CAR T-cell product performance in haematological malignancies before and after marketing authorisation

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Chimeric antigen receptor (CAR) T cells represent a potent new approach to treat haematological malignancies. Two CAR T-cell therapies, tisagenlecleucel and axicabtagene ciloleucel, have been approved in Europe and the USA, as well as several other countries, for the treatment of leukaemia and lymphoma. These approvals marked a major milestone in the field of cell and gene therapies. However, the clinical development and regulatory evaluation of these innovative therapies faced several challenges that are considered important lessons learned for future similar products. Here, we examine the products' non-clinical and clinical data packages to outline the challenges encountered during the regulatory evaluation process in Europe, and to provide an update on their performance after authorisation.

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See **Online** for appendix

Introduction

On Aug 27, 2018, the European Commission granted marketing authorisation to axicabtagene ciloleucel (Yescarta, Kite Pharma [Gilead]; Santa Monica, USA) and tisagenlecleucel (Kymriah, Novartis; Basel, Switzerland). The products are autologous, genetically modified, chimeric antigen receptor (CAR) T cells that were approved for treating various haematological malignancies. Axicabtagene ciloleucel is approved for the treatment of adults with relapsed or refractory diffuse large B-cell lymphoma (DLBCL) and mediastinal large B-cell lymphoma (appendix p 1). Tisagenlecleucel is approved for the treatment of adult relapsed DLBCL, as well as paediatric and young adult (25 years old or younger) acute lymphoblastic leukaemia. In addition to the EU, both products are approved in the USA, Canada, and Switzerland; tisagenlecleucel is also approved in Japan and Australia.

The novelty in CAR T cells lies in part in the genetically engineered chimeric receptor,[1,2] which is a fusion protein with an extracellular antibody-derived domain, known as a single-chain variable fragment (ScFv), and an intracellular signalling component usually comprised of primary and costimulatory signalling domains. The ScFv is responsible for specific antigen recognition on the surface of tumour cells, whereas the intracytoplasmic domains are responsible for T-cell activation, eliciting targeted killing of tumour cells.[1,2] The gene encoding the receptor is delivered to T cells via a viral vector or by membrane permeabilisation techniques such as electroporation. Both products use second-generation CAR constructs with CD19 as the target surface antigen, which is expressed on healthy B cells and in B-cell malignancies (appendix p 2). The primary T-cell signalling domain in both products is CD3ζ. The costimulatory signalling domain in axicabtagene ciloleucel is CD28 and the costimulatory signal in tisagenlecleucel is produced by 4–1BB (CD137, TNSFR9).[3,4] CD28 and 4–1BB are the most widely used costimulatory domains in clinical studies investigating CAR T-cell therapy.[5] CD28 promotes effector T-cell differentiation with an exhausted phenotype (potent, short-lived cells), leading to an initial intense activation and cytokine production that diminishes rapidly,[4] whereas 4–1BB induces differentiation predominantly to memory cell subtypes that promote cellular persistence and less cytokine production.[4,6] By harnessing the specificity of antibodies and the cytotoxicity of T cells, CAR T cells have shown high potency in treating haematological malignancies, with new generations of CAR T cells being tested for the treatment of many subtypes of haematological malignancies and solid tumours.[7,8]

In the EU, CAR T cells are subject to the advanced therapy medicinal product (ATMP) legislation and guidelines. The scientific evaluation of marketing authorisation applications for ATMPs is assessed by the European Medicines Agency (EMA) via a mandatory centralised procedure.[9] Given the complex nature of developing a living drug, meeting the traditional data requirements for marketing authorisation is challenging. As a result, regulatory guidance and incentives have been continuously evolving to address the unique biomanufacturing characteristics of ATMPs, the lack of suitable animal models, and the restrictive nature of the targeted medical indications. For instance, CAR T-cell products aim to treat life threatening or debilitating conditions and thus qualify for multiple regulatory initiatives to accelerate their development,[10] such as the priority medicines scheme (PRIME) and the orphan drug designation programme (appendix p 1). However, some doubts were cast on the completeness and strength of clinical evidence submitted to the EMA to support the marketing authorisation of these products.[11–14] Furthermore, the initial negative evaluation of the products by reimbursement bodies supported the argument that authorisation decisions on these drugs were premature.[11–13] Nevertheless, the EMA tries to strike a balance between timely market availability, patient safety, and postmarketing knowledge gains, by subjecting such products to more stringent postauthorisation measures.

Since their approval in 2018, tisagenlecleucel and axicabtagene ciloleucel are subject to additional monitoring, and their developers are obligated to supplement the safety and efficacy evidence by conducting post-authorisation studies and close follow-up of treated patients for an extended period (between 5 years and 15 years).[15–17] Understanding the added clinical value of

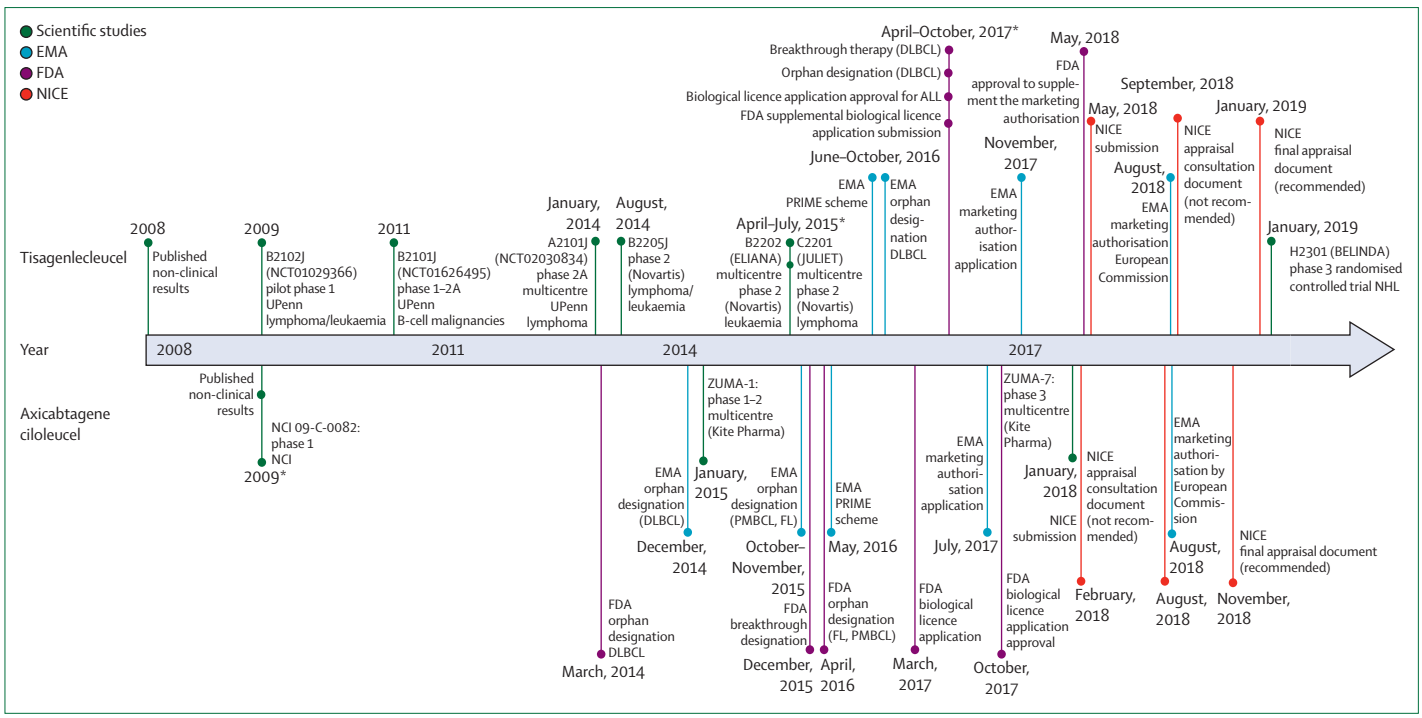


Figure 1: **Development timeline of axicabtagene ciloleucel and tisagenlecleucel**
The spaces between the lines are not to scale. EMA=European Medicines Agency. FDA=US Food and Drug Administration. NICE=National Institute for Health and Care Excellence. UPenn=University of Pennsylvania. NCI=National Cancer Institute. DLBCL=diffuse large B-cell lymphoma. PRIME scheme=Priority Medicines scheme. PMBCL=primary mediastinal B-cell lymphoma. FL=follicular lymphoma. ALL=acute lymphoblastic leukaemia. NHL=non-Hodgkin lymphoma. *Points on the same line represent the events arranged chronologically from top to bottom.

these products, and analysing gaps in evidence, could provide essential information and lessons for future ATMP development. Moreover, having two CAR T-cell products approved at the same time for similar indications created an unprecedented opportunity to scrutinise the ability of these different development pathways to inform clinical and regulatory decisions for orphan oncology therapies (figure 1). In this analysis, we examined the preauthorisation data packages submitted to the EMA to obtain marketing authorisation and then identified regulatory objections and concerns raised during the evaluation of both products. Finally, we present the postauthorisation evidence-generation strategies to fulfil the regulatory requirements and summarise the real-world data available on the use of these products.

Non-clinical proof-of-concept assessment

Our analysis reveals that the majority of the regulatory concerns raised during the evaluation of axicabtagene ciloleucel and tisagenlecleucel pertained to the clinical data and product quality packages, whereas more regulatory flexibility was shown with the non-clinical data (table 1). Nevertheless, animal models provided valuable information about the pharmacokinetics, pharmacodynamics, and some toxicological aspects of the products. Axicabtagene ciloleucel was tested by use of a CD19-expressing 38c13 mouse lymphoma cell line in an immunocompetent syngeneic lymphoma mouse model,[18] whereas tisagenlecleucel treatment studies used an immunodeficient NOD/Shi-scid IL-2Rγ^{null} human leukaemia xenograft mouse model.[6]

The disadvantage of using immunocompetent mice in the axicabtagene ciloleucel studies is that these mice only support the growth of mouse lymphoma, which hampered the efficacy and safety testing of the human-derived CAR T cells. As a result, murine-derived CAR T cells were developed and tested as a surrogate model for the proposed CAR T-cell therapy. The main limitation of these cells is that their manufacturing and cellular dynamics differ from the final human CAR T-cell product. The EMA highlighted this point and accepted the animal studies as a proof-of-concept, deeming the murine model as the most appropriate for testing.[15]

Conversely, the immunodeficient mice used in tisagenlecleucel non-clinical studies could be injected with human acute lymphoblastic leukaemia cells, allowing for the testing of the human CAR T-cell product. However, the absence of an intact immune system in this model less accurately simulates the disease in humans than does a model using immunocompetent mice, and the safety testing of on-target–off-tumour activity and cytokine-release syndrome could not be done.[19] Moreover, several CAR constructs were tested in the leukaemia model (second-generation CD28, second-generation 4–1BB, and third-generation CD28 and

	Tisagenlecleucel	Axicabtagene ciloleucel
Quality aspects		
Major objections	Documentation of GMP compliance	Inconsistent viral transduction
Other concerns	NA	No initial data on comparability and equivalence of the different processes (CLP 1.0. and CLP 2.2.); lower transduction rate in the last manufacturing process
Recommendations	Characterisation and testing of the viral vector, leukapheresis starting material, and the finished product	Enhancing manufacturing process and control of the product
Non-clinical aspects		
Major objections	NA	NA
Other concerns	Not using both CD28 and 4-1BB as the intracellular domain in the CAR construct	NA
Recommendations	NA	NA
Clinical pharmacology		
Major objections	NA	NA
Other concerns	No dose exposure relationship; less proliferation of the cells in patients with DLBCL than patients with ALL; high variability of cellular kinetics in the study groups	No relation between product characteristics and efficacy outcomes; no correlation between biomarkers and positive treatment outcomes
Recommendations	Investigate cellular kinetic parameters	NA
Clinical efficacy		
Major objections	Absence of CD19 tumour expression as a requirement for infusion in the summary of product characteristics	NA
Other concerns	ALL: delayed assessment of the tumour stage after patient enrolment affects baseline characteristics; not reflecting the study population for the submitted indication; DLBCL: testing the null hypothesis of overall response at 20% against the EMA scientific advice recommendation (overall response of 40%); excluding the effect of bridging therapy in the clinical assessment by use of modified intention-to-treat analysis; long time span (54 days) from enrolment to the infusion of tisagenlecleucel due to longer than expected manufacturing time (4–5 weeks); patients dropping out of the study with poor prognostic factors due to disease progression; introducing bias to the efficacy analysis by use of the infused modified intention-to-treat population; not including stable disease and progressive disease populations in the overall survival analysis; different baseline characteristics between non-infused patients and infused patients	DLBCL: Not doing the baseline-PET scan in the prespecified time before conditioning chemotherapy; not reflecting the study population for the submitted indication; absence of comparison with SCHOLAR-1 for a worst-case scenario by excluding patients with an Eastern Cooperative Oncology Group score of 2–4 or unknown
Recommendations	NA	NA
Clinical safety		
Major objections	NA	NA
Other concerns	Severe and life-threatening adverse effects; missing information in several patient groups	High incidence of adverse drug reactions; missing information in several patient groups
Recommendations	NA	NA

GMP=good manufacturing practice. NA=not applicable. CAR=chimeric antigen receptor. DLBCL=diffuse large B-cell lymphoma. ALL=acute lymphoblastic leukaemia. EMA=European Medicines Agency.

Table 1: **Major objections and concerns raised by the EMA during the evaluation of tisagenlecleucel and axicabtagene ciloleucel**

4–1BB).[6] Although CAR T cells with the third-generation CD28 and 4-1BB construct persisted for longer in the tumour-bearing mice, 4–1BB was the construct of choice for clinical testing, a decision that was accepted during the regulatory evaluation process.[16]

Notably, no lymphoma animal model was developed and tested as a proof of concept for tisagenlecleucel. The EMA flagged this observation; nevertheless, the agency found the absence of this animal model acceptable considering the available clinical experience and approved the product for this indication.[16] Overall, the regulatory flexibility in accepting suboptimal non-clinical data packages for both products was evident.

Clinical investigation of CAR T-cell pharmacology

Data on axicabtagene ciloleucel pharmacology were generated by the phase 1–2 ZUMA-1 trial[20] and the supportive National Cancer Institute 09-C-00082 study[21] (figure 1, table 2), whereas tisagenlecleucel relied on the pivotal phase 2 ELIANA trial[22] and supportive studies (Pedi CART19[23] [NCT01626495] and ENSIGN[22] [NCT02228096]) for the acute lymphoblastic leukaemia indication, and the JULIET study[24] for the DLBCL indication (figure 1, table 2). In these trials, proliferation, distribution, and persistence of anti-CD19 CAR T cells were measured in peripheral blood and bone marrow by qPCR and flow cytometry.[25,26]

	ELIANA (NCT02435849)	JULIET (NCT02445248)	ZUMA-1 (NCT02348216)	SCHOLAR-1
Treatment	Tisagenlecleucel	Tisagenlecleucel	Axicabtagene ciloleucel	Salvage chemotherapy
Centres in countries	25 in 11	27 in 10	24 in 1	NA
Study population	Paediatric and young adult patients with relapsed or refractory B-cell ALL	Relapsed or refractory DLBCL after two lines or more of chemotherapy and not eligible for stem cell transplantation	Relapsed or refractory DLBCL, PMLBCL, or FL after two lines or more of chemotherapy or an autologous stem cell transplantation	Refractory aggressive B-cell non-Hodgkin lymphoma (DLBCL, PMBCL, or TFL)
Median age, years (range)	11 (3–23)	59 (22–76)	58 (23–76)	55 (19–81)
Study design	Phase 2, single-arm, open-label, multicentre	Phase 2, single-arm, open-label, multicentre compared with historical data	Phase 2, single-arm, open-label, multicentre compared with historical data	Retrospective meta-analysis
Conditioning chemotherapy	Fludarabine (30 mg/m², intravenous daily for four doses) and cyclophosphamide (500 mg/m², intravenous daily for two doses); cytarabine (500 mg/m² daily for 2 days) and etoposide (150 mg/m² daily for 3 days)	Fludarabine (25 mg/m²) and cyclophosphamide (250 mg/m²); intravenous daily for three doses	Fludarabine (30 mg/m²) and cyclophosphamide (500 mg/m²); intravenous daily for three doses; treatment starts 5 days before infusion of the CAR T cells	NA
Dose	$0{\cdot}2–5{\cdot}0 \times 10^6$ cells per kg (for patients ≤ 50 kg) and $0{\cdot}1–2{\cdot}5 \times 10^8$ cells (for patients >50 kg)	$1{\cdot}0–5{\cdot}0 \times 10^8$ cells single infusion	2×10^6 (± 20%) cells per kg (minimum 1×10^6 cells per kg)	Salvage chemotherapy with an anti-CD20 monoclonal antibody such as rituximab
Enrolled/infused	92/75	165/111	111/101	636/523
Primary endpoints				
Overall response	Best overall disease response as a CR or CRi	Best overall disease response as a CR or PR	Best overall disease response as a CR or PR	..
Response	..	..	..	Best response as a CR or PR
Secondary endpoints	Overall response (CR and CRi) from US manufacturing facilities; percentage of patients with a best overall response of CR or CRi, with negative MRD, from all manufacturing facilities; percentage of patients with a best overall response of CR or CRi, with negative MRD, from US manufacturing facilities	Duration of response, overall survival, time to relapse, event-free survival, progression-free survival	Duration of response, progression-free survival, overall survival	CR and overall survival
Safety endpoints	Incidence of adverse events	Incidence of adverse events	Incidence of adverse events	NA

CAR=chimeric antigen receptor. EMA=European Medicines Agency. NA=not applicable. ALL=acute lymphoblastic leukaemia. DLBCL=diffuse large B-cell lymphoma. PMLBCL=primary mediastinal large B-cell lymphoma. FL=follicular lymphoma. TFL= transformed follicular lymphoma. CR=complete response. CRi=complete response with incomplete haematological recovery. PR=partial response. MRD=minimal residual disease.

Table 2: **Pivotal clinical trials for CAR T-cell products and historical controls submitted in the marketing authorisation application to the EMA**

The non-compartmental analysis of tisagenlecleucel in ELIANA and supportive studies showed an initial rapid expansion of CAR T cells in acute lymphoblastic leukaemia responders, reaching the maximal expansion in peripheral blood (C_{max}) after nearly 10 days (T_{max}).[22,23] Acute lymphoblastic leukaemia responders showed 68% more cellular expansion (C_{max}) and 43% higher exposure (area under the curve for 0–28 days; $AUC_{0–28}$) of tisagenlecleucel than did non-responders. The cells persisted in responders for longer than in non-responders, with the median time until the last measured concentration being 170·0 days in responders versus 28·9 days in non-responders. The pharmacokinetic properties of tisagenlecleucel in the peripheral blood have shown a direct correlation with endpoints in the trial for acute lymphoblastic leukaemia, including event-free survival for more than 3 months and overall response at day 28. Conversely, in the JULIET study,[24] a correlation between the cellular kinetics of tisagenlecleucel in the peripheral blood and treatment efficacy could not be shown in patients with lymphoma as no differences in the geometric means of the C_{max} or $AUC_{0–28}$ were observed between responders and non-responders.

Patients with lymphoma who responded to axicabtagene ciloleucel in the ZUMA-1 trial showed a 205% higher median C_{max} (43·6 cells per µL *vs* 21·2 cells per µL) and two times higher median $AUC_{0–28}$ (7·1 days per cells per µL *vs* 222·0 days per cells per µL) than did non-responders. The number of cells then declined to near background amounts within 3 months, with a median of 0·4 cells per µL (range of 0–15.8 cells per µL). Unlike with tisagenlecleucel, the C_{max} and $AUC_{0–28}$ of axicabtagene ciloleucel directly correlated with the clinical response in patients with lymphoma (responders tended to have more cells and longer exposure). In axicabtagene ciloleucel, the robust cellular proliferation and cytokine release promoted by the CD28 signalling domain might have influenced the high response observed in lymphoma. However, previous studies reported that CD28 CAR T cells might lack

durability and persistence, raising questions about the actual value of treatment, long-term efficacy, and the possible need for subsequent treatment.[4,27,28] The UK National Institute for Health and Care Excellence (NICE) also raised this concern during their health technology assessment of axicabtagene ciloleucel.[29]

Other factors, such as disease burden and location, T-cell phenotype, T-cell subpopulations, conditioning chemotherapy, and the tumour microenvironment have also been reported to affect the cellular kinetics of CAR T cells.[30,31] For instance, differences in the cellular kinetics of CAR T-cells between leukaemia and lymphoma might be attributed in part to the fact that leukaemia cells are often present in peripheral blood, whereas lymphoma cells mostly reside in lymphoid tissues. As noted, 4-1BB costimulation promotes cellular differentiation of memory cell phenotypes leading to longer persistence but weaker initial response compared to CD28 costimulation. Such characteristics of 4-1BB, coupled with the difference in microenvironment, can partially explain the observed variation in tisagenlecleucel's cellular kinetics between lymphoma and leukaemia. These factors prompted the EMA to recommend further characterisation of the cellular kinetics of tisagenlecleucel for both indications as part of the postauthorisation measures. In their efforts to address this point, the developers of tisagenlecleucel established a mixed-effects model describing the effect of tocilizumab and corticosteroids—treatments that are used to manage cytokine-release syndrome—on cellular kinetics.[32] The model can be adapted to characterise the expansion and persistence of CAR T cells across different disease indications, within various cell types, and between different costimulatory domains.[32]

Post-treatment outcomes and analysis of results

We further analysed the European public assessment reports for the submitted clinical data packages of both products.[15,16] Tisagenlecleucel showed a clear efficacy profile in patients with acute lymphoblastic leukaemia. The results of the ELIANA study (data cutoff: April 25, 2017) showed that of 92 patients, 61 (66%) achieved an overall response and 45 (49%) a complete response using the intention-to-treat population, and of 75 patients, 61 (81%) achieved an overall response and 45 (60%) a complete response using the infused modified intention-to-treat population, with a median overall survival of 19·4 months after a median follow-up of 10·5 months.[16] However, when exploring the results of lymphoma clinical trials for tisagenlecleucel, there were more noticeable differences between the intention-to-treat population versus the infused modified intention-to-treat population in the efficacy analysis of JULIET (data cutoff: Dec 8, 2017; figure 2; appendix pp 3–4). These differences also extended to the median overall survival, which was 8·2 months for the intention-to-treat analyses and 11·7 months for the modified intention-to-treat analysis.[16] These differences were not seen for axicabtagene ciloleucel in the ZUMA-1 trial, where overall and complete response proportions were similar between the intention-to-treat and modified intention-to-treat analyses (figure 2), as was median overall survival (17·4 months in the intention-to-treat analysis and not reached in the modified intention-to-treat analysis [data cutoff: Aug 11, 2017]; figure 2; appendix p 4).[15] The results of pivotal trials in lymphoma and leukaemia with both CAR T-cell products met the primary endpoint of best overall response in more than 20% of patients—an endpoint that was decided based on data obtained from historical studies.[23,24,33]

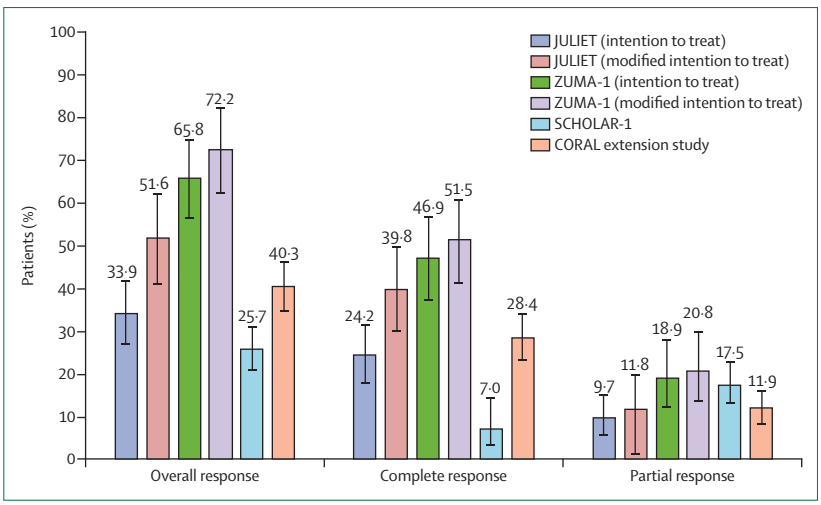
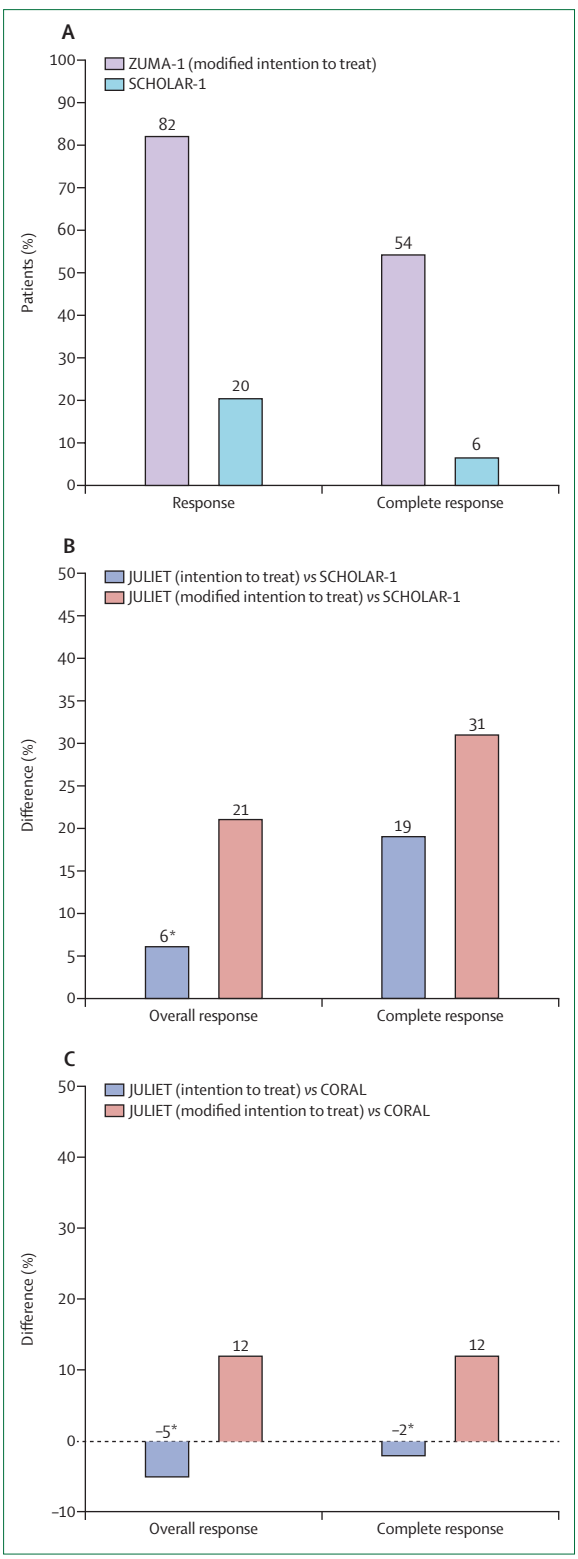


Figure 2: **Unadjusted aggregated efficacy results for JULIET (tisagenlecleucel), ZUMA-1 (axicabtagene ciloleucel), SCHOLAR-1, and CORAL extension studies with different analysis populations for the treatment of DLBCL**

Error bars represent the CI. The number of patients in each study analysis group (n=165, 93, 111, 101, 523, or 278) in order of the key from top to bottom. Data cutoff for ZUMA-1: Aug 11, 2017, with a median follow-up of 15·1 months. Data cutoff for JULIET: Dec 8, 2017, with a median follow-up of 13·9 months. DLBCL=diffuse large B-cell lymphoma.

For the tisagenlecleucel JULIET study, the EMA explored the reasons for the variability seen in the different analyses of clinical outcomes. They found that this variability in results could be attributed to the high dropout (30%), which changed the number of patients included in each analysis. This dropout resulted from a strict inclusion criterion where enrolled patients should not have had any substantial worsening of their disease status before the administration of the cellular product.[16] However, the median time from enrolment to infusion was 54 days due to manufacturing delays, which led to patient deterioration and exclusion from the study.[16] As such, the EMA concluded that selection bias was introduced in the modified intention-to-treat population. Additionally, 20% of patients who dropped out had a response to the bridging chemotherapy that was administered while waiting for product manufacturing (patients in the axicabtagene ciloleucel ZUMA-1 trial did not receive bridging chemotherapy). These observations have prompted the Inter-Committee Scientific Advisory Group on Oncology to advise the EMA that the evaluation of the intervention should be based on the whole treatment regimen, and not only on the infused cellular

product. Taking all these points into consideration, the EMA concluded that the reliability of using the outcomes of the infused modified intention-to-treat population as efficacy estimators was not sufficient to reflect an accurate assessment of clinical benefit (table 1). As such, the EMA used the enrolled intention-to-treat population data to evaluate the differences in outcomes against the historical controls, and to conduct the benefit–risk assessment for both products.[16]



The role of historical controls in evaluating clinical outcomes

The assessment of treatment benefit for both tisagenlecleucel and axicabtagene ciloleucel was supplemented by comparisons with historical control groups. In the case of single-arm studies with no control arms, regulatory and health technology assessment agencies show more flexibility in allowing comparisons with historical data. Analytical tools, such as matching-adjusted indirect comparisons and network meta-analyses, have been introduced for regulatory submissions and health technology assessments.[34,35] However, the choice of a suitable comparator remains challenging, and caution is needed during the interpretation and evaluation of the results.[35] Novartis tried to establish a comparison for the leukaemia indication for tisagenlecleucel by pooling data from their leukaemia studies (ELIANA [NCT02435849], ENSIGN [NCT02228096] and Pedi CART19 [NCT01626495]) and matching the data to other studies of marketed therapies, such as blinatumomab; a combination of clofarabine, cyclophosphamide, and etoposide; and clofarabine monotherapy.[36–40] Despite the potential bias due to small sample size, confounding patient populations, and matching on few variables, tisagenlecleucel showed consistent superiority across all the comparators, endpoints, and sensitivity analyses.[16]

In the lymphoma indication, tisagenlecleucel and axicabtagene ciloleucel were compared with SCHOLAR-1,[41] which is a retrospective, patient-level, pooled analysis of the outcome of currently available standard of care in patients with refractory, aggressive non-Hodgkin lymphoma. The comparison of response in ZUMA-1 with SCHOLAR-1 is shown in figure 2 for the unmatched and unadjusted data. The reliability of SCHOLAR-1 as a comparator with ZUMA-1 was thoroughly assessed during health technology assessments by NICE,[29] and was eventually accepted. This acceptance was attributed to the availability of individual patient data to Kite Pharma

Figure 3: **Matched comparisons of results from axicabtagene ciloleucel and tisagenlecleucel pivotal clinical trials with historical comparators for the treatment of DLBCL**

Figures are reproduced from data presented in the European public assessment reports for both products, and a published article.[15,16,42] (A) Comparison of responses between ZUMA-1 and SCHOLAR-1 (data cutoff ZUMA-1: Aug 11, 2017, median follow-up 15·1 months). (B) The differences in overall response and complete response between JULIET and SCHOLAR-1 by analysis population (data cutoff: Dec 8, 2017, median follow-up 13·9 months). (C) The differences in overall response and complete response between JULIET and CORAL extension studies (data cutoff: Dec 8, 2017, median follow-up 13·9 months).
*No significant difference in responses (p>0·05).

(Gilead), which was the sponsor of SCHOLAR-1, enabling the company to match patients in both trials. In the matched analysis, axicabtagene ciloleucel showed superiority over the standardised historical data, even after adjusting the populations to a stricter baseline in a worst-case scenario analysis (figure 3A).

Since only the published aggregated data of SCHOLAR-1 were available to developers of tisagenlecleucel, other historical comparators were explored. In addition to SCHOLAR-1, the pooled CORAL extension data were used for comparisons (figure 2, appendix p 4).[16] The pooled CORAL extensions emerged from the main CORAL study and were considered by the EMA and NICE as a more suitable comparator than SCHOLAR-1 for evaluating tisagenlecleucel due to similarities in the populations enrolled.[43] The main CORAL study[44] compared salvage chemotherapy regimens followed by stem cell transplantation, whereas the pooled extension studies followed up patients who did not proceed to stem cell transplantation, or had a second relapse after transplantation.[45,46] Novartis used matching-adjusted indirect comparisons to match the individual patients from JULIET to both historical controls. When running the matched analysis with the modified intention-to-treat population, tisagenlecleucel showed a significant difference in overall response and complete response compared with that in both the pooled CORAL extensions and SCHOLAR-1 (figure 3B, C). However, when analysing the intention-to-treat population, the product did not show a significant difference in overall response when compared with the pooled CORAL extensions and SCHOLAR-1 studies (figure 3B, C). Nevertheless, tisagenlecleucel showed a significantly longer median overall survival (10·6 months for intention to treat and 16·3 months for modified intention to treat) compared with the pooled CORAL results where the median overall survival was 5·8 months.

Due to the aforementioned inconsistencies in efficacy analysis for the different populations, 12 members of two EMA committees involved in the evaluation process disagreed with granting authorisation for tisagenlecleucel in the lymphoma indication. Eventually, the product was authorised in lymphoma by taking into account the higher response durability in tisagenlecleucel compared with the controls. Nevertheless, Novartis was mandated to do extensive postauthorisation efficacy studies in the form of data collection on treated patients in dedicated registries and an interventional phase 3, randomised, controlled trial of tisagenlecleucel versus platinum-based immunochemotherapy (BELINDA; NCT03570892; table 3). BELINDA began enrolment in May, 2019, with a target enrolment of 318 patients across the USA, Australia, Germany, Japan, and Spain.

Associated risks and measures to ensure patient safety

Both tisagenlecleucel and axicabtagene ciloleucel used integrating viral vectors (appendix p 2), which might raise the concern of insertional oncogenesis due to semi-random integration patterns. Lentivectors used in tisagenlecleucel are considered safer than γ-retroviral vectors used in axicabtagene ciloleucel, as their integration patterns do not favour transcriptional start sites.[54] However, mature T cells are resistant to malignant transformation after transduction with an integrating viral vector,[55] which was Kite Pharma's (Gilead) justification for using a γ-retroviral vector.[15] Notably, axicabtagene ciloleucel received advice from the EMA in the form of early discussions on the risks of insertional mutagenesis under the PRIME scheme. Another concern of the use of viral vectors is the generation of a replication-competent virus.[56] The risk of replication-competent virus formation was considered low by the EMA as both vectors are replication incompetent and stringently tested for the absence of replication-competent virus.[15,16] Studies have shown that the risk of formation of replication-competent virus either by a lentiviral or retroviral vector is very low.[57] As a result, the US Food and Drug Administration is revising the regulations on testing for replication-competent virus, which might result in a reduction of follow-up testing in the case of vectors where there is substantial experience with safety.[58] Nevertheless, to ensure patient safety and accumulate more data about the products, the EMA requires postauthorisation safety studies where data from patients treated with these products must be collected for a period of up to 15 years to assess the long-term safety of both vector types as part of the risk minimisation plan (table 3).

During clinical testing, all patients infused with either of the two products had adverse events (appendix p 5). Serious adverse events were mainly attributed to cytokine-release syndrome and neurological complications. Other frequent serious adverse events were infections, tumour lysis syndrome, and febrile neutropenia. Axicabtagene ciloleucel showed a higher incidence of cytokine-release syndrome and neurological events than did tisagenlecleucel (appendix p 5), and these events were associated with higher concentrations of cytokines and a higher maximum number of axicabtagene ciloleucel cells in the blood (C_{max}).[33] The clinical management plan for adverse events in both studies was seen as sufficient by the EMA. For instance, CAR T-cell therapies were to be provided only in qualified centres that also had available tocilizumab as a treatment for cytokine-release syndrome. Additionally, the clinical trial sponsors had to offer an educational programme for each participating centre that was targeted towards centre personnel and patients. As part of the postauthorisation measures, each applicant had to collect postauthorisation safety data in dedicated registries. For tisagenlecleucel, the data were collected through the European Society for Blood and Marrow Transplantation and the Center for International Blood and Marrow Transplant Research registries.[59] As part of the ongoing effort, the EMA released the proposed data

	Indication	Primary objective	Obligatory by EMA	Study type	Phase	Control	Randomised	Start date	Number of Patients	Current status
Tisagenlecleucel										
Stein et al (2019)[32]	ALL or DLBCL	Cellular kinetic parameters and the effect of CRS medications	No	Experimental	NA	NA	NA	NA	NA	Published mixed-effects model analysing the effects of CRS medications on cellular kinetics
CCTL019B2401[47]	ALL	Evaluate the efficacy in patients with ALL younger than 3 years	Yes	Observational; registry-based	Phase 4	NA	NA	Q4, 2018	NA	Data from EBMT and CIBMTR registries will be used for the observational study; February, 2019: statistical plan for the study submitted to the committee for advanced therapies
ELIANA[48] (NCT02435849, CCTL019B2202)	ALL	Long-term efficacy and safety of tisagenlecleucel in the ELIANA study	No	Follow-up	Phase 2 multicentre	No	No	April, 2015	97 enrolled, 79 infused at last data cutoff	Official 24-month report of ELIANA (expected Q4, 2019); last published results: April, 2018, data cutoff; 24-month median follow-up; median duration of response not reached; median overall survival not reached; 66% overall survival (modified intention to treat; 24 months)
CCTL019B2401[47]	DLBCL	Evaluate efficacy outcome measures, including the manufacturing time	Yes	Observational; registry-based	Phase 4	No	No	Q4, 2018	NA	February, 2019: statistical plan for the study submitted to the committee for advanced therapies
JULIET[49] (NCT02445248, CCTL019C2201)	DLBCL	Long-term efficacy and safety of tisagenlecleucel in the JULIET study	Yes	Follow-up	Phase 2 multicentre	No	No	July, 2015	167 enrolled, 115 infused	Official 24-month report of JULIET (expected in September, 2019); last published results: May, 2018, data cutoff; 19-month median follow-up; median duration of response not reached; median overall survival of 11·1 months for infused patients; 43% overall survival at 18 months
BELINDA (CCTL019H2301, NCT03570892)	DLBCL	Efficacy of tisagenlecleucel *vs* standard of care in adult patients with refractory or relapsed NHL	Yes	Interventional	Phase 3 multicentre	Yes (active comparator)	Yes	May, 2019	318 (estimated)	Recruiting; primary endpoint: event-free survival
CCTL019B2401[47]	ALL or DLBCL	Long-term safety of tisagenlecleucel in patients with ALL and DLBCL based on disease registry	Yes	Observational; registry-based	Phase 4	NA	NA	Q4, 2018	NA	February, 2019: statistical plan for the study submitted to the committee for advanced therapies
(NCT02445222, CCTL019A2205B)[16]	ALL or DLBCL	Long-term follow-up of patients exposed to lentiviral-based CD19 directed CAR T-cell therapy	Yes	Observational; registry-based	Phase 4	NA	NA	Nov, 2015	620 (estimated)	Follow-up of all the patients who have been infused with tisagenlecleucel for 15 years; annual safety reports and 5-yearly interim reports will be submitted to the EMA; final report of study results in December, 2038
Axicabtagene ciloleucel										
ZUMA-1[50] (NCT02348216)	DLBCL, PMLBCL, or FL	Long-term efficacy and safety of axicabtagene ciloleucel in the ZUMA-1 study	No	Follow-up	Phase 2 multicentre	No	No	January, 2015	111 enrolled, 101 infused	EMA 24-month result update based on intention-to-treat (n=111); 68% overall response; 50% CR; median duration of response not reached; median overall survival of 17·4 months; 48% 24-month overall survival

(Table 3 continues on next page)

	Indication	Primary objective	Obligatory by EMA	Study type	Phase	Control	Randomised	Start date	Number of Patients	Current status
(Continued from previous page)										
Non-interventional registry study[15]	DLBCL	Long-term safety of axicabtagene ciloleucel in the postmarketing setting	Yes	Observational; registry-based	Phase 4	NA	NA	NA	NA	Planned
ZUMA-2 (NCT02601313)	MCL	Efficacy of axicabtagene ciloleucel in patients with refractory or relapsed MCL	No	Interventional	Phase 2 multicentre	No	No	November, 2015	105	Active; expected primary completion date in July, 2019; primary endpoint: overall response
ZUMA-3[51] (NCT02614066)	ALL	Safety and efficacy of axicabtagene ciloleucel in adult participants with refractory or relapsed ALL	No	Interventional	Phase 1–2 multicentre	No	No	March, 2016	100 (estimated)	Recruiting; expected primary completion date in January, 2020; end of phase 1 results: September, 2018, data cutoff; 45 infused patients; 41 evaluable patients; 16-month median follow-up; 68% overall response (CR + CRi); 73% minimal residual disease negative; no DLT
ZUMA-4[52] (NCT02625480)	ALL	Safety and efficacy of axicabtagene ciloleucel in paediatric and adult participants with refractory or relapsed ALL	No	Interventional	Phase 1–2 multicentre	No	No	February, 2016	100	Recruiting; expected primary completion date in July, 2021; end of phase 1 results October, 2018, data cutoff; 24 infused patients; 13-month median follow-up; overall response of 100% (2×10^6), 64% (1×10^6; 68 mL), and 71% (1×10^6; 40 mL) in three dose groups
ZUMA-5 (NCT03105336)	NHL	Safety and efficacy of axicabtagene ciloleucel in patients with indolent refractory or relapsed indolent NHL	No	Interventional	Phase 2 multicentre	No	No	June, 2017	160 (estimated)	Recruiting; expected primary completion date in March, 2020; primary endpoint: overall response
ZUMA-6[53] (NCT02926833)	DLBCL	Safety and efficacy of axicabtagene ciloleucel in combination with atezolizumab in adults with refractory or relapsed DLBCL	No	Interventional	Phase 1–2 multicentre	No	No	September, 2016	37 (estimated)	Active; end of phase 1 results: January, 2018, cutoff; 12 infused patients; 4·4 median follow-up; dose-limiting toxicity in 1 patient; all patients had at least one adverse effect (92%, grade ≥3); overall response in 9 (90%) of 10 evaluable patients
ZUMA-7 (NCT03391466)	DLBCL	Efficacy of axicabtagene ciloleucel against the standard of care in relapsed or refractory DLBCL	No	Interventional	Phase 3 multicentre	Yes	Yes	December, 2017	350 (estimated)	Recruiting; 71 study locations (Europe, North America, Australia, Israel); primary endpoint: event-free survival; secondary endpoints: overall response, overall survival, progression-free survival, duration of response

EMA=European Medicines Agency. ALL=acute lymphoblastic leukaemia. DLBCL=diffuse large B-cell lymphoma. CRS=cytokine release syndrome. NA=not applicable. Q4=fourth quarter of the year (October, November, and December). EBMT=European Society for Blood and Marrow Transplantation. CIBMTR=Center for International Blood and Marrow Transplant Research. NHL=non-Hodgkin lymphoma. CAR=chimeric antigen receptor. PMLBCL=primary mediastinal large B-cell lymphoma. FL=follicular lymphoma. CR=complete response. MCL=mantle cell lymphoma. CRi=complete response with incomplete haematological recovery. DLT=dose limiting toxicity.

Table 3: **Postauthorization studies for tisagenlecleucel and axicabtagene ciloleucel based on the submitted risk management plan**

elements that should be fulfilled by the registries to capture all the necessary information on the safety and efficacy of CAR T-cell products.[60]

Complex logistics and regulatory considerations

Although clear clinical benefits were obtained from clinical trials investigating tisagenlecleucel and axicabta-

gene ciloleucel, issues pertaining to manufacturing and supply chain management should be highlighted. For instance, the locations of the studies might have influenced the outcomes of both treatments and their evaluation by the EMA. ZUMA-1 was done in the USA, except for one patient, who was treated in Israel (table 2). Due to the absence of European patients in ZUMA-1, the developer was advised, under the PRIME scheme, to include European patients in the planned phase 3 trial (ZUMA-7, NCT03391466).[15] Conversely, JULIET was done at 27 sites in ten countries across four continents. Even though clinical, collection, and infusion sites were global, tisagenlecleucel for JULIET was mainly manufactured in the USA, with some manufacturing in Germany. This restricted capacity of the manufacturing might have posed a challenge to the product supply chain and manufacturing coordination, and prolonged the time from enrolment to infusion in the JULIET study. As a result, details on tisagenlecleucel manufacturing turnaround time was required by the EMA as part of the postauthorisation efficacy studies.[16]

Postmarketing performance of CAR T-cell products

The up-to-date clinical follow-up shows that both tisagenlecleucel and axicabtagene ciloleucel elicit a durable response in the approved leukaemia and lymphoma indications (table 3). In patients with leukaemia, the median duration of response and overall survival were not reached at a median follow-up of 24 months in the ELIANA study.[48] In patients with lymphoma, the last update from the tisagenlecleucel JULIET trial showed a median overall survival of 11·1 months, and the median duration of response was not reached (table 3).[49] The 24-month results of the axicabtagene ciloleucel ZUMA-1 study showed a median overall survival of 17·4 months, and the median duration of response was not reached.[15]

Two postmarketing real-world studies were published evaluating patients with non-Hodgkin lymphoma that were treated with standard-of-care axicabtagene ciloleucel in the USA.[61,62] Nastoupil and colleagues[61] reported results of 295 patients treated as of August, 2018, at 17 academic USA centres. 240 of 274 patients had cytokine-release syndrome, of which 18 individuals were grade 3 or worse, and 85 patients had grade 3 or worse neurological complications.[63] Overall response was seen in 81% of patients after a median follow-up of 3·9 months.[61,64] Jacobson and colleagues[62] reported a lower overall response in 67 (71%) of 95 patients infused with axicabtagene ciloleucel, after a median follow-up of 5·6 months.[62,65,66] 95% of the patients had cytokine-release syndrome, of which 17 (16%) patients were grade 3 or worse, whereas neurological complications were reported in 29 (38%) of the treated patients.[65] These real-world experiences extend earlier clinical evidence generated from investigational trials. Further real-world safety and efficacy data on the use of axicabtagene ciloleucel in the USA is expected through the expanded access trial, ZUMA-9 (NCT03153462).

In September, 2019, the European Society for Blood and Marrow Transplantation reported that 155 patients treated with either commercial (80%) or investigational (20%) CAR T cells in 40 centres across nine countries in Europe were registered in their registry.[67] Individual clinical reports on patients receiving CAR T cells in different European countries have also been released. In Germany, of 23 patients who underwent leukapheresis, 20 patients with acute lymphoblastic leukaemia were given tisagenlecleucel, while the remaining 3 patients could not be treated as the manufactured products did not meet the prespecified release criteria.[68] Of these patients, nine (45%) were in remission at the last follow-up visit. The study reported that at a median follow-up of 11 months, the overall survival was 69% and event-free survival was 65%. 17 (74%) of the 23 enrolled patients received tisagenlecleucel either through the expanded access programme (n=6) or as a commercial product (n=11).[68] Grade 4 cytokine-release syndrome was reported in three patients. In Spain, a report released in January, 2019, showed that seven hospitals had treated 84 patients with CAR T cells, out of which only six patients received the product in commercial settings, with the remaining treated in clinical trials.[69] In France, 60 patients with DLBCL were treated with either tisagenlecleucel (n=30) or axicabtagene ciloleucel (n=30) across five centres between April, 2018, and February, 2019, under the temporary authorisation for use programme.[70] Although the actual numbers of treated patients are yet to be disclosed, the uptake of this treatment in Europe has been steady but smaller compared with the USA.

To investigate the activities of specialised treatment centres in adopting CAR T-cell therapies in Europe, a survey study was done between November, 2018, and January, 2019. 566 European Society for Blood and Marrow Transplantation centres were surveyed, of which 134 centres across 22 countries responded.[69] The study showed that 34 centres have already administered CAR T cells to patients, primarily within clinical trials (93% of patients). Furthermore, 57 additional centres located in Europe were planning to administer a CAR T-cell product within the 6 months following the study.[69] In the UK, patients of the National Health Service with acute lymphoblastic leukaemia (children, adolescents, and young adults [up to 25 years old]) can receive CAR T-cell therapy in nine centres and adult patients with DLBCL can receive CAR T-cell products in seven centres, with more centres planning to enrol patients in the future.[71] Although the data indicate a limited number of centres currently available in Europe for commercial CAR T-cell treatments, they also reflect a strong willingness toward the adoption of the therapy.

Ongoing investigations of authorised products in other oncology indications

Tisagenlecleucel and axicabtagene ciloleucel are being investigated for other indications and treatment strategies. A phase 3 trial (OBERON, NCT03628053) is expected to start in late 2019 to further test the efficacy and safety of tisagenlecleucel for the treatment of acute lymphoblastic leukaemia compared with bispecific (blinatumomab) and monoclonal (inotuzumab ozogamicin) antibody-based therapies. Tisagenlecleucel is also being investigated as a treatment for high-risk paediatric acute lymphoblastic leukaemia (positive minimal residual disease at the end of consolidation) in a phase 2 trial (CASSIOPEIA, NCT03876769). Concurrently, axicabtagene ciloleucel is expanding into chronic lymphocytic leukaemia (ZUMA-8, NCT03624036) and acute lymphoblastic leukaemia indications (ZUMA-3, NCT02614066; ZUMA-4, NCT02625480).[51,52] Preliminary results from phase 1 trials were promising,[51,52] and axicabtagene ciloleucel has moved on to phase 2 testing for the treatment of these two conditions (table 3).

In lymphoma, tisagenlecleucel is being tested in combination with pembrolizumab, a PD-1 inhibitor, and with ibrutinib, a BTK inhibitor, in patients with DLBCL (NCT03630159, NCT03876028). The product is also being tested for the treatment of paediatric non-Hodgkin lymphoma (NCT03610724) and relapsed or refractory follicular lymphoma (NCT03568461). In large B-cell lymphoma, axicabtagene ciloleucel is being tested in combination with various anticancer drugs: a PD-1 inhibitor, atezolizumab, with promising results (ZUMA-6, NCT02926833), a 4–1BB agonist (utomilumab; ZUMA-11, NCT03704298), and rituximab or lenalidomide (ZUMA-14, NCT04002401). The developer is also testing axicabtagene ciloleucel as a first-line treatment in high-risk large B-cell lymphoma (ZUMA-12, NCT03761056), and as a treatment for mantle cell lymphoma and indolent non-Hodgkin lymphoma. Data generated from these axicabtagene ciloleucel studies will support the pharmacovigilance plan of this product in Europe.

Conclusion

The two approved CAR T-cell products, tisagenlecleucel and axicabtagene ciloleucel, provided a unique opportunity to explore the effect of choices made by developers during product development on the regulatory evaluation processes. Due to the still undetermined long-term benefits and high price tag, the products face tremendous pressure to have proven long-lasting clinical benefits, particularly when compared with other established treatment options in the market that are more cost-effective, such as haemopoietic stem cell transplantation. The clinical efficacy of the products was identified as the most challenging aspect during development because of the nature of the disease under study, the single-arm study designs, the complex treatment regimens, and the absence of suitable comparators. Both developers were able to implement effective measures to partially mitigate serious adverse events during clinical testing. Further measures were mandated by the regulators in the postmarketing setting to ensure patient safety. The products are being tested for various indications, and more data will further inform their benefit–risk profile. Our analysis suggests that regulatory authorities tend to accept more uncertainty in the evidence generated for CAR T-cell therapies at the time of marketing authorisation submissions compared with small molecules and conventional biologics. Of note, the outlined hurdles and challenges faced by these two products should not discourage more developers from pursuing CAR T-cell therapy development, nor are they intended to call for stricter regulatory assessments. This analysis of the development experiences and regulatory approval processes provide a roadmap to improve the generation of evidence and dossiers for future CAR T-cell therapies, and their integration into routine clinical practice.

Search strategy and selection criteria

We obtained the European public assessment reports from the database of the European Medicines Agency website (accessed April 11, 2019). We extracted the manufacturing and product quality, and non-clinical and clinical data into a spreadsheet. When needed, the relevant scientific literature mentioned in the European public assessment reports was also reviewed. Revision of the clinical data packages in the European public assessment reports relied on datasets with the longest possible follow-up time: 12-month update of the ZUMA-1 clinical study of axicabtagene ciloleucel in lymphoma (data cutoff: Aug 11, 2017), the JULIET clinical study of tisagenlecleucel in lymphoma (data cutoff: Dec 8, 2017), and the ELIANA study of tisagenlecleucel in leukaemia (data cutoff: April 25, 2017). To control for investigator bias, we relied on the results reported by the central independent review committee, rather than the results stated by the investigators. Regarding historical comparators, the SCHOLAR-1 study outcomes used as a comparator for both products were extracted from the axicabtagene ciloleucel European public assessment reports. Outcomes of the pooled CORAL extension studies used as a comparator for tisagenlecleucel were not detailed in the European public assessment reports. To reproduce the pooled analysis of the studies, we extracted the data published in scientific literature that were referenced in the European public assessment reports. The population of the pooled studies comprised patients that had relapsed after a second stem cell transplantation (n=75) and patients who did not proceed to stem cell transplantation (n=203). The responses achieved by patients in the clinical studies and historical comparators were then reproduced with the extracted patient numbers. Postauthorisation studies submitted as additional pharmacovigilance activities were extracted from the risk management plan section in the European public assessment reports for each product. To collect the latest published results of these studies, we searched ClinicalTrials.gov, PubMed, Google, the agendas, minutes, and reports of the Committee for Advanced Therapies using the developers and the ClinicalTrials.gov identifiers of the studies (data cutoff: July, 2019).

Contibutors

ME and MA contributed to the conception and design of the Personal View. ME contributed to data collection and figures, and ME and MA analysed the data. All authors contributed to the literature search, data interpretation, and the writing of the manuscript. MA approved the final manuscript.

Declaration of interests

BLL reports grants and personal fees from Novartis, during the conduct of the work; personal fees from Novartis, Avectas, Brammer Bio,

Incysus, CRC Oncology/Cure Genetics, Novartis, Vycellix, Immuneel, and Ori Biotechand, and equity in Tmunity Therapeutics of which he is a cofounder, outside the submitted work. He has patent methods for the treatment of cancer (US 8906682, US 8916381, US 9101584), patent compositions for the treatment of cancer (US 8911993, US 9102761, US 9102760), a patent method for treating chronic lymphocytic leukaemia (US 9161971), patent compositions and methods for the treatment of cancer (US 9464140, US 9518123, US 9481728, US 9540445), a patent use of CAR-modified T cells to treat cancer (US 9328156, US 9499629), and patent method for assessing the suitability of transduced T cells for administration (US 9572836), with all royalties paid to the University of Pennsylvania. ASW reports advisory board membership and consultation fees from Servier, and grants and consultation fees from Kite Pharma, during the conduct of the work; consultation fees from AbbVie, and grants and consultation fees from Spectrum Pharmaceuticals, outside the submitted work. ME and MA declare no competing interests.

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