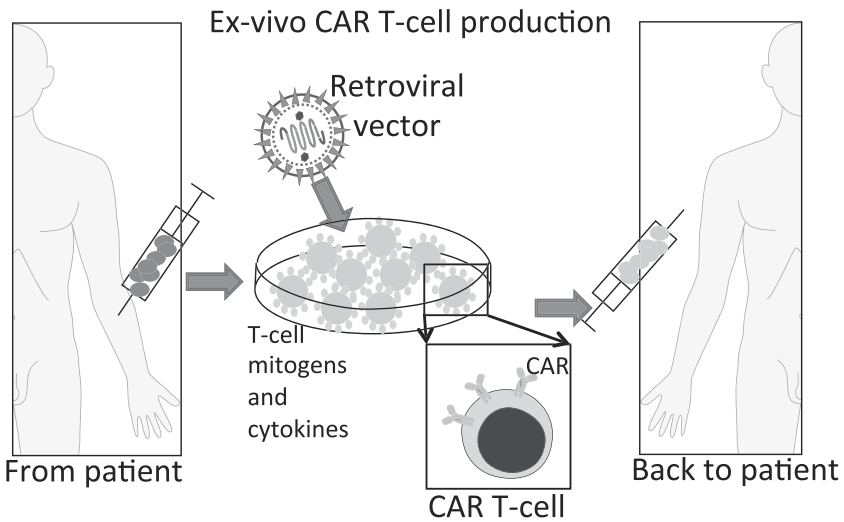

Fig 2. *Ex vivo* chimeric antigen receptor (CAR) T cell production. Autologous CAR T-cells are generated as follows: peripheral blood mononuclear cells (PBMCs) are harvested either from whole blood or, more usually, by leukapheresis.PBMCs are given a mitogenic stimulus, typically using beads coated with anti-CD3/anti-CD28 monoclonal antibodies. Stimulated PBMCs are then exposed to the viral vector and cultured in the presence of cytokine. Since the mitogenic stimulus is T-cell specific, after a few days the cultures typically only contain T-cells and natural killer cells. These cells are expanded for a few days. Expression of CAR is checked usually by flow-cytometry and product is cryopreserved. The cell product is typically thawed at the bedside and administered intravenously.

Selection of T cell subsets for CAR T cell therapy

Most reported clinical studies of CD19 CAR T cell therapy have involved transfer of bulk transduced T cells with wide variation in terms of the CD4: CD8 ratio, proportion of regulatory T cells (Treg) and memory T cell subsets. The ideal T cell type to engineer and transfer is unknown; however, some ground rules are emerging. Co-transfer of CD4 and CD8 T cells with tumour specificity improves efficacy, through both CD4 help and other anti-tumour effects (Adusumilli *et al*, 2014).

Memory T cells rapidly expand upon antigen re-exposure and provide life-long immunity. Therefore, exploitation of specific memory subtypes may lead to improved anti-tumour effects. Effector memory T cells (Tem) give rise to effector T cells adapted for cytotoxic functions but may not persist long term. Central memory T cells (Tcm), on the other hand have a high proliferative potential, persist long term and have the ability to repopulate both memory compartments. Further, Tcm express CCR7, which enables them to penetrate the lymph nodes and bone marrow (Sallusto *et al*, 1999), the niches of haematological malignancies. Non-human primate studies of adoptive transfer of antigen-specific $CD8^+$ T cell clones suggest clones derived from Tcm but not Tem persist long term *in vivo* (Berger *et al*, 2008). Emerging data suggest the percentage of Tcm within the circulating CAR T cell pool may fall from levels within infused product (Kochenderfer *et al*, 2014). Thus, selection of Tcm for CD19 CAR immunotherapy may potentially improve both efficacy and persistence. Other studies have suggested naïve or memory stem cells may be optimal for adoptive immunotherapy (Hinrichs *et al*, 2011; Gattinoni *et al*, 2011). Isolation of distinct T cell subsets using immunomagnetic selection prior to CAR engineering may make the infused product more homogeneous but adds complexity. Studies of adoptive transfer of $CD4^+$ and $CD8^+$ central memory T cells that are separately transduced and infused at a defined ratio are now underway in Seattle (Riddell *et al*, 2014).

An alternative approach to improve persistence of CAR T cells is the use of T cells with anti-viral specificity. Transfer of virus-specific T cells (VSTs) can safely improve anti-viral immunity after allogeneic stem cell transplantation (SCT) (Heslop *et al*, 2010). VSTs mediate anti-viral responses without causing GVHD, expand *in vivo* and can persist long term. Such T cell populations have dual specificity. The endogenous TCR allows physiological T cell activation in the setting of viral infection/reactivation whilst presence of the CAR allows for anti-tumour responses. Transduced memory VSTs may be primed *in vivo* by viral antigens, promoting persistence (Cooper *et al*, 2005). Proof of concept was established with the use of Epstein–Barr virus (EBV) CTLs redirected to a neuroblastoma antigen with a first generation CAR (Pule *et al*, 2008). Patients received two populations of CAR-bearing cells that were distinguishable on a molecular level, one of T cells activated from peripheral blood mononuclear cells using a standard transduction protocol, the other being EBV-specific. The expansion and persistence of the CAR-transduced EBV CTLs was greater than the standard CAR T cells.

This approach to improving persistence may be advantageous over employing newer-generation CARs because signalling through the latter may not be subject to the same degree

of negative regulation as natural TCR signalling. Indeed, supra-physiological immune activation may be responsible for the adverse effects experienced by some patients. A preliminary report from the first clinical trial utilizing VSTs transduced with a second generation CD19 CAR in eight patients with CLL and ALL following allogeneic SCT has reported only limited persistence of up to 12 weeks, with similarly short-lived objective responses in two of six evaluable patients (Cruz *et al*, 2013). In light of this, we are currently investigating whether vaccination with irradiated, EBV-transformed lymphoblastic cell lines will improve the persistence of EBV-specific T cells redirected with a first generation CD19 CAR within a prospective cohort study of paediatric ALL patients relapsing post allogeneic SCT (NCT01195480).

Preparing the patient for CAR T-cells

Previous experience from immunotherapy with melanoma tumour-infiltrating lymphocytes has shown that pre-conditioning patients with lymphodepleting chemotherapy resulted in enhanced engraftment, *in vivo* expansion and anti-tumour efficacy (Rosenberg *et al*, 2011; Restifo *et al*, 2012). Benefit was also shown in animal models of CD19 CAR T cell therapy (Kochenderfer *et al*, 2010). Lymphodepletion, typically with fludarabine and cyclophosphamide, is incorporated into almost all current CD19 CAR studies. Lymphodepletion may facilitate the expansion of adoptively transferred T cells by eliminating Tregs (Antony *et al*, 2005) and may reduce competition for homeostatic cytokines [e.g. interleukin (IL) 2 and IL7] (Gattinoni *et al*, 2005) facilitating CAR T cell engraftment and expansion.

Clinical studies using CD19 CARs (see Table I)

Treatment of NHL and CLL

An initial study of T cells transduced with a first generation CD19 CAR in patients with refractory follicular lymphoma was reported by investigators from the City of Hope Medical Centre (Jensen *et al*, 2010). Despite lymphodepletion and administration of IL2, T cell persistence was limited to <1 week, and no responses or toxicity were seen. This may relate both to the absence of a co-stimulatory domain in the CAR and anti-CAR directed host immune responses, seen in two of four patients.

The first use of a second generation CD19 CAR incorporating a CD28-derived co-stimulatory domain (Kochenderfer *et al*, 2010) was reported by the US National Cancer Institute (NCI) group, in a patient with follicular lymphoma. Lymphodepletion and IL2 were administered. Transduced T cells persisted up to 27 weeks and the patient's lymphoma had an impressive partial response lasting 32 weeks with a similar duration of B cell aplasia. This study suggested persistence may be improved with use of a second generation CAR, a point later confirmed in an elegant study by Savoldo *et al* (2011). They co-transferred autologous T cell populations separately transduced with first and second generation CARs with the same CD19 recognition domain into 6 patients with B-cell NHL. This design allowed comparison of expansion and survival of both T cell populations in the same recipient and showed that these are improved in T cells bearing a second generation CAR. Clinical responses were limited, with two of six patients having stable disease, and the other four suffering progressive disease.

A more recent publication from this group assessed responses in a cohort of 15 patients, mostly with NHL (Kochenderfer *et al*, 2014). The second generation CAR employed was derived from the FMC63 monoclonal antibody and contained a CD28 co-stimulatory domain. T cells were infused following lymphodepletion. Impressive response rates were reported with 12 of 13 evaluable patients achieving a response, of which eight were complete responses (CRs). Persistence of CAR T cells was noted to 75 days and responses were durable, with the best response duration being on-going at 23 months. As discussed below, these responses were associated with significant toxicity, mostly attributable to cytokine release syndrome (CRS).

Investigation of second generation CD19 CARs in the therapy of patients with CLL was first reported from the University of Pennsylvania, with a CD19 CAR containing a 4-1BB co-stimulatory domain (Porter *et al*, 2011). They treated three patients with refractory CLL (Porter *et al*, 2011). Two patients had prolonged complete remissions and the third had a partial response. A similar study in patients with advanced CLL using a second generation CAR containing a CD28 co-stimulatory domain was reported from the group at Memorial Sloane Kettering (Brentjens *et al*, 2011). Of the seven evaluable CLL patients, one achieved a dramatic reduction in lymphadenopathy, which was durable for 6 months, and two patients with previously rapidly progressive disease then experienced stable disease post-T cell infusion. Responses were only obtained in patients who also received lymphodepleting chemotherapy. This study also provided preliminary data that persistence may be inversely related to disease burden. If confirmed, it may be that CAR T cell therapy protocols should be adapted to the setting of minimal rather than bulky disease. In the recent NCI study (Kochenderfer *et al*, 2014), all four patients with advanced CLL responded with three out of four CRs, durable up to 23 months.

Therapy following allogeneic transplantation for CLL has been reported in two studies (Cruz *et al*, 2013; Kochenderfer *et al*, 2013). Neither study utilized conditioning therapy and, of note, the former study expanded and used VSTs as the effector population for CAR transduction. CAR T cell persistence was limited to a month. Of eight patients across these two studies with CLL, there was one durable CR. There was no evidence of GVHD following allogeneic CAR T cell therapy.

Treatment of ALL

The first studies in ALL (Table I) were published in Spring 2013, by groups from Memorial Sloane Kettering (Brentjens *et al*, 2013) and the University of Pennsylvania (Grupp *et al*, 2013). In the report of Grupp *et al* (2013), two children with relapsed, refractory ALL were treated with T cells lentivirally transduced with a second generation CD19 CAR utilizing the FMC63 scFv and incorporating a 4-1BB co-stimulatory domain. Both achieved a molecular CR, which was durable in one case, whereas the other patient relapsed with $CD19^-$ disease 2 months later. An updated report of this study, including 30 patients, has recently been published (Maude *et al*, 2014). Half of these patients had relapsed following allogeneic SCT, two had involvement of the central nervous system (CNS) and two cases were refractory to blinatumomab therapy. 90% achieved a CR at 1 month, 22 of 28 evaluable cases achieved minimal residual disease (MRD) negativity and the 6-month event-free survival rate was 67%. 15 patients received no further therapy after the study. The cases with CNS disease showed clearance of leukaemia from this site. Durable responses appeared to correlate with higher peak levels of circulating CAR-transduced T cells, as well as with the duration of B cell aplasia. In the patients who relapsed following initial response to CAR T cell therapy, there were cases of both $CD19^+$ and $CD19^-$ relapses (4 and 3 cases, respectively). $CD19^+$ relapse was generally associated with loss of circulating CAR T cells and recovery of normal B cells whereas CAR T cells persisted in patients who developed $CD19^-$ leukaemic escape. CRS developed to some extent in all patients, with eight patients (27%) requiring admission to intensive care for organ support. CRS was treatable with tociluzumab, an IL6 receptor blocking antibody, in nine patients and six also required a curtailed course of steroids. Neurological toxicity was noted in 13 patients. Full recovery of CRS and neurological toxicity was noted in all affected patients.

In the adult setting, Brentjens *et al* (2013) treated five ALL patients (two with refractory relapse, two with MRD-positive disease and one who was MRD-negative) with autologous T cells retrovirally transduced to express a CD19 CAR incorporating an scFv derived from the SJ25C1 hybridoma and a CD28 co-stimulatory domain. All of these achieved a deep molecular remission, enabling four of these patients to receive an allogeneic SCT. This precluded assessment of the durability of responses but CAR T cells were only detectable in the blood or bone marrow for 3–8 weeks after infusion. The patient who was not transplanted relapsed at 90 d with $CD19^+$ disease. Subsequently, Davila *et al* (2014) have updated this cohort. Fourteen of 16 adult patients had detectable disease at the point of CAR T cell infusion, despite salvage chemotherapy and cyclophosphamide conditioning. Fourteen of 16 achieved a CR with or without count recovery including seven of nine patients with morphological evidence of detectable MRD after salvage chemotherapy. Twelve of 16 patients achieved MRD negativity and this allowed seven to undergo allogeneic transplantation by the time of publication. Responses were durable in some patients with four of eight non-transplanted patients continuing in morphological remission at up to 24 months follow-up although the survival curves for this cohort are not yet stable.

Most CD19 CAR trials reported to date are single-arm, cohort studies with considerable heterogeneity in disease setting, conditioning chemotherapy, cell dose and the way in which outcomes are reported. A recently published phase 1 dose escalation trial in a cohort of paediatric and young adult patients predominantly with ALL provides the first intention-to-treat analysis of its outcomes. This may help remove the bias inherent in excluding patients who do not receive the anticipated dose of CAR T cells (Lee *et al*, 2014). 21 patients were treated with a CD28 domain-containing second generation CAR. All but two patients received the anticipated T cell dose, highlighting the feasibility of delivering this treatment to those with refractory or multiply-relapsed ALL. The efficacy achieved reinforced the impressive responses seen in the earlier studies mentioned above, with 67% achieving a CR and 60% of those with ALL achieving MRD negativity. It should be noted that 11 out of 18 evaluable patients had CAR T cells in the cerebrospinal fluid (CSF) and CNS disease was cleared in two patients with active CNS involvement.

From the available data, response rates in ALL may be significantly higher than in NHL or CLL. This may reflect the inhibitory nature of the tumour microenvironment in NHL (Yang & Ansell, 2012; Burger & Gribben, 2014), defects in T cell function in CLL (Christopoulos *et al*, 2011) and improved homing of CD19 CAR T cells to the bone marrow compared with lymph nodes and prior therapy. Additionally, comparison of the above patient cohorts suggests differences between the second generation CARs containing 4-1BB and CD28. Persistence of CD28-containing CAR-transduced T cells appears shorter (up to 4 months) (Davila *et al*, 2014) compared to 4-1BB CAR-transduced T cells, which persisted for to up to 2 years (Maude *et al*, 2014). Consistent with this, B cell aplasia after therapy with the 4-1BB CAR constructs lasts longer than in patients treated with CD28 domain-containing constructs, where durations of 1–3 months are more usual. Ultimately, these observations need to be confirmed and current collaborative studies aim to address this (NCT004664531). Differences in the kinetics of CAR T cells may also confer differences in the timing of CRS. This appears to occur earlier (4–6 d after CAR T cell infusion) with CD28-containing second generation CARs (Davila *et al*, 2014) than with 4-1BB containing CARs (Maude *et al*, 2014).

Summary of clinical studies

Overall, the data suggest that response rates of 50–90% can be achieved in patients with ALL, but responses may be

lower in NHL and CLL (see Table I). Responses in patients with chemorefractory disease are possible, as long as such patients receive lymphodepletion, a second generation CAR incorporating a co-stimulatory domain, and a T cell product generated through short term culture. Whilst manufacture of a CAR T cell product for each patient is technically challenging, it is feasible for the majority of patients. There is no clear relationship between T cell dose and efficacy, as even very low doses may expand *in vivo* and mediate anti-tumour effects. Emerging data suggest responses correlate more with expansion of CAR T cells (Xu *et al*, 2013). There are conflicting data on whether response rates correlate with disease burden. *In vivo* detection of CAR T cells in the blood appears to be a pre-requisite for response, though persistence in the blood is variable. Longer follow-up is required to determine the relationship between CAR T cell persistence and response durability. In this regard, the goal of CAR T cell therapy is important: if CAR T cell therapy is used as a bridge to transplantation, persistence of 1–2 months may be sufficient. If, on the other hand, CAR therapy is used as a stand-alone treatment, longer-term persistence is almost certainly required.

Adverse effects

B cell aplasia

This 'on-target, off-tumour' effect is seen both in murine models (Davila *et al*, 2013) and in patients. The duration of B cell aplasia is variable depending on the construct used, as discussed above. Indeed, the absence of circulating B cells appears to be a useful surrogate of the persistence of CD19 CAR T cells. Ideally, CAR T cells should persist long enough to mediate durable eradication of disease but then allow recovery of normal B cells. Persistent B cell aplasia could result in increased risk of infection, however, long-term immune globulin replacement can mitigate infectious complications, as evidenced by patients with X-linked Bruton agammaglobulinaemia.

Cytokine release syndrome

Cytokine release syndrome encompasses a range of inflammatory symptoms ranging from mild 'flu-like symptoms to multi-organ failure with hypotension and respiratory failure. Some degree of CRS occurs commonly in patients treated with CD19 CAR T cells. The frequency of severe CRS is unclear, as diagnostic criteria were lacking until recently, but appears to be approximately 30% (21/73) patients treated in recent cohorts (see Table I). CRS has also been seen in patients treated with blinatumomab, a bi-specific recombinant single-chain antibody recognizing both CD19 and CD3. CRS typically occurs 5–21 d after CAR T cell infusion and the currently available data suggests its severity is proportional to tumour load (Brentjens *et al*, 2011; Davila *et al*, 2014; Maude *et al*, 2014). Whether the severity of CRS correlates to anti-tumour efficacy is not established – many patients with mild CRS show effective anti-tumour responses. The Sloane-Kettering group have retrospectively shown the utility of C-reactive protein and documentation of a fever of 38°C for more than 3 days in predicting severe CRS requiring therapy with tocilizumab (Davila *et al*, 2014). They also formulated diagnostic criteria for severe CRS (see Table II). Such criteria need to be validated prospectively, but may help identify patients needing targeted therapy with simple laboratory parameters.

In patients with frank leukaemic relapse, CRS can be life threatening and require high dependency care. CRS is associated with elevated serum cytokine levels, but levels may not correlate with clinical severity. The cytokines most significantly elevated are IL6, IL10 and interferon gamma (IFNγ). Some clinical manifestations of severe CRS (fever, hepatosplenomegaly, coagulopathy and hyperferritinaemia) overlap with macrophage activation syndrome, suggesting common immunopathological processes are involved. In those developing severe CRS, it seems the peak of IL6 levels coincides with maximal expansion of the transferred T cells (Davila *et al*, 2014). An association between the severity of CRS and expansion of CAR T cells, as well as response has been suggested (Brentjens *et al*, 2011; Kochenderfer *et al*, 2013; Davila *et al*, 2014; Lee *et al*, 2014; Maude *et al*, 2014). At present it is not clear which cell type (CAR T cells, dying tumour cells, or locally-activated macrophages) are responsible for production of the key cytokines, particularly IL6. These questions warrant further investigation.

Therapies given to ameliorate CRS include corticosteroids, but there is concern that by dampening T cell function and proliferation, even short courses may limit the therapeutic efficacy (Brentjens *et al*, 2013; Davila *et al*, 2014). Other potential therapeutic targets include the pro-inflammatory cytokines IL6 and IFNγ. A commercially-available blocking antibody to the IL6 receptor, Tocilizumab, appears highly effective in treating severe CRS, allowing a de-escalation of organ support within days (Grupp *et al*, 2013; Davila *et al*, 2014). In contrast to steroid therapy, tociluzumab may not prevent the expansion of CAR T cells *in vivo* (Davila *et al*, 2014).

Whether interruption of the cytokine cascade leads to abrogation of anti-tumour effects remains unclear and, at present, the optimal timing of targeted therapy is not established. Currently, tocilizumab is generally withheld until established organ dysfunction in case the cytokine storm is critical for supporting maximal T cell expansion.

Neurotoxicity

A number of patients in CD19 CAR studies across institutions have developed transient neurotoxicity with a spectrum of

severity from aphasia to obtundation, delirium and seizures (Davila *et al*, 2014). This appears to be restricted to patients with ALL and a similar syndrome has been documented after blinatumomab therapy. Brain imaging appears normal, CSF may show lymphocytosis, at least partly contributed to by CAR T cells (Davila *et al*, 2014). Interestingly, trafficking to the CSF has been seen in patients without overt CNS disease. Whether neurotoxicity reflects systemic cytokines crossing the blood-brain barrier or cross-reactivity of CAR T cells with a target in the CNS has yet to be defined, but it appears to resolve spontaneously without specific therapy.

Future directions

Strategies to improve safety (Fig 3)

Suicide genes. A 'suicide gene' allows an engineered T-cell to be selectively deleted in the patient in the face of unacceptable toxicity. Various strategies exist: T cells can be engineered to express viral thymidine kinase, rendering them susceptible to thymidine kinase inhibition following administration of ganciclovir (Bonini *et al*, 1997). However, this approach has been hampered by immune responses against the herpes simplex-derived thymidine kinase (Traversari *et al*, 2007), leading to unintended clearance of transduced T cells in some cases. Expression of inducible caspase (Straathof *et al*, 2005; Di Stasi *et al*, 2011) or Fas (Thomis *et al*, 2001) leads to highly efficient induction of cell death after administration of a synthetic dimerization agent. Expression of surface proteins that render T-cells susceptible to existing therapeutic agents e.g. rituximab (Philip *et al*, 2014), or cetuximab (Wang *et al*, 2011) have also been described and are now being tested clinically.

Suicide gene therapy strategies may be useful in switching off long term 'off tumour' toxicities, such as prolonged B cell aplasia, but may be less appropriate for mitigating acute toxicities, such as CRS, because early CAR T cell ablation is likely to compromise therapeutic benefit.

Table II. Suggested diagnostic criteria for severe cytokine release syndrome following CAR T cell therapy.

Fever for ≥3 d
Maximal elevation of serum cytokines (of 2 cytokines by ≥75-fold, or of a single cytokine by ≥250-fold)
At least one clinical manifestation of cytokine release syndrome:
Hypotension, requiring intravenous vasopressor therapy
Hypoxia (pO_2 < 90%)
Neurological disturbance including delirium, obtundation, seizures

Source: Davila, M.L., Riviere, I., Wang, X., Bartido, S., Park, J., Curran, K., Chung, S.S., 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, D.C., Arcila, M.E., Gonen, M., Roboz, G.J., Maslak, P., Douer, D., Frattini, M.G., Giralt, S., Sadelain, M. & Brentjens, R. (2014) Efficacy and toxicity management of 19-28z CAR T cell therapy in B cell acute lymphoblastic leukemia. Science Translational Medicine, 6, 224ra25. Adapted with permission from The American Association for the Advancement of Science (AAAS).

Inducible CAR expression. Instead of permanent CAR T cell ablation, ideally what is needed is a mechanism to regulate CAR expression in a real-time, inducible manner. While drug-inducible transgene expression systems have been extensively used *in vitro*, this technology is not easily translatable as it often relies on xenogeneic transcriptional activators e.g. in the tetracycline-regulated (Tet) gene expression systems (Gossen *et al*, 1995). Other strategies in development include mRNA control of CAR transgene expression (Chen *et al*, 2010) or inclusion of protein degradation domains within the CAR transgene causing conditional degradation of the CAR (Bonger *et al*, 2011). Developing these inducible CAR expression systems represents one of the major challenges for the future in this field but holds the promise of enabling clinicians to switch CAR expression on and off as clinically indicated.

Strategies to improve efficacy (Fig 3)

Polyspecific CAR T cell immunotherapy. In some cases, failure of CD19 CAR T cell therapy has been associated with development of a $CD19^-$ tumour clone. How much of a problem this will be is not clear but it has also been noted in patients treated with blinatumomab (Portell *et al*, 2013). The risk of antigen escape may be reduced if tumour antigens that are essential for survival/proliferation are targeted. Another approach is to engineer T cells to recognize multiple disease-specific B-cell antigens e.g. CD22, CD20 or ROR1. This can be achieved in a number of ways: from designing multiple recognition sites on the same CAR, expression of a number of CARs on the same cell using multi-cistronic vectors, or transducing independent T cell populations with CARs of different specificities. If the technical challenges can be overcome, a multiplexed CAR design may be the most practical approach and proof of principle for this has been established (Grada *et al*, 2013). More exciting still is the possibility of restricting CAR T-cell activation to combinations of antigen expression. Most antigens expressed on tumours are also expressed on normal tissues, however, it is possible to much better define tumour populations by combinations of antigens: either the presence of two antigens together, or the presence of one antigen and the absence of another (Fedorov *et al*, 2013, 2014; Kloss *et al*, 2013).

Strategies to improve efficacy (Fig 3)

Overcoming inhibition by the tumour microenvironment. Tumours are well adapted to overcome productive immune responses with multiple mechanisms for preventing effective T cell persistence, trafficking into tumour and maintenance of functional activity. The importance of co-inhibitory signals in dampening productive immune responses in the setting of solid organ malignancies, e.g., renal cell carci-

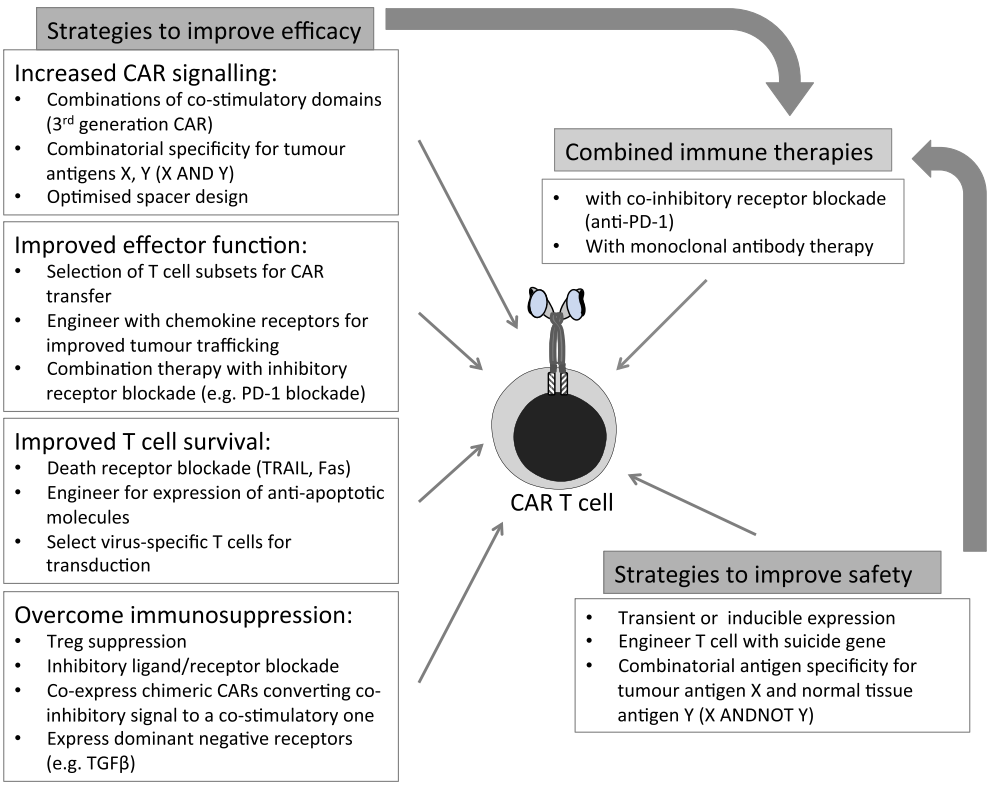

Fig 3. Strategies to improve chimeric antigen receptor (CAR) T cell therapy. There are many potential strategies to improve current CAR T cell therapies, aimed at either increasing CAR T cell efficacy or safety, often through additional genetic modifications. Ultimately, judicious combinations of anti-cancer immunotherapies may allow improved efficacy in the face of more moderate toxicity.

noma, have been highlighted by the regression of advanced tumours upon blockade of signalling through the PD-1/PD-L1 axis and CTLA4. Studies are already underway to combine immune checkpoint blockade (Fig 3) using monoclonal antibodies blocking PD-1 and CTLA4 signalling with CAR T cell therapy (NCT00586391). Solutions engineered into the CAR T-cell have also been proposed: these include engineering T cells with chimeric receptors providing activatory signals upon engagement of inhibitory ligands such as programmed death-ligand1 (PD-L1, CD274) (Prosser *et al*, 2012) or dominant negative TGF-β receptors (Bollard *et al*, 2002). In the future, genomic editing of CAR-transduced T cells may be used to confer resistance to immunosuppressive molecules secreted by the tumour.

Conclusions

CD19 CAR T cells have yielded unprecedented responses across a broad range of B cell malignancies, allowing salvage of cases refractory to conventional chemotherapy or SCT. Because tumour recognition is independent of both HLA restriction and the genetic basis of the tumour, this approach is broadly applicable. Nonetheless, much work remains to maximize efficacy and limit toxicity. The optimal CAR design and effector T cell populations need to be defined, as does the durability of clinical responses. These issues are being addressed in current clinical studies. Future developments in CAR design may overcome antigenic escape and inhibition by the tumour microenvironment, whilst inducible or combinatorial CAR strategies may limit toxicity. Whether long-term toxicity is seen from CD19 CAR T cell therapy and how this compares to the toxicity of standard therapy remains to be determined as the data from on-going clinical studies mature. This will be particularly relevant in the paediatric setting.

In coming years, it will be critical to determine the optimum position of CAR T cell therapy in relation to existing treatments. Whether CAR T cells are best employed in frank relapse or to deepen remissions; whether as a bridge to transplant or a stand-alone therapy needs to be defined. These questions will require appropriately powered, well-designed studies in disease-specific cohorts, ideally based on intention-to-treat analyses. If responses are prolonged without further therapy, it is possible that in some disease cohorts CD19 CAR T cells therapy may replace SCT with its attendant toxicity and cost. Similarly, it will be important to determine the role of CD19 CAR T cells in relation to alternative emerging immune therapies, such as the bi-specific T cell engaging (BiTE) antibody, blinatumomab. *A priori* the potential advantage of CAR T cells is the long-term immune surveillance whereas BiTE antibodies are cleared from the circulation, but improved durability has yet to be formally demonstrated. Ultimately, optimal treatment protocols may employ sequential or combination immunotherapies (Fig 3)

targeting different tumour antigens to prevent antigenic escape (e.g. epratuzumab and CD19 CAR T cells).

The biggest barrier to implementation of CAR T cell therapy is the complexity and prohibitive cost of generating patient-specific cellular therapies. Whilst localized production is feasible in current models, capacity is limited. Even with considerable commercial investment in this area, it will take time to build the infrastructure required to make this therapy available to large numbers of patients.

After more than 20 years in development, CAR T cell therapy is poised to enter the main stream. This represents a triumph of scientific progress. More broadly, this approach may be the beginning of a new era in cancer therapy, where we move from the sledgehammer approach of chemotherapy to engineered, personalized cell therapy with targeted specificity and memory.

Acknowledgements

SG is supported by a grant from the National Institute for Health Research and PJA is a recipient of a National Institute for Health Research Professorship. MP is supported by the National Institute for Health Research University College London Hospitals Biomedical Research Centre. MP collaborates with and receives funding for contract research from Cellectis Therapeutics, Paris. MP owns stock in and is Chief Scientific Officer of Autolus LTD. MP has filed and holds patents in the field for which he is entitled to a revenue share.

Authorship contributions

SG, MP and PJA wrote and edited the manuscript.

References

Adusumilli, P.S., Cherkassky, L., Villena-Vargas, J., Colovos, C., Servais, E., Plotkin, J., Jones, D.R. & Sadelain, M. (2014). Regional delivery of mesothelin-targeted CAR T cell therapy generates potent and long-lasting CD4-dependent tumor immunity. *Science Translational Medicine*, **6**, 261ra151.

Antony, P.A., Piccirillo, C.A., Akpinarli, A., Finkelstein, S.E., Speiss, P.J., Surman, D.R., Palmer, D.C., Chan, C.C., Klebanoff, C.A., Overwijk, W.W., Rosenberg, S.A. & Restifo, N.P. (2005) $CD8^+$ T cell immunity against a tumor/self-antigen is augmented by $CD4^+$ T helper cells and hindered by naturally occurring T regulatory cells. *Journal of Immunology*, **174**, 2591–2601.

Berger, C., Jensen, M.C., Lansdorp, P.M., Gough, M., Elliott, C. & Riddell, S.R. (2008) Adoptive transfer of effector $CD8^+$ T cells derived from central memory cells establishes persistent T cell memory in primates. *Journal of Clinical Investigation*, **118**, 294–305.

Bollard, C.M., Rossig, C., Calonge, M.J., Huls, M.H., Wagner, H.J., Massague, J., Brenner, M.K., Heslop, H.E. & Rooney, C.M. (2002) Adapting a transforming growth factor beta-related tumor protection strategy to enhance antitumor immunity. *Blood*, **99**, 3179–3187.

Bonger, K.M., Chen, L.C., Liu, C.W. & Wandless, T.J. (2011) Small-molecule displacement of a cryptic degron causes conditional protein degradation. *Nature Chemical Biology*, **7**, 531–537.

Bonini, C., Ferrari, G., Verzeletti, S., Servida, P., Zappone, E., Ruggieri, L., Ponzoni, M., Rossini, S., Mavilio, F., Traversari, C. & Bordignon, C. (1997) HSV-TK gene transfer into donor lymphocytes for control of allogeneic graft-versus-leukemia. *Science*, **276**, 1719–1724.

Brentjens, R.J., Latouche, J.B., Santos, E., Marti, F., Gong, M.C., Lyddane, C., King, P.D., Larson, S., Weiss, M., Riviere, I. & Sadelain, M. (2003) Eradication of systemic B-cell tumors by genetically targeted human T lymphocytes co-stimulated by CD80 and interleukin-15. *Nature Medicine*, **9**, 279–286.

Brentjens, R.J., Santos, E., Nikhamin, Y., Yeh, R., Matsushita, M., La Perle, K., Quintas-Cardama, A., Larson, S.M. & Sadelain, M. (2007) Genetically targeted T cells eradicate systemic acute lymphoblastic leukemia xenografts. *Clinical Cancer Research*, **13**, 5426–5435.

Brentjens, R.J., Riviere, I., Park, J.H., Davila, M.L., Wang, X., Stefanski, J., Taylor, C., Yeh, R., Bartido, S., Borquez-Ojeda, O., Olszewska, M., Bernal, Y., Pegram, H., Przybylowski, M., Hollyman, D., Usachenko, Y., Pirraglia, D., Hosey, J., Santos, E., Halton, E., Maslak, P., Scheinberg, D., Jurcic, J., Heaney, M., Heller, G., Frattini, M. & Sadelain, M. (2011) Safety and persistence of adoptively transferred autologous CD19-targeted T cells in patients with relapsed or chemotherapy refractory B-cell leukemias. *Blood*, **118**, 4817–4828.

Brentjens, R.J., Davila, M.L., Riviere, I., Park, J., Wang, X., Cowell, L.G., Bartido, S., Stefanski, J., Taylor, C., Olszewska, M., Borquez-Ojeda, O., Qu, J., Wasielewska, T., He, Q., Bernal, Y., Rijo, I.V., Hedvat, C., Kobos, R., Curran, K., Steinherz, P., Jurcic, J., Rosenblat, T., Maslak, P., Frattini, M. & Sadelain, M. (2013) CD19-targeted T cells rapidly induce molecular remissions in adults with chemotherapy-refractory acute lymphoblastic leukemia. *Science Translational Medicine*, **5**, 177ra38.

Burger, J.A. & Gribben, J.G. (2014) The microenvironment in chronic lymphocytic leukemia (CLL) and other B cell malignancies: insight into disease biology and new targeted therapies. *Seminars in Cancer Biology*, **24**, 71–81.

Carpenito, C., Milone, M.C., Hassan, R., Simonet, J.C., Lakhal, M., Suhoski, M.M., Varela-Rohena, A., Haines, K.M., Heitjan, D.F., Albelda, S.M., Carroll, R.G., Riley, J.L., Pastan, I. & June, C.H. (2009) Control of large, established tumor xenografts with genetically retargeted human T cells containing CD28 and CD137 domains. *Proceedings of the National Academy of Sciences of the United States of America*, **106**, 3360–3365.

Cheadle, E.J., Hawkins, R.E., Batha, H., O'Neill, A.L., Dovedi, S.J. & Gilham, D.E. (2010) Natural expression of the CD19 antigen impacts the long-term engraftment but not antitumor activity of CD19-specific engineered T cells. *Journal of Immunology*, **184**, 1885–1896.

Chen, Y.Y., Jensen, M.C. & Smolke, C.D. (2010) Genetic control of mammalian T-cell proliferation with synthetic RNA regulatory systems. *Proceedings of the National Academy of Sciences of the United States of America*, **107**, 8531–8536.

Christopoulos, P., Pfeifer, D., Bartholome, K., Follo, M., Timmer, J., Fisch, P. & Veelken, H. (2011) Definition and characterization of the systemic T-cell dysregulation in untreated indolent B-cell lymphoma and very early CLL. *Blood*, **117**, 3836–3846.

Cooper, L.J., Topp, M.S., Serrano, L.M., Gonzalez, S., Chang, W.C., Naranjo, A., Wright, C., Popplewell, L., Raubitschek, A., Forman, S.J. & Jensen, M.C. (2003) T-cell clones can be rendered specific for CD19: toward the selective augmentation of the graft-versus-B-lineage leukemia effect. *Blood*, **101**, 1637–1644.

Cooper, L.J., Al-Kadhimi, Z., Serrano, L.M., Pfeiffer, T., Olivares, S., Castro, A., Chang, W.C., Gonzalez, S., Smith, D., Forman, S.J. & Jensen, M.C. (2005) Enhanced antilymphoma efficacy of CD19-redirected influenza MP1-specific CTLs by cotransfer of T cells modified to present influenza MP1. *Blood*, **105**, 1622–1631.

Cruz, C.R., Micklethwaite, K.P., Savoldo, B., Ramos, C.A., Lam, S., Ku, S., Diouf, O., Liu, E., Barrett, A.J., Ito, S., Shpall, E.J., Krance, R.A., Kamble, R.T., Carrum, G., Hosing, C.M., Gee, A.P., Mei, Z., Grilley, B.J., Heslop, H.E., Rooney, C.M., Brenner, M.K., Bollard, C.M. & Dotti, G. (2013) Infusion of donor-derived CD19-redirected virus-specific T cells for B-cell malignancies relapsed after allogeneic stem cell transplant: a phase 1 study. *Blood*, **122**, 2965–2973.

Davila, M.L., Kloss, C.C., Gunset, G. & Sadelain, M. (2013) CD19 CAR-targeted T cells induce long-term remission and B Cell Aplasia in an immunocompetent mouse model of B cell acute lymphoblastic leukemia. *PLoS ONE*, **8**, e61338.

Davila, M.L., Riviere, I., Wang, X., Bartido, S., Park, J., Curran, K., Chung, S.S., 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, D.C., Arcila, M.E., Gonen, M., Roboz, G.J., Maslak, P., Douer, D., Frattini, M.G., Giralt, S., Sadelain, M. & Brentjens, R. (2014) Efficacy and toxicity management of 19-28z CAR T cell therapy in B cell acute lymphoblastic leukemia. *Science Translational Medicine*, **6**, 224ra25.

Di Stasi, A., Tey, S.K., Dotti, G., Fujita, Y., Kennedy-Nasser, A., Martinez, C., Straathof, K., Liu, E., Durett, A.G., Grilley, B., Liu, H., Cruz, C.R., Savoldo, B., Gee, A.P., Schindler, J., Krance, R.A., Heslop, H.E., Spencer, D.M., Rooney, C.M. & Brenner, M.K. (2011) Inducible apoptosis as a safety switch for adoptive cell therapy. *New England Journal of Medicine*, **365**, 1673–1683.

Eshhar, Z., Waks, T., Gross, G. & Schindler, D.G. (1993) 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. *Proceedings of the National Academy of Sciences of the United States of America*, **90**, 720–724.

Fedorov, V.D., Themeli, M. & Sadelain, M. (2013) PD-1- and CTLA-4-based inhibitory chimeric antigen receptors (iCARs) divert off-target immunotherapy responses. *Science Translational Medicine*, **5**, 215ra172.

Fedorov, V.D., Sadelain, M. & Kloss, C.C. (2014) Novel approaches to enhance the specificity and safety of engineered T cells. *Cancer Journal*, **20**, 160–165.

Finney, H.M., Lawson, A.D., Bebbington, C.R. & Weir, A.N. (1998) Chimeric receptors providing both primary and costimulatory signaling in T cells from a single gene product. *Journal of Immunology*, **161**, 2791–2797.

Finney, H.M., Akbar, A.N. & Lawson, A.D. (2004) Activation of resting human primary T cells with chimeric receptors: costimulation from CD28, inducible costimulator, CD134, and CD137 in series with signals from the TCR zeta chain. *Journal of Immunology*, **172**, 104–113.

Gattinoni, L., Finkelstein, S.E., Klebanoff, C.A., Antony, P.A., Palmer, D.C., Spiess, P.J., Hwang, L.N., Yu, Z., Wrzesinski, C., Heimann, D.M., Surh, C.D., Rosenberg, S.A. & Restifo, N.P. (2005) Removal of homeostatic cytokine sinks by lymphodepletion enhances the efficacy of adoptively transferred tumor-specific $CD8^+$ T cells. *Journal of Experimental Medicine*, **202**, 907–912.

Gattinoni, L., Lugli, E., Ji, Y., Pos, Z., Paulos, C.M., Quigley, M.F., Almeida, J.R., Gostick, E., Yu, Z., Carpenito, C., Wang, E., Douek, D.C., Price, D.A., June, C.H., Marincola, F.M., Roederer, M. & Restifo, N.P. (2011) A human memory T cell subset with stem cell-like properties. *Nature Medicine*, **17**, 1290–1297.

Gossen, M., Freundlieb, S., Bender, G., Muller, G., Hillen, W. & Bujard, H. (1995) Transcriptional activation by tetracyclines in mammalian cells. *Science*, **268**, 1766–1769.

Grada, Z., Hegde, M., Byrd, T., Shaffer, D.R., Ghazi, A., Brawley, V.S., Corder, A., Schonfeld, K., Koch, J., Dotti, G., Heslop, H.E., Gottschalk, S., Wels, W.S., Baker, M.L. & Ahmed, N. (2013) TanCAR: a novel bispecific chimeric antigen receptor for cancer immunotherapy. *Molecular Therapy – Nucleic Acids*, **2**, e105.

Grupp, S.A., Kalos, M., Barrett, D., Aplenc, R., Porter, D.L., Rheingold, S.R., Teachey, D.T., Chew, A., Hauck, B., Wright, J.F., Milone, M.C., Levine, B.L. & June, C.H. (2013) Chimeric antigen receptor-modified T cells for acute lymphoid leukemia. *New England Journal of Medicine*, **368**, 1509–1518.

Hacein-Bey-Abina, S., Von Kalle, C., Schmidt, M., McCormack, M.P., Wulffraat, N., Leboulch, P., Lim, A., Osborne, C.S., Pawliuk, R., Morillon, E., Sorensen, R., Forster, A., Fraser, P., Cohen, J.I., de Saint Basile, G., Alexander, I., Wintergerst, U., Frebourg, T., Aurias, A., Stoppa-Lyonnet, D., Romana, S., Radford-Weiss, I., Gross, F., Valensi, F., Delabesse, E., Macintyre, E., Sigaux, F., Soulier, J., Leiva, L.E., Wissler, M., Prinz, C., Rabbitts, T.H., Le Deist, F., Fischer, A. & Cavazzana-Calvo, M. (2003) LMO2-associated clonal T cell proliferation in two patients after gene therapy for SCID-X1. *Science*, **302**, 415–419.

Haso, W., Lee, D.W., Shah, N.N., Stetler-Stevenson, M., Yuan, C.M., Pastan, I.H., Dimitrov, D.S., Morgan, R.A., FitzGerald, D.J., Barrett, D.M., Wayne, A.S., Mackall, C.L. & Orentas, R.J. (2013) Anti-CD22-chimeric antigen receptors targeting B-cell precursor acute lymphoblastic leukemia. *Blood*, **121**, 1165–1174.

Haynes, N.M., Trapani, J.A., Teng, M.W., Jackson, J.T., Cerruti, L., Jane, S.M., Kershaw, M.H., Smyth, M.J. & Darcy, P.K. (2002) Single-chain antigen recognition receptors that costimulate potent rejection of established experimental tumors. *Blood*, **100**, 3155–3163.

Heczey, A., Liu, D., Tian, G., Courtney, A.N., Wei, J., Marinova, E., Gao, X., Guo, L., Yvon, E., Hicks, J., Liu, H., Dotti, G. & Metelitsa, L.S. (2014) Invariant NKT cells with chimeric antigen receptor provide a novel platform for safe and effective cancer immunotherapy. *Blood*, **124**, 2824–2833.

Heslop, H.E., Slobod, K.S., Pule, M.A., Hale, G.A., Rousseau, A., Smith, C.A., Bollard, C.M., Liu, H., Wu, M.F., Rochester, R.J., Amrolia, P.J., Hurwitz, J.L., Brenner, M.K. & Rooney, C.M. (2010) Long-term outcome of EBV-specific T-cell infusions to prevent or treat EBV-related lymphoproliferative disease in transplant recipients. *Blood*, **115**, 925–935.

Hinrichs, C.S., Borman, Z.A., Gattinoni, L., Yu, Z., Burns, W.R., Huang, J., Klebanoff, C.A., Johnson, L.A., Kerkar, S.P., Yang, S., Muranski, P., Palmer, D.C., Scott, C.D., Morgan, R.A., Robbins, P.F., Rosenberg, S.A. & Restifo, N.P. (2011) Human effector $CD8^+$ T cells derived from naive rather than memory subsets possess superior traits for adoptive immunotherapy. *Blood*, **117**, 808–814.

Hombach, A., Sent, D., Schneider, C., Heuser, C., Koch, D., Pohl, C., Seliger, B. & Abken, H. (2001a) T-cell activation by recombinant receptors: CD28 costimulation is required for interleukin 2 secretion and receptor-mediated T-cell proliferation but does not affect receptor-mediated target cell lysis. *Cancer Research*, **61**, 1976–1982.

Hombach, A., Wieczarkowiecz, A., Marquardt, T., Heuser, C., Usai, L., Pohl, C., Seliger, B. & Abken, H. (2001b) Tumor-specific T cell activation by recombinant immunoreceptors: CD3 zeta signaling and CD28 costimulation are simultaneously required for efficient IL-2 secretion and can be integrated into one combined CD28/CD3 zeta signaling receptor molecule. *Journal of Immunology*, **167**, 6123–6131.

Hudecek, M., Lupo-Stanghellini, M.T., Kosasih, P.L., Sommermeyer, D., Jensen, M.C., Rader, C. & Riddell, S.R. (2013) Receptor affinity and extracellular domain modifications affect tumor recognition by ROR1-specific chimeric antigen receptor T cells. *Clinical Cancer Research*, **19**, 3153–3164.

Hudecek, M., Sommermeyer, D., Kosasih, P.L., Silva-Benedict, A., Liu, L., Rader, C., Jensen, M.C. & Riddell, S.R. (2014) The nonsignaling extracellular spacer domain of chimeric antigen receptors is decisive for in vivo antitumor activity. *Cancer Immunology Research*. Epub ahead of print. doi:10.1158/2326-6066.CIR-14-0127

Imai, C., Mihara, K., Andreansky, M., Nicholson, I.C., Pui, C.H., Geiger, T.L. & Campana, D. (2004) Chimeric receptors with 4-1BB signaling capacity provoke potent cytotoxicity against acute lymphoblastic leukemia. *Leukemia*, **18**, 676–684.

Irving, B.A. & Weiss, A. (1991) The cytoplasmic domain of the T cell receptor zeta chain is sufficient to couple to receptor-associated signal transduction pathways. *Cell*, **64**, 891–901.

James, S.E., Greenberg, P.D., Jensen, M.C., Lin, Y., Wang, J., Till, B.G., Raubitschek, A.A., Forman, S.J. & Press, O.W. (2008) Antigen sensitivity of CD22-specific chimeric TCR is modulated by target epitope distance from the cell membrane. *Journal of Immunology*, **180**, 7028–7038.

Jensen, M.C., Popplewell, L., Cooper, L.J., DiGiusto, D., Kalos, M., Ostberg, J.R. & Forman, S.J. (2010) Antitransgene rejection responses contribute to attenuated persistence of adoptively transferred CD20/CD19-specific chimeric antigen receptor redirected T cells in humans. *Biology of Blood and Marrow Transplantation*, **16**, 1245–1256.

Kalos, M., Levine, B.L., Porter, D.L., Katz, S., Grupp, S.A., Bagg, A. & June, C.H. (2011) T

cells with chimeric antigen receptors have potent antitumor effects and can establish memory in patients with advanced leukemia. *Science Translational Medicine*, **3**, 95ra73.

Kershaw, M.H., Westwood, J.A. & Darcy, P.K. (2013) Gene-engineered T cells for cancer therapy. *Nature Reviews. Cancer*, **13**, 525–541.

Klingemann, H. (2014) Are natural killer cells superior CAR drivers? *Oncoimmunology*, **3**, e28147.

Kloss, C.C., Condomines, M., Cartellieri, M., Bachmann, M. & Sadelain, M. (2013) Combinatorial antigen recognition with balanced signaling promotes selective tumor eradication by engineered T cells. *Nature Biotechnology*, **31**, 71–75.

Kochenderfer, J.N., Feldman, S.A., Zhao, Y., Xu, H., Black, M.A., Morgan, R.A., Wilson, W.H. & Rosenberg, S.A. (2009) Construction and preclinical evaluation of an anti-CD19 chimeric antigen receptor. *Journal of Immunotherapy*, **32**, 689–702.

Kochenderfer, J.N., Wilson, W.H., Janik, J.E., Dudley, M.E., Stetler-Stevenson, M., Feldman, S.A., Maric, I., Raffeld, M., Nathan, D.A., Lanier, B.J., Morgan, R.A. & Rosenberg, S.A. (2010) Eradication of B-lineage cells and regression of lymphoma in a patient treated with autologous T cells genetically engineered to recognize CD19. *Blood*, **116**, 4099–4102.

Kochenderfer, J.N., Dudley, M.E., Feldman, S.A., Wilson, W.H., Spaner, D.E., Maric, I., Stetler-Stevenson, M., Phan, G.Q., Hughes, M.S., Sherry, R.M., Yang, J.C., Kammula, U.S., Devillier, L., Carpenter, R., Nathan, D.A., Morgan, R.A., Laurencot, C. & Rosenberg, S.A. (2012) B-cell depletion and remissions of malignancy along with cytokine-associated toxicity in a clinical trial of anti-CD19 chimeric-antigen-receptor-transduced T cells. *Blood*, **119**, 2709–2720.

Kochenderfer, J.N., Dudley, M.E., Carpenter, R.O., Kassim, S.H., Rose, J.J., Telford, W.G., Hakim, F.T., Halverson, D.C., Fowler, D.H., Hardy, N.M., Mato, A.R., Hickstein, D.D., Gea-Banacloche, J.C., Pavletic, S.Z., Sportes, C., Maric, I., Feldman, S.A., Hansen, B.G., Wilder, J.S., Blacklock-Schuver, B., Jena, B., Bishop, M.R., Gress, R.E. & Rosenberg, S.A. (2013) Donor-derived CD19-targeted T cells cause regression of malignancy persisting after allogeneic hematopoietic stem cell transplantation. *Blood*, **122**, 4129–4139.

Kochenderfer, J.N., Dudley, M.E., Kassim, S.H., Somerville, R.P., Carpenter, R.O., Stetler-Stevenson, M., Yang, J.C., Phan, G.Q., Hughes, M.S., Sherry, R.M., Raffeld, M., Feldman, S., Lu, L., Li, Y.F., Ngo, L.T., Goy, A., Feldman, T., Spaner, D.E., Wang, M.L., Chen, C.C., Kranick, S.M., Nath, A., Nathan, D.A., Morton, K.E., Toomey, M.A. & Rosenberg, S.A. (2014) 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. *Journal of Clinical Oncology*. Epub ahead of print, doi:10.1200/JCO.2014.56.2025

Landmeier, S., Altvater, B., Pscherer, S., Meltzer, J., Sebire, N., Pule, M., Vera, J., Hotfilder, M., Juergens, H., Vormoor, J. & Rossig, C. (2010) Cytotoxic T cells transduced with chimeric anti-CD19 receptors prevent engraftment of primary lymphoblastic leukemia in vivo. *Leukemia*, **24**, 1080–1084.

Lee, D.W., Kochenderfer, J.N., Stetler-Stevenson, M., Cui, Y.K., Delbrook, C., Feldman, S.A., Fry, T.J., Orentas, R., Sabatino, M., Shah, N.N., Steinberg, S.M., Stroncek, D., Tschernia, N., Yuan, C., Zhang, H., Zhang, L., Rosenberg, S.A., Wayne, A.S. & Mackall, C.L. (2014) T cells expressing CD19 chimeric antigen receptors for acute lymphoblastic leukaemia in children and young adults: a phase 1 dose-escalation trial. *The Lancet*. Epub ahead of print, doi:10.1016/S0140-6736(14)61403-3

Maher, J., Brentjens, R.J., Gunset, G., Riviere, I. & Sadelain, M. (2002) Human T-lymphocyte cytotoxicity and proliferation directed by a single chimeric TCRzeta/CD28 receptor. *Nature Biotechnology*, **20**, 70–75.

Maude, S.L., Frey, N., Shaw, P.A., Aplenc, R., Barrett, D.M., Bunin, N.J., Chew, A., Gonzalez, V.E., Zheng, Z., Lacey, S.F., Mahnke, Y.D., Melenhorst, J.J., Rheingold, S.R., Shen, A., Teachey, D.T., Levine, B.L., June, C.H., Porter, D.L. & Grupp, S.A. (2014) Chimeric antigen receptor T cells for sustained remissions in leukemia. *New England Journal of Medicine*, **371**, 1507–1517.

Milone, M.C., Fish, J.D., Carpenito, C., Carroll, R.G., Binder, G.K., Teachey, D., Samanta, M., Lakhal, M., Gloss, B., Danet-Desnoyers, G., Campana, D., Riley, J.L., Grupp, S.A. & June, C.H. (2009) Chimeric receptors containing CD137 signal transduction domains mediate enhanced survival of T cells and increased antileukemic efficacy in vivo. *Molecular Therapy*, **17**, 1453–1464.

Philip, B., Kokalaki, E., Mekkaoui, L., Thomas, S., Straathof, K., Flutter, B., Marin, V., Marafioti, T., Chakraverty, R., Linch, D., Quezada, S.A., Peggs, K.S. & Pule, M. (2014) A highly compact epitope-based marker/suicide gene for easier and safer T-cell therapy. *Blood*, **124**, 1277–1287.

Portell, C.A., Wenzell, C.M. & Advani, A.S. (2013) Clinical and pharmacologic aspects of blinatumomab in the treatment of B-cell acute lymphoblastic leukemia. *Clinical Pharmacology: Advances and Applications*, **5**, 5–11.

Porter, D.L., Levine, B.L., Kalos, M., Bagg, A. & June, C.H. (2011) Chimeric antigen receptor-modified T cells in chronic lymphoid leukemia. *New England Journal of Medicine*, **365**, 725–733.

Prosser, M.E., Brown, C.E., Shami, A.F., Forman, S.J. & Jensen, M.C. (2012) Tumor PD-L1 co-stimulates primary human CD8(+) cytotoxic T cells modified to express a PD1:CD28 chimeric receptor. *Molecular Immunology*, **51**, 263–272.

Pule, M., Finney, H. & Lawson, A. (2003) Artificial T-cell receptors. *Cytotherapy*, **5**, 211–226.

Pule, M.A., Straathof, K.C., Dotti, G., Heslop, H.E., Rooney, C.M. & Brenner, M.K. (2005) A chimeric T cell antigen receptor that augments cytokine release and supports clonal expansion of primary human T cells. *Molecular Therapy*, **12**, 933–941.

Pule, M.A., Savoldo, B., Myers, G.D., Rossig, C., Russell, H.V., Dotti, G., Huls, M.H., Liu, E., Gee, A.P., Mei, Z., Yvon, E., Weiss, H.L., Liu, H., Rooney, C.M., Heslop, H.E. & Brenner, M.K. (2008) Virus-specific T cells engineered to coexpress tumor-specific receptors: persistence and antitumor activity in individuals with neuroblastoma. *Nature Medicine*, **14**, 1264–1270.

Restifo, N.P., Dudley, M.E. & Rosenberg, S.A. (2012) Adoptive immunotherapy for cancer: harnessing the T cell response. *Nature Reviews. Immunology*, **12**, 269–281.

Riddell, S.R., Sommermeyer, D., Berger, C., Liu, L.S., Balakrishnan, A., Salter, A., Hudecek, M., Maloney, D.G. & Turtle, C.J. (2014) Adoptive therapy with chimeric antigen receptor-modified T cells of defined subset composition. *Cancer Journal*, **20**, 141–144.

Rosenberg, S.A., Yang, J.C., Sherry, R.M., Kammula, U.S., Hughes, M.S., Phan, G.Q., Citrin, D.E., Restifo, N.P., Robbins, P.F., Wunderlich, J.R., Morton, K.E., Laurencot, C.M., Steinberg, S.M., White, D.E. & Dudley, M.E. (2011) Durable complete responses in heavily pretreated patients with metastatic melanoma using T-cell transfer immunotherapy. *Clinical Cancer Research*, **17**, 4550–4557.

Sallusto, F., Lenig, D., Forster, R., Lipp, M. & Lanzavecchia, A. (1999) Two subsets of memory T lymphocytes with distinct homing potentials and effector functions. *Nature*, **401**, 708–712.

Savoldo, B., Ramos, C.A., Liu, E., Mims, M.P., Keating, M.J., Carrum, G., Kamble, R.T., Bollard, C.M., Gee, A.P., Mei, Z., Liu, H., Grilley, B., Rooney, C.M., Heslop, H.E., Brenner, M.K. & Dotti, G. (2011) CD28 costimulation improves expansion and persistence of chimeric antigen receptor-modified T cells in lymphoma patients. *Journal of Clinical Investigation*, **121**, 1822–1826.

Scholler, J., Brady, T.L., Binder-Scholl, G., Hwang, W.T., Plesa, G., Hege, K.M., Vogel, A.N., Kalos, M., Riley, J.L., Deeks, S.G., Mitsuyasu, R.T., Bernstein, W.B., Aronson, N.E., Levine, B.L., Bushman, F.D. & June, C.H. (2012) Decade-long safety and function of retroviral-modified chimeric antigen receptor T cells. *Science Translational Medicine*, **4**, 132ra53.

Schwartz, R.H. (2003) T cell anergy. *Annual Review of Immunology*, **21**, 305–334.

Straathof, K.C., Pule, M.A., Yotnda, P., Dotti, G., Vanin, E.F., Brenner, M.K., Heslop, H.E., Spencer, D.M. & Rooney, C.M. (2005) An inducible caspase 9 safety switch for T-cell therapy. *Blood*, **105**, 4247–4254.

Thomis, D.C., Marktel, S., Bonini, C., Traversari, C., Gilman, M., Bordignon, C. & Clackson, T. (2001) A Fas-based suicide switch in human T cells for the treatment of graft-versus-host disease. *Blood*, **97**, 1249–1257.

Till, B.G., Jensen, M.C., Wang, J., Qian, X., Gopal, A.K., Maloney, D.G., Lindgren, C.G., Lin, Y., Pagel, J.M., Budde, L.E., Raubitschek, A., Forman, S.J., Greenberg, P.D., Riddell, S.R. & Press, O.W. (2012) CD20-specific adoptive immunotherapy for lymphoma using a chimeric antigen receptor with both CD28 and 4-1BB domains: pilot clinical trial results. *Blood*, **119**, 3940–3950.

Traversari, C., Marktel, S., Magnani, Z., Mangia, P., Russo, V., Ciceri, F., Bonini, C. & Bordignon, C. (2007) The potential immunogenicity of the TK suicide gene does not prevent full clinical benefit associated with the use of TK-transduced donor lymphocytes in HSCT for hematologic malignancies. *Blood*, **109**, 4708–4715.

Wang, X., Chang, W.C., Wong, C.W., Colcher, D., Sherman, M., Ostberg, J.R., Forman, S.J., Riddell, S.R. & Jensen, M.C. (2011) A transgene-encoded cell surface polypeptide for selection, in vivo tracking, and ablation of engineered cells. *Blood*, **118**, 1255–1263.

Xu, X.J., Zhao, H.Z. & Tang, Y.M. (2013) Efficacy and safety of adoptive immunotherapy using anti-CD19 chimeric antigen receptor transduced T-cells: a systematic review of phase I clinical trials. *Leukemia and Lymphoma*, **54**, 255–260.

Yang, Z.Z. & Ansell, S.M. (2012) The tumor microenvironment in follicular lymphoma. *Clinical Advances in Hematology and Oncology*, **10**, 810–818.