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Open access? Widening access to chimeric antigen receptor (CAR) therapy for ALL

Sara Ghorashian[a], Persis Amrolia[b], and Paul Veys[b]

[a]*Developmental Biology and Cancer, University College London Great Ormond Street Institute of Child Health, London, UK;* [b]*Department of Bone Marrow Transplantation, Great Ormond Street Hospital, London, UK*

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T cells that are genetically modified to express chimeric antigen receptors (CARs) specific for CD19 show great promise for the treatment of relapsed/refractory acute lymphoblastic leukemia (ALL). The first U.S. Food and Drug Administration approval of a cellular cancer therapy in 2017, Novartis's CD19-targeting CAR T-cell product Kymriah™ within the context of relapsed/refractory pediatric ALL, followed rapidly by approval of Kite's Yescarta™ and, more recently, Kymriah™ for diffuse large B-cell indications in adults, highlights the pace of progress made in this field. In this review, we will consider the latest evidence from CAR T-cell therapy for B-lineage ALL. We discuss the barriers to CAR T-cell therapy for ALL patients and give a perspective on the strategy we have taken to date to widen access to CAR T-cell therapy for UK pediatric patients with high-risk ALL.

Although standard therapy leads to a long-term event-free survival in childhood acute lymphoblastic leukemia (ALL) approaching 90% [1,2], the outcomes of the 10% that relapse are much poorer, with a predicted long-term survival of 40–50% [3–5]. Within relapsed patients, high-risk groups are identifiable by timing and site of relapse and have a long-term event-free survival of 30% or less even with allogeneic stem cell transplantation (allo-SCT) [3,5,6]. The outcomes of those relapsing following allo-SCT are dismal, with long-term survival of only 10–20% in those undergoing second transplantation procedures [7–9], which are also associated with significant adverse toxicity. For these groups, novel therapies are urgently needed and access to chimeric antigen receptor (CAR) T-cell therapy has been a "game changer" in terms of outcomes.

Redirection of T cells by means of CAR expression enables potentially any cell surface moiety to be recognized in a human leukocyte antigen (HLA)-independent manner. CARs can be engineered with a variety of ligand-binding domains, most commonly antibody-derived single-chain variable fragments (scFvs), but other antigen recognition domains, receptor ligands, can also be employed. Although T cells have been used as the therapeutic effector cell in all of the large-scale studies of CD19 CAR targeting in the United States discussed in this review and summarized in Table 1, other effector cell populations for CAR therapy are actively being investigated. These include CAR engineered natural killer (NK) cells, cytotoxic immune cells that can potentially be infused as an off-the-shelf product, providing a short-lived therapeutic effect [10], and cytokine-induced killer cells, derived from lymphocytes that acquire NK-like cytotoxic potential through the activation and culture process used to manufacture them [11].

Targeting of CD19 by CAR T cells as therapy for B-cell malignancies has served as a model for engineered cellular immunotherapy of cancer. A number of factors have contributed to the success of this approach, including restriction of CD19 expression within the hematopoietic system. Although B-cell aplasia is an expected outcome of CD19 targeting, to date, there has been little evidence of significant infectious complications, suggesting that, at least in the short to medium term, the $CD19^+$ B-cell compartment is dispensable in treated patients. Immunoglobulin replacement has generally been given to those developing hypogammaglobulinemia, but protective humoral immunity can persist despite persistent B-cell aplasia, especially in adults [12]. Targeting of antigens restricted to the

Offprint requests to: Dr. Sara Ghorashian, UCL Great Ormond Street Institute of Child Health Developmental Biology and Cancer, Guilford Street, London NW5 2PN, UK E-mails: s.ghorashian@ucl.ac.uk, sghorashian@fastmail.fm

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hematopoietic system may have advantages, not only for limiting off-tumor cellular damage, but also in modulating the development of tolerance in antigen-specific T cells [13,14].

CD19 is expressed on a broad range of B-cell malignancies, including 95% of B-lineage ALL and, because it forms part of the B-cell signal transduction complex, it is likely to contribute to B-cell survival, although $CD19^{-}$ tumor escape variants are a significant cause of therapeutic failure after CAR T-cell therapy, as is discussed later. CD22 is also expressed on the majority of cells committed to the B lineage [15] and provides a basis for targeting of multiple B-cell antigens with a view to reducing tumor antigenic escape.

A significant challenge to the hemato-oncological community is to now translate the lessons learnt from CD19 targeting to other hematological and solid organ malignancies, where, to date, CAR T-cell therapies have been less successful.

CAR T-cell biology

In most cases, CARs link the antigen specificity of an scFv with the downstream signaling machinery of a T cell (Figure 1) through incorporation of signaling domains derived from the CD3 zeta chain. A spacer region extends the antigen-binding domain from the cell membrane and may be derived from a variety of molecules such as IgG, CD8a, or CD28. The length and derivation of the spacer can be critical for CAR signaling [16,17].

The modular design of CARs has facilitated incorporation of one or more co-stimulatory domains derived from receptors present naturally on T cells and which mediate full activation of T cells upon natural T-cell receptor (TCR) signaling [18,19]. First-generation CARs lacking a co-stimulatory domain (Figure 1) are capable of activating antigen-specific cytolysis, but such CAR T cells lack the ability to proliferate or generate cytokines in response to stimulation, which may contribute to a failure to persist or expand adequately after transfer to recipients [20,21]. In other studies, however, first-generation CAR T cells were noted to persist for more than a decade [22], so other factors are also likely at play. By contrast, second- and later-generation CARs (containing one or more co-stimulatory domains; Figure 1) are capable of mediating full T-cell activation associated with expansion in the host of >1000-fold [23]. In the setting of B-cell malignancies, particularly pediatric ALL, second-generation, CD19-targeting CARs incorporating co-stimulatory domains derived from 4-1BB or CD28 have been shown to be extremely efficacious at mediating long-term remission (Table 1).

In general, CAR T-cell products are made on a patient-specific basis, obtaining peripheral blood mononuclear cells (PBMCs) from the patient or, less commonly, their hematopoietic stem cell donor and using these as a starting material for CAR T-cell manufacture. PBMCs are obtained from whole blood or, more commonly, via leukapheresis (density gradient centrifugation). The obtained PBMCs are then washed and activated using antibodies cross-linking CD3 and CD28 in solution or associated with magnetic beads or colloidal matrices. Prior to activation, T cells may be purified to reduce contaminating populations of granulocytes, monocytes, and red cells, which may improve the quality of the CAR T-cell product generated. A further step enhancing the feasibility of CAR

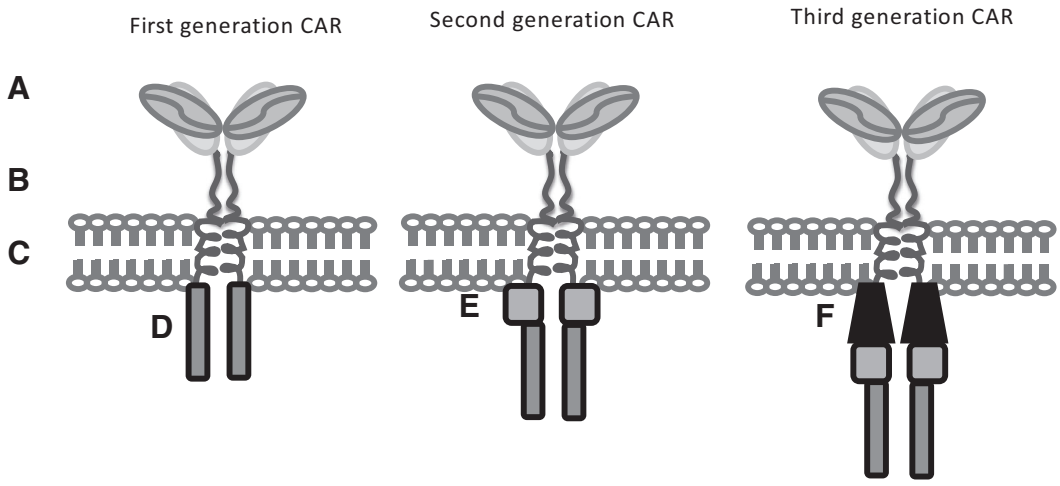

Figure 1. Anatomy of a CAR. (A) Ligand-binding domain most commonly derived from an scFv. (B) Spacer or stalk region derived from a variety of molecules such as the IgG Fc domain or CD8. (C) Transmembrane region commonly derived from the same molecule as the membrane-proximate endodomain. (D) Signaling domain usually derived from CD3 zeta chain. (E,F) Co-stimulatory endodomains that may be derived from a variety of co-stimulatory molecules such as CD28, 4-1BB, or OX40 and may be multiplexed within third-generation CARs.

Table 1. Clinical studies of CAR T-cell therapy for ALL

Site/ Sponsor	*n*	Target/ Binder	Costimulatory Domain	Viral Vector	Preselection	Activation	CR Rate	MRD-Negative CR Rate	Grade 3–5 CRS	Grade 3–5 Neurotoxicity	Allo-SCT as Adjunctive Therapy
Baylor College of Medicine [65]	4 ALL	CD19/FMC63	CD28	Retrovirus	Virus specific CTL	Autologous LCLs	75%	ING	0%	ING	Treated 3-12 months post allo-SCT
MSKCC [31,74]	53	CD19/SJ25C1	CD28	Retrovirus	None	CD3/D28 beads	83%	56%	26%	42%	39%
CHOP/UPenn [29,37]	75	CD19/FMC63	4-1BB	Lentivirus	None	CD3/D28 beads	93%	88%	27%	ING	10% [37]
NCI [30,75]	51	CD19/FMC63	CD28	Retrovirus	None	CD3/D28 beads	60.8%	55%	13.5%	5% [30]	40%
Chinese PLA General Hospital [76]	8 ALL	CD19/FMC63	4-1BB	Lentivirus	None	CD3 antibody	50%	25%	25%	0%	ING
NCI [64]	5 ALL	CD19/FMC63	CD28	Retrovirus	None	CD3 antibody	80%	80%	100%	40% (headache)	20%
FHCRC [25]	32	CD19/FMC63	4-1BB	Lentivirus	CD8 or	CD8Tcm + CD4	CD3/ D28	beads	100%	93%	23% (require ITU)
50%	43%										
MDACC [77]	17 ALL	CD19/ING	CD28	Sleeping beauty transposon	None	None	53%	ING	0%	0%	100%
Seattle Children's [24]	45	CD19/FMC63	4-1BB	Lentivirus	CD8+CD4	CD3/D28 beads	ING	93%	23%	21%	26%
CHOP [70]	36	CD19/ING, humanized	4-1BB	Lentivirus	None	CD3/D28 beads	83%	ING	13%	ING	ING
UCL [21]	11	CD19/FMC63	First generation	Retrovirus	EBV CTL	Autologous LCLs	45%	45%	0%	0%	100%
NCI [72]	21	CD22 /m971 human	4-1BB	Lentivirus	CD3+	CD3/D28 beads	57%	43%	0%	0%	ING
Novartis [34]	75	CD19/FMC63	4-1BB	Lentivirus	None	CD3/D28 beads	81%	81%	46%	13%	11%

ING=information not given, LCL=lymphoblastoid B-cell line

T-cell manufacture where manufacturing slots are limiting is cryopreservation of the leukapheresis product. This potentially allows T cells to be collected at time points prior to administration of chemotherapy, which may detrimentally affect circulating T-cell populations.

Because CAR T-cell products can vary widely in composition, particularly in their CD4:CD8 ratio, and the proportion of cells retaining an early memory phenotype (central memory, naive-like, stem cell memory), researchers at the Fred Hutchison Cancer Center/Seattle Children's Hospital have sought to predefine CAR T-cell populations infused either in terms of a 1:1 CD4:CD8 CAR T-cell ratio [24] or, where available, a 1:1 ratio of CD8 central memory: bulk CD4 CAR T cells [25]. The studies [24,25] utilizing these predefined CAR T-cell populations have demonstrated highly favorable outcomes, which are discussed below. However, it remains unclear whether the additional manufacturing complexity involved, particularly the need for additional expansion, provides an efficacy advantage over unselected CAR T-cell products.

Once activated, T cells are then manipulated to express the CAR through a process of transduction either using viral or nonviral gene delivery systems. The former require culture of packaging cell lines under Good Manufacturing Practice (GMP) conditions for virus production, but have the advantage of very efficient, generally stable transduction of human T cells. Non-viral gene delivery via plasmids, nucleases, or transposon-based technologies are cheaper, but efficiency is variable and cell viability can be compromised, depending on the methods used to introduce nucleic acids into therapeutic cells [26].

Transduced cells are then expanded for a variable length of time to ensure that an adequate dose is generated before impurities are removed and the cellular product is then sampled for quality tests. The CAR T-cell product may be cryopreserved at this point to allow time for release assays to be performed prior to infusion into the patient. These assays include viability tests, stringent sterility tests, tests for purity of the therapeutic cell populations, and assessments of transduction efficiency whether by quantitative polymerase chain reaction for sequences within the transgene or flow cytometry to detect the CAR or a tag introduced with the CAR.

Strategies have been developed to allow generation of "universal" CAR T cells from a third-party donor. This overcomes the need to generate a custom product on a patient-specific basis and means that a single PBMC donation can be used to generate "off-the-shelf" CAR T-cell doses for a number of patients. Additionally, it enables patients who are profoundly lymphopenic and other subgroups (e.g. infant ALL) in whom it has been problematic to generate an autologous product to be treated with CAR T cells. However, in addition to transduction with a transfer vector encoding the CAR, further gene modifications are required to reduce the risk of graft versus host disease (GVHD) mediated by the therapeutic cells, with the potential for associated bone marrow aplasia or hepatic inflammation, as well as to prevent rejection of CAR T cells by the host immune system. At our center, CD19 CAR T cells were generated from a healthy donor by lentivirally mediated transduction with additional modifications including transcription activation-like effector nuclease (TALEN)-mediated disruption of the genes encoding T-cell receptor alpha chain and CD52. The former modification reduces the risk of GVHD against the host and the latter renders the cells resistant to the effect of alemtuzumab, which is used as a transplantation conditioning reagent, in turn promoting persistence of the modified cells. Such an approach for use of off-the-shelf CAR T cells was reported in two infants relapsing after allo-SCT [27], who remain in remission >2 years out, and the approach is now being investigated in the context of studies in pediatric (NCT02808442) and adult (NCT02746952) patients. Other groups have used manipulation of HLA class I to generate universal CAR T cells, with disruption of the HLA-I gene locus and enforced expression of non-classical HLA-I molecules such as HLA-E or HLA-G to prevent NK cell activation and rejection of such universal CAR T cells [28].

Second-generation CAR T cells as therapy for ALL (Table 1)

Efficacy

Three major US centers have published studies of CD19 CAR T-cell therapy for pediatric ALL. Investigators at the University of Pennsylvania conducted a phase 1-2a study of tisangenlecleucel (Kymriah^{TM}) in a cohort of 60 children and young adults, demonstrating a complete remission rate of 93% and a 12 month relapse-free survival of 55% [29]. A phase 2 multicenter study of the same product is in progress and recently reported therapy of 75 patients with an 81% overall remission rate and 50% event-free survival at 1 year. The median persistence of CAR T cells noted was approximately 6 months.

Lee et al. from the National Cancer Institute [30] treated a cohort of 21 patients, defining a feasibility of 90% for generation of a CAR T-cell product within the group. They defined a maximum tolerated dose of 1×10^6 CAR T cells/kg patient weight. Sixty percent of patients achieved a minimal residual disease (MRD)-negative remission and overall survival in this cohort was 52% at 10 months.

Investigators at the Seattle Children's Hospital reported outcomes with therapy of 45 children and young adults with a CD19CAR T-cell product of defined composition (1:1 ratio of CD4:CD8 CAR T cells with high purity of CAR T cells). A 93% MRD-negative remission rate was achieved and the estimated 12-month event-free survival was 50.3% [24]. Median duration of B-cell aplasia, as a correlate of CAR T-cell persistence, was 3 months.

Results in adults [25,31] have been broadly similar. There are 80–90% response rates regardless of cytogenetic risk group. Long-term event-free survival rates are similar to those seen in children at approximately 40–60%. Across both pediatric and adult studies, there are differences in the proportions of patients consolidated with SCT at different centers and this should be taken into account when assessing long-term outcomes.

Relapse may be due to disease that continues to show CD19 expression or arise from $CD19^-$ variant clones. The former type of relapse is generally associated with failure of CAR T-cell persistence and recovery of normal B-cell populations, whereas the latter tends to arise in the ongoing presence of CAR T cells and results from the antigenic selection pressure exerted by them. Investigation of $CD19^-$ relapse has elucidated two main mechanisms by which CD19- variants may arise following therapy with T cells expressing the FMC63 CD19CAR, which binds to exon 2 of CD19. This arises from various mechanisms, including alternatively spliced RNA isoforms that lack exon 2 or hemizygous deletion of this exon [32]. In some cases, it does appear that relapse rates are higher with increased tumor burden [31], which might simply reflect an increased chance of a $CD19^-$ clone emerging from a greater cell mass.

Toxicity

There is a considerable burden of toxicity associated with CAR T-cell therapy for ALL. In particular, these include cytokine release syndrome, CAR T-cell-related encephalopathy syndrome, and B-cell aplasia.

Although generally manageable with good supportive care and, in the case of cytokine release syndrome, the judicious use of a specific agent such as the interleukin-6 (IL-6) receptor antagonist tocilizumab; a few fatal cases of CAR T-cell toxicity have occurred across adult studies utilizing different CAR T-cell platforms. In response to this, the US CARTOX Working Group was established to develop practice guidelines for monitoring, grading, and managing these toxicities in adults [33]. In general, the more severe manifestations of CAR-related toxicity occur in adults and deaths have resulted from severe cytokine release syndrome (CRS) or neurotoxicity.

CRS. This is an inflammatory syndrome akin to sepsis with variable severity in which patients present with fever, hypotension, hypoxia, and potentially develop multiorgan failure. Particularly in children, the manifestations can overlap with hemophagocytic lymphohistiocytosis resulting from immune-mediated macrophage activation. Risk factors for severe CRS include heavier disease burden and presence of comorbidities. The onset generally occurs around the time of peak CAR T-cell expansion (days 2–7 after infusion). There is some influence of CAR design on the time to CRS, being faster with CD28-containing CARs than 4-1BB-containing CARs, a trend mirrored by the times to peak CAR T-cell expansion. Most patients will develop some degree of CRS symptoms; however, there is an incidence of severe (grade 3 or greater) CRS in 23–46% of patients [24,30,34] that requires management in an intensive care setting.

Various groups have studied biomarkers to predict severe CRS [35,36]. In general, these have included high serum levels of proinflammatory cytokines such as IL-6 and interferon-gamma (IFN-γ), as well as of anti-inflammatory cytokines that are induced in concert with these, such as IL-10. However, in general, the predictive value of such biomarker signatures is study specific and may relate to differences in CAR T-cell products or the patient population studied. Severity of CRS has correlated with disease burden, peak of plasma/serum IL-6 [30,35,37], earlier onset of CRS [35,38], and CAR T-cell dose [24,25,30,38] in a number of studies. This latter finding has led to adoption of split [39] or risk-adapted CAR T-cell dosing [25] to mitigate the risk of severe CRS. This is the strategy adopted in a study at our center (the AMELIA study, EudraCT 2016-004680-39), in which a proportion of the total CAR T-cell dose (30%) is delivered initially, with the rest of the dose being administered 5–10 days later contingent upon absence of development of severe toxicity up to this point.

In view of the relationship between disease burden and CRS severity, another strategy to reduce the risk of severe CRS is delivery a cycle of chemotherapy with the aim of debulking disease where possible prior to lymphodepletion. This is not always achievable with highly refractory patients however and repeated chemotherapy cycles may affect CAR T-cell fitness, thus limiting CAR T-cell efficacy. At our center, we took a different approach to mitigating toxicity. The role of the greater affinity of CAR T-cell interactions in mediating greater toxicity compared with TCR-engineered CAR T cells has not been widely explored. By selecting CARs with defined binding kinetics and affinities lower than those that have achieved marketing authorization, it may be possible to obtain therapeutic efficacy with an improved toxicity profile. We have developed a CAR with a lower affinity for CD19 contributed to

by a faster off-rate but equivalent on-rate to the CD19 binder present in Kymriah (FMC63) to investigate the utility of this approach in reducing CAR-related toxicity [40].

There have been a number of recent reviews of optimal clinical care during CRS [33,41,42], so treatment protocols are not considered in detail here. It must be mentioned, though, that there are differing published grading systems and management guidelines for CRS. It is therefore important to agree on a given protocol at each center delivering CAR T-cell therapy to ensure consistency in management of this complication. Important differential diagnoses, namely sepsis, should be managed presumptively until excluded by appropriate investigations.

Current best practice includes closely monitored supportive therapy such as preemptive fluid management, inotrope support, and IL-6 axis blockade upon development of grade 2 CRS. Tocilizumab is a monoclonal antibody blocking the IL-6 receptor that is licensed for the management of CRS as well as inflammatory arthritides in children. Siltuximab, a monoclonal antibody that binds and neutralizes IL-6, is licensed for multicentric Castleman's disease. Both of these are effective therapies for CRS, inducing rapid reversal of CRS symptoms in most patients. Use of tocilizumab with more recent CRS management protocols is required in 25–40% of patients [24,25,31,34].

Short courses of steroids may be added if symptoms persist after tocilizumab administration or repeated IL-6 blockade may be necessary. To date, there appears to be little impact of tocilizumab on CAR T-cell expansion, response rates or durability of response at least on the basis of modeling CAR T-cell kinetics in patients with severe CRS [43], but whether this also holds for patients with lower CRS severity has not been systematically investigated. Because cytokine storm involves elevated circulating levels of a number of proinflammatory mediators, it is likely that novel therapeutic options will be developed for those failing to respond to tocilizumab and steroids [44].

Earlier implementation of IL-6 blockade (e.g., at grade 1 CRS) may be considered in the setting of significant comorbidities. During the peak of CRS, vigilance is required for development of further complications, including CAR T-cell-related encephalopathy syndrome (CRES), cardiac ventricular dysfunction, cardiac arrhythmias, hemophagocytic lymphohistiocytosis, and endothelial activation through appropriate baseline measurements (e.g., cognitive assessment, electroencephalogram, neuroimaging, echocardiography, electrocardiographic telemetry, ferritin, fibrinogen, and d-dimers, respectively) and ongoing monitoring of these parameters if changes subsequently develop.

More recently, an association between severe CRS and coagulopathy/endothelial activation has been highlighted. Investigation of biomarkers of severe CRS in a predominantly pediatric cohort revealed an association between low fibrinogen and grade 4 CRS [35]. This was explored further in an adult cohort treated at the Fred Hutchinson Cancer Center [38,45], where grade 4 or greater CRS was associated with hypofibrinogenemia, increased d-dimers, and reduced platelets, which is consistent with disseminated intravascular coagulation.

Patients with CRES appear encephalopathic with a variety of presenting symptoms including dysphasia, dysgraphia, obtundation, and seizures. The frequency of seizures varies dependent on the CAR design, being more frequent in studies where a CD28-containing CAR was adopted. Symptoms may be of variable severity, but severe neurotoxicity generally develops in those with more severe CRS [24,38] and often after the peak of CRS manifestations [38]. The symptoms of CRES overlap with those of infective encephalopathy and neurological toxicity resulting from methotrexate or fludarabine administration and these are relevant differentials to consider. Neuroimaging and cerebrospinal fluid (CSF) analysis can be highly informative in this regard, with flow cytometry on CSF to demonstrate the nature of any cell populations identified. Demonstration of white matter change is more likely to be associated with drug-related encephalopathy and is generally absent in those with CRES [33].

The mechanism for neurotoxicity is not clearly understood, but in view of the correlation between severity of CRS and that of CRES, the latter does appear to be related to systemic inflammation in the setting of rapid early CAR T-cell expansion, particularly with the rapid upstroke of systemic inflammatory cytokines such as IL-6 [38]. This has led to the implication of rapid transfer of inflammatory cytokines from the systemic circulation into the CSF as a potential mechanism. Further, delineation of endothelial activation in severe neurotoxicity [45] may allow rational drug targeting to ameliorate CRES.

Unfortunately, in some cases, severe neurotoxicity can have a fatal outcome, such as was the case in the development of JCAR015 by Juno therapeutics. A total of five fatal cases of neurotoxicity resulting from cerebral edema led to the closure of this study. Although it was asserted that early rapid CAR T-cell expansion related to the presence of a CD28-derived costimulatory domain may have contributed to the severity of neurotoxicity seen, in reality, it is likely that a number of factors contributed. Indeed, fatal neurotoxicity was reported in 3% of adults treated with a 4-1BB-domain-containing CAR for a variety of B-cell malignancies at the Fred Hutchison Cancer Center in Seattle [45]. There is great interest in determining a biomarker profile capable of predicting high-risk neurotoxicity and, in the same cohort of patients, a combination of fever

≥38.9°C with IL-6 levels of >16 pg/μL and monocyte chemoattractant protein (MCP) 1 >1344 pg/μL in the first 36 hours after CAR T-cell infusion was associated with a very high risk of severe neurotoxicity [45].

An association has been noted between higher CRS and neurotoxicity grades and serum von Willebrand factor (VWF), suggesting greater endothelial activation in both CRS and CRES, and this was corroborated with higher levels of angiopoietin-2 (Ang-2) [38,45]. Importantly, this work also demonstrated that preexisting endothelial activation (prior to CAR T-cell infusion, as determined by increased VWF and Ang-2:Ang-1 ratios) may be associated with increased subsequent CRS severity. Theoretically, then, pharmacological manipulation of endothelial activation may modify CRS severity, providing potential interventions to ameliorate CRS.

There is no specific management of CRES unless it exists concomitantly with CRS, in which case tocilizumab therapy is indicated as above. Siltuximab may also be administered. Although there are less data available on the use of this latter agent, its mode of action in directly neutralizing circulating IL-6 is theoretically advantageous over tocilizumab because serum IL-6 levels often increase after tocilizumab-mediated IL-6R blockade and may contribute to increased CRES severity. However, if encephalopathy develops after CRS, then short-course steroids are often considered as first-line therapy. The delineation of novel mediators implicated in CRES such as increased VWF and Ang-2 may provide rational targets for novel CRES therapies in the future.

B-cell aplasia. Rational targeting of the $CD19^+$ B-cell compartment will result in B-cell aplasia, with the possibility of hypogammaglobulinemia if B-cell aplasia is persistent. In our center, if hypogammaglobulinemia is persistent following CAR T-cell infusion, then prophylactic immunoglobulin replacement is instituted. However, despite the sometimes profound B-cell aplasia sustained after CAR T-cell infusion, the risk of severe infections is low and seems to be associated with the development of severe CRS [46].

Cytopenias. Cytopenias following CAR T-cell therapy are common and contributed to by a number of factors such as intensity of lymphodepletion, number of prior therapy lines, disease burden going into CAR T-cell therapy, and graft function after allo-SCT. For example, patients at our center receiving a combination of 1.5 g/m^2 cyclophosphamide and 150 mg/m^2 fludarabine as lymphodepletion are typically cytopenic for at least 28 days, whereas those undergoing less intensive lymphodepletion (0.9 g/m^2 cyclophosphamide and 90 mg/m^2 fludarabine) tend to be cytopenic for approximately half this interval (PA and SG, unpublished results).

These intervals are not unusual within the field. For example, 15% of patients treated with CD19 CAR T-cell therapy for a range of relapsed/refractory B-cell malignancies were neutropenic beyond 28 days after CAR T-cell therapy [46], and 40–50% of patients treated with tisangenlecleucel/Kymriah demonstrated grade 3-4 cytopenias that persisted beyond 28 days [34]. Further, this degree of cytopenia is not restricted to therapy with CD19-targeting CARs, being also seen, for example, in patients receiving CD22-directed CAR T-cell therapy [47]. These data suggest that it may be a cytokine-mediated rather than an antigen-mediated phenomenon.

Although contributing to the complexity of managing these patients and potentially compounding infection risk in patients who might also have hypogammaglobulinemia, the overall risk of life-threatening infections in relapsed/refractory patients managed with these therapies is low, as mentioned above, at 3–5% [34,46].

Barriers to CAR T-cell therapy

Barriers to CAR T-cell therapy exist at all stages of implementation of these cellular therapeutics. These are discussed below.

CAR T-cell manufacture

Manufacturing CAR T cells is a complex process performed under GMP conditions and represents a key barrier to broader application of this technology. Initially, manufacturing protocols facilitated high levels of T-cell expansion through use of high-dose IL-2 with prolonged ex-vivo culture after transduction. More recent recognition of the importance of retaining T cells within an early differentiation state during CAR T-cell manufacture [48–52] has led to minimizing the expansion phase required so that the total process generally lasts 7–10 days. A further limitation to manufacturing capacity incudes the complexity of the manufacturing process involved, particularly the number of manual processing steps required. Use of semi-automated, closed system manufacturing platforms such as the Miltenyi Biotec's Prodigy™ has been demonstrated to significantly reduce the man hours required for CAR T-cell manufacture [53] and to robustly generate CAR T-cell products suitable for clinical studies [54]. Therefore, the generation of CAR T-cell products for licensed indications beyond B-cell malignancies seems more feasible, although this depends on the flexibility of the automated manufacturing platform employed and the governance issues in providing industry standards for widely diverse processes.

Manufacturing failures are noted, depending on the manufacturing methods and the dose required, but are reported at approximately 10% [30,34]. There are many factors that may have a bearing; for example, the nature of the antileukemic therapy that patients undergo prior to leukapheresis. Certain chemotherapy agents, in particular cyclophosphamide and cytarabine, may be responsible for depleting T cells from early memory subsets that particularly seem to contribute to expansion [55]. By selecting cells from these subsets and then employing expansion steps, it may be possible to eliminate manufacturing failures, as has been the case in some studies [24,25].

Vectors

As discussed above, newer methods of gene transduction may obviate the need to use viral vectors. Although efficient, viral vectors require another costly GMP process for their production prior to incorporation in CAR T-cell manufacture. Different studies have incorporated either lentiviral or retroviral gene transduction methods (Table 1), giving comparable initial response rates, but at present, it is not clear whether one approach is better than the other because studies have also utilized different CAR design and manufacturing methods.

Unlike retroviral vectors, the production of clinical grade lentiviral vector has, to date, involved transient transfection of human embryonic kidney (HEK) 293T cells. This allows only limited batches of lentivirus to be generated on a one-off basis and with a potential for significant batch-to-batch variability in titer, leading in turn to variability in CAR T-cell manufacture. By contrast, use of stable producer cell lines for retroviral vector production leads to a highly scalable process, which is reliable and reproducible producing high yields of viral titer.

However, increasing recognition that differences in the integration profile of retrovirally and lentivirally transduced CAR T cells leads to potentially deleterious differences in CAR expression in terms of the fitness of the CAR T cells generated. This would suggest that, for a given CAR design and targeted tumor antigen, these transduction platforms may not be equivalent in terms of the quality of CAR T-cell product generated [56,57]. Although much research effort is focussed on generating lentiviral producer cell lines for clinical purposes [58,59], at present, viral yields generally remain lower than those generated by transient transfection.

Non-viral transduction methods have been employed in CD19 CAR T-cell studies. These include transposon-based gene transduction methods such as the Sleeping Beauty system that facilitate stable transgene expression, have proven feasible for the generation of clinical CAR T-cell products for patients, and are significantly less costly than the use of viral transduction [26]. Further advantages include a near-random integration pattern rather than preferential targeting of highly expressed genes, as is seen with viral transduction methods. There is a concern that dysregulated gene expression caused by nonrandom transgene insertion may lead to oncogenic potential such as those seen following gene engineering of hematopoietic stem cells [60,61], although in practice, there have been no cases of oncogenesis of this type with transduction of mature T cells across CAR T-cell or other T-cell studies globally. Moreover, the cell manipulation and prolonged expansion used with nonviral transduction may affect the biological fitness of CAR T cells, although prolonged persistence has been documented in early clinical studies of CD19 CAR T-cell therapy in the setting of adjunctive allogeneic or autologous SCT [26]. Data on disease-related outcomes from these studies are eagerly awaited in order to understand whether their therapeutic efficacy is equivalent to virally transduced CAR T cells.

Controlling disease prior to manufacture: a difficult balance

In any patient being considered for CAR T-cell therapy, there is a need to coordinate the therapy required to control disease burden with the requirement for a decent lymphocyte on a given leukapheresis date to facilitate CAR T-cell manufacture, particularly if manufacturing slots are limited. The optimal time is for leukapheresis is unclear: too early after relapse and the blast percentage may be high with an associated risk of transduction and subsequent expansion of CAR-transduced ALL blasts in the recipient [62]. However, the quality of the CAR T-cell product can be affected by prior chemotherapy. For example, certain agents such as cytarabine and cyclophosphamide may have a demonstrable impact on subsequent CAR T-cell fitness [55]. Although other novel agents such as blinatumomab or inotuzumab may also be utilized, it is unclear whether this additional selection pressure prior to CAR T-cell therapy may compound or mitigate the risk of relapse with $CD19^{-}$ escape variants, respectively. Further, an optimal interval from last chemotherapy is required. Although leukapheresis of lymphopenic patients is feasible, contaminating populations within the PBMC pool obtained can cause significant issues by consumption of, for example, cytokines and activating beads, so that measures to remove monocytes and neutrophils such as elutriation, T-cell selection, or plastic adherence [63] can improve the feasibility of generating an effective CAR T-cell dose in all patients. Optimization of relapse protocols incorporating CAR T-cell therapy is required.

If the patient has already undergone allo-SCT, a feasible option is to generate donor-derived CAR T cells. This has

the advantage of overcoming lymphopenia or intrinsic T-cell deficits in the host and allows CAR T-cell manufacture to occur independently of managing the disease itself, which is of particular benefit for those with aggressive disease. Several studies, including one at our institution [21], have highlighted the feasibility and safety of such approach because, at least with CD28-containing CAR constructs and in the absence of lymphodepleting conditioning, the incidence of acute GVHD is low [64,65]. In one study utilizing a 4-1BB-containing CAR, both patients treated with donor-derived CAR T-cell therapy for ALL developed grade 2 acute GVHD (skin and liver) [66]. Although further evaluation in the context of larger clinical studies is warranted, preclinical evidence supports the induction of exhaustion and activation-induced death of allogeneic CD19CAR T cells containing a CD28-containing domain, which abrogates their ability to mediate GVHD. This was not the case after transfer of 4-1BB-containing CD19 CAR T cells, which were capable of mediating GVHD in the same model system [67].

Manufacturing feasibility is a significant consideration in CAR T-cell therapy for infant ALL. The pace of disease may not allow for prolonged intervals between chemotherapy to allow adequate lymphocyte recovery and it appears technically difficult to generate CAR T cells from such patients. It is unclear whether this difficulty reflects differences in the biology of infant T cells or the increased intensity of chemotherapy given in this disease setting. A further challenge facing this population is the frequent association with rearrangement of the MLL gene [68], which provides a particular predilection to evolution of a myeloid leukemic clone leading to subsequent myeloid leukemic relapse [69]. Therefore, therapy of infant ALL remains an ongoing area of unmet clinical need and represents an ideal setting in which to test universal CAR T-cell approaches. The generation of fixed batches of universal CAR T cells from a third party donor allows an off-the-shelf product, as discussed above.

Preventing relapse post CAR T-cell therapy

Because relapses of ALL are contributed to by failure of persistence of CAR T cells, with recovery of normal B cells often heralding relapse usually with $CD19^+$ disease, several groups have investigated the utility of redosing with CAR T cells. In general, this approach has not been effective in prolonging CAR T-cell persistence and, in some cases, a T-cell-mediated immune response against recipient CAR T cells has been identified as mediating poor persistence [25]. Because long-lived T cells capable of mediated immunosurveillance have been identified as being derived from early memory phenotype cells, as discussed above, CAR T-cell manufacturing methods are currently generally designed with the aim of preserving these populations as much as possible. Further efforts to improve outcomes by optimizing CAR T-cell persistence have led to studies utilizing CARs that have been humanized to minimize anti-CAR immune responses, with favorable disease-related outcomes [70], even in patients who have been refractory to standard CAR T-cell therapy.

Finally, in an attempt to circumvent antigenic escape through selection of $CD19^-$ escape variants, studies have investigated the use of other targets for CAR T cells such as CD22 [71]. The utility of this approach was demonstrated, with responses seen in patients having failed prior CD19 targeting CAR T-cell therapy; however, CD22 was downregulated in seven of eight patients who subsequently relapsed, suggesting that single-agent targeting, regardless of the antigen targeted, has the potential for creating antigen escape variants. Therefore, multi-antigenic targeting, for example, with CARs targeting CD19 and CD22, is the subject of a number of studies, including at our center (the AMELIA study, EudraCT 2016-004680-39) and at the National Institutes of Health [72].

Developing an integrated pathway for CAR T-cell therapy: UK approach

At our center, we currently have a number of CD19 CAR T-cell studies open (the CARPALL study NCT02443831, UCART19 NCT02808442, the AMELIA study, EudraCT 2016-004680-39). By having a comprehensive program of CAR T-cell studies, we are able to offer CAR T-cell therapies to a broader range of patients. Patients are identified from a national referral network, promoting equity of access of these therapies to patients across the UK. Patients in whom an autologous product is unlikely to be generated, for example, in those with severe lymphopenia or infant ALL, are treated with universal CAR T cells. The AMELIA study of CD19 and CD22 directed CAR T-cell therapy offers an option for those with $CD19^-$ disease.

Manufacturing capacity for this program is shared across academic (University College London, the CARPALL study) and industrial (Autolus, the AMELIA study; Pfizer, UCART19) partners, ensuring that we are able to treat up to two to three patients per month.

Management of relevant toxicities is carried out with an experienced medical team and in close discussion with the PICU staff. Patients are discussed across the multi-disciplinary team prior to CAR T-cell infusion to facilitate timely transfer to higher dependency care. Clear protocols governing the management of both CRS and neurotoxicity ensure that patients are managed in a consistent fashion.

Conclusion: broadening access and future challenges

Since Eshhar et al. [73] delineated the use of modular chimeric immunoreceptors leading to the development of CARs 15 years ago, progress toward their

therapeutic potential has been rapid. Gilead's recent acquisition of Kite Pharma and, subsequently, Celgene's acquisition of Juno reflects the drive for pharmaceutical giants to position themselves favorably within the developing CAR T-cell therapy market. Until European licenses are granted, however, access to CAR T-cell therapy in Europe currently remains restricted to those eligible for CAR T-cell studies.

Through the establishment of multiple partners in delivering these complex studies of engineered cellular therapeutics, we hope that we have made some progress in providing access to the promise of their therapeutic efficacy in the UK. However, looking forward to the goals for the next 15 years, multiple challenges remain, namely, prevention of $CD19^{-}$ relapse where this antigen alone is targeted, scaling up delivery of this therapy to all eligible patients, and broadening the indications for its use.

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Conflict of interest disclosure

SG: UCL Patents & Royalties, UCL Business
Honoraria: Novartis, Travel Sponsorship: Celgene
PA: UCL: Patents & Royalties: UCL Business
PV: Honoraria: Chimerix, EUSA, Novartis, Gilead, and Jazz pharmaceuticals.

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