CHAPTER 12

CAR-T Cell Clinical Trials Experience – Past, Present and Future

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HISTORY OF CAR-T CELL THERAPY CLINICAL TRIALS

Engineered T cells, as a form of therapy, marks the beginning of a new era of medicine with possible applications in complex diseases such as cancer (Lim and June, 2017). One of the uniquenesses of engineered T cells is the utilisation of 'living cells' as a therapeutic platform that, in contrast to 'small molecules' or 'biologics', is capable of sensing and responding to stimuli. Moreover, they present with challenges in cell manipulation, manufacture and control, thus generating a complex platform with which successful genetic reprogramming can provide transformational therapeutic potential (Lim and June, 2017). Infusion of nonengineered cells, such as T cell subsets of donor origin or donor leucocyte infusions (DLIs), in patients poststem cell transplant has been evidenced for more than two decades. Indeed, the success of adoptive immunotherapy utilising the graft-versus-leukaemia (GVL) effect was first described by Kolb et al. with the use of allogenic DLI for the treatment of recurrent chronic myelogenous leukaemia in marrow transplant patients (Kolb et al., 1990). However, graft-versus-host disease (GVHD) is a significant limitation in the utilisation of donor lymphocyte infusion (Kolb, 2008). Attempts to harness the GVL effect while minimising the risk of GVHD led to the development of adoptive T cell immunotherapy techniques such as chimeric antigen receptor (CAR) T cells and tumour-associated antigen (TAA) T cells for the treatment of haematological malignancies (McLaughlin et al., 2015).

Initial Trials of CAR-T cells

The initial trials reported almost two decades ago, utilised CAR-T cells for the management of human immunodeficiency virus (HIV) infections (Deeks et al., 2002; Mitsuyasu et al., 2000). Cytotoxic T cells engineered to express recombinant chimeric receptors and redirected against HIV-infected cells represented the basis of this new type of immunotherapy. Mitsuyasu et al. reported data from a phase II clinical trial of coinfusion of autologous CD4ζ-modified CD4+ and CD8+ T cells administered with or without

Second Generation Cell and Gene-based Therapies
ISBN 978-0-12-812034-7
https://doi.org/10.1016/B978-0-12-812034-7.00012-1

exogenous interleukin-2 (IL-2) in 24 HIV-infected patients with detectable viral loads (Mitsuyasu et al., 2000). Gene-modified CD4+ and CD8+ T cells at relatively stable levels in the circulation were detected for up to 42 weeks postinfusion. This study constituted a tipping point in the emergence of the new platform technology as it demonstrated the feasibility of adoptive immunotherapy for HIV infection and provided preliminary evidence of tissue homing and antiviral activity against tissue reservoirs of HIV. Furthermore, findings from this study prompted investigators to conduct a second phase II randomised trial that confirmed the safety and feasibility of adoptive therapy with CD4 gene-modified T cells to decrease HIV burden, including in subjects with uncontrolled viremia, and prolonged persistence (Deeks et al., 2002). Although peripheral blood samples verified long-term persistence and retention of CAR expression, the antiviral effects were minimal and further pursuit of the development of CAR therapies for HIV was sidelined. However, in recent years with the identification of broadly neutralising antibodies to enhance the anti-HIV potency of CAR-T cell, the interest in this area has been revisited (Kitchen and Zack, 2016; Liu et al., 2015). Nonetheless, the research is still primarily in the nonclinical phase at the time of writing.

Early experience using CAR-T cells in malignant disorders was attained with the first-generation CAR-T cells, which comprised only of a single-signaling domain, in cancer patients with renal cell carcinoma (Lamers et al., 2006), neuroblastoma (Park et al., 2007) or ovarian cancer (Kershaw et al., 2006). These earlier trials suggested that CAR-T cell therapy could be safely administered, but the limited persistence of this first-generation genetically engineered T cells resulted in a lack of objective clinical activity. Likewise, early experience in the treatment of B-cell malignancies using CD19-targeted first-generation CAR-T cells also demonstrated the feasibility of the approach, but with very limited antitumour effects (Ramos et al., 2014). Taken together, these findings demonstrate that a single-stimulatory domain within the CAR T construct is insufficient to fully activate the killing machinery of chimeric T cells (Ramos et al., 2014). The quest to enhance persistence and durability of CAR-T cells coupled with the need to improve clinical outcomes thus led to the development of second-generation CAR-T cells with additional costimulatory domains such as CD28, 4-1BB and OX40 fused to the CAR protein. This modular CAR design is the one that is the most studied in the clinical setting and available as an approved therapy.

Clinical Trials With CAR-T Cells

Clinical trials with CAR-T cells are conducted mainly in relapsed or refractory malignant disorders, both haematological and nonhaematological. At the time of initiating this project in January 2017, a search in the clinicaltrials.gov website, a 'registry and results database' of publicly and privately supported clinical studies of human participants conducted around the world, evaluating these therapies for relapsed or refractory malignancies (although one of the trials listed was in type 1 diabetes) showed 153 trials. A year

later (as of 15 May 2018) the database reports 288 trials – almost double – suggesting immense interest in this space. Among the 288 trials, 109 were sponsored by industry and 179 are from academia. Most of the trials are in phase I (Table 12.1). According to the Cell Trials Database (celltrials.org *website*) (CAR-Immunotherapy Trials, 2017), there are now 433 clinical trials ongoing globally involving CAR-T cells. Haematological malignancies with CD19-directed CAR-T cell therapies have thus far been the most studied indication (McLaughlin et al., 2015), including for many of the industry-sponsored trials targeting CD19. CD19, a surface antigen expressed in all stages of normal B-cell differentiation, is a particularly interesting antigen as it is also conserved on cells undergoing tumour transformation (Poe et al., 2012). Indeed, the majority of B-cell malignancies express CD19 at normal to high levels (80% of acute lymphoblastic leukaemia (ALL), 88% of B-cell lymphomas and 100% of B-cell leukaemias) (Tedder, 2009).

At the time of initiation of this work, we conducted a systematic PubMed search of all English language articles, published between January 2011 and June 2017, focussing on the clinical trials with CAR-T cell. In addition, we also searched major congress databases such as those of the American Society of Haematology (ASH) or the American Society of Clinical Oncology (ASCO) for any presentations on clinical trials with CAR-T cells in haematological malignancies for this cut-off date. In addition, we also included updates from the 59th ASH annual meeting, December 2017, from selected presentations. At this meeting, CAR-T cell therapy was a major focus, with 323 presentations. Inclusion of all the presentations is beyond the scope of this review. Clinical trials identified through this search were sorted based on indications and are reviewed here. At the time of completion of this project in May 2018, we also updated data from full publications if available for many of the industry-sponsored trials in haematological malignancies.

Table 12.1 Clinical Trials Evaluating CART Therapies as Listed in clinicaltrials.gov.

Phase of Trial	N[a]	Condition Studied
Early Phase I	7	Pancreatic cancer, malignant melanoma, breast cancer, Hodgkin lymphoma with no available curative treatment options that have a limited prognosis, nonsmall cell lung cancer, relapsed or refractory acute myeloid leukaemia
Phase I	174	Glioblastoma, haematological malignancies (diffuse large B-cell lymphoma (DLBCL), non-Hodgkins lymphoma, relapsed/refractory acute lymphoblastic leukaemia), multiple myeloma, solid tumours (HER2 +ve, EGFR +ve, prostate cancer), metastatic pancreatic/colorectal cancer, gastrointestinal cancers, hepatocellular carcinomas
Phase I/Phase II	60	
Phase II	59	
Phase III	1	DLBCL

[a]Reported n's based on information recorded on the phase of the study in the database. In few cases, phase of study was not available.
Source: Clinicaltrials.gov.

In the following section, the clinical outcomes of CD19-targeted CAR-T cells in B-cell haematological malignancies are reviewed.

CLINICAL OUTCOME OF CD19-TARGETED CAR-T CELLS IN B-CELL HAEMATOLOGICAL MALIGNANCIES: CHRONIC LYMPHOCYTIC LEUKAEMIAS

Chronic lymphocytic leukaemia (CLL) was the first B-cell haematological malignancy studied using CAR-modified T cells. Clinical outcomes in patients with high-risk CLL utilising CAR-T cells have been reported from studies conducted at various academic institutions (Brentjens et al., 2011; Kochenderfer et al., 2012, 2015; Porter et al., 2011, 2015, 2016; Turtle et al., 2015) (Table 12.2). Clinicians at the Memorial Sloan Kettering Cancer Center (MSKCC) investigated the new therapy in eight patients with purine analogue-refractory CLL and bulky lymphadenopathy in a phase I dose escalating study (Brentjens et al., 2011). The first cohort of four patients did not receive conditioning chemotherapy prior to the administration of autologous CAR-T cells at a dose of 1.2–3.0×10^7 19-28zeta T cells/kg. Three of these patients died of disease progression. The next four patients received cyclophosphamide (Cy) followed by CAR-T cells (dose ranging from 0.4 to 1.0×10^7 19-28zeta T cells/kg). No patients achieved a complete remission (CR). However, stabilisation of disease (SD) was observed in three patients, with a duration of response ranging from 2 to 6 months.

Likewise, the National Cancer Institute (NCI) reported data in four patients who were infused with their CD-19 targeted 28zeta CAR-T cells at a dose ranging from 0.3 to 2.8×10^7 per kg. Patients had variable anti-CD19 responses including CR of greater than 15 months in duration reported in one patient (Kochenderfer et al., 2012). All patients experienced B-cell aplasia and three patients had toxicities that were consistent with cytokine release syndrome (CRS), including fever, hypotension, fatigue and renal failure, as well as altered mental status. An updated study from the NCI in four CLL patients reported CR in three of the four patients (Kochenderfer et al., 2015). Similar results have been reported from the Fred Hutchinson Cancer Research Center (FHCRC), with three CR and one partial response (PR) of six patients treated (Turtle et al., 2015). The patient with PR died of infection, and the CR patients were in remission 1–10 months following therapy.

Investigators from the University of Pennsylvania reported their first clinical trial of three CLL patients treated with CTL019, a proprietary therapeutic formulation of anti-CD19 CAR-T cells containing the 4-1BB costimulatory domain (Porter et al., 2011; Kalos et al., 2011). Two of the patients reported impressive and durable remissions. The dose of CAR-T cells ranged from 1.4×10^5 to 1.6×10^7 per kg for all patients. As reported with the NCI trial, one of these patients experienced a prolonged B-cell aplasia that lasted for more than 6 months. Treated patients also experienced CRS like symptoms

Table 12.2 Clinical Outcome Reported With CD19-Targeted CAR-T cells in Chronic Lymphocytic Leukemia.

Academic Site	CAR	Patient Population	Conditioning Regimen	Cell Dose	Clinical Outcome	Toxicity
MSKCC (Brentjens et al., 2011)	19-28zeta; γ-retrovirus gene transfer	n = 8, median age 68 years	Cy 0–3 gm/m^2	0.4–3.0 × 10^7/kg	PR/SD 38% CR 0%	Fever, mild hypoxia, no severe CRS
NCI (Kochenderfer et al., 2012)	19-28zeta; γ retrovirus gene transfer	n = 4; median age 59	Cy 60 mg/kg × 2 days + Flu 25 mg/m^2 × 5 days	0.3–3.0 × 10^7/kg	PR/SD (3/4) 75% CR (1/4) 25%	B-cell aplasia, CRS in three patients
NCI-updated study (Kochenderfer et al., 2015)	19-28zeta; γ retrovirus gene transfer	n = 4, median age 62	Cy 60 mg/kg × 2days + Flu 25 mg/m^2 × 5 days	1–5 × 10^6 CAR-T cells/kg	PR (1/4) 25%; CR (3/4) 75%	Grade 3 hypotension, dyspnoea, confusion in two patients
FHCRC (Turtle et al., 2015)	1:1 ratio of CD8+:CD4+ 41BB CAR-T cells; lentivirus gene transfer	n = 6, median age 60 years	Cy, 60 mg/kg ± etoposide or Cy 60 mg/kg and flu 25 mg/m^2 daily for 3–5 days	2 × 10^5, 2 × 10^6 or 2 × 10^7 CAR-T cells/kg	3 CR, 1 PR and 2 no response.	Not reported
University of Pennsylvania (Porter et al., 2015)	19-41BB; lentivirus gene transfer	n = 14, median age 66	Bendamustine 43% Flu/Cy 21% Pentostatin/Cy 36%	1.6 × 10^8 CAR-T cells[a]	CR 28.5%; PR 29%; ORR 57% NR 43%	Fevers, delayed CRS, tumour lysis (n = 2)
University of Pennsylvania dose escalation study (Porter et al., 2016)	19-41BB; lentivirus gene transfer	n = 28 (patients infused) median age 62	Investigators choice	Stage 1: 5 × 10^7 versus 5 × 10^8 CAR-T cells[a] Stage 2: 5 × 10^8 CAR-T Cells[a]	Stage 1: 6/11 responses (4 CR) with high dose versus 4/13 (1 CR) with low dose Stage 2: 6 CR and 3 PR	Delayed CRS in 19 patients; seven grade 3–4 toxicity; manageable with anti-IL6 therapy (tocilizumab)

CR, complete response; *CRS*, cytokine release syndrome; *Cy*, cyclophosphamide; *FHCRC*, Fred Hutchinson Cancer Research Center; *Flu*, Fludarabine; *MSKCC*, Memorial Sloan Kettering Cancer Center; *NCI*, National Cancer Institute; *ORR*, overall response rate; *PR*, partial response.
[a]Per kg dose not reported.

including fever, rigors, dyspnoea and hypotension. An updated publication in 2015, from the same investigators, reported further data from 14 patients with CLL, including the three initial patients discussed above (Porter et al., 2015). Four subjects (29%) achieved a PR within the first month of CTL019 infusion. At a median of 19 months of follow-up, the overall response rate (ORR) was 57%. Four patients (29%) achieved CR with a median duration of response of 40 months. Remarkably, none of these patients relapsed and three of them are reported to be alive (one patient died due to infection-related sepsis). Among those achieving PR, two patients progressed after 4 months and both patients died within 6–27 months. Six subjects (43%) had no response and all six progressed within 1–9 months of CTL019 therapy. The most frequent toxicity that was observed was delayed CRS, which correlated with in vivo CTL019 expansion. Two cases of tumour lysis syndrome (TLS) were also reported. One patient developed ecthyma gangrenosum from a *Pseudomonas* wound infection at the skin biopsy site and died while in remission at 21 months postinfusion (Porter et al., 2015). A subsequent phase II dose optimisation study, where 28 patients with relapsed or refractory CLL received randomly assigned 5×10^7 versus 5×10^8 CTL019, reported 6/11 responses (four CR) with high dose versus 4/13 (one CR) with low dose (Porter et al., 2016). In a total of 18 patients treated with the optimal dose of 5×10^8 CTL019, six CR and three PR were reported. Delayed CRS was reported in 19 patients (grade 3 – four toxicities in seven patients) associated with high levels of IL-6 and interferon-gamma (IFN-γ). However, the CTL019 dose was not correlated to CRS development or severity. Tocilizumab successfully reversed CRS in 4 patients; 15 did not require intervention.

The experience with CAR-T cell therapy for CLL reported thus far by the various academic institutions has been attained in a small number of patients; with fewer than 10 patients in most cases and the largest experience from University of Pennsylvania. There were differences in clinical outcomes noted among the various institutions. These differences could be ascribed to various factors, including variation in the potency of the CAR-T cell formulations used, clinical differences in the patient population, disease states and the conditioning regimen that were used (Fraietta et al., 2016c). Nevertheless, despite being very encouraging, the CR rates reported are, at best, modest responses, ranging from 25% to 50% in most cases. The hostile CLL microenvironment has been hypothesised to be the major limiting factor for cell expansion and efficacy (Geyer and Brentjens, 2016). Several strategies to enhance the efficacy of CAR-T cells for CLL are currently being investigated, including the utilisation of appropriate preconditioning with lymphodepletion therapy (Fraietta et al., 2016c; Geyer and Brentjens, 2016). Of note, at the 58th ASH Annual Meeting, investigators from MSKCC and FHCRC reported improved efficacy of CAR-T cell therapies in patients who previously failed ibrutinib, with ORR of 76% (Turtle et al., 2016d) or received CAR-T cells concurrently with ibrutinib (Geyer et al., 2016), thereby confirming the validity of a treatment strategy previously reported by the University of Pennsylvania group (Fraietta et al., 2016a).

Furthermore, at the 2017 ASCO annual meeting, investigators from University of Pennsylvania reported a 89% MRD-negative marrow CR in patients with high-risk CLL (not in CR despite therapy with ibrutinib for at least 6 months) using a well-tolerated combination of CAR-T cells comprising CD3z, 4-1BB and humanised anti-CD19 single-chain variable fragment (scFv) (CTL119) with ibrutinib (Gill et al., 2017).

CLINICAL OUTCOME OF CD19-TARGETED CAR-T CELLS IN B-CELL HAEMATOLOGICAL MALIGNANCIES: ADULT ACUTE LYMPHOBLASTIC LEUKAEMIA

The body of evidence using CAR-T cell therapy for adult ALL is summarised in Table 12.3. The first clinical trial reported in this setting originated from MSKCC, using the CAR containing a CD28 costimulatory domain (19-28zeta) in adult patients with relapsed/refractory (r/r) ALL (JCAR015). Investigators presented updated results of their phase I trial in 51 patients at the 2016 ASCO meeting (Geyer and Brentjens, 2016; Park et al., 2016b). Enrolled patients had high-risk features, including Philadelphia chromosome positive (Ph+) B-ALL (n = 15) and prior allogeneic haematopoietic stem cell transplantation (alloHSCT, n = 18) and ≥3 prior lines of treatment (n = 31). Following lymphodepleting chemotherapy consisting of Cy alone or Cy in combination with fludarabine (Flu), all patients received a 19-28zeta CAR-T cell infusion at a dose of 3×10^6 cells/kg. Due to treatment-related toxicities, the CAR-T cell dose was adjusted based on disease burden, and patients with morphologic disease received 1×10^6 CAR-T cells/kg, whereas patients with minimal disease continued to receive the dose of 3×10^6 cells/kg. CR and minimal residual disease-negative CR (MRD-CR) rates were 91% and 71% in the minimal disease cohort and 75% and 65% in the morphologic disease cohort, respectively (Park et al., 2016b). In the entire cohort, inclusive of patients receiving all T cell dose levels, 41 patients (82%) achieved CR. At a median follow-up of 12 months (range, 1–45), the estimated 6-month overall survival (OS) rates for all patients in the minimal disease and morphologic disease cohorts were 73% and 57%, respectively. Sixteen of 41 patients who achieved CR underwent alloHSCT. Severe CRS exclusively occurred in patients with morphologic disease (44% vs. 0%), but grade 3/4 neurotoxicity was observed in 14% of patients with minimal disease versus 40% with morphologic disease. Eighteen patients relapsed during follow-up, including three patients relapsing with undetectable CD19 expression (Park et al., 2016a). In the updated results presented at the 2017 ASCO annual meeting, and in a follow-up publication, investigators reported that in patients with morphologic disease with a median follow-up of 18 months, duration of median event-free survival and OS was 6.3 and 17 months, respectively, versus zero in the minimal disease cohort. At a median follow-up of 29 months, the median event-free survival was 6.1 months and OS was 12.9 months. Allogeneic HSCT in either cohort did not improve survival. The authors concluded that the durability of 19-28zeta CAR-T cell mediated remissions and survival in

Table 12.3 Clinical Outcome Reported With CD19-Targeted CAR-T cells in Acute Lymphoblastic Leukaemia.

Academic/ Industry Site	CAR	Patient Population	Conditioning Regimen	Cell Dose	Clinical Outcome	Toxicity
Adult ALL						
MSKCC (Geyer and Brentjens, 2016; Park et al., 2016a, 2017b)	19-28zeta; γ-retrovirus gene transfer	n = 51, age range 22–74 years	Cy (n = 42) or Cy/Flu (n = 9)	1×10^6 to 3×10^6 CAR-T cells/kg	CR: 91% minimal disease cohort; 75% in morphologic disease cohort; 6-month OS: 73% minimal disease cohort; 57% in morphologic disease cohort	Severe CRS in pts with morphological disease 44%; minimal disease 0%; grade 3/4 neurotoxicity 40% in pats with morphological disease versus 14% minimal disease
FHCRC (Turtle et al., 2016a)	1:1 ratio of CD8+:CD4+ 41BB CAR-T cells; lentivirus gene transfer	n = 30, median age 40	Cy 2–4 g/m^2 ± etoposide 100 mg/m^2/day × 3 days or Cy 30–60 mg/kg + Flu 25/mg/m^2/day × 3–5 day	2×10^5, 2×10^6, and 2×10^7 CAR-T cells/kg; 1:1 CD4+:CD8+	CR: 86%; MRD-negative CR: 10/12 among patients receiving Cy monotherapy; 16/17 among patients receiving Flu/Cy	CRS in 25 of 30 patients between 6 h and 9 days after CAR–T cell infusion, 7 of 25 severe CRS; Neurotoxicity in 15 of 30 patients. 2 deaths due to toxicity at high dose
NCI (Brudno et al., 2016)	19-28zeta; γ retrovirus gene transfer	n = 5, age range 25–68	None (CART infusion following alloHSCT)	4.2–7.1 × 10^{6}T cells/kg	CR: 80% (4/5, all MRD negative)	Grade 3–4 toxicity including fever, anaemia, neutropenia, hypophosphatemia
University of Pennsylvania (Frey et al., 2016)	19-41BB; lentivirus gene transfer	n = 27, median age 44	Investigators choice	5×10^7–5×10^8 CAR-T cells[a]	CR: 33% low dose ORR: 83% fractionated high dose	Grade 3–4 CRS manageable with anticytokines with fractionated high dose 3 death with CRS with single high-dose infusion
Kite Pharmaceuticals (Shah et al., 2017a,b,c)	19-28zeta; γ retrovirus gene transfer	n = 11	25 mg/m^2/day Flu for 3 days and 900 mg/m^2/day Cy given on the last day.	1×10^6 or 2×10^6 CAR-T cells/kg	CR or CR with partial haematopoietic recovery in 8 (73%); blast free BM 1 (9%)	Grade ≥3 CRS: 25%; neurological events (63%); controlled with Toci (94%); steroids (75%)

Paediatric ALL						
NCI (Lee et al., 2015)	19-28zeta; γ retrovirus gene transfer	n = 20, median age 15	Cy 900 mg/m^2 and Flu 25 mg/m^2/day × 3 days	1 × 10^6 CAR-T cells/kg or 3 × 10^6 CAR-T cells/kg	CR: 70% (MRD-negative in 60% of those who achieved CR) OS: 52% at 7.8 months 10 of 12 in MRD-negative CR underwent alloHSCT	Dose limiting toxicity (grade 3–4 CRS) was observed at the higher dose of 3 × 10^6 CAR-T cells/kg. With the dose of 1 × 10^6 CAR-T cells/kg, grade 3 or 4 CRS occurred in four patients. Reversible neurotoxicity in 6 patients
NCI-updated data (Lee et al., 2016)		n = 51	Cy 900 mg/m^2 and Flu 25 mg/m^2/day × 3 days (Low-dose Cy/Flu) Flu (30 mg/m^2/day, 3–6 days) and Cy (1200 mg/m^2/day, 3–4 days) (High-dose Cy/Flu; n = 8) FLAG (n = 2) ifosfamide/etoposide (n = 6)	1 × 10^6 CAR-T cells/kg	CR: 60.8% (31/51); MRD −ve: 90% (28/31) 21 of 28 subjects achieving MRD− CR, had a subsequent HSCT	CRS combined for a severe CRS incidence of 13.5%. 3 grade-3 neurotoxicities (1 each: dysphasia, delirium, headache) and 2 seizures (one grade 1, one grade 2) occurred
MSKCC multicentre trial (Curran et al., 2015a,b)	19-28zeta; γ retrovirus gene transfer	n = 9; median age 15	Investigators choice	1–3 × 10^6 CAR-T cells/kg	CR: 5/9 (55%)	Not reported
CHOP/ University of Pennsylvania (Maude et al., 2016a)	19-41BB; lentivirus gene transfer	n = 59; 20 months–24 years	Cy 300–500 mg/m^2/day × 2–3 days + Flu 30 mg/m^2/day × 3–4 days or Cy 440 mg/m^2/day × 2 days + etoposide 100 mg/m^2/day × 2 days or other	1 × 10^7 to 1 × 10^8 cells/kg	CR: 93% 55/59; OS 79% at 12 months	CRS 88%; severe CRS 27% associated with high disease burden, and was reversed with the anti-IL6R agent tocilizumab.

Continued

Table 12.3 Clinical Outcome Reported With CD19-Targeted CAR-T cells in Acute Lymphoblastic Leukaemia.—cont'd

Academic/ Industry Site	CAR	Patient Population	Conditioning Regimen	Cell Dose	Clinical Outcome	Toxicity
CHOP/ University of Pennsylvania – first US multicentre trial (Maude et al., 2016c)	19-41BB; lentivirus gene transfer	n = 29, median age 12 years	Flu + Cy	Target dose: 2.0–5.0 × 10^6 cells/kg for ≤50 kg; 1.0–2.5 × 10^8 cells for >50 kg	CR/CRi (maintained at 2 evaluations ≥28 days apart), - 69.0% (20/29)	Serious adverse events occurred in 79.3% of pts within 8 weeks of infusion.; CRS (26/29) 89.7%-reversible; severe CRS 37.9% (11/29); reversible neuropsychiatric events 31% (9/29)
Novartis/ University of Pennsylvania (pivotal trial) (Maude et al., 2018)	19-41BB; lentivirus gene transfer	n = 75, median age 11 years	Flu + Cy	Median weight–adjusted dose of 3.1 × 10^6 transduced viable T cells per kilogramme of body weight (range, 0.2 × 10^6 to 5.4 × 10^6 cells per kilogram)	ORR 81% (within 3 months) CR: 60% (45 patients) Cri: 21% (16 patients)	CRS-77% of patients 48% received anticytokine therapy Neurologic events (40%); 13% grade 3; encephalopathy (11%), confusional state (9%), delirium (9%), tremor (8%), agitation (7%), and somnolence (7%)

ALL, Acute Lymphoblastic Leukaemia; *CR*, complete response; *CRS*, cytokine release syndrome; *Cy*, cyclophosphamide; *FHCRC*, Fred Hutchinson Cancer Research Center; *Flu*, Fludarabine; *MSKCC*, Memorial Sloan Kettering Cancer Center; *NCI*, National Cancer Institute; *ORR*, overall response rate; *OS*, overall survival; *PR*, partial response.
[a]Per kg dose not reported.

adult patients with relapsed B-ALL positively correlated to a low disease burden. Among patients with a low disease burden, the median OS was 20.1 months and was accompanied by a markedly lower incidence of the CRS and neurotoxic events supporting the early incorporation of CD19 CAR therapy (Park et al., 2017a, 2018).

Investigators from FHCRC, reported results from their phase I/II trial of 30 patients utilising their CD19 CAR containing a 4-1BB costimulatory domain and T cells expanded in vitro with a defined 1:1 ratio of CD4+:CD8+ CAR-T cells (Turtle et al., 2016a). Twenty-nine of 30 patients who received CAR-T cells survived for more than 21 days. All patients (100%) had no detectable leukaemia in the bone marrow (BM) by morphology, and in 27 of 29 patients (93%), leukaemia was undetectable by high-resolution flow cytometry. Moreover, 25 of 29 patients (86%) achieved CR without evidence of MRD by flow cytometry. The investigators also conducted an analysis on the influence of intensity of lymphodepleting chemotherapy. Thirteen patients received lymphodepleting chemotherapy consisting of Cy without Flu; 10 of 12 evaluable patients achieved CR by flow cytometry (83%), although 7 of 10 experienced relapse at a median of 66 days following CAR T cell infusion. Flu 25 mg/m^2 per day for 3–5 days was added along with Cy before CAR-T cell infusion, and investigators observed BM CR by flow cytometry and cytogenetic studies in 16 of 17 treated patients. Higher CAR-T cells and improved disease-free survival was observed in patients receiving Flu/Cy versus Cy alone (Turtle et al., 2016a,b). The most common toxicity that was observed in the first 14 days after CAR-T cell infusion was CRS, characterised by fever and/or hypotension and elevated serum levels of IL-6 and IFN-γ. Overall, 25 of 30 patients developed CRS between 6 h and 9 days after CAR-T cell infusion, and 7 of these 25 patients had severe CRS requiring ICU care. Severe neurotoxicity occurred in 15 of 30 patients. Two patients died due to toxicity. Severe toxicity due to CAR-T cells was predominantly seen in patients with 20% or more BM blasts and occurred more often after infusion of higher CAR-T cell doses (Turtle et al., 2016a).

Using a similar costimulatory domain as that used by clinicians at FHCRC for their CAR-T cell, CTL019, the investigators from University of Pennsylvania reported their findings in 27 adult patients with r/r ALL at the 2016 ASCO annual meeting (Frey et al., 2016) Following investigator's choice of lymphodepleting chemotherapy, CTL019 at a dose of 5×10^7 to 5×10^8 was infused either as a one-time infusion or fractionated infusions over 3 days. Six patients each in both of the dose groups received a one-time infusion. Across both dose groups, 15/27 patients (55%) achieved CR. Among patients receiving fractionated infusion at the higher dose of 5×10^8, ORR was 83% (9/12 CR and 1 PR). CR with the low dose of 5×10^7 was considerably low (33%). CRS refractory to corticosteroid and tocilizumab was a cause of death in three patients receiving one-time infusion with the high dose of 5×10^8T cells. In patients receiving the fractionated dose of 5×10^8, 9/12 had manageable grade 3–4 CRS (Frey et al., 2016).

Recently, NCI investigators reported their data in five adult ALL patients with progressive disease postalloHSCT, who received a single infusion of CAR-T cells containing

a CD28 costimulatory domain, and a CD3zeta T cell activation domain. No lymphodepleting chemotherapy was administered, and 4/5 patients achieved MRD-negative CR (Brudno et al., 2016).

The results of these clinical trials performed in the academia setting in adult ALL are overall very promising for CAR-T cell therapies; however, it must be emphasised that severe toxicity and fatal outcomes were observed. Investigators from University of Pennsylvania report that 'there is a high risk of fatal outcome with concurrent sepsis and CRS and measures to prevent infection and intervene early for CRS are warranted' (Frey et al., 2016). Two industry-initiated trials with CAR therapy for adult r/r ALL have also been reported. In June 2015, Juno Therapeutics Inc. in collaboration with MSKCC initiated their phase II trial, ROCKET, NCT02535364, using a CAR containing the 28zeta costimulatory domain. However, this trial is now suspended following 4 deaths from cerebral oedema (Dangi-Garimella, 2017; Lerman, 2016). Of interest is to note that cerebral oedema, as an adverse event, was not reported in any of the academia trials. At the 2016 ASH annual meeting, Kite Pharmaceuticals Inc. reported preliminary findings from their ZUMA-3 trial (NCT02614066) that enrolled three adult ALL patients (aged ≥18 years) treated with KTE-C19, also known as axicabtagene ciloleucel or axi-cel (Shah et al., 2016a). KTE-C19 utilises the same CAR construct as the NCI trial but is centrally manufactured in a streamlined 6–8 day process, with approximately a 2-week turnaround time from the time of aphaeresis to the delivery of KTE-C19 to the treatment site for patient infusion (Better et al., 2016). KTE-C19 was administered at a target dose of either 1 or 2×10^6 anti-CD19 CAR-T cells/kg after low-dose conditioning with Flu (25 mg/m^2 per day for 3 days) and Cy (900 mg/m^2 per day). MRD-negative remission was observed in all three patients. No patient experienced a dose-limiting toxicity. CRS and neurotoxicity were reported in all patients and was managed to resolution with tocilizumab, corticosteroids and/or siltuximab in addition to other supportive care. Updated results in 11 enrolled patients, including the 3 patients reported above, were presented at the 2017 ASCO annual meeting (Shah et al., 2017a). One patient experienced serious AE prior to KTE-C19 administration and 10 patients received KTE-C19. At the dose of 2×10^6 anti-CD19 CAR-T cells/kg, one patient experienced grade 5 AE due to CRS and multiorgan failure. Subsequently six patients received the low-dose 1×10^6 anti-CD19 CAR-T cells/kg. MRD-negative complete response (CR or CR+ partial or incomplete haematopoietic recovery) was reported in six patients. Manufacturing was successful in all patients. Overall, 3 of 11 (27%) patients had grade 3 or higher CRS, and 6 of 11 (55%) had grade 3 or higher neurologic events. These adverse events were generally reversible. At the ASH 2017 annual meeting, investigators presented the updated data reporting comparable efficacy between the two doses, i.e., 1×10^6 and 2×10^6 CAR-T cells/kg and data from patients (n = 8) treated at a lower dose of 0.5×10^6 CAR-T cells/kg. Results from 24 evaluable patients demonstrated a CR of 71% (n = 17/24), with 100% of responders having no detectable minimal residual disease, including in those with high tumour burden and high risk genetic

abnormalities (Shah et al., 2017b). The phase I portions of ZUMA-3 are ongoing with planned expansion to phase II. Initial results, although promising and showing the feasibility of a central manufacturing and logistics approach, remain insufficient and more data need to be generated and to become available to derive any conclusion (Table 12.4).

CLINICAL OUTCOME OF CD19-TARGETED CAR-T CELLS IN B-CELL HAEMATOLOGICAL MALIGNANCIES: PAEDIATRIC ACUTE LYMPHOBLASTIC LEUKAEMIA

Paediatric ALL is by far the most studied indication with CAR-T cell therapy. NCI, MSKCC and Children's Hospital Of Philadelphia – CHOP (I n collaboration with University of Pennsylvania are the academic institutes that have studied CAR-T cell therapy the most extensively for paediatric relapsing/refractory (r/r) ALL (Table 12.3). NCI conducted a phase I dose escalation study in 21 children and young adults (aged 1–30 years) with r/r CD19+ B-ALL (n = 20) or B-non-Hodgkin lymphoma (NHL) (n = 1) that utilised CAR with the CD28 costimulatory domain (Lee et al., 2015). Patients with high-risk features including primary refractory disease (n = 6), prior alloHSCT (n = 8) and active CNS leukaemia (n = 2) at the time of treatment were enrolled. Median BM blast content was 26%, and most (n = 16) had ≥5% BM blasts at the time of therapy. Following lymphodepletion with Flu and Cy, patients received CAR-T cells at a dose of either 1×10^6 CAR-T cells/kg (dose 1) or 3×10^6 CAR-T cells/kg (dose 2) or the entire CAR-T cell product (if sufficient numbers of cells to meet the assigned dose were not generated) using a 3 + 3 dose escalation scheme. The maximum tolerated dose was 1×10^6 CAR-T cells/kg. Of the 20 patients, CR was obtained in 14/20 patients (70%); with 12/20 patients (60%) achieving MRD-negative CR. OS was 51.6% at 9.7 months, median follow-up was 10 months and leukaemia-free survival was 78.8% at 4.8 months among the 12 patients who achieved MRD-negative CR. Furthermore, of the 12 patients who achieved MRD-negative CR, 10 underwent alloHSCT and remained in CR. Two patients who achieved an MRD-negative CR were deemed medically ineligible for HSCT and subsequently relapsed with CD19-negative disease. Of the six nonresponders, three were administered a second infusion of CAR-T cell and were nonresponsive to retreatment. Dose limiting toxicity (grade 3–4 CRS) was observed at the higher dose of 3×10^6 CAR-T cells/kg. With the dose of 1×10^6 CAR-T cells/kg, grade 3 or 4 CRS occurred in four patients. Reversible neurotoxicity was observed in six patients and included grade 1 visual hallucinations (n = 5) and transient dysphasia (n = 1). T cells were higher in patients who developed neurotoxicity than in those who did not. No CAR-T cells were detected in any patient after Day 68 (Lee et al., 2015). At the ASH 2016 annual meeting, the investigators presented updated data from 51 children and young adults (including the 21 patients reported above) with r/r ALL. Patients had a median follow-up of 18.7 months (Lee et al., 2016).

Table 12.4 Clinical Outcome Reported With CD19-Targeted CAR-T cells in NHL.

Academic Site	CAR	Patient Population	Conditioning Regimen
NCI (Kochenderfer et al., 2015)	19-28zeta; γ retrovirus gene transfer	n = 9 DLBCL; age range 42–60	Cy 60 mg/kg × 1–2 days + Flu 25 mg/m^2 × 5 days
NCI (Brudno et al., 2016)	19-28zeta; γ retrovirus gene transfer	n = 10 (DLBCL or MCL)	None; administered following alloHSCT
FHCRC (Turtle et al., 2016c)	1:1 ratio of CD8+:CD4+ 41BB CAR-T cells; lentivirus gene transfer	n = 32; median age = 58	Cy, 2–4 g/m^2 IV on day 1 + etoposide, 100–200 mg/m^2 per day IV on days 1–3 (Cy/E) or Cy, 60 mg/kg IV on day 1 and fludarabine 25 mg/m^2/day IV on days 2–4 or days 2–6 (Cy/Flu)
University of Pennsylvania (Chong et al., 2016; Schuster et al., 2016a,b, 2017a)	19-41BB; lentivirus gene transfer (CTL019)	n=30 (43 enrolled; 30 with CTL019 infusion; 14 DLBCL; 14 FL); median age = 56	Bendamustine, Cy, Cy + Flu; radiation + Cy, EPOCH and carboplatin-gemcitabine (only FL patients)
Kite Pharmaceuticals (Locke et al., 2017; Neelapu et al., 2016, 2017)	KTE-C19 (same construct as NCI) 19-28zeta; γ retrovirus gene transfer; centralised, closed and streamlined process of approximately 8 days	n = 7 (phase 1); n = 51 (phase 2); age range 29–69	Low-dose conditioning chemotherapy with Cy 500 mg/m^2 + Flu 30 mg/m^2 for 3 days
Novartis/ University of Pennsylvania (Pivotal trial) (Kymriah PI)	19-41BB; lentivirus gene transfer	n = 106	Flu (25 mg/m^2 IV daily for 3 days) and Cy (250 mg/m^2 IV daily for 3 days starting with the first dose of flu) Alternate: bendamustine 90 mg/m^2 IV daily for 2 days

DLBCL, diffuse large B-cell lymphoma; *MCL*, mantle cell lymphoma; *Cy*, cyclophosphamide; *NCI*, National Cancer Institute; *FHCRC*, Fred Hutchinson Cancer Research Center; *Flu*, fludarabine; *PR*, partial response; *CR*, complete response; *ORR*, overall response rate; *OS*, overall survival; *CRS*, cytokine release syndrome; *SD*, stable disease.

Cell Dose	Clinical Outcome	Toxicity
$1–5 \times 10^6$ CAR-T cells/kg	CR = 4/7, durable in three patients, 9–22 months; PR = 2, 1 SD	Grade 3–4 toxicities including hypotension, fever and neurotoxicity including confusion and obtundation
$2–10 \times 10^6$ CAR-T cells/kg	CR = 1; PR = 1; SD = 8	Grade 3–4 toxicities including hypotension, neutropenia, fever observed only in three patients
2×10^5 CAR-T cells/kg, 2×10^6 CAR-T cells/kg or 2×10^7 CAR-T cells/kg	Cy/Flu: CR = 50%; ORR7 = 2%; Cy/E: CR = 8%; ORR = 50% Cy/Flu with maximum tolerated dose of 2×10^6 CAR-T cells/kg (n = 11); CR = 64% (ORR = 82%)	Severe CRS and grade ≥3 neurotoxicity were observed in 13% and 28% of all patients, respectively
$1–5 \times 10^8$ CTL019 cells	DLBCL, CR was 43% at 6 months FL, CR rate 71% at 6 months	Overall, severe CRS occurred in five patients (18%); serious encephalopathy occurred in three patients (11%); two cases were self-limiting and one case was fatal (FL patient) DLBCL patients: CRS in nine patients (eight grade 2; one grade 3); transient neurotoxicity including delirium in 2/13 (1 grade 2; 1 grade 3) and cognitive disturbance in 1/13 (grade 1) FL patients: CRS in six patients (four grade 2; one grade 3, one grade 4); one episode of grade 5 encephalitis
2×10^6 CAR-T cells/kg (minimum dose 1×10^6 CAR-T cells/kg)	Phase 1: ORR 71% (n = 5/7) and CR 57% (n = 4/7); three patients CR ongoing 12+ months Phase 2: Overall ORR was 82%, with a 54% CR DLBCL: ORR was 82%; with 38% CR and 25% PR	Phase 1: Grade 3 toxicity 57%; grade 4 14%; one dose-limiting toxicity leading to death not related to KTE-C19 Phase 2: most common grade ≥3 treatment-emergent AEs were neutropenia (78%), anemia (43%), thrombocytopenia (38%); CRS occurred in 94 patients (93%) with grade ≥3 CRS reported in 13%; neurologic events (64%); with grade ≥3 reported in 28%; most common events of grade≥3 were encephalopathy (21%), confusional state (9%), aphasia (7%) and somnolence (7%) One death due to hemophagocytic lymphohistiocytosis
Median dose 3.5×10^8 CAR-positive viable T cells (range: 1.0 to 5.2×10^8 cells)	ORR = 50%; CR = 32%; PR = 18%	CRS (74%); with grade≥ 3 in 23% of the 78 patients, 16 (21%) received systemic tocilizumab or corticosteroids. Neurological toxicities 62 (58%); with grade ≥3 in 18% The most common neurological toxicities were headache (21%), encephalopathy (16%), delirium (6%), anxiety (9%), sleep disorders (9%), dizziness (11%), tremor (7%) and peripheral neuropathy (8%)

The additional 32 patients enrolled in the trial received lymphodepleting chemotherapy based on disease burden. Subjects with low burden ALL (low ALL; <25% marrow blasts) received low-dose Flu/Cy, wherease those with high burden disease (high ALL; >25% marrow blasts or lymphomatous disease) received an alternative regimen, including ifosfamide/etoposide or high-dose Flu/Cy. Of the total 51 patients, 31 (60.8%) achieved a CR with 28/31 (90%) of responders negative for MRD. Subjects with low ALL had a significantly higher CR rate (18/21; 85.7%) than those with high ALL (13/32; 40.6%) ($P = .0011$) and use of a Flu/Cy regimen correlated with higher response (29/44; 65.9% vs. 2/8; 25%; $P = .0301$). Severe CRS, grade 3 and 4 combined was reported in 13.5%. Three grade 3 neurotoxicities (1 each: dysphasia, delirium, headache) and two seizures (one grade 1, one grade 2) were reported (Lee et al., 2016).

At the ASCO 2016 annual meeting, investigators from Seattle Children's institute in collaboration with FHCRC reported their findings from a phase I trial using a 4-1BB costimulatory domain (with a 1:1 ratio of CD4+ and CD8+) CAR-T cell. In a cohort of 36 children and young adults with aggressive ALL, MRD-negative CR was reported in 33/36, 91% patients. The investigators also reported that the durability of remissions was influenced by the duration of CAR-T cell functional persistence of at least 63 days (Gardner et al., 2016c).

MSKCC reported data from their multicentre trial in children and young adults with relapsed CD19+ B-ALL utilising the 19-28zeta CAR-T cell at the 2015 ASH annual meeting (Curran et al., 2015b). The objective of this trial was to assess tolerability of the technology. A total of nine patients (median age 15 years) received CAR-T cells at a dose of $1–3 \times 10^6$ CAR-T cells/kg. CR was reported in 55% (5/9) patients. Development of fever and CRS occurred in responders, including grade 1–2 (n = 2) and grade 3–4 (n = 3), and was well controlled by systemic immunosuppressants (corticosteroids or anti-IL6 therapy, tocilizumab). Lower disease burden and greater expansion following in vitro CD3/CD28 bead activation correlated with responses. Peak CAR-T cells were observed in 1–2 weeks with loss of detection in 1–2 months (Curran et al., 2015a).

CHOP investigators in collaboration with University of Pennsylvania have generated the largest amount of data in r/r paediatric ALL using the 4-1BB costimulatory domain CAR, CTL019. Their initial report with CTL019 was in two children, where CR was reported and ongoing 11 months after treatment in one patient (Grupp et al., 2013). The second patient had a CD19-negative relapse within 2 months after treatment. In their first report on 30 patients (5 adults and 25 paediatric patients, 5–22 years of age), including 18 patients (all children) with relapsed disease after alloHSCT, and mostly CNS status 1 (no detectable blast cells in cerebrospinal fluid) receiving CTL019, CR was reported in 90% of patients (Maude et al., 2014). Considerable in vivo expansion of CTL019 cells occurred in responders and was detected in the blood, BM and cerebrospinal fluid. Sustained remission was achieved with 6-month event-free survival rate of 67% and an OS of 78%. The most common toxicity was CRS occurring in all patients with severe CRS in eight (27%) patients. This first report did not tease out efficacy and safety data in paediatric versus adults. The investigators

reported updated findings at the 2016 ASCO annual meeting, reporting on 59 children/young adults (including the 25 patients from the first report) with r/r B-ALL treated with CTL019 (Maude et al., 2016c). Patients aged 20 month–24 years with CD19+ ALL, 39 of whom relapsed with prior alloHSCT, received CTL019 at a dose of 1×10^7–1×10^8 cells/kg. At 1 month after infusion, 55/59 (93%) were in CR. CTL019 cells were detected in the CSF and no CNS relapses were observed. With a median follow-up of 12 months, 34 patients had ongoing CR, with only 6 receiving a subsequent therapy (5 HSCT, 1 donor lymphocyte infusion). OS was 79% at 12 months. CTL019 persistence was accompanied by B-cell aplasia, which continued in 24/34 patients with ongoing CR. CRS was observed in 88% of patients. Notably, severe CRS requiring haemodynamic or respiratory support occurred in 27% was associated with high disease burden and was reversed with the anti-IL6 therapy, tocilizumab (Maude et al., 2016c). Relapse was reported in 20 patients, 13 of whom had a CD19-negative relapse. Among patients with CD19-positive relapse, early loss of CTL019 or lack of persistence was reported (Maude et al., 2014). It is hypothesised that scFv domains of murine origin causing antimouse reactivity could potentially cause immune-mediated rejection and early loss of CTL019 (Maude et al., 2016a; Hucks et al., 2017; Song et al., 2015). A pilot phase I trial utilising humanised CAR-T cell (CTL119), where scFv domains of murine origin in CTL019 is replaced by humanised anti-CD19 scFv domain, in non-responders of previously treated murine CAR-T cells, was reported to have a high rate of CR of 64% (Maude et al., 2016a). Updated data presented at the 2017 ASH meeting including 38 children and young adults with relapsed/refractory B-ALL or lymphoblastic lymphoma (B-LL) reported a CR of 56% in patients with poor or transient response to prior murine CD19-directed CAR-T cells and 100% in CAR naïve patients (Maude et al., 2017). Further investigations with humanised CAR-T cell, CTL119, are ongoing.

CHOP/University of Pennsylvania conducted a Phase II US multicentre trial, the first of its kind with a centralised manufacturing process, and data from an interim analysis were presented at the ASH 2016 annual meeting (Maude et al., 2016b). CTL019 cells at a target dose 2.0–5.0×10^6 cells/kg for ≤50 kg and 1.0–2.5×10^8 cells for >50 kg was infused in 29 of the 35 enrolled paediatric patients (median age 12 years) with r/r ALL. The target cell dose was met in 24 out of 29 patients. ORR maintained at two evaluations ≥28 days apart was 69.0% (20/29). Of the five patients who received CTL019 below the target dose, two patients achieved CR/Cri (CR with incomplete blood count recovery). Relapse-free survival and OS at 6 months were 66.4% and 75.7%, respectively. Two patients died before Day 28 (the causes of death were not attributed to CTL019), six did not respond and one patient achieved CRi at Day 28 but relapsed 17 days later. Of the 20 patients who achieved a CR/CRi, 8 patients relapsed 1.7–7.6 months after the onset of remission and two of these were CD19 negative. CRS was the most common adverse event occurring in 26 (89.7%) patients and was reversible; 11 patients (37.9%) had grade 3 or 4 CRS that was managed by anti-IL6 therapy (tocilizumab), ventilation and vasopressors. Reversible neuropsychiatric events, including seizure (two patients) was

reported in 9 (31%) patients. The rate of grade 3 or 4 CRS reported was comparable to that measured during the single centre study, and standardised management of CRS was successful in a multicentre trial with no deaths attributable to CRS. The efficacy rate in this study, however, was lower than that obtained from the single centre. Nevertheless, the study showed the feasibility of manufacturing CTL019 at a central location, its distribution to treatment sites while maintaining central logistics with the infusion being administered at the treatment site level. Moreover, with standardised training of hospital staff, the adverse events were well managed at the site level. Currently this study is ongoing under a Novartis investigational new drug (IND) (NCT02228096).

The findings from the various academic institutes in the setting of paediatric r/r ALL are promising for CAR-T cell therapy. Based on the encouraging findings from CHOP/University of Pennsylvania, Novartis, in partnership with University of Pennsylvania, conducted their first global registration trial using CTL019 (tisagenlecleucel-T) across 25 centres in the United States, European Union, Canada, Australia and Japan (ELIANA, NCT02435849). Preliminary findings from this pivotal trial were presented at the 2016 ASH annual meeting (Grupp et al., 2016). CTL019 was manufactured from patient peripheral blood mononuclear cells under GMP conditions in the United States, at a centralised 'sponsor-owned' manufacturing facility, and supplied to all sites. As of March 2016, 57 patients were enrolled. At the time of reporting, there were 3 manufacturing failures (5%), 5 patients were not infused due to death or adverse events (9%) and 15 patients were pending infusion. After Flu/Cy lymphodepleting chemotherapy in the majority of the patients, 34 patients (median age 11 years and 50% with prior HSCT) were infused with a single dose of CTL019 at a median dose of 2.9×10^6 transduced CTL019 cells/kg. The primary end point of ORR (CR+ CRi within 3 months) was achieved in 83% (24/29) of patients. CRS was experienced in 82% of patients including grade 3 (21%, 7 patients) and grade 4 (24%, 8 patients) events; 44% patients with CRS received tocilizumab with or without other anticytokine therapy, resulting in complete resolution. Grade 3 or 4 neuropsychiatric events including confusion, delirium, encephalopathy, agitation and seizure were reported in 21% of patients. The preliminary findings from this global multicentre trial confirmed findings from the single centre experience showing high level of efficacy and manageable safety profile (Grupp et al., 2016). Updated data from 88 enrolled patients presented at the 2017 European Haematology Association (EHA) Annual Meeting reported 75% relapse-free probability at 6 months following remission onset, with a probability of 89% survival at 6 months and 79% survival at 12 months (Buechner et al., 2017). Manufacturing failures were reported in 7 (8%) patients. A total of 11 deaths were reported, 2 died within 30 days of infusion (ALL progression, n = 1; cerebral haemorrhage, n = 1), and 9 patients died >30 days after infusion (ALL relapse/progression, n = 6; HHV-6 encephalitis, pneumonia, systemic mycosis, n = 1 each). CRS occurred in 78% of pts (21% grade 3; 27% grade 4); no CRS-associated deaths occurred and 38% of patients received tocilizumab for

treatment of CRS with or without other anticytokine therapy. Grade 3 neuropsychiatric AEs were reported in 15% of patients, with no grade 4 events and no cerebral oedema. Grade 3/4 neutropenia with high (>38.3°C) fever occurred in 60% of patients. Quality of life (QoL) data from 3 to 6 months following the one-time infusion was recently presented (Dietz et al., 2017). Improved QoL as measured by change from baseline in EQVAS scores was obtained in 82% of patients at Day 28 and in 86% of patients at month 3 (Dietz et al., 2017). Recently updated data from 92 enrolled patients of whom 75 patients receiving an infusion of tisagenlecleucel (median weight-adjusted dose of 3.1×10^6; median total dose of transduced viable T cells 1.0×10^8), with a median follow-up time of 13.1 months was published (Maude et al., 2018). In this updated analysis, the primary end point OR was 81% with CR in 45 patients (60%) and CRi in 16 patients (21%). The rate of relapse-free survival among patients with a response to treatment was 80% at 6 months and 59% at 12 months. In this updated analysis, CRS occurred in 77% of patients, 48% of whom received tocilizumab. Neurologic events were reported in 40% of patients, were transient, mainly occurred in patients with higher-grade CRS and were managed with supportive care; no cerebral oedema was reported. Death was reported in 19 patients (2 within 30 days of infusion and 17 post 30 days infusion).

In August 2017, Novartis received Food and Drug Administration (FDA) approval for tisagenlecleucel (Kymriah) for the treatment of patients up to 25 years of age with B-cell precursor ALL that is refractory or in second or later relapse.

In addition to Novartis, Kite Pharmaceuticals is also actively pursuing this indication with their CAR-T cells, and preliminary data from their ongoing ZUMA-4 (NCT02625480) trial in two paediatric patients with ALL using their KTE-C19 CAR T (axicabtagene ciloleucel or axi-cel) at a dose of 2×10^6 CAR-T cells/kg were presented at the ASH 2016 annual meeting (Shah et al., 2016a). MRD-negative remission was observed by Day 28 in both patients. CRS (grade 2) was reported in both patients and was managed to resolution either tocilizumab, corticosteroids, and/or siltuximab in addition to other supportive care (Shah et al., 2016a). Updated results, where two additional patients received KTE-C19, were presented at the 2017 EHA Annual Meeting (Lee et al., 2017). All patients receiving KTE-C19 achieved an MRD-negative remission. CRS of grade 3 or less severity that was resolved with tocilizumab, corticosteroids, and/or siltuximab plus other supportive care with a median duration of 8.5 (range, 4–16) days was reported in all 4 patients. Grade 3 neurologic events were reported in one patient.

CLINICAL OUTCOME OF CD19-TARGETED CAR-T CELLS IN B-CELL HAEMATOLOGICAL MALIGNANCIES: NON-HODGKIN'S LYMPHOMA

In a pilot study of patients with refractory indolent B-cell malignancies, including four patients with Follicular Lymphoma (FL), and one patient with splenic Marginal Zone Lymphoma, treated with CAR-T cells along with IL-2, NCI investigators

reported partial remissions in four patients (Kochenderfer et al., 2012). Patients received lymphodepleting chemotherapy consisting of Cy and Flu followed by a single infusion of $0.3–3.0 \times 10^7$ CAR-T cells/kg and administration of IL-2 every 8 h until toxicity precluded further administration. Patients experienced severe toxicity, including hypotension, fevers, fatigue, renal failure and obtundation, possibly associated with elevations in the levels of the inflammatory cytokines IFN-γ and tumour necrosis factor (TNF) within 10 days of CAR-T cell administration (Kochenderfer et al., 2012). In another trial conducted by NCI investigators, in 10 patients with diffuse large B-cell lymphoma (DLBCL) or mantle cell lymphoma, patients received anti-CD19 CAR-T cells containing a CD28 costimulatory domain, at a dose of $2–10 \times 10^6$ T cells/kg, following progression after alloHSCT (Brudno et al., 2016). No lymphodepleting chemotherapy was administered. One patient had CR and the remaining patients had SD (Brudno et al., 2016). Successful treatment of DLBCL with anti-CD19 CAR-T cells containing a CD28 costimulatory domain (without IL-2) was first reported by NCI investigators in nine patients who received conditioning chemotherapy (Kochenderfer et al., 2015). Following lymphodepletion with Cy at a total dose of either 120 or 60 mg/kg + Flu 25 mg/m^2 for 5 days, CAR-T cells were administered at a dose of $1–5 \times 10^6$ CD19-targeted CAR-T cells/kg, without postinfusion IL-2. Peak CAR-T cell levels postinfusion varied considerably, with highest levels detected 7–17 days after infusion and ranging between 9 and 777 CAR+ cells/μL and declining thereafter. Of the seven evaluable patients with DLBCL, four obtained CR (durable in three patients, 9–22 months), two obtained PR and one had SD after infusion of CAR-T cells. All patients with DLBCL experienced grade 3–4 toxicities including hypotension, fever and neurotoxicity (confusion and obtundation) and toxicities resolved within 3 weeks with appropriate management. One patient died of an unknown cause 16 days after cell infusion.

Investigators at FHCRC reported data from their anti-CD19 CAR T with a 4-1BB costimulatory domain, using lentivirus for transduction and CAR-T cells manufactured from defined T cell subsets and administered in a 1:1 CD4+/CD8+ ratio to 32 adults, median age 53 years, with r/r B-cell NHL after Cy-based lymphodepletion chemotherapy with or without Flu (Turtle et al., 2016c). CAR-T cells were administered at one of the three dose levels: 2×10^5, 2×10^6 or 2×10^7 cells/kg. Patients receiving Cy/Flu lymphodepletion had increased CAR-T cell expansion and persistence and higher response rates (50% CR, 72% ORR) compared with patients receiving Cy-based lymphodepletion without Flu (8% CR, 50% ORR). At the tolerable dose of 2×10^6 per kg, CR and ORR rates were 64% and 82%, respectively. Severe CRS and grade ≥3 neurotoxicity were observed in 13% and 28% of all patients, respectively. Severe toxicity in patients receiving Cy/Flu lymphodepletion was predominantly seen at the dose of 2×10^7 cells/kg (Turtle et al., 2016c).

Data from University of Pennsylvania utilising their CAR-T cell containing 4-1BB costimulatory domain, CTL109, were reported at the ASCO 2016 (Schuster et al., 2016a) and ASH 2016 annual meeting (Chong et al., 2016; Schuster et al., 2016b). Forty-three patients of median age 56 years, 14 (33%) of whom received prior alloHSCT were enrolled in the study. Thirty patients received CTL019; responses were evaluable in 29 patients. Data from 28 patients (14 DLBCL and 14 FL) are published (Schuster et al., 2017c). Lymphodepleting chemotherapy regimens included bendamustine, Cy + Flu, radiation + Cy, EPOCH (etoposide-prednisone-Oncovin-cyclophosphamide-hydroxydaunorubicin regimen) and carboplatin-gemcitabine (only FL patients). CTL019 cells at a dose of $1.0–5.0 \times 10^8$ were infused 1–4 days after the completion of lymphodepleting chemotherapy. Among patients with DLBCL, 7 of 14 had a response (50%) at 3 months, and at 6 months CR was 43%. One patient with DLBCL who had a PR at 3 months had a CR by 6 months, whereas another patient, who had a PR at 3 and 6 months, ultimately had progressive disease. Among the FL patients, ORR at 3 months was 79% (11/14) with CR rate 50% (7/14). At 6 months the CR was 71%. Two FL patients with PR at 3 months converted to CR at 6 months. One FL patient with PR at 3 month remained in PR at 6 and 9 months and had progression of disease at 13 months. All patients achieving CR by 6 months remained in remission at 7.7–37.9 months (median, 29.3 months) after induction. Among subtypes of DLBCL patients in whom the cell of origin was known as germinal centre (GC, n = 7), or nongerminal centre (NGC, n = 5), ORR at 3 month 71% (5/7) and 40% (2/5), respectively were reported. CR at 3 month for GC patients was 43% (3/7) and NGC patients was 40% (2/5) (Schuster et al., 2016b). Overall, severe CRS occurred in five patients (18%). Serious encephalopathy occurred in three patients (11%); two cases were self-limiting and one case was fatal (FL patient) (Schuster et al., 2017c). In patients with DLBCL, CRS occurred in nine patients (8 grade 2; 1 grade 3) and was not predictive of response. Transient neurotoxicity including delirium in 2/13 (1 grade 2; 1 grade 3) and cognitive disturbance in 1/13 (grade 1) was reported (Schuster et al., 2016b). CRS occurred in six FL patients (4 grade 2; 1 grade 3, 1 grade 4) and was not predictive of response. Other toxicities in FL patients included one episode of grade 5 encephalitis, possibly related to therapy; this patient remained in CR at the time of death (Chong et al., 2016).

From the industry-sponsored trials, data from the Kite Pharmaceuticals ZUMA I (NCT02348216), phase 2 extension of the study, with the CAR-T cell KTE-C19 (axicabtagene ciloleucel; axi-cel), was presented at the ASH 2016 annual meeting, and results of the phase 1 dose-finding study and the phase 2 extension were recently published (Locke et al., 2017; Neelapu et al., 2016, 2017). ZUMA-1 is the first multicentre study evaluating the safety and efficacy of anti-CD19 CAR-T cells in patients with refractory NHL. KTE-C19 uses the same construct as the NCI CAR-T cell (autologous CD3z/CD28) but is manufactured in a centralised, closed and streamlined process of approximately 8 days (Locke et al., 2017). Seven patients with refractory aggressive NHL, aged

29–69 years, were treated in the phase 1 study that evaluated the dose-limiting toxicity with KTE-C19. Following low-dose conditioning chemotherapy with Cy 500 mg/m^2 + Flu 30 mg/m^2 for 3 days, KTE-C19 was administered at a target dose of 2×10^6 CAR-T cells/kg (minimum dose 1×10^6 CAR-T cells/kg). Adverse events occurred within 30 days of KTE-C19 infusion in all seven patients. One patient experienced a dose-limiting toxicity of grade 4 CRS and neurotoxicity; however, death was deemed unrelated to KTE-C19. Grade 3 and 4 events were reported in four (57%) and one (14%) patient(s), respectively, and resolved within 1 month. The ORR was 71% (5/7) and CR rate was 57% (4/7). CR was ongoing for 12+ months in three patients. CAR-T cells demonstrated peak expansion within 2 weeks and continued to be detectable in patients with ongoing CR (Locke et al., 2017). Interim analysis from phase 2 DLBCL cohort was presented at the ASH 2016 annual meeting (Neelapu et al., 2016) and recently published (Neelapu et al., 2017). In the phase 2 trial, 111 patients from 22 institutions were enrolled. Following leukapheresis, 101 patients (77 with DLBCL and 24 with primary mediastinal B-cell lymphoma or transformed FL) received KTE-C19. KTE-C19 was successfully manufactured in 99% of enrolled patients (1 unsuccessful manufacture) and the average turnaround time from aphaeresis to receipt of KTE-C19 at the clinical site was 17.4 days.

Among the 101 patients at 6 month follow-up, the ORR was 82%, with a 54% CR. Of these, 77 evaluable patients were available in the DLBCL cohort. ORR was 82%; with 38% CR and 25% PR. The most common grade ≥3 treatment-emergent AEs were neutropenia (78%), anaemia (43%), thrombocytopenia (38%). CRS occurred in 94 patients (93%) with grade ≥3 CRS reported in 13%. All the events associated with CRS resolved except for one event of grade 5 hemophagocytic lymphohistiocytosis. An event of grade 5 cardiac arrest occurred in a patient with the CRS. Neurologic events occurred in 65 patients (64%), with grade ≥3 reported in 28%. The most common events of grade ≥3 were encephalopathy (21%), confusional state (9%), aphasia (7%) and somnolence (7%). Rates of CRS and neurologic events decreased over the course of the study. Forty-three percent of patients received tocilizumab and 27% received glucocorticoids for the management of the CRS, neurologic events or both (Neelapu et al., 2017).

Kite Pharmaceuticals completed their submission of a BLA for KTE-C19 (axicabtagene ciloleucel/Yescarta) with the FDA in March 2017 and in October 2017 was granted regular approval as the first CAR T therapy for the treatment of adult patients with relapsed or refractory large B-cell lymphoma after two or more lines of systemic therapy, including DLBCL not otherwise specified, primary mediastinal large B-cell lymphoma (PMBCL), high-grade B-cell lymphoma and DLBCL arising from FL (transformed follicular lymphoma, or TFL). A Marketing Authorisation Application for axicabtagene ciloleucel is currently under review with the European Medicines Agency (EMA) and potential approval is expected in the first half of 2018. In addition, Kite Pharmaceuticals is also pursuing a phase 2 trial, ZUMA-5, in patients with relapsed/refractory indolent B-cell non-Hodgkin lymphoma.

Interim results from the multicentre Phase 2 JULIET study (NCT02445248) of CTL019 (tisagenlecleucel) in adult patients with relapsed or refractory DLBCL was presented by Novartis at the 2017 International Conference on Malignant Lymphoma (Schuster et al., 2017a). Among 51 patients with at least 3 month follow-up (or earlier discontinuation) that were evaluated, best ORR was 59% with 43% CR and 16% PR. CR and PR rates at 3 months were 37% and 8%, respectively. All patients in CR at 3 months remained in CR at data cut-off. The study met its primary objective at interim analysis. CRS occurred in 57% of infused patients with 17% grade, 3% and 9% grade 4; no CRS-associated deaths were reported. Sixteen percent of patients received tocilizumab for CRS management. Grade 3–4 neurologic adverse events (AEs) were reported in 13% patients and was managed with supportive care. No cerebral oedema was reported. At ASH 2017, data from 81 infused patients with at least 46 patients completing 6-month follow-up were presented (Schuster et al., 2017b). Durable response with an ORR of 37%, with 30% achieving a CR and 7% achieving a PR, was reported, indicating that those who were cancer-free at 3 months remained relapse-free at 6 months and beyond. Updated data as reported in the package insert report 160 patients enrolled, and 106 patients receiving tisagenlecleucel, including 92 patients who received product manufactured in the United States, and were followed for at least 3 months or discontinued earlier (KYMRIAH™, 2018). Eleven out of 160 patients enrolled did not receive tisagenlecleucel due to manufacturing failure. A retrospectively identified subgroup of 68 patients (had no bridging chemotherapy or had imaging that showed measurable disease after completion of bridging chemotherapy before infusion) was evaluable for the major efficacy outcome measures. Among these patients, 78% had primary DLBCL 22% had DLBCL following transformation from FL. The median dose was 3.5×10^8 CAR-positive viable T cells (range: 1.0 to 5.2×10^8 cells). ORR was 50%, with a CR of 32% and PR of 18%. CRS was reported in 78 (74%) patients, with grade ≥3 in 23%. Of these, 16 (21%) received systemic tocilizumab or corticosteroids. Six (8%) patients received a single dose of tocilizumab, 10 (13%) patients received two doses of tocilizumab and 10 (13%) patients received corticosteroids in addition to tocilizumab. Neurological toxicities including severe or life-threatening reactions were reported in 62 (58%) patients (grade ≥3 in 18%). Median time for development of neurological AEs was 14 days. The most common neurological toxicities were headache (21%), encephalopathy (16%), delirium (6%), anxiety (9%), sleep disorders (9%), dizziness (11%), tremor (7%) and peripheral neuropathy (8%).

Based on the data from JULIET study, in April 2017, the US FDA granted 'Breakthrough Therapy' designation to CTL019. In May 2018, tisagenlecleucel (Kymriah) was approved for the treatment of adult patients with relapsed or refractory (r/r) large B-cell lymphoma after two or more lines of systemic therapy including DLBCL, high grade B-cell lymphoma and DLBCL arising from FL.

Juno Therapeutics conducted the TRANSCEND NHL 001 trial using JCAR017 (lisocabtagene maraleucel) in relapsed/refractory B-cell NHL (NCT02631044), and the

preliminary results were presented at the ASH 2017 meeting (Abramson et al., 2017). The FULL data set consisted of patients in the DLBCL cohort (DLBCL, NOS, PMBCL, FL grade 3B) treated with JCAR017, and CORE data set included only patients meeting planned inclusion of DLBCL NOS (de novo or transformed from FL), ECOG 0-1, no prior allo-SCT (stem cell transplantation) in into the pivotal cohort. Results from patients in the CORE data set showed a 3 month OR of 74% (14/19) and a 3 month CR of 68% (13/19) in those receiving a dose of 100 million cells. In patients who had reached 6 months of follow-up, CR was 50% (7/14). Severe CRS was reported in 1% of patient of both CORE group (1/67) and FULL data set (1/91) and neurotoxicity was reported in 15% and 12%, respectively, in the CORE group and FULL data set (Abramson et al., 2017). Due to the low toxicity, potential for use of JCAR017 as an outpatient administered therapy was also discussed (Maloney et al., 2017).

It is worth noting that among the CD19-targeted industry-sponsored studies, the staff at the participating clinical sites had no prior experience with CAR-T cell therapies. A standardised training programme on aphaeresis, infusion of cell therapy and management of expected adverse events resulted in the successful utilisation of these emerging therapies at these investigational sites (Maude et al., 2016b; Grupp et al., 2016). The success of this training approach of the medical staff is particularly notable as a standard training protocol will also need to be developed and provided at each hospital where the new therapeutics will be provided.

CAR-T CELL THERAPIES TARGETING ANTIGENS OTHER THAN CD19 FOR HAEMATOLOGICAL MALIGNANCIES

With the success of CD19 as a target in haematological malignancies, the field of CAR-T cell therapy is expanding to other targets of high unmet medical need, such as B-cell maturation antigen (BCMA) and CD138 for plasma malignancies; CD33 and CD123 for myeloid malignancies and targets such as CD20 and CD22 for haematological malignancies in patients who do not express CD19 or who experience relapse with CD19-negative mutant variants (Jackson et al., 2016). Target antigens to overcome CD19-negative relapse is discussed under the *Challenges section*. Here, we focus on studies with CAR T cell treatment for acute myeloid leukaemia (AML) and multiple myeloma.

Acute Myeloid Leukaemia

The feasibility of CAR-T cell therapies in AML directed against Lewis Y antigen and CD33 has been investigated in pilot studies in several academic institutes (Ritchie et al., 2013; Wang et al., 2015). In a study of four AML patients utilising CAR-T cell directed against Lewis Y antigen, two patients achieved SD, one patient achieved a transient reduction in blasts and a fourth patient showed transient cytogenetic remission. Grade 3–4 toxicity as reported with CD19 targeted haematological malignancies was not

observed (Ritchie et al., 2013). Data on one patient with refractory AML have been reported with the anti-CD33 CAR-T cell. A marked decrease of blasts in the BM was observed on examination 2 weeks after therapy. Grade 4 chills and fever along with elevation in cytokines occurred with infusion of the CAR-T cell (Wang et al., 2015). The findings are preliminary and more data are awaited from these ongoing trials. Recently, investigators from University of Pennsylvania assessed the feasibility of RNA CART123 in AML. Although the CAR-T cell therapy was safe, the trial was terminated early, due to lack of efficacy (Cummins et al., 2017).

Multiple Myeloma

CD19 is not considered as a valid target for developing immunotherapeutic treatments for multiple myeloma. However, it has been reported that a minor component of the multiple myeloma clone with drug-resistant, disease-propagating properties has a B cell that is a CD19-positive phenotype (Garfall et al., 2015b). This prompted investigators at University of Pennsylvania to evaluate their CAR, CTL019, for multiple myeloma. Preliminary data in patients with refractory multiple myeloma, with a single infusion of CTL019 in conjunction with standard treatment for multiple myeloma (consisting of high-dose melphalan and autologous haematopoietic stem cell transplantation), were presented at ASCO 2015 annual meeting. Four of the five patients responded to therapy, and 2/4 patients had posttransplant responses that lasted longer than they achieved after their first transplant (Garfall et al., 2015a). Four of the first five patients experienced transient hypogammaglobulinemia, and one patient experienced mild CRS. Although preliminary, these data are encouraging; additional data are awaited.

On the other hand, CD138 has been identified as a target for multiple myeloma and researchers from China reported data on five patients with advanced, progressive multiple myeloma treated with chemotherapy followed by an infusion of CAR T-138 cells. Stable disease was maintained in 4/5 patients (Guo et al., 2016a).

Similarly, the BCMA was explored as a target for multiple myeloma, and CAR-T cells that express an anti-BCMA chimeric antigen receptor (CAR-BCMA) were used; results were presented at the ASH 2015 annual meeting, where NCI investigators described the preliminary results of the first-in-humans study in myeloma with BCMA CAR (Ali et al., 2015, 2016). Following lymphodepleting chemotherapy with 300 mg/m^2 of Cy and 30 mg/m^2 of Flu for 3 days, 12 patients with multiple myeloma were treated with CAR-BCMA T cells at one of the four dose levels, 0.3×10^6, 1×10^6, 3×10^6 and 9×10^6 CAR-T cells/kg. Among 12 treated patients, investigators observed a stringent CR (sCR) (n = 1, 9×10^6 dose) that was ongoing at more than 14 weeks, a very good PR (n = 1, at 3×10^6 dose), PR (n = 2) and SD (n = 8). Toxicities with CAR-BCMA T cells were similar to toxicities observed with anti-CD19 CAR-T cells. Two patients treated with the highest dose level of 9×10^6 CAR-T cells/kg had toxicity consistent with CRS including fever, hypotension and dyspnoea. Both patients had prolonged cytopenias (Ali et al.,

2016). Recently, University of Pennsylvania (in collaboration with Novartis) investigators presented their preliminary data from the 4-1BB BCMA CAR at the ASH 2016 annual meeting (Cohen et al., 2016). In this ongoing study, three cohorts are planned: cohort 1: $1–5 \times 10^8$ CAR-T cells alone without lymphodepletion; cohort 2: Cy $1.5\,g/m^2 + 1–5 \times 10^7$ CAR-T cells and cohort 3: Cy $1.5\,g/m^2 + 1–5 \times 10^8$ CART cells. Results from six treated patients in cohort one were presented. Significant expansion of CAR-T cells occurred in 2/6 patients. These two patients also had significant responses, a very good partial response (VGPR) and a sCR, with the sCR ongoing at 7 months, but the patient with the VGPR relapsed after 5 months. Two patients had modest expansion and minimal response, and two other patients had minimal expansion and no response. CRS occurred in five patients: two patients with grade 3 requiring tocilizumab, one patient with grade 2 and two patients with grade 1 toxicity. Dose-limiting toxicity, grade 4 posterior reversible encephalopathy syndrome, occurred in one patient who experienced severe delirium, recurrent seizures, obtundation and cerebral oedema on MRI. This resolved after antiepileptics, high-dose methylprednisolone and cyclophosphamide, without long-term neurologic sequelae (Cohen et al., 2016). Updated data from 21 infused patients (nine in cohort 1, five in cohort 2 and seven in cohort 3) were presented at the ASH 2017 meeting (Cohen et al., 2017). Toxicities in cohort 1 included CRS in eight patients (3 grade 3/4, with four receiving tocilizumab) and neurotoxicity (grade 4 encephalopathy) in two patients. In Cohorts 2 and 3, CRS has occurred in nine patients (3 grade 3, 0 grade 4, none requiring tocilizumab) and neurotoxicity in 1 patient (grade 2 confusion/aphasia), with no unexpected/dose-limiting toxicities, and no treatment-related deaths. Efficacy was lower at the 10^7 dose (cohort 2), compared with 10^8 dose, with response observed in only 2/5 patients (1 PR, 1 MR) and both progressed at four and 2 months, respectively. Trial is ongoing with patients enrolled in cohort 3 (Cohen et al., 2017).

Bluebird bio presented their phase I data on BCMA CAR-T cell, bb2121, at the 2016 EORTC-NCI-AACR Molecular Targets and Cancer Therapies Symposium in Munich, Germany, (Berdeja et al., 2016) and updated results with data from 11 patients at the 2017 ASCO and 2017 EHA (Lin et al., 2017). Following lymphodepletion with Cy $300\,mg/m^2$ + Flu $30\,mg/m^2$ for 3 days, CAR-T cells were administered at a dose ranging from 5 to 120×10^7 T cells. Data were reported on 11 patients infused at three dose levels: 5×10^7, 15×10^7 and 45×10^7 T cells. All patients treated with doses of 15×10^7 or higher remained in the study and ORR in the nine evaluable patients was 100%, including two sCRs and two MRD-negative responses (CR and very good PR). Toxicity was mild with grade 1–2 CRS reported in 73% (8/11) patients. No dose-limiting toxicity was reported. At the ASH 2017 annual meeting, data from 18 evaluable patients (21 infused) were presented. ORR was reported at 100% in patients treated with doses of 150×10^6T cells or higher. Two patients had grade 3 CRS (grade 1 or 2 reported in 71% patients) that resolved in 24 h (4 patients received tocilizumab, one with steroids). CRS was more common in the higher dose groups but was not related to tumour burden.

One patient with a history of an extensive cardiac history died more than 4 months after bb2121 infusion due to cardiopulmonary arrest, and this was reported as unrelated to bb2121 (Berdeja et al., 2017). bluebird bio has initiated a global phase 2 KarMMa trial (NCT03361748) that will serve as the basis for a regulatory submission for bb2121.

Positive results were also reported by Legend Biotech (China) with the use of LCAR-B38M CAR-T, targeting principally BCMA at 2017 ASCO (Fan et al., 2017). In an ongoing phase 1 clinical trial in China in patients with relapsed or treatment-resistant (refractory) multiple myeloma, ORR was 100%, and 33 (94%) patients had an evident clinical remission of myeloma (CR or very good PR) within 2 months of receiving CAR-T cells. In 19 patients, having a follow-up of more than 4 months, 14 reached sCR, one patient has reached PR and four patients achieved very good PR. Only two patients experienced grade 3 CRS that responded to tocilizumab. No neurologic toxicities were reported (Fan et al., 2017).

At the ASH 2017 meeting, Juno Therapeutics announced development of their BCMA CAR T, JCARH125, for multiple myeloma, and a phase 1 trial is planned in 2018 (Harrington et al., 2017).

Taken together, among all targets tested, the use of BCMA CAR-T cell shows promise in the management of multiple myeloma. More data from the ongoing studies are awaited to validate these preliminary findings.

CAR-T CELLS IN SOLID TUMOURS

Unlike the success in haematological malignancies, clinical trial results with CAR-T cells in solid tumours are less encouraging (Fousek and Ahmed, 2015; Gill et al., 2016; Guo et al., 2016b; Jindal et al., 2018; Newick et al., 2016). Initial trials using first-generation CAR-T cells directed towards antigens that are specific towards solid tumours, including ovarian cancer, neuroblastoma, colon cancer and mesothelioma showed inadequate anti-tumour responses with lack of persistence of the CAR-T cells and 'off-tumour/on-target' toxicity (Kershaw et al., 2006; Beatty et al., 2014; Morgan et al., 2010; Pule et al., 2008). Experts contend that one of the greatest challenges in solid tumours is identifying a suitable target antigen (Gill et al., 2016; Newick et al., 2016). Solid tumours present a significant amount of heterogeneity and are more often characterised based on combinations of anatomic location, histology or immunohistochemical stains, all of which makes it complicated to yield a CAR target (Gill et al., 2016). Furthermore, unlike CD19 that is consistently expressed on haematological tumour cells, and only on 'dispensable' B cells (Newick et al., 2016), the likelihood of identifying a specific antigen in solid tumours that is expressed in all tumour cells is very small, and the risk of 'off-tumour/on-target toxicity', when the CAR-T cells attack healthy tissues, is very high (Guo et al., 2016b). Another challenge is the penetration of CAR-T cells into solid tumours. The large size of typical solid tumours, along with their locations in heavily

restricted areas, may make it difficult for the T cells to reach sufficient concentrations and eradicate the full tumour (Fousek and Ahmed, 2015). Lastly, the complex microenvironment, including physical and anatomical barriers as in stroma that is associated with high tissue pressure, prevents CAR-T cell penetration; last but not least, the metabolic environment, which includes hypoxia and lack of availability of important metabolites, results in the inability of the T cells to fully activate and proliferate, leading to reduced antitumour activity (Guo et al., 2016b; Newick et al., 2016). Nevertheless, researchers have invented novel approaches to overcome these hurdles with the exploration of new targets, measures to improve CAR-T cell accumulation to tumour sites, and withstanding the immunosuppressive tumour microenvironment, as well as addition of safety on/off or cell suicide gene switches to improve safety (Zhang et al., 2016a).

Exploring Potential Target Antigens

Historically, CAR-T cells have been developed to recognise and target 'self-antigens'. However, recent efforts have shifted towards 'mutated antigens' (i.e., 'neoantigens') created by cancer cell genome mutations that can be rapidly identified and expressed in several tumours (Guo et al., 2016b). Unlike self-antigens, which are expressed in both normal and tumour tissues, neoantigens are specific to tumours (Guo et al., 2016b). As a result, using a strategy for CAR-T cells to specifically target neoantigens may offer the best potential therapeutic treatment for solid tumours without severe target-mediated toxicity. EGFR variant 3 (EGFRvIII) is one such example, which is only expressed on malignant tumour cells and mainly on glioblastomas (Sampson et al., 2008). EGFRvIII CARs have shown promise in preclinical models of glioblastomas, and clinical trials testing the efficacy of EGFRvIII CAR in patients with glioblastomas are currently underway (NCT02209376, NCT01454596) (Guo et al., 2016b). Another specific target includes the prostate-specific membrane antigen (PSMA) that is highly expressed in prostate cancer and correlates with advanced disease and metastasis, while it is only minimally expressed in the normal endothelium (Westdorp et al., 2014). PSMA in combination with a suicide gene, the herpes simplex virus thymidine kinase, is being studied at MSKCC (NCT01140373) (Gill et al., 2016). A third target, mesothelin, a glycoprotein overexpressed in mesothelioma and in ovarian and pancreatic carcinomas, combined with low expression on peritoneal, pleural and pericardial surfaces, has made it an attractive target for CAR therapy (Yu et al., 2017). University of Pennsylvania is currently investigating their murine-based scFv, meso-CAR T in patients with metastatic pancreatic cancer, serous epithelial ovarian cancer or pleural mesothelioma (NCT02159716). MSKCC is investigating their fully humanised meso-CAR T in a clinical trial (NCT02414269) (Newick et al., 2016; Yu et al., 2017). Likewise, GD2 is a disialoganglioside that is expressed on tumours of neuroectodermal origin, including human neuroblastoma and melanoma, with highly restricted expression on normal tissues, principally to the cerebellum and peripheral nerves in humans. GD2 is being targeted with CAR

T cells at Baylor for the treatment of neuroblastoma (NCT01822652). However, due to target expression on peripheral nerves, significant pain syndrome can be an adverse event and investigators have included a fast-acting suicide gene into the CAR-modified T cells (Gill et al., 2016).

Optimisation of the CAR-T cell to Improve Antitumour Efficacy

Efforts ranging from genetically modifying CAR-T cells to express CD40 ligand (a type II transmembrane protein belonging to the TNF gene superfamily) that has the potential to enhance tumour-specific T cell function (Curran et al., 2015b) to enhancement of in vivo persistence through delivery of modified mRNA encoding telomerase reverse transcriptase (Bai et al., 2015) have been tried in preclinical models. Preclinical data also show that blocking the inhibitory immune-checkpoint pathways, such as programmed cell death protein 1 (PD1) is likely to enhance the survival of CAR-T cells by relieving the immunosuppressive effects of the tumour microenvironment (Gill et al., 2016; Zhang et al., 2016a). Other measures include affecting the tumour stroma directly with CAR-T cells targeting fibroblast activation protein (Petrausch et al., 2012). However, in preclinical studies this approach was associated with severe toxicities including cachexia and myelosuppression (Gill et al., 2016).

Regarding clinical trials, MSKCC is currently conducting a trial in ovarian cancer, NCT02498912, which utilises their second-generation CAR (CD28/CD3ζ) expressing IL-12, binding to the ectodomain of mucin-16 (MUC-16) that is overexpressed on 70% of ovarian cancer cells. Investigators in the United Kingdom are conducting a trial in head and neck cancer (NCT01818323) using a second-generation CAR-T cell to express TIE28z, which provides cytokine-mediated growth stimulation to the T cell in the tumour microenvironment (Jackson et al., 2016). Both these studies are currently recruiting. Local instillation of CARs to improve trafficking of the CAR-T cells to the tumour site are also investigated in these trials (Newick et al., 2016).

Addition of a Safety Switch to Manage Toxicity

Preclinical studies show that encoding suicide genes in CAR-T cells could ensure their safety for solid tumour treatments, avoiding unwanted and severe adverse events and increasing on-tumour specificity (Guo et al., 2016b). One such strategy is to include the inducible caspase-9 suicide gene system as a 'safety switch' to limit on-target, off-tumour toxicities (Gargett and Brown, 2014). The CD28-costimulated mesotargeting CARs used in MSKCC's ongoing trial, NCT02414269, in patients with mesoexpressing solid tumours, incorporates the safety 'off' switch, icaspase-9.

T Cell Receptor Gene Therapy to Treat Solid Tumours

In recent years, there is renewed interest for T cell receptors (TCRs) that bind tumour-associated antigens (TAAs) with optimal affinity, particularly in treating solid tumours (Fesnak et al., 2016; Johnson and June, 2017; Sharpe and Mount, 2015). Feasibility trials

conducted with TCR gene therapy demonstrated significant clinical responses in patients with metastatic melanoma, colorectal carcinoma and synovial sarcoma, using high-affinity TCR; however, the initial clinical response was hampered by treatment-related toxicity and tumour relapse (Johnson and June, 2017; Kunert et al., 2013). TCR therapies are also challenged due to the fact that unlike CARTs they require peptide-bound Major Histocompatibility (pMHC) restriction antigen presentation for T cell engagement and activation. The binding of TCRs to pMHC leads to a series of signaling events culminating in cellular responses such as proliferation, differentiation and secretion of cytokines and growth factors (Choudhuri et al., 2005).

Based on research with the identification of TAAs, cancer-testis antigen (CTAs) and neoantigens represent the best available choices for therapy with TCR engineered T cells (Kunert et al., 2013). Studies conducted at NCI, using TCR-encoding activity against the CTA, or New York oesophageal squamous cell carcinoma-1 (NY-ESO1), in patients with melanoma or synovial cell carcinoma, reported PRs in 66% of sarcoma patients and 27% of melanoma patients (Kershaw et al., 2014; Robbins et al., 2011). University of Pennsylvania investigators studied TCR directed towards NY-ESO1 using lentivirus vector for delivery in their phase I/II study and reported clinical outcomes of 80% with notably high levels of long-term persistence of TCR-positive cells in patient blood (Rapoport et al., 2015). Nevertheless, not all CTAs are safe targets for therapy. For example, studies with TCR directed towards MAGE-A3/A9/A12 in nine cancer patients reported regression of tumour in 5/9 patients; however, two patients died of neurotoxicity. Analysis revealed that the expression of MAGE-A12 in human brain tissue was the likely cause of the observed toxicity (Morgan et al., 2013). Likewise, another phase I trial using TCR targeting MAGE-A3 for the treatment of melanoma or myeloma reported death in two patients with cardiovascular toxicity (Cameron et al., 2013; Linette et al., 2013). In recent years, investigations of TCR targeting a viral antigen from the human papillomavirus (HPV), specifically the E6 or E7 oncoprotein, is being pursued. HPV E6 TCR immunotherapy is being investigated for use against HPV-associated vaginal, cervical, anal, penile and oropharyngeal cancers in a trial led by NCI investigators (Draper et al., 2015).

From an industry perspective, many biotechnology companies such as Kite Pharmaceuticals (in collaboration with NCI) or Adaptimmune are conducting phase 1 trials with their TCRs targeted towards CTA-MAGE A3/A6 (Kite/NCI) or MAGE A4 (Adaptimmune), or directed towards neoantigens- HPV-16 E6 & E7 (Kite/NCI) for cervical, head and neck cancer. The arrival of CRISPR/Cas9 gene editing systems has led to great interest in the application of this latest gene editing technology to therapeutic application. In June 2016, the Recombinant DNA Advisory Committee (RAC) at the United States National Institutes of Health (NIH) reviewed the first application of a TCR using CRISPR/Cas-9 gene editing. Investigators led by a team at the University of Pennsylvania and collaborating with MD Anderson Cancer Center in Texas, the

University of California in San Francisco announced plans to proceed with a CRISPR gene editing trial using autologous T cells entitled 'Phase I Trial of Autologous T Cells Engineered to Express NY-ESO-1 TCR and Gene Edited to Eliminate Endogenous TCR and PD-1' (Baylis and McLeod, 2017).

CHALLENGES WITH CAR-T CELL THERAPIES

The promising results attained to this date with CAR-T cell therapies in haematological malignancies with improved CR of 70%–90% (Lee et al., 2015; Maude et al., 2014, 2016c; Grupp et al., 2016) have brought opportunities to conceptualise personalised medicine. Nevertheless, these therapies are associated with challenges. Because the CAR-T cells currently studied target the CD19 antigen, one would assume that the response rates across haematological malignancies would be similar. However, investigators from the same academic centre report lower CR rates of 40%–50% in NHL or CLL compared with those observed for ALL (Porter et al., 2011, 2015; Turtle et al., 2016b; Schuster et al., 2016b). Reasons for this discrepancy in CAR-T cell efficacy between the various malignancies remain unclear; however, differences in access to tumour antigen or immune suppression in the tumour microenvironment in each disease has been proposed (Porter et al., 2015; Turtle et al., 2016c). The differences in the functional abilities of the autologous T cells in ALL patients vs. CLL or NHL patients has also been speculated to contribute to the observed discrepancy in efficacy (Porter et al., 2015). In addition to the variability in efficacy, there are few other challenges with the use of CAR T cell, including relapse, adverse events and challenges associated with manufacturing these engineered cells.

Relapse

Relapse after CAR-T cell therapy inclusive of both CD19-positive and-negative types has been reported in at least 37%–45% of the patients, occurring immediately postinfusion or after CR is achieved (Maude et al., 2016c; Park et al., 2016b). Reinfusion of CAR-T cell therapy in patients with CD19-positive relapse may be a possibility; however, whether this will show any objective response is not clear (Lee et al., 2015; Maude et al., 2016a). Of concern is the CD19-negative relapse that has been reported during long-term follow-up in at least 30% of the treated patients. This relapse is characterised by a loss of CD19 antigen, rendering the malignant cells invisible to CD19-specific immunotherapies (Ruella et al., 2016a), and has been reported regardless of the CAR construct (41BB vs. CD28 costimulatory domain) and/or variations in the clinical protocol (in vivo expansion, lymphodepleting chemotherapy) (Ruella and Maus, 2016). It has been speculated that the long-term persistence of CAR-T cells and the absence of treating patients with subsequent alloHSCT may increase the chances of antigen-loss relapse (Ruella and Maus, 2016). Likewise, although there was no clear risk factor

identified, prior exposure to blinatumomab, a bispecific antibody targeting CD19, influenced the loss of CD19 antigen (Ruella and Maus, 2016). The mechanisms causing the loss of CD19 is unclear, although one group reported mutations affecting the CD19 gene resulting in alternative exon splicing of CD19, leading to the loss of the CD19 epitope that is recognised by CAR-T cells (Sotillo et al., 2015). Another group of investigators reported the acquisition of a clonally related myeloid phenotype associated with CD19-negative escape after anti-CD19 CAR-T cell immunotherapy (Gardner et al., 2016b). Other mechanisms postulated include preexisting CD19-negative subpopulation that can be selected under strong pressure with CART T cell therapy targeting CD19 or the occurrence of two different treatment-resistant clonally related diseases resulting in a cell that lacks B-cell differentiation and utilising divergent lymphoid differentiation such that it evades recognition (Ruella and Maus, 2016). Regardless of the mechanism, the prognosis of CD19 negative relapse is poor.

Strategies to Manage CD19-Negative Relapse

One of the hypothesised strategies to avoid CD19-negative relapse include treating patients with alloHSCT following CAR-T cell therapy; however, there are currently no randomised clinical trials conducted testing this hypothesis (Ruella and Maus, 2016). Recent investigations on management strategies for CD19-negative relapse have focused on the use of an alternative target antigen such as CD22. Investigators from NCI reported preliminary findings using this strategy at the ASH 2016 annual meeting (Shah et al., 2016b). In this first-in-human investigation of nine patients who had previously undergone at least one prior alloHSCT (two patients with two prior HSCT), including prior anti-CD19 CAR-T cell therapy in seven (six of whom had a CD19 negative/dim antigen escape); patients received infusions of the transduced T cells at one of three dose levels, 3×10^5 transduced T cells/kg (DL-1), 1×10^6 per kg (DL-2) and 3×10^6 per kg (DL-3) following lymphodepletion with Flu and Cy. MRD-negative CR was reported in all evaluable patients (4/9). Three of the remissions were comparatively durable, with one lasting more than a year. CRS of maximum grade 2 was reported in six patients (Shah et al., 2016b) and neurotoxicity was mild to moderate (Shalabi et al., 2016). Updated results presented at the ASH 2017 annual meeting reports confirmation of the activity of CD22 CAR-T cells with high remission induction rates in relapsed/refractory ALL, including responses in patients previously treated with CD19 CAR-T cells (Shah et al., 2017c). While the results are encouraging and the study is still accruing patients, these early results raise new questions on when to use anti-CD22 CAR. For example, is it following relapse with an anti-CD19 CAR or is it as a combined therapy. CD22 as a target is also being studied by investigators at University of Pennsylvania/CHOP (NCT02588456; NCT02650414). More data are needed to understand the optimal timing for the utilisation of anti-CD22 CAR-T cell therapy. Using preclinical models, investigators from University of Pennsylvania have also reported cotargeting CD123 along with CD19 to reduce the antigen loss (Ruella et al., 2016a). Other strategies, such as use

of trivalent CAR-T cells (T cells targeting CD19, CD20 and CD22) to mitigate CD19 negative relapse, have been proposed (Fousek et al., 2017).

CAR-T Cell Induced Toxicities

The engineered CAR-T cell therapy is known to act precisely on the target antigens exhibited by the tumour, thus theoretically they have a potential to cause less toxicity compared with small molecules or biologics. Nevertheless, toxicities with CAR-T cells have been reported (Bonifant et al., 2016; Brudno and Kochenderfer, 2016).

As reported in many clinical trials with CAR-T cell therapy in haematological malignancies, important toxicities include CRS, macrophage activation syndrome and neurotoxicity (Ruella and June, 2016). Other toxicities such as TLS occurring due to direct effects of tumour destruction and B-cell aplasia due to CAR-T cell therapies targeting normal B cells expressing CD19 have also been reported (Barrett et al., 2015). CAR-T cell-mediated GVHD has not been reported in clinical trial setting that uses autologous T cells and perhaps this will be encountered with 'off-the-shelf' allogenic T cell products (Bedoya et al., 2017).

Cytokine Release Syndrome

CRS is by far the most prevalent toxicity reported. This serious adverse event manifests itself with symptoms such as high fever, rigors, sweating, anorexia, headache, altered mental status, myalgia/arthralgia, nausea/vomiting and can also progress to life-threatening capillary leak with hypoxia and hypotension and multiorgan dysfunction (Bonifant et al., 2016; Ruella and June, 2016; Frey and Porter, 2016). The clinical signs of CRS correlate with T cell expansion and progressive immune activation of the transferred cells (Bonifant et al., 2016) and high levels of cytokines, including IL-6. CRS is generally milder in CLL and DLBCL, but it is more prominent in ALL (Barrett et al., 2015).

The grading of CRS as mild, moderate, severe or life threatening varies depending on the CRS scaling used. Each academic institute utilises a different grading scale, making it difficult for subjective comparison of the severity of CRS observed by various CAR-T cell therapies across trials. The Common Terminology Criteria for Adverse Events Version 4.0 (CTCAE) grading scale includes a description CRS-related adverse events caused by immunotherapies and was developed for toxicity grading of acute infusion-related toxicities of monoclonal antibodies (Brudno and Kochenderfer, 2016). The CTCAE v 4.0 grading is not only based on symptoms but also takes into account the need for intervention of therapy. Because CAR-T cell therapy is unique as it involves only one infusion, newer grading systems have been developed (Frey and Porter, 2016). A modified CTCAE grading scale based on input and consensus from several programmes was developed by NCI and utilised in their clinical trials (Lee et al., 2014). University of Pennsylvania/CHOP developed a third-modified grading-scale that has been utilised in their trials (Frey and Porter, 2016). A comparison of the various grading scales is listed in Table 12.5. Utilising the various scales, a patient receiving anti-CD19 CAR-T cell therapy for ALL, exhibiting clinical symptoms of hypotension requiring

Table 12.5 Grading Scale for CRS (Frey and Porter, 2016).

Grading Scale	CTCAE v 4.0	2014 Consensus NCI	University of Pennsylvania/CHOP	Comparisons
Grade 1	Mild; no infusion interruption; no intervention	Symptoms are not life threatening and require symptomatic treatment only, e.g., fever, nausea, fatigue, headache, myalgias, malaise	Mild reaction treated with supportive care only	CTCAE: linked to infusion of drug, not applicable to cellular therapy Grade 3 NCI permits more severe hypotension compared with a University of Pennsylvania/CHOP grade 3 Life-threatening hypoxia (mechanical ventilation) similar across scales; NCI and University of Pennsylvania/CHOP allow for symptom management, whereas CTCAE does not
Grade 2	Infusion interruption indicated but responds promptly to symptomatic treatment (e.g., antihistamines, NSAIDs, narcotics, intravenous fluids); prophylactic medications indicated for ≤24 h	Symptoms require and respond to moderate intervention; oxygen requirement <40% or hypotension responsive to fluids or low-dose pressors or grade 2 organ toxicity	Moderate reaction requiring IV therapies or parenteral nutrition; mild signs of organ dysfunction (creatinine ≤grade 2 or liver function tests ≤grade 3) Hospitalisation for CRS or febrile neutropenia	CTCAE: linked to infusion/withholding of a drug, not applicable to cellular therapy Grade 2 NCI permits more severe hypoxia and hypotension compared with a UPENN/CHOP grade 2
Grade 3	Prolonged (e.g., not rapidly responsive to symptomatic medications and/or brief interruption of infusion); recurrence of symptoms after initial improvement; hospitalisation indicated for clinical sequelae (e.g., renal impairment, pulmonary infiltrates)	Symptoms require and respond to aggressive intervention; oxygen requirement <40% or hypotension requiring high dose or multiple pressors or grade 3 organ toxicity or grade 4 transaminitis	More severe reaction, requiring hospitalisation; moderate signs of organ dysfunction (grade 3 creatinine or grade 4 liver function tests) related to CRS; hypotension treated with intravenous fluids or low-dose pressors; hypoxaemia requiring oxygenation, bilevel positive airway pressure or continuous positive airway pressure	Grade 3 NCI permits more severe hypotension compared with a University of Pennsylvania/CHOP grade 3
Grade 4	Life-threatening consequences; pressor or ventilator support	Life-threatening symptoms; requirement for ventilator support or grade 4 organ toxicity (excluding transaminitis)	Life-threatening complications, including hypotension requiring high-dose vasoactives or hypoxaemia requiring mechanical ventilation	Life-threatening hypoxia (mechanical ventilation) similar across scales

CHOP, Children's Hospital of Philadelphia; *CTCAE*, Common Terminology Criteria for Adverse Events; *NASAIDs*, nonsteroidal antiinflammatory drugs; *NCI*, National Cancer Institute.

low-dose pressors for haemodynamic support, would have grade 4 CRS on the CTCAE v4.0 scale; grade 2 CRS on the NCI scale and grade 3 CRS on the U PENN/CHOP scale (Frey and Porter, 2016). Thus, clearly a consensus is needed regarding the choice of scale to utilise when grading CRS and influencing its management. Members of the RAC at the NIH have discussed this topic during one of their meetings in June 2015 (Sadelain, 2015). Although the development of a consensus recommendation to manage serious toxicities such as CRS has been reported (Neelapu et al., 2018), such a generalised recommendation will not be feasible as the magnitude and timing of the toxicities associated with CAR-T cell therapy vary considerably, between different CAR-T cell constructs, as well as across different diseases (acute lymphoblastic leukemia (ALL) vs. NHL) (Teachey et al., 2018).

IL-6 blockade with the use of IL-6 antagonist, tocilizumab, has generally been used in most clinical trials for the management of severe CRS (Bonifant et al., 2016; Barrett et al., 2015; Frey and Porter, 2016) and is now considered as standard of care for severe CRS following CAR-T cell therapy (Frey and Porter, 2016). On 30 August 2017, the US FDA approved Actemra (tocilizumab, Genentech, Inc., South San Francisco, CA) for the treatment of severe or life-threatening CAR-T cell-induced CRS in adults and in paediatric patients 2 years of age and older (Le et al., 2018). Corticosteroids may negatively impact the antitumour effects of CAR-T cell therapy and thus are preferentially not used (Frey and Porter, 2016). However, MSKCC has utilised corticosteroids for the management of severe CRS (Geyer and Brentjens, 2016; Park et al., 2016a). At NCI, the use of corticosteroids is reserved for patients not responding to tocilizumab (Brudno and Kochenderfer, 2016).

Other measures to prevent CRS or strategies to manage severe CRS have also been explored. At the ASH 2016 annual meeting, investigators from China reported that the anti-CD19-CD3z-4-1BB CAR that was cultured for 5–6 days in serum-free media containing IL2, IL7, IL15 and IL21 is in general well tolerated (Deng et al., 2016). In this study, 15 patients with B-ALL were treated with a CAR-T cell dose of 10×10^4 cells/kg that was infused following lymphodepletion with Cy 250 mg/m^2 per day × 3 days + Flu 30 mg/m^2 per day × 3 days; all patients achieved CR without severe CRS. Grade 1–2 CRS was the maximum grade reported. At the same meeting, another group of investigators from China reported a low incidence of CRS with a fourth generation CAR T cell therapy, 4SCAR19, which is generated from a safety-engineered lentivector CAR containing four intracellular signaling domains: CD19-scFv/CD28/CD137/CD27/CD3ζ-iCasp9 (4SCAR19) (Chang et al., 2016).

CRS prophylaxis via the preemptive administration of tocilizumab and dexamethasone with the goal to lessen the occurrence of severe CRS was reported by the investigators from Seattle Children's Hospital at the ASH 2016 annual meeting (Gardner et al., 2016a). In this phase I (PLAT-02) study (NCT02028455), which compared an early intervention with tocilizumab + steroid to a later intervention after the occurrence of

severe CRS, the early intervention approach with immunomodulation appeared to decrease the rates of severe CRS (15% vs. 30% in patients without early tocilizumab) while preserving the high rates of MRD-negative CR (Gardner et al., 2016a). The investigators at University of Pennsylvania/CHOP are conducting a similar study to investigate what would be the optimal timing of administration of tocilizumab (NCT02906371). The outcome of preclinical studies conducted by investigators from University of Pennsylvania highlights the potential for ruxolitinib (JAK/STAT inhibitor) and ibrutinib (tyrosine kinase inhibitor) in preventing the development of severe CRS without impairing the antitumour effect of CAR-T cells (Kenderian et al., 2016; Ruella et al., 2016b), although this observation would have to be tested in a clinical trial setting.

Neurological Toxicity

The development of neurologic toxicities including confusion, delirium, expressive aphasia, obtundation, myoclonus and seizure has been reported in clinical trials using CAR-T cell therapies (Maude et al., 2018; Neelapu et al., 2017; Schuster et al., 2017c; Bonifant et al., 2016). The aetiology remains unclear and an association with CRS is also not clear in that the onset of neurological symptoms do not correlate with the clinical course of CRS and do not respond to tocilizumab (Frey and Porter, 2016). In a majority of cases, the observed neurologic toxicity was reversible (Maude et al., 2018; Neelapu et al., 2017; Schuster et al., 2017c; Bonifant et al., 2016), except with the ROCKET trial conducted by Juno therapeutics where deaths due to cerebral encephalopathy was reported resulting in the halting of the trial. Based on the findings from an analysis of patients treated with the 19-28z CAR-T cell therapy, investigators from MSKCC recently reported that the incidence of severe neurotoxicity was associated with high disease burden, defined as ≥50% blasts at the time of T cell infusion ($P = 0.0045$) and with posttreatment CRS ≥grade 3 ($P = .0010$). There was a lack of correlation between severe neurotoxicity and the CAR-T cell dose; however, peak CAR-T cell expansion was associated with both CRS and neurotoxicity. Elevated levels of IL-5 and IL-2 at Day 3 was very specific for the development of severe neurotoxicity. The findings from their analysis also showed that a platelet count <60 or mean corpuscular haemoglobin concentration >33.2% and the presence of morphologic disease, defined as >5% blasts at baseline, could identify patients at the risk of developing severe neurotoxicity with 95% sensitivity and 70% specificity (Park et al., 2017b).

On Target Toxicity

B-cell aplasia is an 'on target/off tumour' toxicity that has been repeatedly reported with anti-CD19 CAR-T cells directed towards B-cell malignancies. To manage this treatment-related toxicity, the infusion of immunoglobulin to prevent infection-related complications is typically required (Bonifant et al., 2016; Barrett et al., 2015). Because B-cell

aplasia will be present as long as anti-CD19 CAR-T cells are present, investigators at CHOP/University of Pennsylvania have recommended to use B-cell aplasia as a biomarker of the persistence of functional CD19-targeted T cells (Maude et al., 2014). Lethal 'on target/off tumour' toxicities have also been reported in solid tumours with CAR-T cells targeted towards ERBB2 (Morgan et al., 2010) as well as with engineered TCR targeting MAGE-A3 (Linette et al., 2013). Notably, the search for specific neoantigens and the development of safer CAR-T cells or TCR for solid tumours continues.

Insertional Oncogenesis and Replication-Competent Viruses

Based on findings from studies using retrovirus-mediated gene transfer for chromosome-linked severe combined immunodeficiency (SCIDX1), where a few patients developed T cell leukaemia, a perceived risk of insertional oncogenesis caused by transgene integration was of concern in the early clinical trials with CAR-T cell therapy (Bedoya et al., 2017; Hacein-Bey-Abina et al., 2008). However, there have been to this date not a single reported case of cellular transformation caused by any of the viral vectors used to modify T cells. Indeed, long-term results from three clinical trials to evaluate gammaretroviral vector-engineered T cells for HIV promote the view that CAR-T cells persist in 98% of these patients for upward of 11 years without any evidence of transformation (Scholler et al., 2012).

The oncogenic potential of experimental treatments could be worsened when a replication-competent retrovirus or lentivirus (RCR/RCL) is used as a vector. However, current retroviral and lentiviral delivery systems are designed to only transduce cells, and no cases of replication-competent viruses were identified in cell products or patients (Bedoya et al., 2017). Nevertheless, an FDA 2006 guidance treats lentiviral and retroviral vectors as being potentially oncogenic and thus recommends testing for RCR/RCL at multiple stages throughout their manufacturing, as well as the monitoring of patients for RCR/RCL pretreatment, 3 months, 6 months, 1 year posttreatment and yearly thereafter (FDA Supplemental Guidance on Testing, 2006; The US FDA Guidance for Industry, 2006). In addition, the FDA also recommends long-term follow-up observational studies for up to 15 years to monitor any potential delayed adverse event caused by the persistence of these vectors.

Manufacturing Challenges

The experience with CAR-T cell manufacturing has been driven mainly by academic centres supporting most of the phase 1/2 clinical trials conducted worldwide. Initially, engineered T cells were manufactured in facilities that were in close proximity to the academic clinical trial centre (Cooper, 2015). With an intent to pivot to multicentre trials globally and to broadly commercialise these therapies to cover the unmet medical needs of multiple recipients, manufacturing has moved from academia to industry in a centralised manufacturing facility, along with the custody of cells to ensure appropriate

patient delivery. This shift is associated with many challenges, including stringent regulations and monitoring not only of the GMP facility but also of the raw materials utilised in cell processing. To this date, no clear guidance from the FDA or other regulatory agencies (e.g., EMA) for a regulated product is available, given this field of immune-oncology is still emerging; agencies work hand-in-hand with pharmaceutical and biotechnology companies towards the goal of bringing these therapies to patients faster but without compromising on reaching appropriate confidence in safety and in efficacy. The manufacturing of T cells from autologous T cells is the primary root cause of batch-to-batch variability and heterogeneity that are observed; it is critical to manage this issue adequately because such variations may have an important impact on the efficacy of the T cell drug (Turtle and Maloney, 2016). What is more, each of the academic centres implement a different process for manufacturing CAR-T cells, for example by using different transducing vector or different methods to increase the ex vivo expanded product. This lack of homogeneity in manufacturing processes combined with the complexity of standardising the process in an industry-owned central manufacturing facility can influence the production time and in turn the end-to-end time of delivery back to the patient. Of note, Novartis Pharmaceuticals Ltd. was the first company to have achieved the successful transfer of this new technology from academia (Boyd et al., 2015). Notably, in their pivotal trials of CTL019 (tisagenlecleucel-T) for the approved indications, T cell manufacturing was conducted at the Novartis manufacturing facility (Grupp et al., 2016), a site that was acquired from Dendreon and retrofitted to fulfil that very purpose. Likewise, Kite Pharmaceuticals opened its manufacturing facility in June of 2016 to manufacture their CAR-T cell therapy, KTE-C19.

BIOMARKERS

Recent advances in the field of immuno-oncology indicate that the tumour microenvironment mediates a complex and heterogenous interaction between the tumour cell and the immune system of the patient; this interaction is unique to each individual tumour and to patient (Yuan et al., 2016). Thus, biomarker-based research has already become essential and has taken centre stage to enable the implementation of the precision medicine concept, which defines a road map for identifying effective therapies and enabling predictive toxicology (Kalos, 2011). Typically, biomarker studies for small molecules or biologics have focused on the impact of the treatment on the target tissue(s). As a result, typically a biomarker is a measure of specific products that are secreted from tumour cells. Because CAR-T cells are biological entities, the transferred T cells need to be both present and functional for the treatment to be efficacious (Kalos, 2011). The presence of T cells is evaluated primarily by flow cytometry, whereas functional competence can be measured by the presence of surface and activation markers and T cell bioactivity by the measurement of cytokines (Kalos, 2011). In this context, the MIATA (Minimum

Information About T cell Assays) initiative provides recommendations to specifically facilitate the identification of the relevant parameters important to document and report about T cell assays (Janetzki et al., 2009). With the implementation of MIATA recommendations one can enhance the consistency of data reporting across laboratories and thereby increase data reproducibility (Britten et al., 2012).

Can Biomarkers Predict the Efficacy of CAR-T cells?

As emphasised earlier, CAR-T cell therapies have different treating potentials, as observed particularly in the area of haematological malignancies where these agents have already been well studied. For example, the response rates are significantly lower for CLL or NHL as compared with those observed for ALL. Could biomarkers predict the efficacy of CAR-T cells and aid in identifying responders? This is a question that investigators are currently evaluating. For example, B-cell aplasia as a surrogate marker for response to CAR-T cell therapy has been reported by University of Pennsylvania investigators (Maude et al., 2014). The first report on the use of biomarkers to predict efficacy was provided by the investigators from NCI in collaboration with Kite Pharmaceuticals at the 2015 ASCO annual meeting (Bot et al., 2015). In their preliminary findings, these investigators reported that on coculture with CD19+ tumour cells, CAR-T cells produce T1, T2, proinflammatory, homoeostatic and effector cytokines and chemokines. The induction of key homoeostatic and inflammatory cytokines on conditioning, as well as increased levels of immune effector molecules (granzymes, perforin), was seen during the first 2 weeks after CAR-T cell infusion. These researchers concluded that detailed biomarker analysis from patients treated with anti-CD19 CAR-T cells may provide insights into clinical outcomes and thus guide the design of future T cell therapies. Similar findings were made during the ZUMA-1 study, utilising the CAR-T cell KTE-C19 in subjects with refractory aggressive NHL, where an increase in proinflammatory, immune-homoeostatic cytokines and chemokines that peaked within 2 weeks of infusion was observed (Rossi et al., 2015).

Furthermore, similar findings were also reported by China-based clinical investigators, where, in a case study of three patients with B-ALL, they reported the presence of long-term and discontinuous increases in serum cytokines (mainly IL-6 and C-reactive protein, CRP) as being suggestive of the persistence of the functional activity of CAR-T cells (Zhang et al., 2016b).

Investigators from University of Pennsylvania reported data from a study that evaluated several biomarkers to predict clinical responses to CTL019 in CLL (Fraietta et al., 2016b, 2018). In this study of 41 patients with advanced heavily pretreated and high-risk CLL who received at least one dose of CTL019 cells, durable remissions were associated with transcriptomic signatures of early memory T cells, while T cells from nonresponding patients were enriched in genes belonging to known pathways of terminal differentiation and exhaustion. Indeed, memory T cells are known to contribute to the long-term

persistence of CAR-T cells (Kalos et al., 2011) and thus their presence correlate with durable remissions. In the study, highly functional CAR-T cells from CLL patients produced signal transducer and activator of transcription (STAT) 3-related cytokines and serum IL-6 correlated with CAR-T cell expansion (Fraietta et al., 2018). Combined PD1 and CD27 expression on CD8+ CTL019 cells in the infusion product accurately predicted treatment responses and tumour control. Moreover, CTL019 cells from complete responders secreted significantly high levels of several cytokines, including CCL20, IL-21, IL-22, IL-17 and IL-6. In addition, unbiased analyses of the biomarker panel revealed that the frequency of CD27+ CD45RO cells in the CD8+ T cell population significantly correlated with complete and durable responses to this therapy. In nonresponders, flow cytometry demonstrated higher level of T cell exhaustion markers and reduced CD27 expression. Investigators conclude that the findings underscore the potential of using pretreatment biomarkers of response to advance immunotherapies.

Can Biomarkers Predict Toxicity?

With an intent to bring CAR-T cell therapies to patients, a major goal is to efficiently manage toxicities. As discussed above, one of the most common adverse events with CAR-T cell therapies in haematological malignancies is CRS. Standardising management of CRS, including identifying patients who have a potential to develop CRS, is indeed a priority for the successful utilisation of these therapies. In recent years, a major research focus has thus been to identify characteristics and biomarkers that can accurately predict patients developing severe CRS (Rouce and Heslop, 2016).

Elevated cytokine levels, including IL-6, are the hallmark of CRS; as a result the use of the IL-6 antagonist tocilizumab has been adopted as a first-line therapy for managing severe CRS (Frey and Porter, 2016). Whether these elevated cytokine levels can accurately predict the development of CRS, in particular severe CRS, was investigated by researchers at University of Pennsylvania (Teachey et al., 2016). In their study of 51 patients (39 paediatrics and 12 adults) with B-ALL treated with CTL019, 48 patients developed CRS with 35% (18/51) grade 1–2; 31% (16/51) grade 3 and 27% grade 4–5. Peak levels of 24 cytokines, including CRP, ferritin, IFN-γ, IL-6, sgp130 and sIL6R, in the first month after infusion were highly associated with severe CRS. Although peak levels of cytokines correlated with severe CRS, none of these cytokines were helpful in predicting severe CRS in the first 3 days (that is, before the development of clinical symptoms). However, two cytokines, IFN-γ and sgp130, were observed to have much higher levels in severe CRS as compared with nonsevere CRS patients in the first 3 days after infusion (that is, prior to the patients becoming critically ill) after adjusting for multiple comparisons. Using a logistic regression model, the elevated levels of these two cytokines accurately predicted patients developing grade 4–5 CRS with a sensitivity of 86% and a specificity of 89%. Notably, IL-6, that is, the cytokine which is the most strongly associated with severe CRS (and thus justifying the use of the IL-6 antagonist,

tocilizumab, to manage these events) did not predict severe CRS in the first 3 days of infusion (Teachey et al., 2016). This is in contrast to the findings reported by investigators from FHCRC. In this latter evaluation, the investigators reported elevated levels of IL-6 (along with IFN-γ and TNF-α) as early as Day 1 following infusion and also noted higher IL-6 levels in patients who developed severe-grade CRS and neurotoxicity (Turtle et al., 2016a). Additional analyses evaluating CRS grades and neurotoxicity, as well as correlative biomarkers through 28 days after infusion of the anti-CD19 CAR-T cells, with 1:1 ratio of CD8+:CD4+ CAR-T cells, in 127 adults with ALL, NHL or CLL (Turtle et al., 2016e) were conducted by the same investigators who presented the results of their studies at the ASH 2016 annual meeting. Findings showed significantly higher peak levels of IL-15, IL-6, IL-2, IFN-γ, CRP and ferritin in both ALL and NHL cohorts in those with grade 3–5 CRS as compared with grade 0–2 CRS. Furthermore, in a univariate analysis, the levels of IL-15, IL-6, IL-8, IL-10, soluble TNF receptor type 1 and IFN-γ were significantly higher on Day 1 after CAR-T cell infusion in patients who developed grade 3–5 CRS (Turtle et al., 2016e). In an updated publication, the investigators from FHCRC reported correlation between serum levels of monocyte chemoattractant protein-1 (MCP-1) and high risk of severe CRS, with the best sensitivity and specificity obtained by testing serum MCP-1 in patients with fever (≥38.9°C) within 36 h of infusion (Hay et al., 2017). These investigators also found that biomarkers of endothelial activation (angiopoietin-2 and von Willebrand factor) were increased during severe CRS and also before lymphodepletion in patients who subsequently developed CRS, suggesting that preexisting endothelial activation might be an unrecognised risk factor for severe CRS (Hay et al., 2017).

At the ASH 2016 annual meeting, investigators from NCI presented data illustrating correlation between a rise in CRP and severe CRS (Ishii et al., 2016). However, these investigators also reported that in one patient who was unresponsive to tocilizumab, substantially higher serum IL-2 (35 pg/mL vs. median 6.1 (range, 1.2–13.5)) and Granulocyte-Macrophage Colony Stimulating Factor, GM-CSF level (28 pg/mL vs. median 1.0 (range, 0–6.1)) 12-h post infusion was noted. Subsequent CRP elevation was not accompanied by a rise in IL-6, explaining the lack of response to tocilizumab (Ishii et al., 2016).

Based on the data thus far available, it is clear that there is a need to identify patients who may potentially develop serious CRS to initiate treatment. The findings from independent laboratories at various institutes validate this need. However, different key prediction biomarkers were reported from the FHCRC and the studies from the University of Pennsylvania study. The University of Pennsylvania data were obtained mainly from paediatric patients, whereas those generated by the FHCRC were generated in adults. The possibility of age influencing the biological features of CRS and its predictive markers has been hypothesised (Rouce and Heslop, 2016). Nevertheless, the important question remains whether the prophylactic use of tocilizumab is effective in reducing

morbidity and mortality from CRS; studies are still ongoing to identify the optimal timing for the administration of tocilizumab.

Investigators from University of Pennsylvania also describe another potential use of biomarkers to distinguish CRS resulting from sepsis and CRS resulting from CAR T cell treatments (Lacey et al., 2016). In some patients treated with CAR-T cells, concurrent infectious complications potentially can fuel underlying CRS leading to hypotension and hypoxia that are refractory to anticytokine therapies (Brudno and Kochenderfer, 2016). Furthermore, therapies used for CRS, including anticytokines or corticosteroids, can exacerbate infection and worsen the sepsis. Thus, the use of biomarkers to distinguish the two CRS events would be therapeutically relevant (Lacey et al., 2016).

Current Status in the Field of Biomarker Use

Currently, the study of biomarkers in immunotherapy is in its exploratory phase and needs to be validated in future large clinical trials. To bring the routine use of biomarkers in clinical practice, the Society for Immunotherapy of Cancer Immune Biomarkers Task Force was formed with a purpose to review the current state of the science, identify current challenges to advance the field and make recommendations regarding biomarkers for immunotherapy agents. This task force is composed of experts whose role is to make recommendations to the field in the areas of (1) validation of candidate biomarkers, (2) identification of the most promising technologies, (3) testing of high-throughput immune signatures and (4) investigation of the pretreatment tumour microenvironment. Further information can be found at the following Internet site: https://www.sitcancer.org/research/biomarkers.

ACADEMIA-INDUSTRY PARTNERSHIPS

Most of the work in the CAR-T cell space has been initiated in an academic setting. Bridging the gap between the scientific discoveries that are made at academic centres and bringing them to patients and in the clinical practice is the underlying pillar for academic–industry collaborations. In 2015, a handful of collaborations in the CAR T cell space have been implemented between academia and industry: (1) University of Pennsylvania/Novartis, (2) MSKCC/Juno Therapeutics, (3) FHCRC and NCI/Juno Therapeutics, (4) NCI/Kite Pharmaceuticals, (5) MD Anderson/ZIOPHARM Oncology and (6) Baylor college of Medicine/bluebird bio. A 2016 report from EP vantage shows that following this initial set of collaborations, the list has significantly grown (Table 12.6). http://info.evaluategroup.com/2016-06-CAR-T-Report-EPV.html.

Biotechnology and pharmaceutical companies like Novartis and Kite Pharmaceuticals have successfully transferred the relevant CAR-T technology from academic

Table 12.6 Academia/Industry Collaboration to Commercialise CAR-T cell Therapy (Excluding China).

Academia	Industry	Project	Proposed Indication
Baylor college of Medicine	Cell Medica	Functionally enhanced CAR-modified NKT cells; genetically engineered T cell receptor (TCR) for use in NKT cells and T cells	Next generation cellular immunotherapy for solid tumours
	Aurora Biopharma	CAR T HER2 (AU101) Bispecific CAR T cell AU105	Osteosarcoma, Glioblastoma
	Bellicum	BPX-601 that includes GoCART (proprietary) dual costimulatory domain MC (inducible MyD88/CD40) activation switch	Solid tumours
	bluebird bio/Celgene	BCMA targeting CAR-T cell bb2121	Multiple myeloma
	Celgene	CD30 targeting CAR-T cell	NHL
Christie Hospital NHS Foundation Trust	Cellular therapeutics Ltd.	Development of TILs	
City of Hope	Mustang Bio, Inc., a Fortress Biotech Company	CAR-T cells targeting the high-affinity IL-13 receptor, IL13Rα2, that is overexpressed in a majority of glioblastomas	Glioblastoma
FHCRC/NCI	Juno Therapeutics	JCAR014 targeting CD19 (more closely resembles JCAR017)	r/r lymphoma; with ibrutinib for CLL; with durvalumab for NHL
FHCRC	Juno Therapeutics	JCAR024 targeting ROR-1	For advanced ROR1+ malignancies with defined subsets of autologous T cells engineered to express a ROR1-specific CAR-T cell
		JTCR016, WT-1-directed, TCR cell product	Patients with high risk or relapsed AML, MDS or CML, WT1-expressing nonsmall cell lung cancer and mesothelioma
MSKCC	Juno Therapeutics	JCAR015 targeting CD19-CD3+ enriched PBMCs, retroviral transduction, CD28 costimulatory domain, scFv-SJ25C1	Adult ALL ROCKET trial halted
		JCAR020 targeting MUC-16 with IL-12 secretion	Autologous T cells genetically engineered to secrete IL-12 and to target the MUC16ecto antigen in patients with recurrent MUC16ecto+ epithelial ovarian, fallopian tube or primary peritoneal cancer

Continued

Table 12.6 Academia/Industry Collaboration to Commercialise CAR-T cell Therapy (Excluding China).—cont'd

Academia	Industry	Project	Proposed Indication
Seattle Gen Hospital	Juno Therapeutics	JCAR017 targeting CD19-fixed ratio of CD4+ and CD8+ T-lymphocytes; lentiviral transduction, 4-1BB costimulatory domain, scFv-FMC63	Paediatric ALL
		JCAR023 targeting L-1CAM (CD171)	Recurrent/refractory neuroblastoma using autologous T cells lentivirally transduced to express CD171-specific CAR-T cells
None	Celgene/Juno Therapeutics	JCAR017	NHL
NCI	Juno therapeutics via Opus Bio	JCAR018 (developed under cooperative research development agreement, CRADA) targeting CD22	Paediatric and young adults with recurrent or refractory CD22-expressing B-cell malignancies
NCI	Kite Pharmaceuticals	KTE-C19 targeting CD19	NHL
		TCR therapy candidate targeting HPV-16 E7 and MAGE A3	Solid tumours
		Human anti-CD19 CAR-T cell	Haematological malignancies
Kings College London	Leucid Bio	T1E28zeta CAR T targeting eight of nine homo- and heterodimers formed by the ErbB family	Solid tumours
University college London	Cellectis/Servier/ Pfizer	UCART19 allogeneic therapies where T cells from healthy donors are genetically edited with proprietary technology TALEN to seek and destroy cancer cells.	ALL
University of Pennsylvania	Novartis Pharmaceuticals	CTL019 CAR-T cell targeting haematological malignancies	ALL, DLBCL
		CT-119 humanised CAR-T cell	Haematological malignancies
		CAR T-22 targeting CD22	Haematological malignancies that relapse with anti-CD19 CAR-T cells
		CAR T-EGFRvIII	Glioblastoma
		CAR T-meso	Solid tumours
MD Anderson Cancer Center	Ziopharm Oncology Inc./Intrexon	CD33 CAR-T cell	AML
		Nonviral Sleeping Beauty System to Express CD19-specific CAR in T cells	Haematological malignancies
		Nonviral Sleeping Beauty System CAR-T cell	Various solid tumours

ALL, acute lymphoblastic leukaemia; *AML*, acute myeloid leukaemia; *BCMA*, B-cell maturation antigen; *DLBCL*, diffuse large B-cell lymphoma; *FHCRC*, Fred Hutchinson Cancer Research Center; *MSKCC*, Memorial Sloan Kettering Cancer Center; *NCI*, National Cancer Institute; *NHL*, non-Hodgkin lymphoma; *TALEN*, transcription activator-like effector nucleases.

Source: EP Vantage and company websites.

laboratories to their own R&D facilities, and indeed in their respective multicentre pivotal studies, the cells are manufactured in their respective centralised facilities. Although, two CAR T therapies are approved, several challenges in terms of process improvement to further reduce the manufacturing time and the broad use of the new technology beyond haematological malignancies, including solid tumours, still remain (Walker and Johnson, 2016). To standardise the process and to commercialise at a reasonable cost, industry may need to implement incremental process optimisations and capture economies of scale. Automating as many of the steps that are currently performed manually would achieve two important goals: cost reductions and human error reductions (Walker and Johnson, 2016). Decreasing raw material inputs to improve the overall cost-effectiveness of the process is another important path to explore. It is worth noting here that a fundamental difference between CAR-T cell therapies and small molecules or biologics in that the T cells being alive are not only expanded ex vivo during processing but also expanded in vivo following their administration. Although lymphodepleting chemotherapy and the tumour microenvironment have been shown to influence the expansion in vivo, the quality of the starting material obtained from leukapheresis plays a key role in the performance of the ex vivo expansion step. Currently, most of the CAR-T cell technologies use autologous cells. Thus, establishing product uniformity, even within an industrial manufacturing suite, can be challenging due to inevitable batch-to-batch and patient-to-patient variations. Failure to obtain a satisfactory ex vivo expansion from a leukapherised material can on the one hand drive cost increases, and on the other increase the burden to the receiving patient, as a second leukapheresis sample would typically need to be obtained; what is more, the patient would have to be placed on an additional waiting period to receive the CAR-T cell product while the underlying disease is progressing. When should a patient be leukapherised is often a challenging question and what holds true for one population (e.g., children) may not be the case for another (e.g., adults). An approach to overcome part of these challenges has been tried with the development of 'off-the-shelf' cell-based therapy where cells are manufactured from donors ahead of the need and infused on demand (Torikai and Cooper, 2016). Cellectis in partnership with Servier and Pfizer as an industry team collaborated with University College London, on U CAR T 19 (Universal CAR T) as an 'off-the-shelf' therapy without generating GVHD reaction. This allogeneic therapy combines the promise of a CAR-T cell using a gene editing technique (TALENs) to suppress 'off-target' toxicities by way of eliminating endogenous TCRs and provide a therapy with improved efficacy and reduced toxicity (Valton et al., 2015). This technology can also help in reducing cost by reducing the lead time (Walker and Johnson, 2016). Data with the use of U CAR T 19 are to this date still very limited with reports from five children (three males and two females) aged between 8 months and 16.4 years, including two infants, aged 11 and 8 months (Qasim et al., 2017a,b). By Day 28–42, all patients had achieved CR. All patients experienced reversible CRS (1 patient with grade 3 requiring

two doses of tocilizumab). Although results of these adoptive therapies remain still preliminary, two participants developed grade 1 skin manifestations of GVHD 2 months after infusion, with the said GVHD resolving with use of topical steroid treatments. Four children experienced viral complications related to lymphodepletion, and all experienced neutropenia, which was prolonged in two patients. All patients proceeded to conditioned allo-SCT between 7–9 weeks after UCART19 infusion. Among the five children only two subjects remained in molecular remission 2 and 2.5 months posttransplant. Two children relapsed 3 months after transplantation (one CD19− and one CD19+) and died 7 and 8 months after UCART19 infusion, respectively. One subject died in remission from transplant-related complications including thrombotic microangiopathy and BK virus induced hemorrhagic cystitis and nephritis. Preliminary results from the first-in-human trial of UCART19 treatment in a high risk relapsed/refractory B-ALL adult population was also presented at the 2017 ASH annual meeting (Graham et al., 2017). Of the six patients treated, four achieved a CRi with MRD negativity at Day 28 (MRD −ve, defined as a tumour burden <0.01% assessed by flow cytometry and/or qPCR), one was refractory to treatment at Day 28 and one died at Day 15. All four patients underwent a subsequent alloSCT, three of them within 3 months of UCART19 infusion and one following retreatment. Post alloSCT one patient relapsed at 100 days with CD19+ disease, one died from infection and two remain in CR (Graham et al., 2017). As already highlighted above, these data remain too recent at the time of this writing to make any conclusion on the promise of the 'Universal CAR-T cell therapy'; as a result further trials and long term surveillance data are still eagerly awaited.

Researchers worldwide, from industry and academia, are also striving to innovate on the broad use of CAR-T cell technology and to expand its applications to the management of solid tumours, notably via industry-academia strategic alliances as reported in Table 12.6.

RESEARCH IN CHINA

One area of the world that adds invaluable insights into the benefit-risk of CAR T therapies is China. Adoptive immune cell therapy as a medical technology in the clinical trial setting is growing very rapidly in China (Liu et al., 2017). The number of clinical trials conducted with CAR-T cell therapies has progressively increased over the years and to date 138 trials investigating the use of CAR-T cell therapies listed in clinicaltrials.gov were conducted in China, and 38 of these trials listed in Chinese clinical registry (Table 12.7), making it by far the leading country investing in CAR-T cell therapy research today (Liu et al., 2017). Indeed, the first-in-human trial of a CRISPR therapy, that is, injecting genome-edited immune cells into a patient with advanced nonsmall cell lung cancer, was initiated by a group of researchers in China (Cyranoski, 2016). Among the CAR T-trials, CD19 is the most commonly targeted antigen (Table 12.7), with 81

Table 12.7 Clinical Trials in China.

CAR-Target	Indication	Trial Identifier	Age Group	Study Phase	Status
BCMA	Multiple myeloma, lymphoma	ChiCTR-OPC-16009113	Adults	1	Recruiting
		NCT02954445	Paediatric; adults	1/2	Recruiting
	Multiple myeloma	NCT03093168	Adults	1	Recruiting
BCMA/CD38	Multiple myeloma	NCT03090659	Adults	1/2	Enrol by invite
BCMA and CD19	Multiple myeloma	ChiCTR-OIC-17011272	Adults	2	Recruiting
CD123	AML	NCT02937103	Paediatric; adults	1/2	Recruiting
CD133	Multiple solid cancers, haematological malignancies	NCT02541370	Adults	1	Recruiting
CD138	Multiple myeloma	NCT01886976	Adults	1/2	Recruiting
		ChiCTR-INh-16008198	Adults	Unknown	Pending
CD138 and CD19	Multiple myeloma	ChiCTR-ONN-17011517	Adults	Unknown	Pending
CD19	CLL, lymphomas, B-ALL	NCT01864889	Paediatric; adults	Unknown	Recruiting
	NHL	NCT02081937	Adults	1/2	Recruiting
	Lymphomas	NCT02247609	Adults	1/2	Recruiting
	B-ALL	NCT02186860	Adults	1	Recruiting
	CLL, ALL, NHL	NCT02349698	Paediatric; adults	1	Recruiting
	CLL, ALL, lymphomas	NCT02456350	Paediatric; adults	1	Recruiting
	CD19+ leukaemia, lymphoma	NCT02537977	Paediatric; adults	1/2	Recruiting
	Lymphomas	NCT02547948	Adults	1/2	Recruiting
	ALL, CLL, NHL	NCT02546739	Adults	1	Not yet open
	B-cell malignancies	NCT02644655	Adults	1/2	Recruiting
	Lymphomas	NCT02652910	Adults	1/2	Recruiting
	B-cell leukaemias	NCT02672501	Paediatric; adults	1/2	Recruiting

Continued

Table 12.7 Clinical Trials in China.—cont'd

CAR-Target	Indication	Trial Identifier	Age Group	Study Phase	Status
	Lymphomas, CLL, ALL	NCT02656147	Adults	1	Not yet open
	ALL, CLL, lymphomas	NCT02685670	Paediatric; adults	1/2	Recruiting
	DLBCL	NCT02728882	Paediatric; adults	1/2	Recruiting
	B-cell malignancies	NCT02782351	Paediatric; adults	1/2	Recruiting
	ALL, CLL, NHL	NCT02813837	Paediatric; adults	Unknown	Recruiting
	ALL	NCT02810223	Paediatric; adults	1	Recruiting
	B-ALL	NCT02822326	Paediatric; adults	1	Recruiting
	B-ALL	NCT02799550	Adults	1	Recruiting
	ALL, CLL, lymphomas	NCT02819583	Adults	1	Recruiting
	Lymphoma	NCT02842138	Adults	1	Recruiting
	ALL, CLL, lymphomas	NCT02851589	Paediatric; adults	1/2	Recruiting
	CLL, B-ALL, lymphomas	NCT02892695	Paediatric; adults	1/2	Recruiting
	B-ALL	NCT02924753	Paediatric; adults	1	Recruiting
	CD19+ B-cell malignancies	NCT02933775	Adults	1	Not yet open
	B-ALL	NCT02975687	Adults	1	Recruiting
	B-ALL lymphomas	NCT02965092	Adults	1	Recruiting
	DLBCL	NCT02976857	Adults	1	Recruiting
	ALL	NCT02968472	Paediatric; adults	1	Recruiting
	Lymphoma	NCT02992834	Adults	4	Not yet open
	Acute non–T--lymphocytic leukemia	NCT02735291	Paediatric; adults	1/2	Recruiting
	B-cell leukaemias and B-cell lymphoma	NCT02963038	Paediatric; adults	1/2	Recruiting
	NHL	NCT03029338	Adults	1	Recruiting

B-ALL	NCT03018093	Adults	1	Recruiting
Systemic lupus erythematosus	NCT03030976	Adults	1	Recruiting
B-ALL	NCT03027739	Paediatric; adults	2/3	Recruiting
B-ALL, CNS complications	NCT03064269	Paediatric; adults	1	Recruiting
B-cell malignancies	NCT03050190	Paediatric; adults	1/2	Recruiting
ALL, CLL, lymphomas	NCT03076437	Paediatric; adults	1/2	Recruiting
Lymphomas	NCT03086954	Paediatric; adults	1	Not yet open
B-cell lymphoma	NCT03101709	Adults	1	Recruiting
B-ALL, CLL	NCT03110640	Paediatric; adults	1	Not yet open
Lymphomas	NCT03118180	Paediatric; adults	1/2	Recruiting
B-ALL, lymphomas	NCT03156101	Paediatric; adults	1/2	Recruiting
NHL	NCT03154775	Adults	1	Recruiting
B-cell lymphoma	NCT03146533	Adults	1/2	Recruiting
B-ALL	NCT03173417	Paediatric; adults	1/2	Recruiting
Lymphomas	NCT03208556	Adults	1	Recruiting
B-cell malignancies	NCT03191773	Paediatric; adults	1	Recruiting
NHL	ChiCTR-OIC-17011310	Unknown	1/2	recruiting
B-cell malignancies	ChiCTR-OIC-17011271	Paediatric; adults	2	Recruiting
B-cell malignancies	ChiCTR-ONC-17011211	Paediatric; adults	Unknown	Recruiting
B-cell malignancies	ChiCTR-OIC-17011180	Unknown	1	recruiting
ALL	ChiCTR-ORN-16008948	Paediatric; adults	Unknown	Recruiting
B-ALL	ChiCTR-ONC-16009889	Paediatric	unknown	Pending
B-ALL, lymphoma	ChiCTR-ONN-16009862	Paediatric; adults	Unknown	Recruiting
B-ALL	ChiCTR-OIC-16009259	Paediatric; adults	2	recruiting
B-ALL	ChiCTR-IIh-16008711	Paediatric; adults	1	Recruiting
B-cell (CD19+) leukaemias	ChiCTR-OCB-15006379	Paediatric; adults	1	Recruiting
ALL, lymphoma	ChiCTR-OCC-15007008	Paediatric; adults	1	recruiting

Continued

Table 12.7 Clinical Trials in China.—cont'd

CAR-Target	Indication	Trial Identifier	Age Group	Study Phase	Status
	B-cell malignancies	ChiCTR-OIN-15007668	adults	1	Ongoing
	B-cell malignancies	ChiCTR-OOC-16007779	Adults	Unknown	Recruiting
	CD19+ B cell malignancies	ChiCTR-OIN-16007723	Paediatric; Adults	1/2	Recruiting
	ALL	ChiCTR-OOC-16008448	Paediatric; Adults	2	Recruiting
	ALL	ChiCTR-OOC-16008447	Paediatric; adults	2	Recruiting
	NHL	ChiCTR-ONC-16009027	Paediatric; adults	Case series	Pending
	B-cell lymphoma	ChiCTR-ONC-16008911	Paediatric; adults	Case series	Recruiting
	HIV related lymphoma	ChiCTR-ONC-16009567	Adults	Case series	Pending
CD19 and CD20	DLBCL	NCT02737085	Paediatric; adults	1/2	Ongoing, not recruiting
CD19 or CD20	CLL, lymphomas	NCT02846584	Paediatric; adults	2	Ongoing, not recruiting
CD19+ CD20	Lymphomas	NCT03207178	Adults	1/2	Recruiting
CD19/CD20	B-ALL, CLL, lymphomas	NCT03097770	Paediatric; adults	Unknown	Recruiting
CD19/CD22	B-ALL, NHL	NCT03098355	Paediatric	1/2	Recruiting
	B-cell malignancies	NCT03185494	Paediatric; adults	1/2	Recruiting
CD19 and CD22	Lymphomas, multiple myeloma	ChiCTR-OPN-16008526	Adults	1	Recruiting
	B-cell malignancies	NCT02903810	Adults	1/2	Recruiting
	Lymphoma, multiple myeloma, ALL, CLL	ChiCTR-OPN-16009847	Adults	1	Recruiting
CD19/CD20/ CD22/CD30	NHL	NCT03196830	Paediatric	2	Recruiting
CD19+ CD20 or CD22 or CD38 or CD123	B-cell malignancies	NCT03125577	Paediatric; adults	1/2	Recruiting
CD19 or CD30	B-cell malignancies	ChiCTR-ONC-16008405	Paediatric; adults	Unknown	Recruiting
CD20	B-ALL, CLL, lymphomas	NCT01735604	Adults	1/2	Recruiting
	CD20+ B-cell lymphoma	NCT02965157	Adults	1/2	Recruiting

CD22	Lymphomas	NCT02721407	Adults	1	Recruiting
	B-cell malignancies	NCT02794961	Paediatric; adults	1/2	Recruiting
	B-cell malignancies	NCT02935153	Paediatric; adults	1/2	Recruiting
CD30	Lymphoma	NCT02274584	Adults	1/2	Recruiting
	Lymphoma	NCT02259556	Paediatric; adults	1/2	Recruiting
	CD30+ lymphoproliferative diseases	ChiCTR-OPN-16009069	Adults	1	Recruiting
	CD30+ lymphocyte malignancies, lymphoma	NCT02958410	Paediatric; adults	1/2	Recruiting
CD33	AML	NCT01864902	Paediatric; adults	1/2	Recruiting
	AML	NCT02799680	Adults	1	Recruiting
	AML	ChiCTR-OPC-16009097	Adults	1	Recruiting
	Myeloid malignancies	NCT02958397	Paediatric; adults	1/2	Recruiting
	AML	NCT02944162	Paediatric; adults	1/2	Recruiting
CD7	CD7+ AML, other leukaemias and lymphomas	NCT02742727	Adults	1/2	Recruiting
CD80/CD86, PD-L1	Lung cancer	NCT03060343	Adults	1	Recruiting
CEA	Multiple solid cancer	NCT02349724	Adults	1	Recruiting
	Colorectal cancer	ChiCTR-OIN-16008146	Adults	1	Ongoing
CEpCAM	Nasopharyngeal carcinoma or breast cancer	NCT02915445	Adults	1	Recruiting
CAR-CLD18	Pancreatic cancer	NCT03159819	Adults	Unknown	Not yet open
EGFR	Multiple solid cancers EGFR+	NCT01869166	Adults	1/2	Recruiting
	Glioma	NCT02331693	Adults	1	Unknown
	Colorectal cancer EGFR+	NCT03152435	Adults	1/2	Recruiting
	Multiple solid tumours	NCT03182816	Adults	1/2	Recruiting

Continued

Table 12.7 Clinical Trials in China.—cont'd

CAR-Target	Indication	Trial Identifier	Age Group	Study Phase	Status
EGFRvIII	Glioblastoma	ChiCTR-OIN-16008252	Adults	1	Pending
	Glioblastoma	NCT02844062	Adults	1	Recruiting
	Glioblastoma	NCT03170141	Adults	1/2	Enrolment by invitation
EPCAM	Liver cancer	NCT02729493	Paediatric; adults	1/2	Recruiting
	Stomach cancer	NCT02725125	Paediatric; adults	1/2	Recruiting
	Gastrointestinal cancers	ChiCTR-ONC-16008278	Adults	Unknown	Recruiting
	Multiple solid cancers	NCT03013712	Adults	1/2	Recruiting
EphA2	Malignant Glioma	NCT02575261	Adults	1/2	Recruiting
GD2	Neuroblastoma	NCT02765243	Paediatric	2	Recruiting
	Neuroblastoma	NCT02919046	Paediatric	1/2	Recruiting
	Multiple solid cancers	NCT02992210	Paediatric; adults	1/2	Recruiting
GPC3	Liver cancer	NCT02395250	Adults	1	Terminated
	Liver cancer	NCT02715362	Adults	1/2	Recruiting
	Liver cancer	NCT02723942	Adults	1/2	Recruiting
	Lung cancer	NCT02876978	Adults	1	Recruiting
	Liver and lung cancer	ChiCTR-OID-16009515	Adults	1	Pending
	Lung, gastric and breast cancer	ChiCTR-OPN-16009943	Adults	Unknown	Recruiting
	Liver cancer	ChiCTR-OPN-16009897	Adults	Unknown	Recruiting
	Liver cancer	NCT03084380	Adults	1/2	Not yet open
	Hepatocellular carcinoma	NCT03146234	Adults	Unknown	Recruiting
	Hepatocellular carcinoma	NCT03130712	Adults	1/2	Recruiting
	Lung cancer, liver cancer	NCT03198546	Adults	1	Recruiting
GPC3. meso-, CEA	Colorectal, pancreatic, liver cancers	NCT02959151	Adults	1/2	Recruiting

HER2	Multiple solid cancers HER2+	NCT01935843	Adults	1/2	Recruiting
	Breast cancer	NCT02547961	Adults	1/2	Recruiting
	Multiple solid cancers HER2+	NCT02713984	Adults	1/2	Recruiting
LeY	Myeloid malignancies	NCT02958384	Paediatric; adults	1/2	Recruiting
LMP1	EBV+ nasopharyngeal neoplasms	NCT02980315	Paediatric; adults	1/2	Recruiting
Mesothelin	Multiple solid cancers	NCT02580747	Adults	1	Recruiting
	Pancreatic cancer	NCT02706782	Adults	1	Recruiting
	Meso+ solid cancers	NCT02930993	Adults	1	Recruiting
	Multiple solid cancers (mesothelin+)	NCT03030001	Adults	1/2	Recruiting
	Multiple solid tumours	NCT03182803	Adults	1/2	Recruiting
Mesothelin, EpCAM, MUC16, L1CAM	Gynaecological cancers	ChiCTR-OOC-16008377	Adults	Unknown	Pending
MG7 (CEA)	Liver Metastases	NCT02862704	Adults	1/2	Recruiting
MUC1	Multiple solid cancers	NCT02587689	Adults	1/2	Recruiting
	Multiple solid cancers	NCT02617134	Adults	1/2	Recruiting
	Multiple solid cancers	NCT02839954	Adults	1/2	Recruiting
	Multiple solid cancers	NCT03179007	Adults	1/2	Recruiting

Continued

Table 12.7 Clinical Trials in China.—cont'd

CAR-Target	Indication	Trial Identifier	Age Group	Study Phase	Status
Multiple (CD19, CD22, CD30, CD7, BCMA)	Relapsed or refractory haematopoietic and lymphoid malignancies	NCT03121625	Paediatric; adults	1	Recruiting
NKG2D	Colorectal cancer	ChiCTR-IID-17011603	Adults	1	Not yet open
PD1	EGFR+ cancers	NCT02873390	Adults	1/2	Recruiting
	EGFR+ cancers	NCT02862028	Adults	1/2	Recruiting
PD-L1	PD-L1+ solid cancers	NCT02930967	Adults	1	Recruiting
	Glioblastoma	NCT02937844	Adults	1	Recruiting
PSCA/MUC1/ PD-L1 or CD80/86	Lung cancer	NCT03198052	Adults	1	Recruiting
PSMA	Bladder cancer	ChiCTR-OIN-17011414	Adults	1/2	Recruiting
PSMA/Fra	Bladder cancer	NCT03185468	Adults	1/2	Recruiting
Unknown target	AML	ChiCTR-ONC-16009558	Paediatric; adults	Unknown	Pending

ALL, acute lymphoblastic leukaemia; *AML*, acute myeloid leukaemia; *BCMA*, B-cell maturation antigen; *CLL*, chronic lymphocytic leukaemia; *DLBCL*, diffuse large B-cell lymphoma; *NHL*, non-Hodgkin lymphoma.
Source: Celltrials data. Celltrials.org website.

trials with an anti-CD-19 CAR T and 83 clinical trials targeting non-CD19 antigens, including CD20, CD22, CD30, CD33, CD38, CD123, CD138, BCMA and Lewis Y for haematological malignancies and other targets such as mesothelin, EGFR, CEA, MUC-1, GD2 for solid tumours. Many trials are also investigating dual or multi-antigen targets (CAR-Immunotherapy Trials, 2017). At the ASH 2017 annual meeting, 54 presentations on CAR-T cell therapy represented research from China.

Advancements, including the development of fourth generation CAR-T cells that notably include a safety switch, and investing in manufacturing facilities are making China a hot bed for the industry. The changes implemented in 2015 in the Chinese regulations directed towards improved governance for conducting clinical trials with stem cells, which also applies to CAR-T cell research, had several important positive outcomes comprising strengthened innovation, facilitated collaborations with foreign researchers and joint applications for the approval of candidate therapies at drug regulatory authorities in China and in other countries (Rosemann and Sleeboom-Faulkner, 2016). Today, many US-based pharmaceutical and biotechnology companies have partnered with biotechnology companies within China (Kite Pharmaceuticals with Shangai-based Fosun Pharmaceuticals; Juno Therapeutics and WuXi AppTec). Likewise, industry-academia collaborations to generate CAR-T cell therapies with enhanced safety and efficacy have been flourishing.

REGULATORY CHALLENGES AND SOLUTIONS

Among the various CAR-T cell therapies targeting CD19 studied in haematological malignancies, many, including those from Novartis, Juno Therapeutics and Kite Pharmaceuticals, have received a breakthrough designation (and orphan drug designation for axicabtagene ciloleucel) from the FDA or a PRIME designation from the EMA. Moreover, in 2017, the US FDA approved the first CAR T by Novartis, Kymriah (tisagenlecleucel) for the treatment of patients up to 25 years of age with B-cell precursor ALL that is refractory or in second or later relapse and the CAR T from Kite Pharmaceuticals (now Gilead), Yescarta (axicabtagene ciloleucel) for the treatment of adult patients with relapsed or refractory large B-Cell Lymphoma after two or more lines of systemic therapy (KYMRIAH™, 2018;YESCARTA™ PI, 2017). In May 2018, the US FDA approved the second indication for tisagenlecleucel in adult patients with relapsed or refractory (r/r) large B-cell lymphoma after two or more lines of systemic therapy including DLBCL not otherwise specified, high-grade B-cell lymphoma and DLBCL arising from FL (KYMRIAH™, 2018). As of March 2016, the US FDA has received 105 INDs for genetically engineered T cell therapies, 36 of which are specifically for anti-CD19 CAR-T cells and originate from more than 15 different sponsors (*See more at*: http://www.raps.org/Regulatory-Focus/News/2016/03/16/24549/FDA-Proposes-New-Databases-to-Monitor-CAR-T-Cell-Safety-Across-INDs/#sthash.NErYPhnq.dpuf).

From a regulatory perspective, challenges in CAR-T cell therapies include both manufacturing and clinical aspects. Manufacturing issues mainly consist of product consistency, patient-dependent variations in T cell transfection efficiency, optimal T cell types for CAR transfection, product tracking and labelling; key clinical challenges include dosing issues and safety (Kim et al., 2016).

As discussed earlier, many of the trials in the CAR-T cell space were initiated at academic centres. While moving towards obtaining licensure for commercialisation, regulatory challenges also include the testing and utilisation of GMP-grade materials required for cell production, the strength of the supply chain to procure these materials, and the comparability of product characteristics and clinical efficacy between those obtained from academia as compared with those obtained from industry (Levine et al., 2017). One other challenge that any industry sponsor filing for licensure at multiple countries needs to take into consideration is that the guidelines recommended for gene and cell therapies by various countries are not harmonised; as a result, one needs to meet all the requirements recommended by each regulatory authority of the jurisdictions of interest (Levine et al., 2017). In an effort to harmonise regulations across the globe in the area of cell and gene therapies, nine members of the global regulatory community, including Brazil ANVISA; the EMA; Health Canada; the India National Institute of Biologicals (NIB); the Japan Ministry of Health, Welfare and Labour/Pharmaceutical and Medical Devices Agency; the South Korea Ministry of Food and Drug Safety (KFDA, previously known as the FDA); the Singapore Health Sciences Authority; Swissmedic; and the US FDA, convened to form an integrated group with the goal to discuss best practices in the regulation of cell- and gene-based therapies and to support and tend achieve greater harmonisation. Nevertheless, until such harmonised guidelines are available, industry sponsors can expect significant challenges to meet the requirements of individual health authorities and thus should have a thorough understanding of the regulatory landscape while considering filing requirements and devising their market access and regulatory strategies (Levine et al., 2017).

To address some of these challenges, the FDA proposed the creation of two new databases that will allow to access safety and manufacturing information across the multiple applications received for anti-CD19 CAR modified T cells. From the IND applications received thus far, the FDA has data on at least 275 patients who have been treated with CAR-T cell therapies targeting CD19 and who have on aggregate received more than 500 individual doses. During a meeting of the RAC, organised by the NIH Office of Biotechnology Activities, to oversee the clinical development of gene therapies, the medical officer, Maura O'Leary, in the FDA's Office of Cellular, Tissue and Gene Therapies at the Center for Biologics Evaluation and Research, explained that by analysing safety and chemistry, manufacturing and controls (CMC) data across multiple investigational new drug applications (INDs), the FDA could 'establish a safety profile for anti-CD19 CAR-T cell therapies'. The main goal of this project is to collect trial data

across all submitted INDs, analyse them and utilise the same for the benefit of the industry sponsors and FDA (Mezher, 2016).

One of the main drivers of the FDA's desire to build this database is that the sample sizes of studies supporting individual INDs for anti-CD19 CAR-T cell therapies are too small to paint a complete picture of their safety versus their benefit characteristics. However, by building a central database for safety and CMC across all INDs within the same class, the FDA would be able to 'build risk-prediction and risk-mitigation models (to) advise sponsors on these safety issues' (Mezher, 2016). The CMC data will enable to identify certain steps in the manufacturing process that might significantly influence the intrinsic safety of a product. Through the database, the FDA hopes to answer some lingering questions about CAR-T cell therapies, such as optimal and maximal tolerated doses, response based on disease type, disease state and percent BM involvement, as well as any correlation that exist between CAR-T cell levels and cytokine levels which would in turn affect patient outcomes and adverse events (mainly CRS and neurological events that have been reported with CAR-T cell therapies). Other key questions that could be answered include whether there are any interaction between demographic features and disease characteristics that would predispose to a specific adverse event or whether concomitant treatments, such as tocilizumab, etanercept or corticosteroids, influence the characteristics of adverse events.

The FDA plans to roll out the pilot in three overlapping phases (Mezher, 2016):

- Phase I – data collection (underway)
- Phase II – storage of the data in High performance Integrated Virtual Environment (HIVE) database using an integrated data format that enables cross study/cross IND data queries
- Phase III – data analysis from HIVE database from cross study/cross IND by FDA

Currently, the FDA is in the testing phase on the use of this database for safety queries. Once this initial testing is complete, the FDA will ask additional sponsors to provide the clinical data previously collected through IND safety reporting such that these data could be included in the database.

The above is an example of how regulatory authorities are willing and committed to work towards the same goal of getting these highly innovative therapies to patients faster.

COMMERCIAL, VALUE AND ACCESS CHALLENGES FOR THE SUCCESSFUL ADOPTION OF CAR-T CELL THERAPIES

At the time of writing, the initial CAR T products that received US FDA approval have been available less than a year. When thinking of the future commercial and business challenges, many practitioners in the field look to an important historical case study that of the cell-based therapy Provenge (sipuleucel-T), an autologous dendritic cellular immunotherapy for patients with advanced castrate-resistant prostate cancer, which

received regulatory approval but which ultimately failed commercially due to manufacturing challenges coupled with questionable patient benefits given the unavoidable risk of the new treatment, a questionable market access and reimbursement model, as well as the approval shortly thereafter of conventional drugs, abiraterone acetate (Zytiga), for example, from J&J for the same indication and patient population; all these factors resulted in Dendreon, the manufacturer of Provenge, quickly filing for bankruptcy after the launch of the new product (Grover, 2014).

While the main current commercial focus is on autologous CAR T therapies that involve the use of either academic or centralised manufacturing centres, some in the field are more focused on the creation of 'off the shelf' allogeneic CAR T products and have questioned the merit of centralised manufacturing. This is leading to a degree of uncertainty as to what the ultimate commercial model will look like for the next generation of CAR T and adoptive cell therapies. It is the opinion of these authors that regardless of the type of therapy platform (autologous vs. allogeneic), the key drivers for the successful adoption of CAR-T cell therapy will be the creation of an integrated and viable commercial model in terms of manufacturing, supply chain, reimbursement, clinical and translational access. Few business models have been proposed for the commercialisation of CAR-T cell therapies that can be categorised broadly under centralised or decentralised model of manufacturing options (Walker and Johnson, 2016; Malik and Durdy, 2016). The advantages of the centralised model include manufacturing scalability, reproducibility of cell processing, limited regulatory hurdles, moderate capital expenditure and gain in economies with scalability (Malik and Durdy, 2016). However, this model also adds to the complexity of supply chain and logistics around product shelf life, stability and transportation (Malik and Durdy, 2016) (Walker and Johnson, 2016). A decentralised model with a single manufacturing centre colocated with the treatment facility enables the use of fresh, noncryopreserved products and tight control of the supply chain and logistics but has the disadvantages of limited scalability; lack of reproducibility of cell processing from centre to centre; regulatory hurdles of comparability data and validity of process changes; as well as high capital investment to set up multiple centres. A hybrid model for globalisation that utilises a centralised manufacturing unit to cater the needs of several regions or even countries (e.g., units serving the markets in North America, Europe or Asia) can eliminate some of the challenges posed by each model comprising regulatory hurdles and cross border shipping logistics. Regardless of the philosophy of the models that will evolve, the fundamentals will have to focus on efforts to radically reduce the overall manufacturing costs, incorporating and gaining regulatory comfort with aspects of adaptive manufacturing, setting new standards and guidelines on quality systems and embedding lessons from the clinical and translational data that will emerge from the initial commercial experiences.

In terms of pricing CAR T therapies, a natural comparator for pricing will be the cost of stem cell transplantation in patients with refractory/relapsing disease in

haematological malignancies. In the United States, the cost is cited as $350,000–400,000 USD for autologous transplants and up to $900,000 USD in allogeneic transplant (Bentley and Phillips, 2017). At the time of writing, the price of Kymriah in the treatment of paediatric refractory/relapsing ALL is $475,000 USD and for adults diagnosed with relapsed or refractory (r/r) large B-cell lymphoma after two or more lines of systemic therapy including DLBCL, high grade B-cell lymphoma and DLBCL arising from FL is to be finalised. The price of Yescarta in the treatment of adult patients with relapsed or refractory large B-cell lymphoma after two or more lines of systemic therapy, including DLBCL not otherwise specified, PMBCL, high-grade B-cell lymphoma and DLBCL arising from FL (TFL) is $373,000 USD (Beasley, 2018). Whether CAR-T cell therapy constitutes a bridge to transplantation or whether it will replace haematopoietic SCT to treat various forms of haematological malignancies is yet to be seen. In clinical trials, few patients who relapsed received SCT post CAR-T cell therapy. The cost of the new therapy for modeling or reference purposes should include not only all the direct costs, for example, manufacturing and raw material costs, but also the costs of prerequisite treatments such as lymphodepleting chemotherapy that is typically performed during the aphaeresis step, as well as hospitalisation costs and numbers of days of stay, the management of adverse events, and any other follow-up costs.

Will CAR-T cell therapy costs be accepted by payers in a highly budget-constrained healthcare environment? This is a key question that needs to be answered while considering the long-term benefits of these therapies. The approval of CAR T-therapies was on the basis of phase II trials in relatively small patient samples, without long-term, real-world follow-up. Durability of response is crucial to estimate the potential value of these therapies. To complicate this further, reimbursement can pose challenges as the bulk of payment needs to be dealt with upfront while the patient benefits are seen only later. On the day of FDA approval, Novartis announced a 'pay per performance' type of arrangement where CMS (Medicaid) needs not reimburse if a patient fails to respond in the first 28 days of treatment (Caffrey, 2018). The success of this model can be seen only with data available over time. Gilead reported that commercial payers were willing to cover the treatment for their CAR T therapy. However, there can be long waiting period for patients awaiting confirmation from their payers (Caffrey, 2018).

Currently the majority of the data available in the CAR-T cell arena has been generated in haematological malignancies utilising autologous T cells. In 2016, the reported treatment-related deaths in the Juno trial evaluating JCAR015 led to the termination of that CAR T (ROCKET) (Dangi-Garimella, 2017; Lerman, 2016), as well as emerging treatment related adverse events from other studies (Harris, 2017) created concerns in the field and prompted questioning of benefit risk for the CAR T platform as a whole. However, the US FDA evaluations and subsequent CAR T approvals has placed these findings into context, given the benefits to patients dramatically outweigh the risks in such indications with a very poor survival prognosis otherwise. Lastly, to sustain the

commercial model, pharmaceutical and biotechnology companies will need to continue to invest in optimizing and developing safe and effective CAR-T cells, work closely with regulators to develop relevant biomarkers and companion diagnostics to identify the patient subpopulations that are the most likely to benefit from the new treatment or to be the most at risk for treatment-related death, improve on manufacturing to radically reduce the cost of goods of the therapy to maximise their affordability while maintaining appropriate returns for adoptive cell product. In addition, developers also need to invest in the next generation of transformational therapies and pursue indications to include other malignant states, including solid tumours. While ongoing research in this space focuses on improving the safety and efficacy of autologous CAR-T cells to enable commercial success, the 'off-the shelf' use of CAR-T cells could reduce treatment lead times and reduce production costs; advances in this particular segment of research is already showing great promises (Walker and Johnson, 2016). However, to date, data on allogeneic CAR-T cells remain highly limited and thus it still is to be seen if remissions provided by allogeneic CAR-T cells are durable over long periods of time.

PERSPECTIVES

The year 2017 ushered in a new excitement in the field of cell and gene therapies with the approval of two CAR-T cell therapies in the United States in patients with haematological refractory/relapsing malignancies. In 2018 and beyond, we await to see how other global regulators assess the benefit risk of these potentially curative products and view the associated challenges described earlier. Unlike small molecules or biologics, where numerous development and regulatory precedent exist, CAR therapies need additional investigation in many areas including optimizing and understanding differences in modular architecture of the various constructs, defining target antigen, impact of manipulating the tumour microenvironment (especially in solid tumours), optimizing the cell manufacturing process, identifying optimal conditioning regimens for in vivo cell expansion and function, discover biomarkers to identify appropriate patients that will respond to these therapies as well as help manage their treatment and improving the overall CAR-T cell therapeutic potential, for example, by increasing the in vivo persistence of the engineered T cells and preventing cell fatigue.

To date, the success established in clinical trials with CAR-T cell therapies has been demonstrated most prominently in haematological malignancies targeted towards CD19. Within haematological malignancies, data in paediatric ALL have been striking and studies in patients with DLBCL have also been highly promising. The patient fatalities reported from the ROCKET trial (Juno Therapeutics) and the ZUMA-1 trial (Kite Pharmaceuticals) due to cerebral oedema in adult patients have led some to question the benefit-to-risk advantages, in particular, in conditions such as adult ALL. However, there is wide consensus that much more research is needed to understand the many different

factors that lead to an optimal and safe response with a CAR-T cell therapy, including research on the use of biomarkers to predict efficacy and adverse events (mainly CRS and neurotoxicity) and the influence of conditional chemotherapy on the in vivo expansion of T cells and response. It is emerging that the toxicity with these agents is likely a class effect as evidenced by on-target toxicities, with CRS and neurotoxicity reported with all of the agents currently undergoing clinical trial evaluation. Although the symptoms of CRS and timing of occurrence are somewhat consistent across the clinical trials, there is no clear consensus in the grading of CRS. Likewise, for neurotoxicity, the timing of occurrence and what percentage of patients will develop this, is not clear. Based on emerging clinical experience from global multicentre trials, many treatment centres have already reported success in adequately managing CRS. However, time and greater patient experience is needed to fully characterise the real-world safety profiles of these highly powerful therapies. Understanding the observed neurotoxicities, which are fatal in some extreme cases, remains a challenge, and more research is needed to decipher whether these are caused by the CAR-T cells themselves (e.g., variations in costimulatory domain, the ability of certain CAR-T cell therapies to cross the blood–brain barrier, use of conditioning agents, etc.) or the tumour microenvironment. Thus, there are opportunities to improve outcomes and toxicities, including standardisation of the preparatory lymphodepleting chemotherapy and the development of more informative grading scales for measuring CRS and its management. Although the use of biomarkers, beyond monitoring patient interleukin levels, to predict toxicity seems appealing, the need to develop much more sophisticated correlative science research and reverse translate from patients in real time in clinical trial settings is needed.

The arrival of novel gene editing tools such as CRISPR/Cas9 has now found their way in to the creation of the next generation of CAR T and other adoptive cell therapies (Ren and Zhao, 2017). How these novel approaches will be embedded into the creation of next generation products will be closely watched both from a scientific and societal/ethical viewpoint.

In China, adoptive cell therapy, as a medical technology in the clinical trial setting, is growing very rapidly (Liu et al., 2017). The number of clinical trials conducted with CAR-T cell therapies has progressively increased over the years, and currently, there are over 300 clinical trials investigating the use of CAR-T cell therapies in China, making it by far the leading country investing in CAR-T cell therapy research today (Celltrials.org website). In the United States and in Europe there have been within the past 5 years significant investments by pharmaceutical and biotechnology companies working collaboratively with academic centres to pivot from single-centre clinical trials with the objective to submit regulatory applications for approval of novel CAR-T cell therapies and ultimately commercialising them. 2017 and 2018 saw seismic activity in the life sciences biotechnology markets with the acquisition of two of the leading companies in the space of CAR T, Kite Pharmaceuticals by Gilead Sciences (Gilead Sciences Press

Release, 2017) and Juno Therapeutics by Celgene (Celgene Corporation Press Release, 2018), further validating that CAR T therapies are now being accepted as modalities that can be seen as attractive by large pharmaceutical organisations to deliver future growth and revenue for these companies. This activity has now led to other large pharmaceutical players entering the space, for example, Johnson & Johnson (Janssen Press Release, 2017).

The traditional pharmaceutical business and commercial model is being challenged with the advent of advanced cellular therapies. To date, the conventional big pharmaceutical company model to supply the market with small molecules and biologics has relied on a tried and tested method of developing and commercialising 'blockbuster' therapies, namely a rigorous process of research and development, in parallel engaging regulators at well-defined points in the development cycle, as well as established models to determine pricing and reimbursement and finally working with established systems of healthcare to provide those therapies. However, this model may not apply in the exact same way to CAR-T cell therapies. It is likely that companies vested in the space will have to rethink this model and devise other types of market access models. As an example, companies will need to think through the different elements that make up a service model for advanced cellular therapies as the 'product' is the 'process'. The end-to-end commercial process, starting with apheresing a patient to obtain T cells for autologous use, cryopreserving and shipping them to a centralised manufacturing facility, undergoing a process of cellular reprogramming, and eventually sending a finished, high-grade quality product back to the same patient, needs an integrated 'chain of identity' that is controlled end-to-end by the company producing the therapy and ensuring at the same time that the 'service model' is wired efficiently into the end user (in the case of CAR-T cell therapies in haematological malignancies, these will be for the majority of the BM transplant centres).

Although, at the time of this writing, two of the CAR T therapies were approved, and the manufacturing processes employed by each of the companies are inherently different, process improvement for cell selection, stimulation, enrichment, expansion, and scalability at a reduced cost and reduced lead time are areas that still remain to be improved. To this effect, the use of 'off-the shelf' CAR-T cells is speculated to offer the greatest promise to achieve the reduction of lead time and cost. However, much more clinical and safety data are still required to build on the initial experience in the handful of patients who have been treated with such allogenic CAR-T cell therapies, in particular to better understand the true risks for these patients to develop GVHD, as well as the persistence and durability of responses in patients to prevent disease relapses.

Another area that will require a change of industry mindset will be in terms of manufacturing processes and scale. Firstly, unlike small molecules and biologics, the concept of what is a dose of a CAR-T cell is not as simple as a threshold of a minimum number of cells. The quality of the incoming material from the patient will heavily determine the manufacturing outcome. As an example, in a patient with high blast disease and heavy

tumour burden, a more protracted manufacturing process may be required as compared to a patient with a lower disease burden. Likewise, patients with a low T cell count is a major contributor for manufacturing failures as reported in clinical trials (Bersenev, 2016). In such examples, aspects of 'adaptive' manufacturing may be required, tailoring the process to the patients' needs. This is very different to 'fixed' manufacturing for small molecules and biologics. As CAR-T cell therapies gain traction in the market, there will be a demand to make these therapies available globally, thus driving the need to focus on aspects of reducing the cost of goods and automating the current CAR-T cell process, which requires the use of expensive raw materials and a limitation on the number of patients cells that can be processed at any one facility, as well as being resource and capital intensive.

Whether these therapies will replace SCT or be used as a 'bridge' to transplantation to prolong the life of the patients' will determine the overall cost of treatment. Thus, long-term durability data of 5 year or more is needed. In addition, to sustain the commercial model, aside of continuous efforts to reduce costs as described above, expanding the utilisation in indications beyond haematological malignancies will be crucial. There is now a focused effort to develop both CAR-T cell and TCR therapies in solid tumour settings. However, very high hurdles remain that need to be overcome, both at the level of the constructs of the CARs and TCRs themselves, for example, making T cells more resistant to fatigue, enhancing T-memory cell subpopulations, as well as overcoming the more difficult tumour microenvironment challenges, both at the level of neoantigen presentation by the tumour cells and better managing HLA restriction (in the TCR setting).

In conclusion, over the last 5 years, we have witnessed a renaissance in adoptive cellular therapies, culminating with the approval of the first CAR-T cell products in the United States and further validation of this platform technology, at least initially in the setting of haematological malignancies, as a potential to transform the treatment of patients who have little hope left in their battle with refractory or relapsing malignancies, such as certain leukaemias and types of lymphoma. While the approval of two of the CAR-T cell therapies is monumental, noting that they have been decades in the making (starting with pioneering work in the HIV space), numerous challenges still lie ahead for practitioners and providers of these therapies. With these recent approvals of the first CAR T products and the continued interest in investing in clinical trials with these therapies, the biotechnology and pharmaceutical industries will continue their quest to better improve the current generation of CAR-T cell products, not only in terms of improved benefit-risk outcomes but also with more attractive models of improved manufacturing of these therapies. As these therapies gain acceptance in the armamentarium of existing treatment modalities, the improved commercial delivery of these products will follow for the ultimate benefits of patients.

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