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Tisagenlecleucel therapy for relapsed or refractory B-cell acute lymphoblastic leukaemia in infants and children younger than 3 years of age at screening: an international, multicentre, retrospective cohort study

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Summary

Background Children aged younger than 3 years were excluded from the ELIANA phase 2 trial of tisagenlecleucel in children with acute lymphoblastic leukaemia. The feasibility, safety, and activity of tisagenlecleucel have not been defined in this group, the majority of whom have high-risk (*KMT2A*-rearranged) infant acute lymphoblastic leukaemia and historically poor outcomes despite intensification of chemotherapy, and for whom novel therapies are urgently needed. We aimed to provide real-world outcome analysis of the feasibility, activity, and safety of tisagenlecleucel in younger children and infants with acute lymphoblastic leukaemia.

Methods We did an international, multicentre, retrospective cohort study at 15 hospitals across ten countries in Europe. Eligible patients were children aged younger than 3 years at screening between Sept 1, 2018, and Sept 1, 2021, who were screened for tisagenlecleucel therapy for relapsed or refractory B-cell precursor acute lymphoblastic leukaemia according to licensed indications. Patients received a single intravenous infusion of tisagenlecleucel. We tracked chimeric antigen receptor T-cell therapy outcomes using a standardised data reporting form. Overall survival, event-free survival, stringent event-free survival, B-cell aplasia, and toxicity were assessed in all patients who received a tisagenlecleucel infusion.

Findings 38 eligible patients were screened, of whom 35 (92%) received a tisagenlecleucel infusion. 29 (76%) of 38 patients had *KMT2A*-rearranged acute lymphoblastic leukaemia, and 25 (66%) had relapsed after previous allogeneic haematopoietic stem-cell transplantation (HSCT). Patients had previously received a median of 2 lines (IQR 2–3) of (non-HSCT) therapy. Seven (18%) of 38 patients had received inotuzumab and 14 (37%) had received blinatumomab. After a median of 14 months (IQR 9–21) of follow-up, overall survival at 12 months after tisagenlecleucel infusion was 84% (64–93; five patients had died), event-free survival was 69% (47–83; nine events), and stringent event-free survival was 41% (23–58; 18 events). The probability of ongoing B-cell aplasia was 70% (95% CI 46–84; seven events) at 12 months. Adverse events included cytokine release syndrome, which occurred at any grade in 21 (60%) of 35 patients and at grade 3 or worse in five (14%), and neurotoxicity at any grade in nine (26%), none of which were severe. Measurable residual disease-negative complete response with or without haematological recovery occurred in 24 (86%) of 28 patients who had measurable disease.

Interpretation These data suggest that tisagenlecleucel has antitumour activity and has an acceptable safety profile for young children and infants with B-cell precursor acute lymphoblastic leukaemia.

Funding None.

Introduction

The majority of very young children with high-risk B-cell precursor acute lymphoblastic leukaemia have infant acute lymphoblastic leukaemia, a rare form of haematological malignancy that carries a poor prognosis with standard chemotherapy, despite access to allogeneic haematopoietic stem-cell transplantation (HSCT) for children with high-risk disease. In general, outcomes of successive cooperative studies have not shown substantial improvement. The Interfant-99 cohort[1] showed a 6-year event-free survival of 46·5% (SE 2·3) and 6-year overall survival of 53·8% (2·5), and the Interfant-06 cohort[2] showed a 6-year event-free survival of 46·1% (2·1) and a 6-year overall survival of 58·2% (2·0). High-risk disease, defined in the Interfant-06 protocol as being *KMT2A*-rearranged leukaemia in patients aged younger than 6 months at diagnosis, with a white blood cell count of at least 300×10^9 cells per L or a poor response to

Research in context

Evidence before this study

We searched PubMed on April 10, 2022, using the terms "chimeric antigen receptor", "CAR-T", "CAR", "acute lymphoblastic leukaemia", and "ALL", with no language or date restrictions. We searched for publications that included children aged younger than 3 years who were treated with CD19-targeted chimeric antigen receptor (CAR) T-cell therapy for B-cell precursor acute lymphoblastic leukaemia, with outcomes reported specifically for this age group. There were three case reports, and three prospective studies each including one to four children aged younger than 3 years. These papers showed variable responses with CAR T-cell therapy, including no response, early relapse, and durable responses that were maintained for up to 23 months after infusion. The results of two studies (NCT02028455 and NCT03330691) with a total cohort of 18 infants with acute lymphoblastic leukaemia were reported in conference proceedings and showed safety and feasibility of CAR T-cell therapy, with 14 (93%) of 15 evaluable patients reaching measurable residual disease-negative complete response. All of these reports were on experimental CD19-targeting CAR T-cell products. One retrospective study reported outcomes of 12 children with infant and *KMT2A*-rearranged acute lymphoblastic leukaemia who were treated with either experimental or licensed CD19-targeting CAR T-cell products. The study showed there was no difference in relapse-free survival or overall survival in this subgroup compared with the total cohort of 231 patients aged 29 years or younger, but did not provide data on toxicity and characteristics of CAR T-cell products in children aged younger than 3 years, or on the feasibility of manufacturing these products. We concluded that there was a paucity of data to guide clinicians and families on the feasibility, toxicity, and outcomes of licensed anti-CD19 CAR T-cell therapy (tisagenlecleucel) in children aged younger than 3 years.

Added value of this study

The ELIANA phase 2 trial of tisagenlecleucel in children and young adults with B-cell precursor acute lymphoblastic leukaemia excluded children aged younger than 3 years at screening. There is therefore a paucity of information on outcomes with tisagenlecleucel in this age range, although it has been licensed, for example, in the USA, Europe, Canada, Australia, and Japan, for this age group. To our knowledge, our retrospective cohort study, conducted across a network of 15 hospitals in Europe, represents the largest study of CD19-targeted CAR T-cell therapy in children aged younger than 3 years and is the first report to show that manufacturing success rates, safety, and disease-related outcomes with tisagenlecleucel in this age range are equivalent to those reported in older children in ELIANA.

Implications of all the available evidence

Given the limitations of retrospective reporting in rare leukaemias, there is a clear suggestion of equivalent outcomes with the use of tisagenlecleucel in children aged younger than 3 years compared with older children who were treated on ELIANA, which extends the findings from experimental CD19-targeting CAR T-cell studies and support the use of tisagenlecleucel across the approved age range. Children with high-risk infant acute lymphoblastic leukaemia have poor outcomes and intensification of chemotherapy has had little impact over decades. This information will benefit clinicians and families evaluating CAR T-cell therapy as a treatment for this indication.

prednisolone during the pre-phase, carries a particularly poor prognosis, despite available chemotherapy and allogeneic HSCT. In the high-risk cohort of the Interfant-06 study,[2] 6-year event-free survival was 20·9% (having been 20·8% in the cohort with high-risk disease in the Interfant-99 study[1]), although this was improved in children who received allogeneic HSCT, in whom the 4-year disease-free survival was 44·0% (SE 6·0). However, only 76 (53%) of 143 patients in the intended cohort proceeded to allogeneic HSCT owing to early events (mainly relapses). A report from the Japanese cooperative group showed somewhat better outcomes in high-risk infant acute lymphoblastic leukaemia, with 3-year event-free survival of 56·6% (SE 6·8) and 3-year overall survival of 80·2% (SE 5·4).[3] This improvement was potentially attributable to the inclusion of high-dose cytarabine and L-asparaginase in early consolidation, intensified supportive care, and broader indications for allogeneic HSCT, such that 38 (78%) of 49 patients with high-risk disease and complete response proceeded to this therapy. Although these findings might suggest there is scope for improved outcomes with alternative front-line regimens, outcomes among infants with relapsed acute lymphoblastic leukaemia, such as those considered for chimeric antigen receptor (CAR) T-cell therapy, are extremely poor, with a 3-year overall survival of around 20%.[4] Novel therapies are being investigated in this context, including bispecific T cell-engaging antibody therapy, such as blinatumomab,[5,6] and CAR T-cell therapy.[7]

The pivotal ELIANA phase 2 trial[8] evaluated the activity and safety of tisagenlecleucel in children and young adults aged 3–25 years with relapsed or refractory B-cell lineage acute lymphoblastic leukaemia, paving the way for regulatory approval by the US Food and Drug Administration and European Medicines Agency. Children aged younger than 3 years were excluded from the study, so outcomes of tisagenlecleucel in younger children, including those with historically poorer outcomes (ie, with relapsed or refractory infant acute lymphoblastic leukaemia) and those expected to have a better outcome (eg, being aged older than 1 year at

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diagnosis but younger than 3 years at screening), were not established. However, in some countries (eg, the USA, Europe, Canada, Australia, and Japan), tisagenlecleucel is licensed for use in patients up to the age of 25 years, with no lower limit. We therefore aimed to provide real-world outcome analysis of the feasibility, safety, and activity of tisagenlecleucel in younger children and infants with acute lymphoblastic leukaemia.

Methods

Study design and participants

We conducted an international, multicentre, retrospective cohort study at 15 hospitals across ten countries in Europe (appendix p 1). Eligible patients were children aged younger than 3 years at screening between Sept 1, 2018, and Sept 1, 2021, for tisagenlecleucel therapy of relapsed or refractory B-cell precursor acute lymphoblastic leukaemia according to licensed indications at the study centre. Eligibility was assessed on the basis of the summary of product characteristics for tisagenlecleucel—ie, in the context of refractory disease, post-transplant relapse, or second or greater relapse of B-cell acute lymphoblastic leukaemia. Patients or their parents consented to deidentified or pseudoanonymised data collection and sharing according to institutional policy. Ethical approval was not mandatory as this data collection was done as part of routine service outcome evaluation in each centre.

See Online for appendix

Procedures

Data relevant to CAR T-cell therapy (such as disease characteristics, leucapheresis product, bridging therapy, CAR T-cell dose, toxicities and disease response, and long-term outcomes) were collected through a standardised data reporting form circulated to all centres associated with the Resistant Disease Committee of the International Berlin-Frankfurt-Münster study group and authorised for CAR T-cell therapy. Centres submitted data on all patients who met the eligibility criteria and who were intended to receive tisagenlecleucel. CAR T-cell therapy and follow-up was delivered according to local protocols. Patients received a single intravenous infusion of tisagenlecleucel. Follow-up was conducted as per institutional policy for delivery of licensed CAR T-cell therapy, and ended on Oct 1, 2021. Data extraction from the institutional medical reporting system was performed by the treating physician at each centre, in accordance with local protocols. We retrospectively collected data on specific toxicities on a per-patient basis. In the ELIANA study,[7] The UPenn grading criteria were used for reporting cytokine release syndrome and Common Terminology Criteria for Adverse Events (CTCAE; version 4.03) grading was used for neurotoxicity assessments. CTCAE (version 5) grading was used for all other toxicities.

Measurable residual disease was evaluated by quantitative PCR of leukaemia-specific immunoglobulin and T-cell receptor rearrangements, or flow-cytometric measurable residual disease analysis, or both, depending on local protocol. Relapse in this case was defined in accordance with the Ponte di Legno consensus guidelines.[9] Cytokine release syndrome and immune effector cell-associated neurotoxicity syndrome were defined and graded according to the 2019 American Society for Transplantation and Cellular Therapy consensus guidelines.[10] Response was analysed by the treating physician according to local or national guidelines.

Outcomes

The main outcomes were manufacturing success, overall survival, event-free survival, duration of B-cell aplasia, and toxicity. Overall survival was defined as the time from infusion of CAR T cells (tisagenlecleucel) to death. Patients were otherwise censored at the date of last follow-up.

Two definitions of event-free survival were used. The first definition was identical to that used in the ELIANA trial,[8] in which events were failure to respond, morphological relapse before response was maintained for at least 28 days, morphological relapse (with >5% blasts) after reaching complete response with or without haematological recovery, and death. With this definition, patients were censored if they received further therapy. We also applied a more stringent event-free survival definition, in which events were the same as in the first definition but additionally included measurable residual disease emergence after reaching complete response with or without haematological recovery, and receipt of further anti-leukaemia therapy, including allogeneic HSCT. Measurable residual disease emergence was defined as the presence of detectable measurable residual disease measured by flow cytometry or quantitative PCR-based measurable residual disease assessment within the quantitative range for that assay and disease with less than 1% of leukaemic cells. Cumulative incidence of relapse in patients who had a response was calculated on the basis of the time from infusion to morphological relapse or bone marrow disease of greater than 1% of leukaemic cells confirmed by two measurable residual disease assessment methods. Competing events for relapse included death in response, although, in practice, this did not occur in our cohort during the study period.

B-cell aplasia was defined by the absence of CD19-positive disease in the bone marrow and a peripheral blood B lymphocyte concentration of less than 10 cells per µL. Loss of B-cell aplasia was therefore defined as presence of peripheral blood B lymphocytes concentration of 10 cells per µL or greater. Hypogammaglobulinaemia was defined as IgG concentration of less than 5 g/L. Ongoing B-cell depletion was defined as B-cell aplasia and the absence of CD19-positive disease at any site.

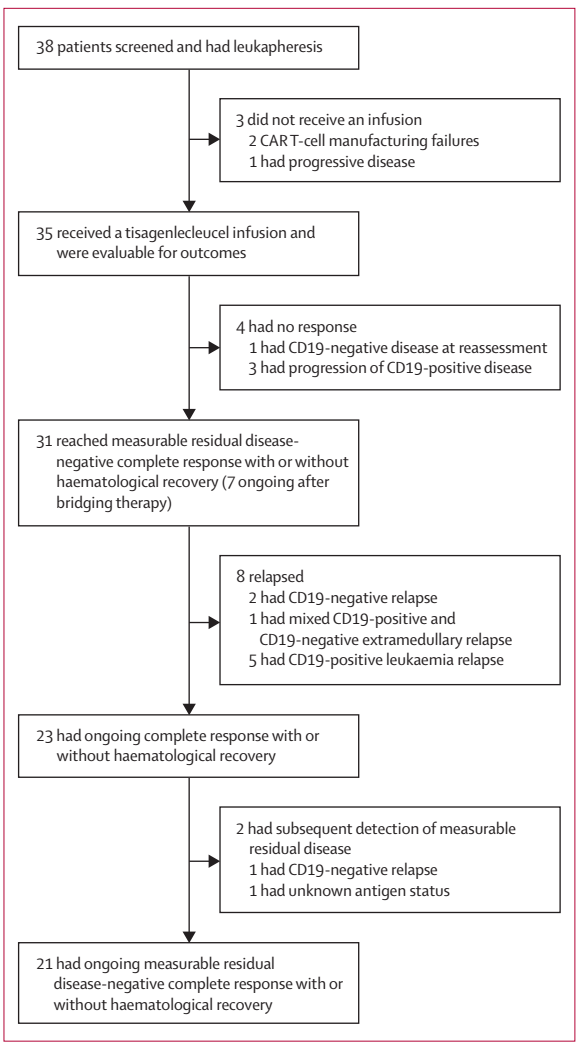

Figure 1: **Study profile**
Measurable residual disease was assessed by flow cytometry or PCR depending on local practice. CAR=chimeric antigen receptor.

	Participants
Whole cohort (n=38)	
Age at diagnosis, months	5·2 (2·6–7·6)
Sex	
Female	17 (45%)
Male	21 (55%)
White blood cell count at diagnosis, ×10⁹ cells per L	375 (130–797)*
Presenting with CNS involvement	18/32 (47%)
Treated according to Interfant-06 protocol	31 (82%)
KMT2A rearrangement	29 (76%)
Refractory to one or more previous treatment lines	19 (50%)
Previous HSCT	25 (66%)
Number of previous lines of therapy not including HSCT	2 (2–3)
Previous inotuzumab	7 (18%)
Previous blinatumomab	14 (37%)
Participants who received a tisagenlecleucel infusion (n=35)	
Median age at infusion, months	17·0 (14·9–24·6)
Bone marrow disease burden before lymphodepletion	
Median (IQR)	5% (0·2–31·0)
Measurable residual disease negative	7 (20%)
0–<1%	5 (14%)
1–<5%	5 (14%)
5–<10%	2 (6%)
10–<50%	9 (26%)
50–100%	7 (20%)
CNS disease before lymphodepletion	1 (3%)

Data are median (IQR), n (%), or n/N (%). Data on race or ethnicity were not collected. HSCT=haematopoietic stem-cell transplantation. *n=34.

Table 1: **Baseline characteristics**

Statistical analysis

All outcomes were assessed in all patients who received a tisagenlecleucel infusion (the evaluable population). Survival (or inverse survival) curves were estimated using the Kaplan-Meier method for overall survival, event-free survival and stringent event-free survival, cumulative risk of relapse, probability of ongoing B-cell aplasia, and separately for the univariate comparisons of three prespecified treatment risk factors on relapse. These risk factors were bone marrow disease burden (≥5% before CAR T-cell infusion), *KMT2A*-rearranged disease, and previous blinatumomab therapy. A Cox proportional hazards model including all three risk factors was next fitted to estimate the adjusted risk of relapse. 95% CIs were calculated based on the log hazard.

Given that children aged 1 year or older at diagnosis are likely to have a better prognosis than those meeting the definition for infant acute lymphoblastic leukaemia, in a post-hoc analysis, we separately considered the outcomes of these children.

All statistical analyses were performed using R (version 4.1.1).

Role of the funding source

There was no funding source for this study.

Results

38 eligible patients were screened and had leukapheresis (figure 1). 35 (92%) patients received tisagenlecleucel infusion and were evaluated for toxicity and disease-related outcomes. Two patients did not receive a tisagenlecleucel infusion because CAR T-cell manufacturing failed and one because disease progressed; all three died.

The median age at diagnosis in the full cohort was 5·2 months (IQR 2·6–7·6) and at tisagenlecleucel infusion was 17·0 months (14·9–24·6; table 1). 31 (82%) of 38 patients had been previously treated according to the Interfant-06 protocol and 29 (76%) had *KMT2A*-rearranged acute lymphoblastic leukaemia. As expected in this age range, the most frequent *KMT2A* partners were *AFF1* and

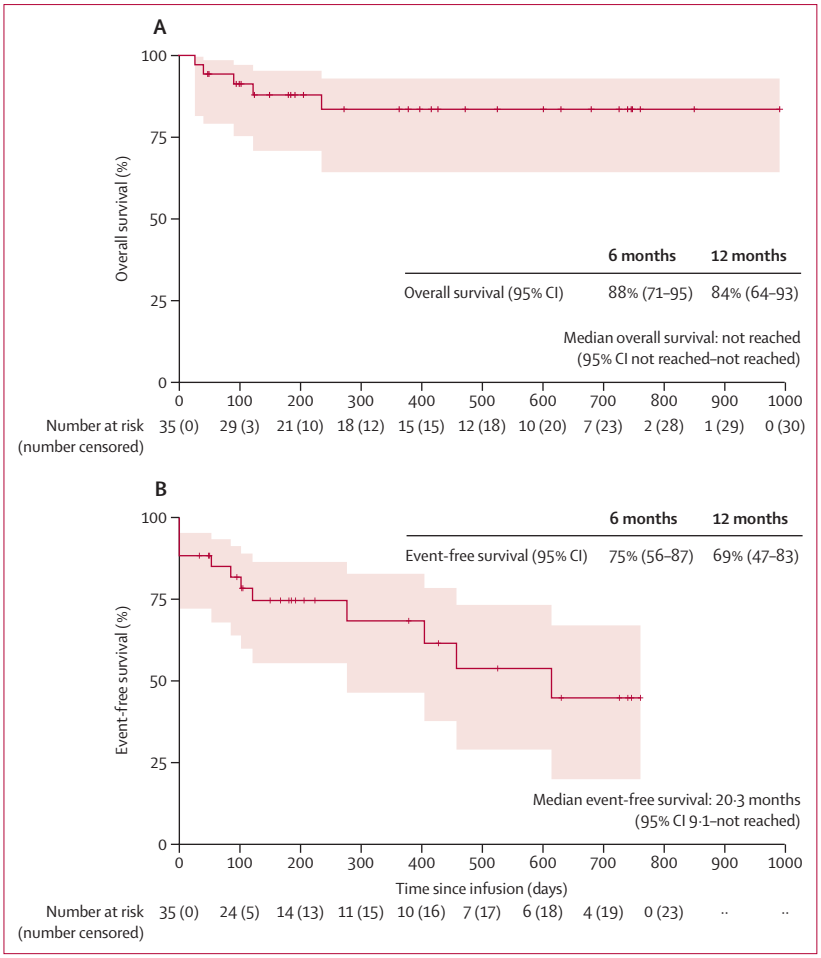

Figure 2: **Overall survival and event-free survival**
(A) Overall survival. (B) Event-free survival as defined in the ELIANA trial.[7] Shaded areas are 95% CIs.

KMT2AT1, and each were observed in nine (31%) of 29 patients. Further details of the *KMT2A* partner genes and karyotype of non-*KMT2A* rearranged leukaemias are provided in the appendix (p 2). 25 (66%) of 38 patients had relapsed after previous allogeneic HSCT, and the median number of lines of previous (non-HSCT) therapy was 2 (IQR 2–3). The proportion of patients who had received previous allogeneic HSCT was similar to that in the ELIANA trial.[7] The majority had received previous immunotherapy; seven (18%) of 38 children had received previous inotuzumab ozogamicin and 14 (37%) had received blinatumomab. The median bone marrow blast percentage before lymphodepletion in patients who received tisagenlecleucel was 5% (IQR 0·2–31·0); seven (20%) of 35 patients had undetectable measurable residual disease, ten (29%) had measurable residual disease that was detectable at up to 5% blasts, and 18 (51%) had a disease burden of 5% blasts or more, including seven who had a disease burden of greater than 50% blasts.

The median $CD3^+$ cell count of leukapheresis products obtained from patients in the whole cohort was $1{\cdot}1\times10^9$ cells (IQR 0·8–1·9) and the median $CD45^+$ or total nucleated cell count was $3{\cdot}4\times10^9$ cells (2·0–5·3; appendix p 3). The two patients for whom manufacturing failed had $CD3^+$ cell counts of $0{\cdot}6\times10^9$ cells and $1{\cdot}0\times10^9$ $CD45^+$ or total nucleated cells. The median time required for leukapheresis collection was 1 day (IQR 1–2). Six (16%) of 38 patients required 2 days of harvest for leukapheresis, and one of these patients did not receive a tisagenlecleucel infusion due to manufacturing failure. There were no manufacturing failures in the seven (18%) of 38 patients who required 3–4 days of harvest. Within the cohort, no out-of-specification products were generated. The median tisagenlecleucel dose manufactured was $2{\cdot}3\times10^6$ cells per kg patient weight (IQR 2·0–4·4).

Among the 28 patients who received an infusion and in whom response could be assessed, 24 (86%) had a complete response with or without haematological recovery. All of these responses were reached by 30 days after tisagenlecleucel infusion and were associated with measurable residual disease negativity. Seven patients received the infusion after attaining a measurable residual disease-negative bone marrow status after bridging therapy and without measurable extramedullary disease; all of these patients remained measurable residual disease-negative 30 days after the infusion. All four patients who did not have a response subsequently died of disease progression within 3 months after the infusion. After a median of 14 months (IQR 9–21) of follow-up, overall survival at 6 months was 88% (95% CI 71–95; four of 35 patients had died) and at 12 months was 84% (64–93; five patients had died; figure 2A). Event-free survival, as defined in ELIANA, at 6 months was 75% (95% CI 56–87; eight events) and at 12 months was 69% (47–83; nine events; figure 2B). Stringent event-free survival (which included institution of further therapy or emergence of measurable residual disease) at 6 months was 63% (95% CI 44–77; 12 events) and at 12 months was 41% (23–58; 18 events; figure 3A). Within the patients who received an infusion and had no measurable residual disease (n=7), event-free survival was 100% at 6 months and 12 months; however, stringent event-free survival was 83% (95% CI 28–97; one event) at 6 months and 67% (19–90; two events) at 12 months. Two patients had B-cell recovery before 6 months, which prompted further therapy in one patient. One patient had emergence of measurable residual disease and received further therapy at 6 months. Two patients had late (>1 year after the infusion) disease relapses and went on to receive further therapy.

Survival analysis (of overall survival, event-free survival, and stringent event-free survival) showed no significant association between pre-treatment risk factors (bone marrow disease burden of ≥5% leukaemic cells, previous blinatumomab therapy, or *KMT2A*-rearranged disease) and any outcome measure (appendix p 5). Relapse risk was not significantly increased in patients with *KMT2A*-rearranged leukaemia (appendix pp 2, 5).

Eight (26%) of 31 patients who reached complete response had a subsequent relapse during follow-up; two had relapses involving evolution to CD19-negative disease and one had a mixture of CD19-positive and CD19-negative blasts on biopsy of an extramedullary chloroma (figure 1). There were no cases of lineage switch as a cause for emergence of CD19-negative disease in patients who received a tisagenlecleucel infusion. The other five relapses were CD19-positive. Among the eight patients with frank relapse, three relapses occurred at extramedullary sites (brain chloromas, periorbital soft tissue infiltration, and lymphadenopathy of the cervical and lower limbs). Six relapsed patients received further therapy; of these, one patient had died due to the disease by the time of data cutoff. The cumulative incidence of relapse among patients who received a tisagenlecleucel infusion was 19% (95% CI 9–37; six events) at 6 months and 25% (12–44; seven events) at 12 months (appendix p 4). Two patients had emergence of measurable residual disease but did not meet the criteria for relapse; evidence of CD19-negative disease was noted in one and one had CD19-positive disease, both were detected at 6 months after the infusion. Both patients with detection of measurable residual disease following the CAR T-cell infusion proceeded to receive further therapy (not HSCT) and remained alive, albeit with short follow-up.

15 (43%) of 35 patients received further therapy during follow-up. In six patients, this was for frank relapse (in one patient for non-response to CAR T-cell therapy; in three patients for early B-cell recovery at 6 months or less after CAR T-cell infusion; and in two patients for re-emergence of measurable residual disease). Six (17%) of 35 patients received allogeneic HSCT (in five of whom this was their second HSCT); due to relapse in two patients, early B-cell recovery (at 3 months and 6 months, respectively) in two patients, and physician preference in two patients who had measurable residual disease-negative response with ongoing B-cell aplasia.

Of four children who were aged 1 year or older at diagnosis, one had *KMT2A*-rearranged disease, did not receive a CAR T-cell infusion due to manufacturing failure, and subsequently died due to the disease. The other three children received an infusion of tisagenlecleucel. One patient went on to receive allogeneic HSCT after the CAR-T cell infusion because of short CAR T-cell persistence, one received further therapy for emergence of measurable residual disease, and one remained in remission without further therapy.

Cytokine release syndrome of any grade occurred in 21 (60%) of 35 patients who received a tisagenlecleucel infusion (table 2). Severe cytokine release syndrome (grade 3 or worse) occurred in five (14%) of 35 patients. The median duration of cytokine release syndrome was 1·5 days (IQR 0·0–4·0), compared with 8 days (range 1–36) in ELIANA. Eight (23%) of 35 patients received tocilizumab. Nine (26%) children were managed in an intensive care setting, compared with 35 (47%) of 75 in ELIANA. The median duration of intensive care unit (ICU) stay was 2 days (IQR 2–10), compared with 7 days (range 1–34) in ELIANA. Neurotoxicity or immune effector cell-associated neurotoxicity syndrome occurred in nine (26%) of 35 patients, but was severe in none.

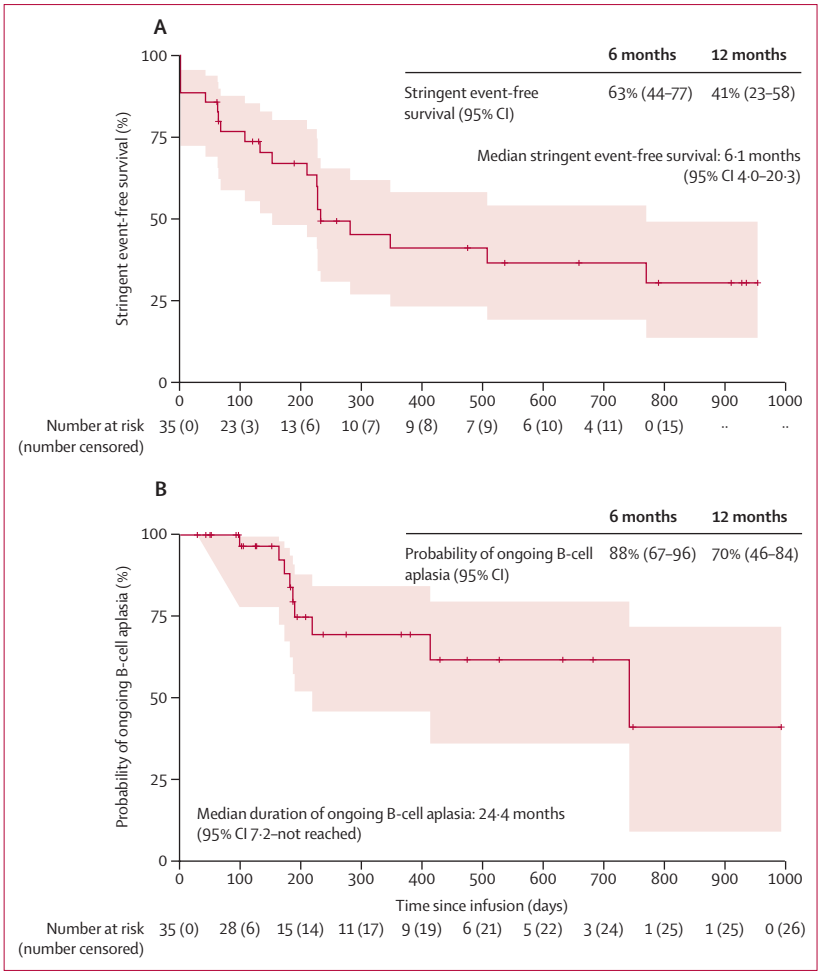

Figure 3: **Stringent event-free survival and probability of ongoing B-cell aplasia**
(A) Stringent event-free survival. (B) Ongoing B-cell aplasia. Shaded areas are 95% CIs.

Prolonged cytopenia (after day 30) was observed in 15 (65%) of 23 patients with evaluable data (table 2), and this was severe (grade 3 or worse) in 12 (52%). Infections were recorded in ten (29%) of 34 patients, nine of which were grade 3 or worse. Infections included bacteraemia in seven (21%) patients, bacterial meningitis in one (3%), and abdominal infections in two (6%). There were two cases of febrile neutropenia (6%). There were no deaths due to toxicity (only deaths due to progressive or relapsed disease).

The probability of ongoing B-cell depletion, which might correlate with CAR T-cell persistence, was 88% (95% CI 67–96; three events) at 6 months and 70% (95% CI 46–84; seven events) at 12 months (figure 3B). Hypogammaglobulinaemia was reported in 27 (87%) of 31 evaluable patients (excluding those who did not

	Patients who received a tisagenlecleucel infusion (n=35)	ELIANA cohort (n=75)
Cytokine release syndrome		
Any grade	21 (60%)	58 (77%)
Grade 1–2	16 (46%)	23 (31%)
Grade 3	3 (9%)	16 (21%)
Grade 4	2 (6%)	19 (25%)
Median duration, days	1·5 (0·0–4·0)	8·0 (NR–NR)
Tocilizumab administered	8 (23%)	28 (37%)
Managed in ICU	9 (26%)	35 (47%)
Median duration in ICU, days	2 (2–10)	7 (NR–NR)
Neurotoxicity or immune effector cell-associated neurotoxicity syndrome		
Any grade	9 (26%)	30 (40%)
Grade 1–2	9 (26%)	20 (27%)
Grade 3	0	10 (13%)
Grade 4	0	0
Cytopenia for ≥30 days		
Any grade	15/23 (65%)	28 (37%)
Grade 1–2	3/23 (13%)	4 (5%)
Grade 3	9/23 (39%)	12 (16%)
Grade 4	3/23 (13%)	12 (16%)
Hypogammaglobulinaemia	27/31 (87%)	NR
Infection		
Any grade	10/34 (29%)	32 (43%)
Grade 1–2	2/34 (6%)	14 (19%)
Grade 3	8/34 (24%)	16 (21%)
Grade 4	0/34	2 (3%)

Data are median (IQR), n (%), or n/N (%). ICU=intensive care unit. NR=not reported.

Table 2: **Toxicity of tisagenlecleucel therapy**

proceed to infusion and those who had no response), all of whom were treated with immunoglobulin replacement therapy in accordance with local protocols.

Discussion

The ELIANA trial[8] excluded patients aged younger than 3 years at screening and therefore, to date, outcomes of patients in this age range receiving tisagenlecleucel have not been shown. This report provides real-world data on the use of tisagenlecleucel in children aged younger than 3 years at assessment for this therapy. Our data show the safety and activity profile in this younger age range was consistent with that observed in the older children or young adults enrolled in ELIANA. Responses were durable in a substantial proportion of patients, with a median event-free survival of 20·3 months. To our knowledge, this is the largest and most comprehensively reported cohort of younger children treated with licensed CD19-targeting CAR T-cell therapy published to date, and the only study to systematically report the use of a licensed CAR T-cell therapy for this indication in this age group.

Theoretically, there are several reasons why outcomes in very young patients with predominantly infant acute lymphoblastic leukaemia might be different to those of older patients infused with the same CAR T-cell product. The feasibility of leukapheresis in very young children weighing less than 10 kg requires special consideration. Furthermore, inherent differences in the more naive lymphocyte pool obtained from younger children, and differences in front-line chemotherapy regimens, might affect the manufacture, therapeutic efficacy, expansion, and persistence of CAR T cells. Infant acute lymphoblastic leukaemia with *KMT2A* rearrangement is particularly aggressive compared with acute lymphoblastic leukaemia in older children and results in poorer outcomes with intensive chemotherapy.[1,2] Although older patients with high-risk cytogenetic lesions, including *KMT2A* rearrangement, were included in ELIANA, participant numbers were too small to provide rearrangement-specific outcomes.

Feasibility and early activity of experimental CAR T-cell therapy for infant acute lymphoblastic leukaemia were reported in a conference abstract in a cohort of 18 patients with a median age of 23 months (range 14·5–40·1) treated with two CAR T-cell products, targeting either CD19 or CD19 and CD22.[7] 16 (94%) of 17 patients received an infusion. Of 15 evaluable patients, 14 (93%) had a measurable residual disease-negative complete response, six of whom received a subsequent HSCT. One patient who received HSCT had CD19-negative relapse. Among the eight patients who did not receive HSCT, there was one case of lineage switch relapse, and two cases of CD19-positive relapse. The estimated 1-year leukaemia-free survival was 66·7%, and 1-year overall survival was 71·4%. In real-world cohorts, as well as in a combined single-cente retrospective analysis of children treated on CAR T-cell studies as well as with the licensed product,[11–13] the clinical characteristics and outcomes for younger children, including manufacturing feasibility, toxicity, and further therapy, were not systematically reported. In a small cohort of 14 patients treated with tisagenlecleucel for infant acute lymphoblastic leukaemia and reported by the US Pediatric Real World CAR Consortium,[14] the reported outcomes were worse than those presented here, with a 6-month event-free survival of 48%, 6-month overall survival of 71% (CIs were not reported), and lineage switch relapse in four (29%) of 14 patients who received an infusion. This report did not consider outcomes for children aged older than 1 year at diagnosis and, as for all the retrospective studies considered above, data on clinically relevant outcome measures, such as the need for further therapy in complete response or emergence of measurable residual disease, were not provided. Therefore, our data on clinically relevant outcomes, such as stringent event-free survival, from the largest real-world cohort of children in this age range are of particular relevance in guiding clinicians delivering licensed CAR T-cell therapy for acute lymphoblastic

leukaemia in very young children, as well as for their families.

We noted an excellent feasibility of manufacture of tisagenlecleucel, despite the aggressive nature of relapsed or refractory infant acute lymphoblastic leukaemia, with 35 (92%) of 38 patients who were screened receiving an infusion. This feasibility might be associated with the proportion of patients who had received a previous allogeneic HSCT (25 [66%] of 38), such that donor-derived T cells were collected in the majority, rather than more heavily chemotherapy-exposed autologous T cells. It is not possible to exclude that patients with accelerated relapse and high blast counts were diverted to other treatments before screening. Despite the young age of our cohort, a leukapheresis meeting manufacturer's specifications was obtained with a 1-day harvest in the majority (57%) of patients. However, seven patients required 3 or 4 days of harvest, which is an indication that collection of a sufficient lymphocyte number can be challenging. There were two manufacturing failures, giving a rate of 5%, compared with 8% in ELIANA. The median dose of CAR T cells delivered ($2 \cdot 3 \times 10^6$ cells per kg patient weight) was robustly in the middle of the licensed dose range, with no out-of-specification products, which suggests that, having achieved an adequate leukapheresis, CAR T-cell manufacturing for younger children and infants is as feasible as that for older children and young adults.

The proportion of patients with a complete response with or without haematological recovery of 86% (24 of 28 patients) noted in this study is similar to that noted in older children and young adults in ELIANA (81%),[8] and in the various reported real-world consortia data (85–96%).[11,12,15,16] Furthermore, the overall survival and event-free survival we report compare favourably with that reported in ELIANA (6-month overall survival 90% [95% CI 81–95] *vs* 88% [71–95], 12-month overall survival 76% [63–86] *vs* 83 [64–93], 6-month event-free survival 73% [60–82] *vs* 75 [56–87], 12-month event-free survival 50% [35–64] *vs* 69 [47–83]) and with the real-world consortia data. Thus, our data suggest that responses following tisagenlecleucel therapy in younger patients with predominantly high-risk relapsed infant acute lymphoblastic leukaemia are similar to those in the older age range; additionally, most responses are durable, albeit within a median follow-up of 14 months.

Factors associated with poorer outcomes after tisagenlecleucel infusion have been identified through retrospective and registry studies, including higher disease burden.[12,13] Initial studies indicated that previous blinatumomab therapy is associated with poorer outcomes to subsequent CD19-targeted CAR T-cell therapy.[16–18] In a further large study,[19] worse outcomes were confined to patients who showed no response to blinatumomab, suggesting such patients might have intrinsic T-cell dysfunction. Furthermore, although *KMT2A*-rearranged leukaemias have a propensity for lineage switch as a mechanism of CD19-negative leukaemic escape,[20] it is not clear if the rate of relapse is higher overall than for leukaemias in which *KMT2A* is germline. One report has suggested this is not the case,[7] and, similarly, outcomes for patients with *KMT2A*-rearranged leukaemias were not significantly different to those without *KMT2A* rearrangement in our study. Contrary to a congress report of patients with *KMT2A*-rearranged acute lymphoblastic leukaemia of all ages who were treated with tisagenlecleucel,[21] in which a larger proportion of patients had myeloid lineage switch (nine of 38 patients), there were no reports of myeloid lineage switch in our cohort. It is noteworthy that, of three patients who had emergence of CD19-negative disease (one non-response and two relapses), two were in patients with *KMT2A*-germline leukaemia, and only one had been treated with blinatumomab before receiving CAR T-cell therapy. Two of three patients had a high disease burden at the point of CAR T-cell infusion, which was noted to be associated with increased risk of CD19-negative disease burden in other studies.[16,19]

The toxicity profile noted in our cohort of younger children was similar to that seen in the older age range in ELIANA.[8] Given the differences in the cytokine release syndrome scoring systems used (American Society for Transplantation and Cellular Therapy consensus guidelines were used in this study versus the UPenn cytokine release syndrome score in ELIANA), one would expect a lower incidence of grade 3 or worse cytokine release syndrome in the cohort reported here, which was indeed observed. Severe cytokine release syndrome was reported in five (14%) of 35 patients, compared with 35 (46%) of 75 patients in ELIANA. However, other more comparable measures of CAR T-cell-associated toxicity, such as the proportion of patients with any-grade cytokine release syndrome (60% in our cohort *vs* 77% in ELIANA), median duration of cytokine release syndrome ($1 \cdot 5$ days *vs* $8 \cdot 0$ days), proportion of children managed in ICU (26% *vs* 47%), median duration of ICU stay (2 days *vs* 7 days), and proportion of patients who received tocilizumab (23% *vs* 37%), compared favourably with those in ELIANA. These toxicity data are reassuring for clinicians treating children in a younger age range. The lower incidence of severe cytokine release syndrome could be due to an inherently reduced severity or duration of cytokine release syndrome in the younger age range, but it is also possible that differences in practice between the ELIANA study and a real-world setting (eg, earlier referral, intensity of bridging therapy, lower disease burden at the time of CAR T-cell infusion, and earlier use of tocilizumab) might also have had a role.

The incidence and severity of neurotoxicity observed in this cohort of younger children again compared favourably with those seen in ELIANA (any-grade neurotoxicity 26% *vs* 40%, grade 3–4 neurotoxicity 0% *vs* 13%). Although a contribution by the grading system used (Common Terminology Criteria for Adverse Events

for ELIANA and American Society for Transplantation and Cellular Therapy consensus grading for our cohort) cannot be excluded, the absence of severe neurotoxicity is reassuring.

Prolonged cytopenias were frequent in this cohort and were more likely to be severe than in ELIANA. 15 (65%) of 23 evaluable patients had any grade of cytopenia persisting beyond the first month, 12 (52%) of whom had severe cytopenia. However, because data were only provided for some patients, it is possible that a reporting bias was introduced towards those more severely affected. This interpretation is supported by the incidence of infections (any grade in 12 [35%] of 34) and severe infections (ten [29%]), which did not differ from those seen in older children in ELIANA. Given that this was a heavily pretreated cohort, who had generally relapsed after HSCT or receiving multiple lines of intensive chemotherapy, the manageable incidence of infection, despite the incidence and prolonged nature of the cytopenias documented, is again encouraging. Importantly, there were no deaths due to infection.

These data support the use of tisagenlecleucel for licensed indications among children aged younger than 3 years at screening for CAR T-cell therapy. The application of a novel, stringent event-free survival outcome measure provides additional benefit to clinicians advising patients on tisagenlecleucel therapy. We think this measure more accurately reflects therapy failure than the standard event-free survival definition applied in ELIANA, because it more completely captures clinically relevant endpoints, such as the need for further anti-leukaemia therapy and emergence of measurable residual disease. As such, we advocate for wider adoption of this outcome measure in future clinical studies of CAR T-cell therapy, because we believe it will better inform the community of outcomes that have impact on patients. Limitations of this study were its retrospective nature, and the small cohort size with regard to performing subgroup analyses, which might have contributed to the inability to identify significant differences in outcomes between the subgroups of interest analysed. Furthermore, because contributing centres provided data on a voluntary basis, it is possible that centre self-selection introduced bias. However, because this is the largest cohort we are aware of worldwide, a substantial proportion of eligible European patients were likely to be included.

Our findings are encouraging with respect to the historical outcomes of infant acute lymphoblastic leukaemia following HSCT, and, if confirmed with longer follow-up, they support the development of prospective studies to compare the efficacy of tisagenlecleucel against HSCT earlier in the course of therapy in this population.

Contributors

SG, EJ, and AB conceived the project and coordinated data collection. All authors except for SH provided data. All authors were involved with writing or revising the manuscript and interpreting the findings. SG and SH did the data analysis. SG and SH accessed and verified the data. All authors had full access to all the data in the study and had final responsibility for the decision to submit for publication.

Declaration of interests

SG, PB, AMQ, and AA report personal fees from Novartis, outside of the submitted work. EJ, BDM, SR, and PB report honoraria from Novartis. SR and AB report personal fees from Novartis, and honoraria from Novartis, during the conduct of the study. SR and PB report personal and honoraria from Servier, Celgene, and Bristol Myers Squibb. SR and AA report personal fees from Kite, Gilead, JAZZ Pharma, and Amgen. SR reports honoraria from Kite, Novartis, Celgene, and Servier-Cellectis, outside of the submitted work. SG reports non-financial support from Novartis, outside of the submitted work. SG, PB, BH, and BG report personal fees and non-financial support from Amgen, outside of the submitted work. BDM, PB, BH, and BG report non-financial and honoraria from Jazz, outside of the submitted work. AA reports personal fees from Takeda, Johnson and Johnson, and MSD outside of the submitted work. BH and BG report non-financial and honoraria from Servier. AB reports grants and personal fees from Servier, personal fees from AstraZeneca, non-financial support from Sanofi, personal fees from Jazz, grants and personal fees from Servier, and non-financial support from Sanofi, outside of the submitted work. AA reports SG has a University College London Business patent (PCT/GB2016/050574 WO 2016/139487) with royalties paid. PB has a patent Medacs with royalties paid (14 177 312.7) and reports grants from Novartis, Neovii, Riemser, and Medacs. All other authors declare no competing interests.

Data sharing

Deidentified data from this study will be made available on request to the corresponding author.

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