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Introducing innovative cellular therapies into the clinic: a 2-year retrospective experience of a chimeric antigen receptor T-cell programme at a single centre in Switzerland

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Summary

AIM OF THE STUDY: Chimeric antigen receptor T (CAR-T) cells are a powerful form of immune-cell therapy for patients with relapsed/refractory B-cell lymphoma and acute B lymphoblastic leukaemia. CAR-T cells have been commercially available in Switzerland since 2018. Because of the complexity and costs of this treatment it is critical to review patient outcomes in real-world settings, to examine whether the promising results from pivotal trials can be reproduced and to identify clinical parameters that determine their efficacy.

METHODS: Here we present results of a retrospective study analysing outcomes of patients treated with CAR-T cells in a single academic centre in Switzerland during the first two years after commercial approval (BASEC-No. 2020-02271). Cytokine release syndrome (CRS), immune-cell associated neurotoxicity syndrome (ICANS), responses to treatment, ancillary laboratory studies and administrative specifics of CAR-T treatment were examined and are discussed.

RESULTS: From October 2018 to August 2020 CAR-T cell therapy was evaluated in 34 patients, mostly with relapsed/refractory aggressive B-cell lymphoma (87% had refractory disease). Thirty-one patients underwent leukapheresis. Three of 31 patients (9.6%) died of rapid disease progression before the CAR-T cell product was delivered, two patients were enrolled into a clinical trial, three patients were not given CAR-T cells for other reasons. Ultimately, 23 patients were infused with a commercial CAR-T cell product and included in this analysis. Fourteen (61%) patients received bridging therapy while waiting for a median of 41 days (range 31–62) for delivery of the CAR-T cell product. Toxicity and severe side effects were rare (CRS >3 in 13%, ICANS > grade 3 in 10% of patients), manageable and resolved completely thereafter. The best overall response rate was 65%, with complete responses in 38% of lymphoma patients. At 12 months postinfusion, 61% of patients were alive and 35% progression free. With a median follow-up of 14 months, 13/23 (56%) patients were alive at the time of writing.

CONCLUSION: CAR-T cell therapy proved to be safe and manageable under adequate hospital conditions. Outcomes resemble results from pivotal trials. The majority of patients was heavily pretreated and refractory at the time of CAR-T cell infusion. Patient selection, time point of leukapheresis, bridging strategies and timing of CAR-T cell infusion may be critical to further improve outcomes.

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Introduction

Patients with aggressive non-Hodgkin's lymphoma, including diffuse large B-cell lymphoma (DLBCL) and high-grade B-cell lymphoma, who relapse after or are refractory to first-line immunochemotherapy have an unfavourable prognosis with <50% being cured with high-dose chemotherapy and autologous stem cell transplantation (ASCT) [1]. A large proportion of patients are not eligible for ASCT because of comorbidities, chemorefractory disease, or relapse following prior ASCT [2]. For these patients there is an unmet need to improve outcomes [3,4]. CD19-directed chimeric antigen receptor-T (CAR-T) cell therapy provides a new approach as clinical trials have demonstrated high response rates after failure of several lines of conventional chemotherapy and suggest that some patients may even be cured [5–8].

In Switzerland, the first CAR-T cell product, tisagenlecleucel (tisa-cel; CTL019; Kymriah®), was approved in October 2018 by Swissmedic, the national regulatory and supervisory authority for drug and medicinal products. Based on the pivotal phase II JULIET trial [9], tisa-cel is currently available for patients with DLBCL after two or more lines of immunochemotherapy including anthracyclines and a CD20-antibody. Concurrently, tisa-cel has been approved for paediatric patients and young adults aged ≤25 years with relapsed/refractory acute B-lymphoblastic leukaemia (B-ALL), on the basis of the ELIANA trial

cohort [8]. In April 2019, axicabtagene ciloleucel (axicel, Yescarta®) was approved for patients with DLBCL and primary mediastinal large B-cell lymphoma, based on the results of the ZUMA-1[10]. Within the last year, two additional CAR-T cell products have been approved in Switzerland, brexucabtagene autoleucel (KTE-X19; Tecartus®) for relapsed/refractory mantle cell lymphoma, based on the results of the ZUMA-2 study [12], and idecabtagene vicleucel (ide-cel; Abecma®) for relapsed/refractory multiple myeloma, based on the results of the KarMMA trial [13]. Finally, in March 2022, lisocabtagene maraleucel (liso-Cel; Breyanzi®) was approved for aggressive lymphomas based on the results of the TRANSCEND study [11].

All currently commercially available CAR-T cell products are generated from patients' autologous mononucleated or selected T cells, which are obtained by leukapheresis at the treatment centre. Cells are shipped fresh or frozen to the manufacturing plant, where they are genetically modified by retro- or lentiviral transduction and equipped with the so-called chimeric antigen receptor. The CAR acts as a receptor protein that combines antigen binding via an extracellular single-chain variable fragment with T-cell activating functions via the intracellular T-cell signalling domain, augmented by a costimulatory molecule to enhance T-cell potency. As mode of action, the autologous CAR-T cell is able to identify and attack malignant as well healthy antigen-expressing cells – regardless of the prior specificity of the endogenous T-cell receptor and without its interaction with human leucocyte antigen (HLA) molecules [14]. Once infused following lymphodepleting chemotherapy, activated CAR-T cells massively expand *in vivo*, which may be accompanied by an excessive immune response, called cytokine release syndrome (CRS), and other side effects.

Many aspects of this cellular therapy are novel to all involved parties and demand a close interaction and collaboration between (1) patient, referring haemato-oncologist, haemato-oncologist and cellular therapy team at the CAR-T cell treatment site, including an apheresis unit and clinical cell laboratory; (2) physicians of several specialties at the treatment site involved in risk management; (3) treatment site, pharmaceutical companies and insurance companies; and finally (4) insurance companies and pharmaceutical companies. Whereas initially only a few selected centres were able to offer this new form of cellular treatment, by now several centres in Switzerland are committed to becoming CAR-T cell treatment sites in the near future. A broader availability of CAR-T cell sites may be necessary since the number of approved CAR-T products is rapidly increasing, opening this treatment opportunity to a broader patient population.

Here, we present the experience of establishing a CAR-T cell programme at a large University Hospital in Switzerland. We highlight administrative, regulatory and medical hurdles encountered and report outcomes of consecutive patients treated with CAR-T cells during the first 2 years after CAR-T cell approval.

Methods

Adult patients evaluated for anti-CD19 CAR-T cell therapy at the University Hospital Zurich between October 2018 (date of first approval of CAR-T cell therapy in Switzerland) and August 2020 were retrospectively analysed. The use of CAR-T cell therapies was in accordance with the official Swissmedic approval of the respective CAR-T cell product. Basic information was retrieved from the documentation files of the data management for cellular therapies. Data were supplemented with information from the CAR-T cell therapy coordinators and patient charts. The study was approved by the local ethics committee (BASEC Nr. 2020-02271) and conducted in accordance with the Declaration of Helsinki. Patients <18 years of age or who refused their general research consent were excluded.

Handling of cells

Leukapheresis to collect mononucleated cells for CAR-T cell production was performed using a Spectra Optia device (Terumo BCT, Lakewood, USA) according to the requirements stated by the company. CAR-T cells were produced at the manufacturing plant of the respective company. CAR-T cells were thawed and infused 48–72 hours after a 3-day course of lymphodepletion with fludarabine (25 mg/m^2) and cyclophosphamide (250 mg/m^2). The target dose of 2 × 10^6 CAR-T cells per kilogram body weight was reached in all patients.

Factors potentially affecting outcomes

The following clinical variables were retrospectively analysed:

- Time-span from addressing insurance companies (date of initial correspondence) until approval of cost coverage of the treatment.
- Time from placing the order until receipt of the CAR-T cell product at the hospital.
- Number of prior treatment lines; bridging therapy between leukapheresis and CAR-T cell infusion.
- Disease status at the time of patient evaluation and initiation of the CAR-T cell process.

Outcomes following CAR-T cell therapy

Response to treatment and remission status for B-cell lymphoma was based on positron emission-computed tomography (PET-CT) results 3 months after CAR-T treatment according to Lugano staging [15]. Among patients with B-ALL, complete remission was defined as complete morphological response (<5% blasts in bone marrow).

Side effects of CAR-T cells and their treatment

Cytokine release syndrome (CRS) and immune cell associated neurotoxicity syndrome (ICANS) were assessed and graded according to the consensus criteria of the American Society for Transplantation and Cellular Therapy (ASTCT) twice per day during hospitalisation (minimum of 10 days after CAR-T infusion) [15]. CRS was diagnosed and scored based on the occurrence of fevers, hypotension and hypoxia. Grading ICANS involved assessment of the 10-point "immune effector cell-associated encephalopathy" score, levels of consciousness, occurrence of seizures, motor findings and increased intracerebral pressure. CRS, ICANS and other toxicities were treated according to

ASTCT recommendations with the anti-interleukin-6 (IL-6) receptor antibody tocilizumab and/or steroids.

Ancillary laboratory assessments

The following laboratory parameters were regularly assessed within routine laboratory studies: IL-6 (serum upper limit <7pg/ml (Cobas 8000, Roche, Rotkreuz, Switzerland)); c-reactive protein (CRP) (normal <5mg/l); lactate dehydrogenase (LDH) (normal 240–480 U/l). Measurements of peak circulating CAR-T cells in the blood were performed by flow cytometry (CAR Detection Reagent, Miltenyi Biotec, Bergisch Gladbach, Germany; CAR = chimeric antigen receptor), once this kit was commercially available in 2020.

Statistical analysis

Statistical analyses were performed using Prism 8.0 (GraphPad Software, San Diego, USA) and R version 4.0.3. For comparison of ordinal data we applied the Mann-Whitney-U test and Wilcoxon rank sum exact test. The alpha level was set at 0.05. Estimation of survival was done by the Kaplan-Meier method using R.

Results

General aspects of CAR-T cell therapy

Figure 1 displays key processes and interactions around CAR-T cell therapy.

In Switzerland CAR-T cell treatment sites are required to be JACIE (Joint Accreditation Committee ISTC EBMT) accredited. Currently, site selection for specific CAR-T cell products is directly negotiated between the pharmaceutical company and the hospital. Treatment centres must have access to an apheresis facility, a cellular processing laboratory and an appropriate storage facility for gene-modified cellular products. The complex onboarding process involves contract negotiations (regulating order and cancellation conditions; billing; data protection of health-related patient data), training of site staff and audits of the facilities. Finally, the hospital has to perform intensive risk management training, as side effects are common and often require interdisciplinary management including neurological assessments and intensive care treatment.

Patient selection and characteristics

In total, 34 patients were evaluated for CAR-T cell therapy at our centre from 10 October 2018 to 08 August 2020. As displayed in figure 2, 23/34 patients were ultimately infused with a commercial CAR-T product, whereas two pa-

Figure 1: General aspects of establishing a CAR-T cell programme include the qualification and onboarding procedures, which involve both treatment centre and pharmaceutical company (pink); reimbursement issues, patient selection and referral strategies, interdisciplinary risk management, which involve mostly the CAR-T cell treatment centre (blue); and approval, reimbursement issues, registries, as well as the production process *per se*, which is organised and managed by the pharmaceutical company.

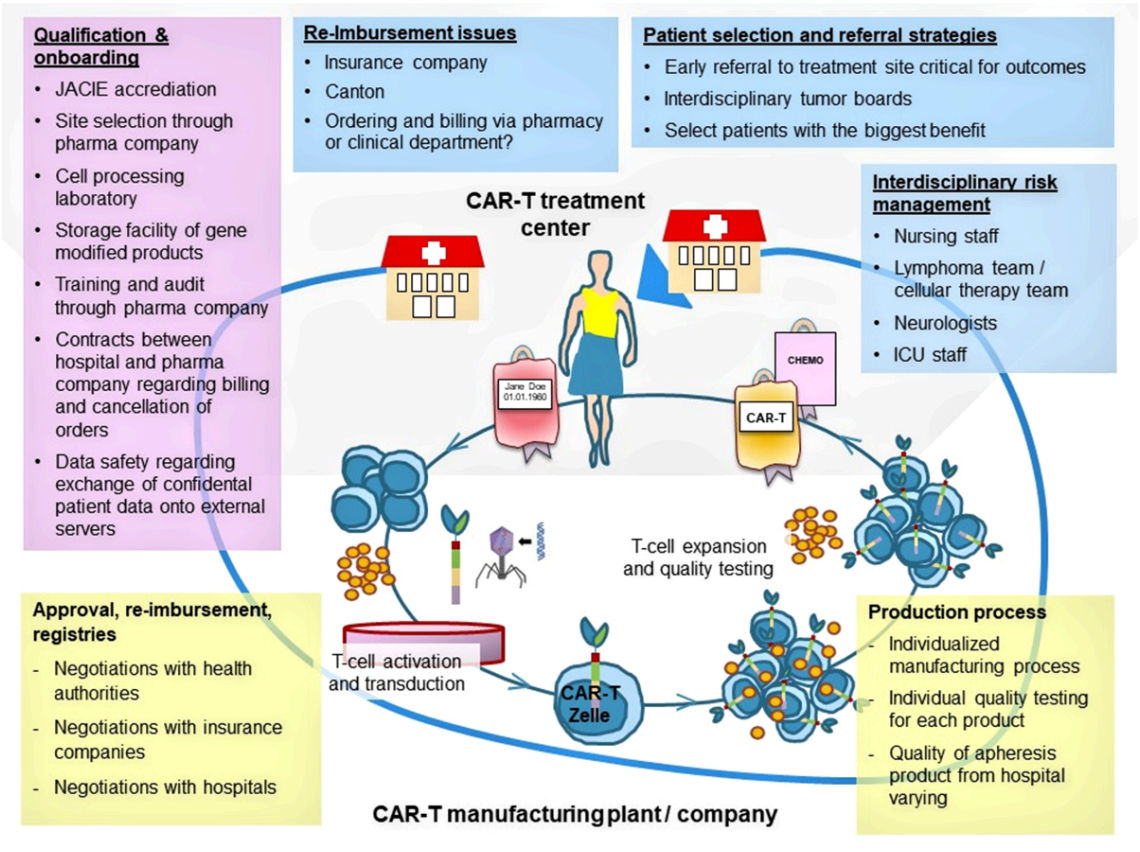
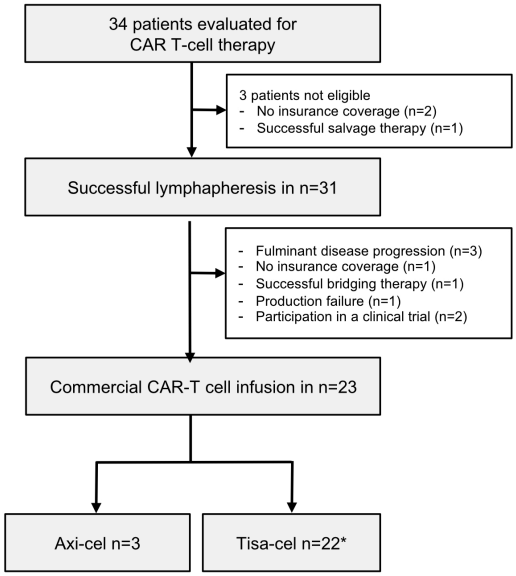


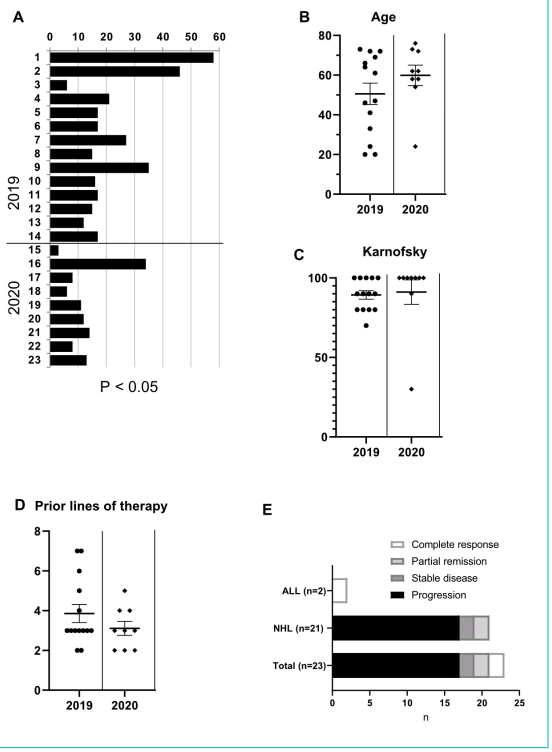
Figure 2: Patient evaluation, selection, leukapheresis, and treatment with CAR-T cells at our single centre after the approval of CAR-T cells, between November 2018 and August 2020. Of 34 patients who were evaluated for CAR-T cell therapy, 3 patients did not undergo lymphapheresis because of denial of insurance coverage (n = 2) or successful salvage therapy (n = 1). Thirty-one patients underwent lymphapheresis. Twenty-three of 31 patients were infused with CAR-T cells, and 8 patients did not reach the infusion date for various reasons.



tients achieved a complete remission with salvage/bridging therapy and were not taken to CAR-T cell consolidation; three patients were denied insurance coverage (one of them still underwent leukapheresis); three had rapid lymphoma progression and died before infusion of the CAR-T cell product, in one patient the production of CAR-T cells failed, and two patients were enrolled into a phase III clinical trial and were thus excluded from this analysis. Of the remaining 23 patients, 20 received tisa-cel, and 3 were treated with axi-cel.

The imbalance of tisa-cel and axi-cel in our patient cohort was due to the fact that tisa-cel was approved 6 months earlier, and our site was opened for axi-cel only later. Of note, certain clinical and logistic factors, such as central nervous system (CNS) involvement and flexibility in timing of the leukapheresis may influence treatment decisions regarding the choice of the CAR-T cell product.

Figure 3: (**A)** Time from requesting reimbursement of CAR-T cell therapy until confirmation of cost coverage by the health insurance. The Y-axis is arranged in chronological order. Patients 1—14 were treated before January 2020, the date of the decision of the Federal Office of Public Health of the Swiss Confederation to include CAR-T cell therapy in the list of obligatory benefits to be covered by the insurance. **(B)** Age. (**C)** Karnofsky performance status. **(D)** Prior lines of therapy in patients treated in 2019 vs 2020. **(E)** Remission status at the time of CAR-T cell infusion in patients with acute lymphoblastic leukaemia (ALL), non-Hodgkin's lymphoma (NHL) and the total cohort.



When CAR-T cells first became available in Switzerland, the situation regarding their reimbursement was not clarified, and securing cost coverage from the insurance company and cantonal office was time-consuming with a median of 17 days in 2019 (range 6–58) from requesting to obtaining cost coverage. This process improved in 2020 with inclusion of CAR-T cell therapies in the Federal Office of Public Health's list of reimbursed treatments to be covered by the obligatory health insurance (median 11 days, range 3–34 days) (fig. 3A).

Table 1:
Patient characteristics.

		Total (n = 23)	NHL (n = 21)	B-ALL (n = 2)
Median age (range)		61 (20–76)	62 (20–76)	22 (20–24)
Female, n (%)		5	5 (23.9 %)	0
Disease status at CAR-T cell infusion, n (%)				
Disease status at CAR-T cell infusion, n (%)	Progressive disease	17 (73)	17 (80)	0 (0)
	Stable disease	3 (13)	2 (10))	1 (50)
	Partial remission	2 (9)	2 (10)	0 (0)
	Complete remission	1 (4)	0 (0)	1 (50)
Number of prior treatment lines, mean (range)		3.6 (2–7)	3.6 (2–7)	4 (3–5)
History of CNS involvement		2	2	0
CAR-T cell product	tisa-cel	20	17	2
	axi-cel	3	3	0
Median observation period, d (range)		412 (4–798)	403 (4–798)	593 (412–775)

B-ALL: B-cell acute lymphoblastic leukaemia; CNS: centralö nervous system; NHL: non-Hodgkin's lymphoma

Table 1 and Figure 3B-3E provide details on patient characteristics by year.

The median age of the total cohort was 61 years (range 20–76), and 76% of patients were male. Most patients had lymphoma (21/23), whereas only two B-ALL patients were treated with CAR-T cells during this time period. Two of the lymphoma patients had a history of CNS involvement (but no active disease at the time of CAR-T cell infusion); the two B-ALL patients had no CNS involvement. Karnofsky performance status was between 70 and 100%, except for one patient with morbid obesity and fulminant disease, who was severely disabled (formally Karnofsky 30%) (fig. 3C). Patients were heavily pretreated with an average number of 3.6 prior lines of therapy (range 2–7) for NHL and 4 (range 3–5) for B-ALL. The maximum number of prior lines of therapy was higher in 2019 (average 3.8, range 2–7) than in 2020 (average 3.2, range 1–4) (fig. 3D, p = 0.546). Sixty-one percent of patients (14/23) required bridging therapy because of symptoms or progression of disease. Yet at the time of CAR-T cell infusion, 87% (20/23) of the patients had refractory disease (17 patients with progressive and 3 with stable disease).Two patients achieved a partial remission and one a complete remission with bridging treatment (fig. 3E).

Leukapheresis and manufacturing of CAR-T cells

CAR-T cell products are ordered via an online portal of the respective pharmaceutical company. Currently, for production of tisa-cel, cells are cryopreserved at the treatment site before shipping to manufacturing facilities in the United States or Europe (including a site in Switzerland). For all other approved (and soon to be approved) CAR-T cell products fresh cells are picked up by a courier and frozen at a centralised facility in Europe prior to shipment to the manufacturing plant, or in the US at the manufacturing site. The timing of leukapheresis is a critical factor in the treatment process: collection of T cells for CAR-T cell production should be performed at a time period without chemotherapy and other T-cell suppressive/depleting agents (minimum 3–5 days for steroids; low-dose cytotoxic agents: 14 days; lymphotoxic chemotherapeutic agents such as clofarabine, fludarabine: 2 months; immunosuppressive agents 14 days). Identifying the optimal time point for lymphocyte collection in patients who are often heavily pretreated, present with severe lymphopenia and may urgently require lymphoma-directed treatment can be challenging. In our patient cohort the median absolute number of T cells per microlitre blood at the time of lymphocyte collection was 498 (range 193–2746; normal range: 530–1790) (fig. 4A).

Of note, in the majority of patients there was a shifted, abnormal CD4/CD8 ratio with a dominance of $CD8^+$ T cells in the blood (fig. 4B). In our cohort, in all but one patient one single apheresis session yielded sufficient T cells (median 5.43 x 10^9 $CD3^+$ T cells absolute; range 0.76–28.8 x 10^9, fig. 4C). Product information was available only for tisa-cel (for axi-cel all product features were "in range"). A median of 2.971 x 10^6 CAR-T cells/kg bodyweight (range 1.6–12.96 x 10^6, fig. 4D) with a median viability of 86% were delivered for administration. CAR-T cell production failed in one patient.

A median of 41 days (range 31–62) passed from placing the order to the day of CAR-T cell delivery to the treatment centre (fig. 4E). The time from production start to delivery of the CAR-T cell product to the treatment site was shorter, overall very consistent (3.5–4 weeks) and includes *in vitro* manufacturing of approximately 9–10 days, quality testing, product release for infusion and shipment logistics.

CAR-T cell therapy outcomes

The overall response rate of all treated patients was 47% (11/23) at 3months after CAR-T infusion (fig. 5A), with a complete response rate of 28.5% (6/21) for lymphoma patients.

Both patients with ALL were in complete remission with minimal residual disease negate at this time. The best overall response rate to CAR-T cell treatment for all patients achieved at any time point was 65% (15/23) and best complete response rate was 43% (10/23). These outcomes are in line with what has been reported in for the pivotal studies of tisa-cel, axi-cel, and liso-cel (table 2), and published real-world experiences (table 3).

Of note, of the 81% (17/21) of lymphoma patients with disease progression at the time of CAR-T cell infusion, 23% (4/17) achieved a complete response at 3 months after the infusion. The number of patients who converted from partial to complete response at 3 months after CAR-T cell infusion was 66 % (2/3). Within the first year after CAR-T cell therapy, 65% (15/23) of the patients suffered from disease progression (fig. 5B). Overall survival at 1 year after CAR-T cell infusion was 61% for lymphoma patients (fig. 5C). The median observation period was 412 days (range 4–798).Seven patients never responded to CAR-T cells and two patients had disease progression after showing at least a partial response at 3 months. All deaths were related

Figure 4: (A) Absolute T-cell counts in the peripheral blood prior to leukapheresis for CAR-T cell production. Grey marks the normal range (530–1790 T cells per microlitre). (B) CD4/CD8 ratio displaying a dominance of CD8+ T cells in the blood. Grey marks the normal range (1–5). (C) T-cell yield after leukapheresis. (D) Number of transduced CAR-T cells/kg bodyweight infused into patients treated with tisa-cel. These numbers are not available for patients treated with axi-cel.). Turn-around time from placing the order to delivery of the CAR-T cell product to the treatment site in 2019 and 2020.

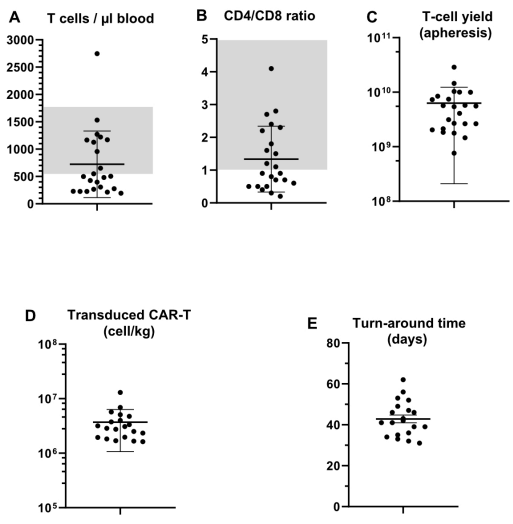


Table 2:
Response to CAR-T cell therapy in clinical trials.

Author	Trial	CAR-T product	n	Median FU	ORR	CR	Median OS	OS	Median PFS	PFS	med DOR
Locke	ZUMA-1, 2 y FU	Axi-cel	ITT n = 119; mITT n = 108; Evaluable n = 101	27.1 m	83%	58%	Not reached		5.9 m		11.1 m
Jacobson ASH 2020 1187	ZUMA-1, 4 y FU			51.1 m			ITT 17.4 m; mITT 25.8	ITT @4 y 41%; mITT @ 4y 44%			
Schuster 2019	JULIET	Tisa-cel	Infused n = 93	14 m	52%	40%				@12 m 65% (CR pts: 79%)	
Jaeger ASH 2020 1192	JULIET		Infused n=115	40.3 m			All: 11.1 m; CR pts: NR	@12 m: 48.2%; @ 24 m 40.4%; @36m 36.2%	CR pts: NR	@24 m 33%; @ 36 m 31%; Responders: @24+30 m: 60.4%	
Abramson 2020	TRANSCEND NHL 001	Liso-cel	L'aph n = 344; Infused n = 294; Liso-cel n = 269; OOS n = 25; Evaluable n = 256	18.8 m (for all 344) from L'aph	73%	53%	21.1 m	@6 m 74.5%; @12 m 57.9%	6.8 m	@6 m 51.4%; @12 m 44.1%	NR

L'aph: leukapheresed; m: months; ITT: intent-to-treat; mITT: modified intent-to-treat; FU: follow-up; ORR: overall response rate; CR: complete remission; OS: overall survival; PFS: median progression-free survival; DOR: duration of response; NR: not reached

to progression or relapse of the underlying B-cell neoplasia. Treatment of relapsed disease after CAR-T cell therapy was based on individual decisions and included conventional regimens (e.g., R-Gem-Ox, radiotherapy) and more targeted approaches (e.g., venetoclax, polatuzumab, pembrolizumab).

In order to stratify patients retrospectively into those with the biggest benefit versus those who may not achieve disease remission we analysed baseline LDH levels before CAR-T cell infusion, as an indicator of tumour burden. Maximum LDH levels in the week prior to CAR-T cell infusion were significantly higher in non-responders (mean 1008 U/l, range 197–2176) compared with responders (mean 471 U/l; range 293–1222, $p < 0.05$, fig. 5D). Other laboratory baseline characteristics such as IL-6, ferritin or CRP showed no significant difference between patients with disease progression at 3 months and patients in remission (data not shown).

Side effects and toxicity of CAR-T cells

CRS of any grade was found in 74% (17/23) of the patients, CRS of grade ≥3 in 13% (3/23). Two of the latter three patients received tocilizumab. The median onset of CRS was 2 days after CAR-T infusion (range 1–10). After day 10, none of the patients experienced CRS symptoms. Within our cohort, 30% (6/20) of tisa-cel patients developed ICANS (1/20 with grade 3), and oneof the threepatients treated with axi-cel (grade 3). ICANS was always accompanied by CRS. Overall, 13% (3/23) of patients were transferred temporarily to the intensive care unit. IL-6

Table 3:
Response to CAR-T cell therapy in "real-world" data collections.

Author	Group	CAR-T product	n	Median FU	ORR	CR	Median OS	OS	Median PFS	PFS	med DOR
Nastoupil LJ 2020	US Lymphoma CAR-T consortium	Axi-cel	L'aph n = 298; Infused n = 275	12.9 m from infusion	82%	64%	NR	@12 m 68%	8.3 m	@12 m 47%	NR
Jacobson 2020	Retrospective multi-centre	Axi-cel	Infused n = 122	10.4 m	70%	50%: @6 m 41%	NR	@12 m 67%	4.5; CR pts NR		11m
Vercellino 2020	French Lymphoma Study Association	Tisa-cel n = 49; Axi-cel n = 67	n = 116 identified	8.2 m	52.5%*	NA	NA	@6 m 78.5%; @12 m 67%	7.4 m	@12 m 47.2%	
Iacoboni 2021	GETH, GELTAMO Spanish Groups	Tisa-cel	L'aph n = 91; Infused n = 75	14.1 from infusion	60%	32%	10.7 m	CR pts; @12 m 93%	3 m	CR pts. @12m 87%; All: @6 m 33.3%; @12 m 31.7%	8.9 m in responders (CR/PR)
Sesques 2020	Single centre France	Tisa-cel n = 33; Axi-cel n = 28	L'aph n = 70; Infused n = 61	5.7 m from infusion	@1 m 63%; @3 m 45%; Best ORR 66%	@1 m 48%; @3 m 39%	11.8; CR pts. NR	@6 m 68%	3 m	@6 m 44%	PR pts: 1.8m; CR pts NR
Pasquini 2020	US cellular therapy registry CIBMTR	Tisa-cel	NHL: n = 155	11.9 m	61.8%	39.5	13.06	@6 m 70.7	4.21	@6 m 38.7	6.32
Stolz 2021	Single centre University Hospital Zurich	Axicel; Tisa-cel	L'aph: n = 31; Infused n = 25; Analysed n = 23	Total: 13.5 m; L: 13.2 m; ALL: 19.7 m	Total 65%; L: 61.9%; ALL: 100%	Total 43%; L: 38%; ALL: 100%	L: 17.2m; ALL: NR	L: @ 1y: 61.5%; @2 y: 44.9%; ALL: NR			

L'aph: leukapheresed; m: months; FU: follow-up; ORR: overall response rate; CR: complete remission; OS: overall survival; PFS: progression-free survival; DOR: duration of response; NA: not available; NR: not reached; L: lymphoma; ALL: acute lymphoblastic leukaemia

levels were monitored daily following CAR-T cell infusion and reached a peak between day 5 to 7. Baseline IL-6 levels (2.7 to 130 pg/ml) on the day of CAR-T cell infusion were not associated with the risk of developing CRS (data not shown). Patients with CRS had significantly higher IL-6 peak levels compared with those without CRS (median IL-6 peak 177.8 pg/ml in CRS patients vs 21.27 pg/ml in non-CRS patients, $p < 0.01$, fig. 5E). The median IL-6 peak in patients with ICANS was 1271 pg/ml and significantly higher than in those without ICANS (42.9 pg/ml, $p < 0.01$). All side effects resolved with adequate therapy without residual problems.

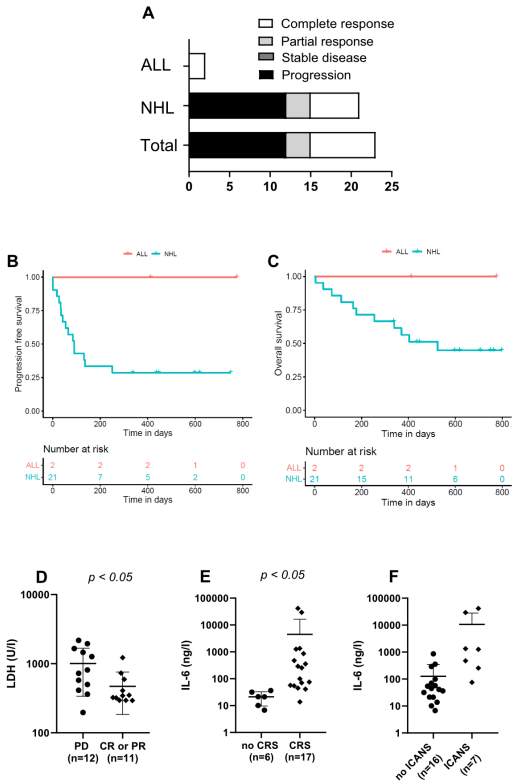
Monitoring CAR-T cells

Before monitoring CAR-T cells in the blood of patients by flow cytometry or PCR testing was established at our centre, we performed retroviral human immunodeficiency virus (HIV) PCR measurements as an indicator of retroviral DNA. Data are available for 17 patients between days 3 and 30 after CAR-T therapy, in 12 of whom we detected a positive retroviral signal at PCR level at least once during the observation period. We report this result as patients and physicians need to be aware and informed that following CAR-T therapy HIV screening may turn "false" positive.

From 2020 on, CAR-T specific flow cytometric assays for tisa-cel and axi-cel were established at our site. We present data of four patients to illustrate the dynamics of CAR-T expansion in the peripheral blood. To put CAR-T levels into clinical context the corresponding IL-6 and CRP levels, CRS grading and clinical parameters are also displayed. Figure 6A shows a 76-year-old patient with a relapsed lymphoma following ASCT who was treated with axi-cel.

He had an increase of IL-6 and CRP in the days following CAR-T cell infusion and developed CRS grade 1 and ICANS grade 3. Simultaneously, an expansion of CAR-T cells, comprising up to 57.4% of T cells in the blood, was detectable. He responded well to treatment and has been in a complete remission for >15 months at the time of writing. Figure 6B displays images and clinical parameters of a 58-year-old patient with chemorefractory disease. She tolerated tisa-cel treatment well with no CRS and no ICANS. CRP and IL-6 were both elevated, without a clear infectious focus, but CAR-T cells were detected only at lower levels in the blood. Her large lymphoma mass in the psoas major muscle resolved initially, but there was an early relapse at 3 months after cellular therapy. Patient C was a 69-year-old patient with relapsed DLBCL following ASCT, who was given tisa-cel following two cycles of salvage therapy with R-ICE, who had neither elevated inflammatory markers nor high levels of CAR-T cells in the blood, nor signs of toxicity. Yet he responded well to treatment and has been in a sustained complete remission for >20 months (fig. 6C). Patient D was a 24-year-old man with B-ALL, who suffered from a molecular relapse of disease following allogeneic haematopoietic cell transplantation. He did not respond to blinatumomab and donor lymphocyte infusions, but has been in a complete molecular remission for more than one year since he was infused with a single dose of tisa-cel (fig. 6D). He developed clinical signs of CRS, but displayed no increase of inflammatory blood markers, nor high numbers of CAR-T cells in the blood. These examples illustrate that there is no simple association that can be generalised between IL-6 levels, CRP, CAR-T cell counts, CRS manifestations and response to treatment.

Figure 5: (A) Response to treatment at 3 months after CAR-T cell infusion in patients with acute lymphoblastic leukaemia (ALL), non-Hodgkin's lymphoma (NHL) and the total cohort. (B) Kaplan-Meier estimates of progression-free survival, and (C) overall survival in patients with ALL and NHL. (D) Baseline levels of lactate dehydrogenase (LDH) in responders (complete [CR] and partial remission [PR] at 3 months after CAR-T cell infusion) versus patients with progressive disease at this time point. LDH level was obtained between day –7 and day –1 prior to CAR-T cell infusion. (E+F) Peak levels of interleukin-6 (IL-6) following CAR-T cell infusion in patients who developed cytokine release syndrome (CRS) (E) and immune-cell associated neurotoxicity syndrome (ICANS) (F) versus those without CRS/ICANS.



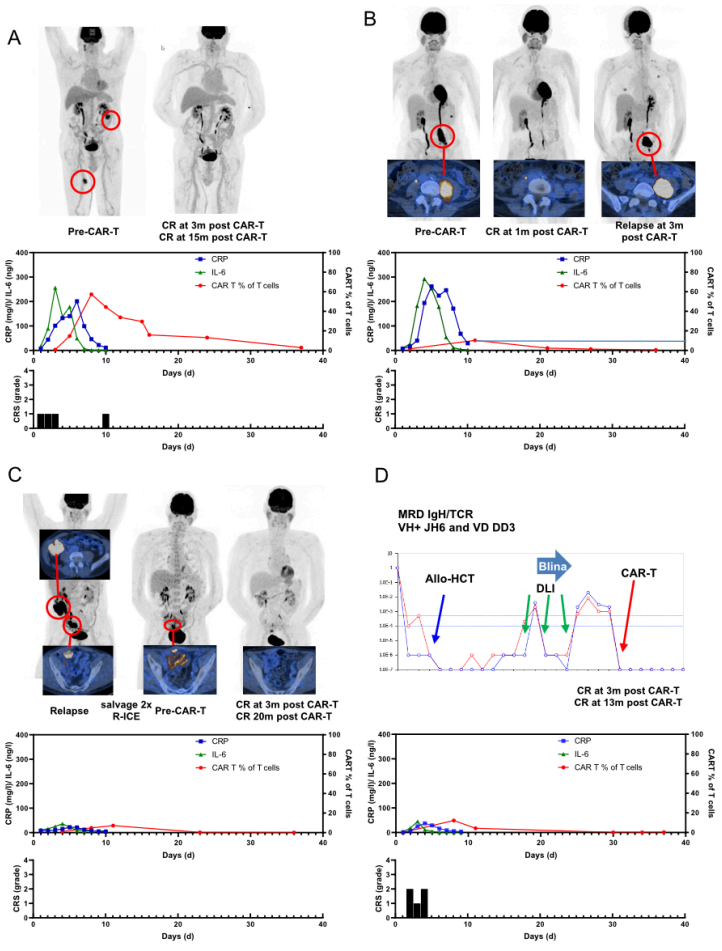
Discussion

CAR-T cells are a milestone in improving therapies for patients with relapsed and/or chemo-refractory B-cell malignancies, and may soon move into earlier lines of therapy. Available data from the pivotal studies and real-world registry data confirm the proof-of-principle that CAR-T cells can be highly effective and potentially even curative in some patients. Their efficacy and safety was reproducible in our cohort. Yet there remains room for improvement to increase the rate of those achieving long-term remissions following CAR-T cell treatment. One strategy for improving outcomes with currently available CAR-T cell products will rely on patient selection and timing of the treatment. Particularly patients with poor-risk genetics (e.g., double-hit, triple-hit) who do not respond to first-line chemotherapy have a very high chance of also not responding to salvage therapy and should be evaluated early for CAR-T cell treatment. Higher cumulative doses of lymphotoxic treat-

ments presumably impair the endogenous functions of autologous T cells, which are the basic ingredient for CAR-T cell production.

First published real-world data, in comparison with trial results, underline the importance of patient selection: patients with poor performance status and high LDH, both indicators of high tumour burden, who mostly require bridging therapy, have worse outcomes compared with those with ECOG <1, normal LDH and controlled disease [10]. Consistent with these reports, in our cohort high LDH levels pre-CAR-T-cell treatment were also predictive of disease progression at 3 months. Particularly during the first year of access to CAR-T cells, patients were heavily pretreated with up toseven lines of prior therapy. The high proportion of refractory patients in our lymphoma population may explain the shorter progression-free survival of 35% for lymphoma patients at 12 months compared with 65% in the JULIET-trial. The overall response rate at 3 months was 47% and best overall response rate was 65% in our cohort, which is comparable to results from the JULIET study. Best overall response rate was higher in ZUMA-1 (83%). Such differences between the ZUMA-1, JULIET and TRANSCEND trials, but also between trial and real world data, can partly be explained by patient selection strategies, and whether patients who needed bridging therapy were in- or excluded from the study. Accordingly, the proportion of patients with refractory disease was 30% in ZUMA-1 and 55% in the JULIET study, but as high as 87% in our unselected patient cohort. The need for bridging therapy is based on clinical judgement to decrease tumour burden and achieve symptom relief – but also the anticipated turn-over time from confirming the indication for CAR-T cell treatment until delivery of the finished product. Sixty-one percent of our patients were given bridging therapy. In comparison, 92% of the JULIET study cohort received bridging therapy whereas the ZUMA-1 study did not include patients in need of bridging treatment.

Figure 6: Time course of CAR-T cell detection in the blood during the first 40 days in representative patients. **(A)** 76-year-old pa-tient with relapsed lymphoma, treated with axi-cel and displayed an expansion of CAR-T cells up to 57.4% in the blood. (**B**) 58-year.old patient with chemorefractory disease, who had elevated CRP and IL-6, but not CRS and no ICANS following tisa-cel. CAR-T cells reached a maximum of 9.8% in the blood. **(C)** 69-year-old patient with relapsed DLBCL, treated with tisa-cel, who had neither elevated inflammatory markers nor high levels of CAR-T cells in the blood, nor signs of toxicity. (**D**) 24-year-old pa-tient with B-ALL, suffering from molecular relapse (as indicated by the IgG/TCR markers of minimal residual disease) following allo-geneic haematopoietic cell transplantation; he did not respond to blinatumomab and donor lymphocyte infusionswhen he was given a single dose of tisa-cel. He developed clinical signs of CRS, but displayed no increase of inflammatory blood markers, nor high numbers of CAR-T cells in the blood.Figure 6Time course of CAR-T cell detection in the blood during the first 40 days in representa-tive patients. (A) 76-year-old patient with relapsed lymphoma, treated with axi-cel and displayed an expansion of CAR-T cells up to 57.4% in the blood. (B) 58-year.old patient with chemorefractory disease, who had elevated CRP and IL-6, but not CRS and no ICANS following tisa-cel. CAR-T cells reached a maximum of 9.8% in the blood. (C) 69-year-old patient with relapsed DLBCL, treated with tisa-cel, who had neither elevated inflammatory markers nor high levels of CAR-T cells in the blood, nor signs of toxicity. **(D)** 24-year-old patient with B-ALL, suffering from molecular re-lapse (as indicated by the IgG/TCR markers of minimal residual disease) following allogeneic haematopoietic cell transplantation; he did not respond to blinatumomab and donor lymphocyte infu-sionswhen he was given a single dose of tisa-cel. He developed clinical signs of CRS, but displayed no increase of inflammatory blood markers, nor high numbers of CAR-T cells in the blood.



Three to 12 days after infusion, CAR-T cells become activated, expand *in vivo* and circulate through the bloodstream. The dynamics of *in vivo* expansion do not adhere to the pharmacokinetics of common drugs, but rather depend on tumour mass, lymphodepleting chemotherapy, and also multiple humoral inflammatory cytokines and growth factors, as well as endogenous T-cell parameters of immune reactivity [16]. Flow cytometry or PCR tests are tools to monitor CAR-T cells in the weeks and months following treatment [17, 18] and may help to guide treatment decisions in those not achieving a complete response [19]. We and other centres have started monitoring CAR-T cell activity *in vivo* either indirectly by assessing B-cell aplasia or hypogammaglobulinaemia, or directly by flow cytometry or PCR tests. As of now, the meaning of detecting or not detecting CAR-T cells in the blood stream at distinct time points is not fully clarified. We anticipate that in the near future such information may guide treatment decisions regarding subsequent salvage treatments in the case of CAR-T cell loss and failures, or immune modulating strategies in the case of CAR-T cell persistence but insufficient immune activity.

Toxicities, including CRS and ICANS, vary considerably among individual patients and CAR-T cell products. In

general, risk factors for CRS include high tumour burden and the presence of active infection at the time of infusion [20–22]. In patients with DLBCL, evidence is emerging that the interaction of several factors such as tumour burden, individual immune status, IL-6 levels and peak numbers of CAR-T cells will help to predict and manage treatment-related toxicities more effectively [23]. Also, in our patient cohort the mean IL-6 level was significantly higher in patients with CRS or ICANS than in those without. In general though, the incidence of severe forms of CRS (grade ≥3) in our experience was low and well manageable using established guidelines [24]. Our patient cohort also included patients with a history of CNS involvement – and these patients did not experience high-grade ICANS. This is in line with increasing experiences on the use of CAR-T cells in patients with CNS involvement, indicating that toxicity rates and outcomes are comparable in those with and without CNS involvement [25, 26].

In conclusion, CAR-T cell therapy is safe and well manageable in adequate hospital settings with a trained interdisciplinary team. Selected patients can benefit greatly from this potent treatment, but at the same time, CAR-T cells pose a strain on the health system. Now is a critical time for the community of haemato-oncologists to learn how to use these powerful therapies in a responsible manner and the most efficient way to bring greatest benefit at the lowest rate of complications to those in biggest need.

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Potential competing interests
All authors have completed and submitted the International Committee of Medical Journal Editors form for disclosure of potential conflicts of interest. AMM received speaker's honoraria as well as honoraria for consulting, advisory boards from Novartis, Kite/Gilead, Celgene/BMS, Janssen. No potential other conflict of interest was disclosed.

References

1. Philip T, Guglielmi C, Hagenbeek A, Somers R, Van der Lelie H, Bron D, et al. Autologous bone marrow transplantation as compared with salvage chemotherapy in relapses of chemotherapy-sensitive non-Hodgkin's lymphoma. N Engl J Med. 1995 Dec;333(23):1540–5. http://dx.doi.org/10.1056/NEJM199512073332305. PubMed. 0028-4793
2. Gisselbrecht C, Schmitz N, Mounier N, Singh Gill D, Linch DC, Trneny M, et al. Rituximab maintenance therapy after autologous stem-cell transplantation in patients with relapsed CD20(+) diffuse large B-cell lymphoma: final analysis of the collaborative trial in relapsed aggressive lymphoma. J Clin Oncol. 2012 Dec;30(36):4462–9. http://dx.doi.org/10.1200/JCO.2012.41.9416. PubMed. 1527-7755
3. Crump M, Neelapu SS, Farooq U, Van Den Neste E, Kuruvilla J, Westin J, et al. Outcomes in refractory diffuse large B-cell lymphoma: results from the international SCHOLAR-1 study. Blood. 2017 Oct;130(16):1800–8. http://dx.doi.org/10.1182/blood-2017-03-769620. PubMed. 1528-0020
4. Van Den Neste E, Schmitz N, Mounier N, Gill D, Linch D, Trneny M, et al. Outcome of patients with relapsed diffuse large B-cell lymphoma who fail second-line salvage regimens in the International CORAL study. Bone Marrow Transplant. 2016 Jan;51(1):51–7. http://dx.doi.org/10.1038/bmt.2015.213. PubMed. 1476-5365
5. Kochenderfer JN, Dudley ME, Kassim SH, Somerville RP, Carpenter RO, Stetler-Stevenson M, et al. Chemotherapy-refractory diffuse large B-cell lymphoma and indolent B-cell malignancies can be effectively treated with autologous T cells expressing an anti-CD19 chimeric antigen receptor. J Clin Oncol. 2015 Feb;33(6):540–9. http://dx.doi.org/10.1200/JCO.2014.56.2025. PubMed. 1527-7755
6. Kochenderfer JN, Somerville RP, Lu T, Yang JC, Sherry RM, Feldman SA, et al. Long-Duration Complete Remissions of Diffuse Large B Cell Lymphoma after Anti-CD19 Chimeric Antigen Receptor T Cell Therapy. Mol Ther. 2017 Oct;25(10):2245–53. http://dx.doi.org/10.1016/j.ymthe.2017.07.004. PubMed. 1525-0024
7. Neelapu SS, Locke FL, Bartlett NL, Lekakis LJ, Miklos DB, Jacobson CA, et al. Axicabtagene Ciloleucel CAR T-Cell Therapy in Refractory Large B-Cell Lymphoma. N Engl J Med. 2017 Dec;377(26):2531–44. http://dx.doi.org/10.1056/NEJMoa1707447. PubMed. 1533-4406
8. Maude SL, Laetsch TW, Buechner J, Rives S, Boyer M, Bittencourt H, et al. Tisagenlecleucel in Children and Young Adults with B-Cell Lymphoblastic Leukemia. N Engl J Med. 2018 Feb;378(5):439–48. http://dx.doi.org/10.1056/NEJMoa1709866. PubMed. 1533-4406
9. Schuster SJ, Svoboda J, Dwivedy Nasta S, Porter DL, Chong EA, Mahnke Y, et al. Phase IIa Trial of Chimeric Antigen Receptor Modified T Cells Directed Against CD19 (CTL019) in Patients with Relapsed or Refractory CD19+ Lymphomas. Blood. 2014 Dec;124(21):3087–. http://dx.doi.org/10.1182/blood.V124.21.3087.3087. 0006-4971
10. Locke FL, Ghobadi A, Jacobson CA, Miklos DB, Lekakis LJ, Oluwole OO, et al. Long-term safety and activity of axicabtagene ciloleucel in refractory large B-cell lymphoma (ZUMA-1): a single-arm, multicentre, phase 1-2 trial. Lancet Oncol. 2019 Jan;20(1):31–42. http://dx.doi.org/10.1016/S1470-2045(18)30864-7. PubMed. 1474-5488
11. Abramson JS, Palomba ML, Gordon LI, Lunning MA, Wang M, Arnason J, et al. Lisocabtagene maraleucel for patients with relapsed or refractory large B-cell lymphomas (TRANSCEND NHL 001): a multicentre seamless design study. Lancet. 2020 Sep;396(10254):839–52. http://dx.doi.org/10.1016/S0140-6736(20)31366-0. PubMed. 1474-547X
12. Wang M, Munoz J, Goy A, Locke FL, Jacobson CA, Hill BT, et al. KTE-X19 CAR T-Cell Therapy in Relapsed or Refractory Mantle-Cell Lymphoma. N Engl J Med. 2020 Apr;382(14):1331–42. http://dx.doi.org/10.1056/NEJMoa1914347. PubMed. 1533-4406
13. Munshi NC, Anderson LD Jr, Shah N, Madduri D, Berdeja J, Lonial S, et al. Idecabtagene Vicleucel in Relapsed and Refractory Multiple Myeloma. N Engl J Med. 2021 Feb;384(8):705–16. http://dx.doi.org/10.1056/NEJMoa2024850. PubMed. 1533-4406
14. Chmielewski M, Hombach AA, Abken H. Antigen-Specific T-Cell Activation Independently of the MHC: Chimeric Antigen Receptor-Redirected T Cells. Front Immunol. 2013 Nov;4:371. http://dx.doi.org/10.3389/fimmu.2013.00371. PubMed. 1664-3224
15. Lee DW, Santomasso BD, Locke FL, Ghobadi A, Turtle CJ, Brudno JN, et al. ASTCT Consensus Grading for Cytokine Release Syndrome and Neurologic Toxicity Associated with Immune Effector Cells. Biol Blood Marrow Transplant. 2019 Apr;25(4):625–38. http://dx.doi.org/10.1016/j.bbmt.2018.12.758. PubMed. 1523-6536
16. Neelapu SS, Tummala S, Kebriaei P, Wierda W, Gutierrez C, Locke FL, et al. Chimeric antigen receptor T-cell therapy - assessment and management of toxicities. Nat Rev Clin Oncol. 2018 Jan;15(1):47–62. http://dx.doi.org/10.1038/nrclinonc.2017.148. PubMed. 1759-4782
17. O'Hara MH, Stashwick C, Plesa G, Tanyi JL. Overcoming barriers of car T-cell therapy in patients with mesothelin-expressing cancers. Immunotherapy. 2017 Aug;9(9):767–80. http://dx.doi.org/10.2217/imt-2017-0026. PubMed. 1750-7448
18. Mueller KT, Maude SL, Porter DL, Frey N, Wood P, Han X, et al. Cellular kinetics of CTL019 in relapsed/refractory B-cell acute lymphoblastic leukemia and chronic lymphocytic leukemia. Blood. 2017 Nov;130(21):2317–25. http://dx.doi.org/10.1182/blood-2017-06-786129. PubMed. 1528-0020
19. Novo M, Sidiqi MH, Paludo J, Gandhi S, Kubusek J, Truong T, et al. Peak Lymphocyte Count after CAR T Infusion Is a Clinically Accessible Test That Correlates with Clinical Response in Axicabtagene Ciloleucel Therapy for Lymphoma. Blood. 2019 Nov;134 Supplement_1:4106 . http://dx.doi.org/10.1182/blood-2019-127094. 0006-4971
20. Davila ML, Brentjens RJ. CD19-Targeted CAR T cells as novel cancer immunotherapy for relapsed or refractory B-cell acute lymphoblastic leukemia. Clin Adv Hematol Oncol. 2016 Oct;14(10):802–8. PubMed. 1543-0790
21. Santomasso BD, Park JH, Salloum D, Riviere I, Flynn J, Mead E, et al. Clinical and Biological Correlates of Neurotoxicity Associated with CAR T-cell Therapy in Patients with B-cell Acute Lymphoblastic Leukemia. Cancer Discov. 2018 Aug;8(8):958–71. http://dx.doi.org/10.1158/2159-8290.CD-17-1319. PubMed. 2159-8290
22. Brudno JN, Kochenderfer JN. Recent advances in CAR T-cell toxicity: Mechanisms, manifestations and management. Blood Rev. 2019 Mar;34:45–55. http://dx.doi.org/10.1016/j.blre.2018.11.002. PubMed. 1532-1681
23. Hay KA, Hanafi LA, Li D, Gust J, Liles WC, Wurfel MM, et al. Kinetics and biomarkers of severe cytokine release syndrome after CD19

chimeric antigen receptor-modified T-cell therapy. Blood. 2017 Nov;130(21):2295–306. http://dx.doi.org/10.1182/blood-2017-06-793141. PubMed. 1528-0020

24. Yakoub-Agha I, Chabannon C, Bader P, Basak GW, Bonig H, Ciceri F, et al. Management of adults and children undergoing chimeric antigen receptor T-cell therapy: best practice recommendations of the European Society for Blood and Marrow Transplantation (EBMT) and the Joint Accreditation Committee of ISCT and EBMT (JACIE). Haematologica. 2020 Jan;105(2):297–316. http://dx.doi.org/10.3324/haematol.2019.229781. PubMed. 1592-8721
25. Abbasi A, Peeke S, Shah N, Mustafa J, Khatun F, Lombardo A, et al. Axicabtagene ciloleucel CD19 CAR-T cell therapy results in high rates of systemic and neurologic remissions in ten patients with refractory large B cell lymphoma including two with HIV and viral hepatitis. J Hematol Oncol. 2020 Jan;13(1):1. http://dx.doi.org/10.1186/s13045-019-0838-y. PubMed. 1756-8722
26. Bennani NN, Maurer MJ, Nastoupil LJ, Jain MD, Chavez JC, Cashen AF, et al. Experience with Axicabtagene Ciloleucel (Axi-cel) in Patients with Secondary CNS Involvement: Results from the US Lymphoma CAR T Consortium. Blood. 2019 Nov;134 Supplement_1:763 . http://dx.doi.org/10.1182/blood-2019-129097. 0006-4971