Characterization of second primary malignancies post CAR T-cell therapy: real-world insights from the two global pharmacovigilance databases of FAERS and VigiBase

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Summary

Background The FDA's alerts regarding the T-cell lymphoma risk post CAR-T therapy has garnered global attention, yet a comprehensive profile of second primary malignancies (SPMs) following CAR-T treatment is lacking.

Methods We extracted adverse event reports of hematological malignancies (HMs) patients with clearly definable SPMs from the FAERS and VigiBase databases (2017–2023). Disproportionality analysis using reporting odds ratio (ROR) and adjusted ROR was performed to assess associations between SPMs and CAR-T therapy. Time-to-onset analysis explored factors affecting SPM manifestation.

Findings SPMs post CAR T-cell therapy include HMs and solid tumors. T-cell lymphoma and myelodysplastic syndromes were consistently identified as positive signals across the overall and subgroup analyses. Hematological SPMs showed earlier onset with increasing annual incidence post CAR-T therapy, whereas solid tumors exhibit delayed manifestation. SPMs in CAR-T recipients had significantly earlier onset than non-recipients. Furthermore, age-specific characteristics reveal earlier SPM manifestations in pediatric, adolescent, and young adult populations compared to older populations post CAR-T therapy.

Interpretation The current SPM profile highlights the necessity of long-term safety monitoring for all CAR-T recipients given the observed yearly increase of SPMs. Customizing long-term SPM screening across different age groups may enhance early detection and intervention strategies, ultimately improving patient outcomes in the follow-up of CAR-T recipients.

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Research in context

Evidence before this study

Chimeric antigen receptor T-cell (CAR-T) therapy has demonstrated remarkable efficacy in managing refractory or relapsed hematological malignancies, nonetheless, the FDA's recent alerts regarding the risk of T-cell lymphoma associated with CAR-T therapy have raised global concerns. Prior to this study, we have searched PubMed using the following terms "CAR-T", "second primary malignancy", with- and without either "FAERS" or "VigiBase" from inception to February 1st 2024, without language restrictions. There are only a scarcity of case reports and retrospective studies with limited sample size reporting second primary malignancies (SPMs) post CAR-T therapy, however, there lacks a comprehensive SPM profile after CAR-T treatment utilizing the post-marketing real-world data from the pharmacovigilance databases.

Added value of this study

We found increased rate of hematological malignancies and solid tumors post CAR-T therapy, with T-cell lymphoma and myelodysplastic syndromes consistently emerging as safety signals across the overall and subgroup analyses. Our time-to-onset analysis revealed distinct manifestation patterns, with hematological SPMs showing earlier onset and increasing annual incidence, while SPMs of solid tumors exhibiting delayed occurrence. Additionally, we found the onset time of SPMs was significantly earlier in CAR-T recipients than non-recipients, with age-specific characteristics of earlier SPM manifestations in younger populations under 40-years old.

Implications of all the available evidence

Our study suggests the necessity of long-term and customized SPM screening across different age groups in the follow-up of CAR-T recipients. Large population-based prospective studies are warranted to comprehensively investigate SPMs post CAR-T therapy and develop more precise monitoring and management strategies in the future.

Introduction

Hematological malignancies (HMs), generally classified into leukemia, lymphoma, and multiple myeloma (MM), constitute approximately 10% of all cancer cases globally,[1] while refractory or relapsed (r/r) HMs lead to a poor prognosis.[1,2] Chimeric antigen receptor T-cell (CAR-T) therapy has emerged as a transformative approach in r/r HMs treatment, yielding significantly improved complete remission rates ranging from 70 to 90% in r/r B-cell acute lymphoblastic leukemia (B-ALL) and MM, and 40–50% in r/r Non-Hodgkin lymphoma (NHL).[3] Overall, CAR-T therapy stands out as a distinguished therapeutic approach of precision medicine, for its notable achievements including high response rates, lowered relapse rates, sustained remissions, and synergistic effects when combined with other treatment modalities in the management of r/r HMs.[4,5]

Apart from the considerable clinical benefits, the safety of CAR-T therapy has been under continuous monitoring, with cytokine release syndrome (CRS) and immune effector cell-associated neurotoxicity syndrome (ICANS) being the most prevalent adverse events (AEs).[6] Second primary malignancy (SPM) of T cell lymphoma, a clinically rare but impactful AE following CAR-T therapy, has drawn sustained worldwide attention. The U.S. Food and Drug Administration (FDA) has announced receiving 22 AE cases of T-cell lymphoma from patients within two years after CAR-T infusion, and three were confirmed to harbor the CAR transgene in the malignant clone, underscoring the highly suspected role of CAR-T in developing SPMs.[6,7] From a mechanistic perspective, a specific vector integration site of CAR-T might enhance the anti-cancer ability, however, as a double-edged sword, it might also increase the risk of insertional carcinogenesis.[6,8] Consequently, the FDA has mandated lifelong monitoring of SPMs in all commercially available CAR-T products since November 2023.[9,10]

SPMs are among the leading death causes in the first primary malignancy (FPM) survivors[11]; however, our understanding of SPMs post CAR-T therapy is still incomplete, primarily from a scarcity of case reports or retrospective studies with limited sample size.[12–14] To address this knowledge gap, this study aimed to comprehensively elucidate the current landscape of SPMs post CAR-T therapy using real-world, large-scale data from global pharmacovigilance databases. Such efforts will be instrumental in guiding the lifelong safety monitoring of CAR-T products in the future.

Methods

Data source

We endeavored to harness data mining techniques employed in our previous pharmacovigilance studies[15,16] and the published literature[17] to characterize SPMs following CAR-T therapy. To be specific, we extracted SPMs reports within patients with HM from the two largest pharmacovigilance databases in the world, the

FDA Adverse Event Reporting System (FAERS) and World Health Organization's global Individual Case Safety Report database (VigiBase). FAERS is a publicly available database of drug safety reports submitted by patients, healthcare professionals, and pharmaceutical companies.[18] VigiBase, developed and maintained by the Uppsala Monitoring Center, is the world's largest pharmacovigilance database containing potential side effects of medicines reported globally.[19] The reports we analyzed were submitted to these two databases between January 2017 (the year when the first CAR-T product Tisagenlecleucel (Kymriah®; Novartis Pharma Schweiz AG) officially approved by the FDA) and December 2023. For cases from FAERS, patients' diagnosis of HMs were based on the reported drug indications, which were compared and matched with the preferred term (PT) from the Medical Dictionary for Regulatory Activities (MedDRA) version 25.1 (Supplementary Table S1). For cases from VigiBase, MedDRA 25.1, International Classification of Diseases-9 (ICD-9) and ICD-10, were all used to match the reported drug indication with patients' diagnosis. For the ICD system, terms categorized under "Malignant neoplasms, stated or presumed to be primary, of lymphoid, haematopoietic and related tissue (C81–C96)" were employed to screen for HM patients. CAR-T products, including commercially available anti-CD19 (axicabtagene ciloleucel, brexucabtagene autoleucel, lisocabtagene maraleucel, tisagenlecleucel) and anti-BCMA CAR-T (idecabtagene vicleucel, ciltacabtagene autoleucel), were used to search for all the cases who received CAR-T therapy. In this study, SPM was defined as the presence of two distinct malignancies between the reported drug indication and the AE following CAR-T therapy or the AE was originally reported as the "second primary malignancy" to FAERS and/or VigiBase. Notably, for the analysis of SPMs after CAR-T treatment, we only considered cases where CAR-T was identified as the "primary suspect (PS)" drug in FAERS or a "suspect" drug in VigiBase.

Data processing procedure

We deduplicated CAR-T reports obtained from FAERS and VigiBase. In FAERS, we eliminated the duplicated reports with all the same values in the fields "Sex", "Age", "Country", "Event Date", "Adverse Reaction", "Drug", and "Indication".[20] In VigiBase, we eliminated duplicated reports based on the standardized VigiBase inherent algorithms.[21] Additionally, cases without a clear inference of SPM as we defined were excluded from further analysis. In this study, we inferred a second primary malignancy by comparing the reported patient's AEs with drug indications. Records that could not be accurately inferred as SPMs were excluded prior to the further analysis. Specifically, Supplementary Table S2 provides examples that could not be accurately inferred as SPMs. Following the previously detailed steps of deduplication and the exclusion of data containing undefinable SPMs, a total of 492,845 AE reports from patients with HMs from FAERS and 457,337 reports from VigiBase were maintained for further analysis. Among these, there were 6370 and 6942 AE reports under CAR-T products in FAERS and VigiBase, respectively. The flow chart of data processing is detailed in Fig. 1.

Annual incidence calculation of SPMs post CAR-T therapy

To determine the annual incidence rate of SPMs following CAR-T therapy, we divided the number of

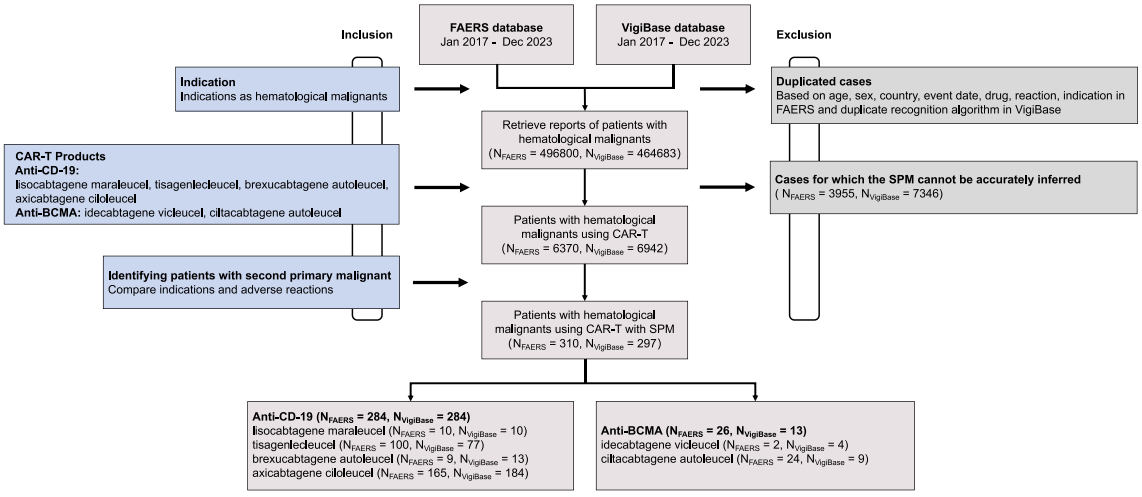

Fig. 1: Data processing flowchart delineating the selection process of SPMs post CAR-T therapy. Cases lacking accurate inference as SPMs are excluded; refer to Supplementary Table S2 for examples. FAERS indicates the FDA adverse event reporting system, SPM indicates secondary primary malignancies.

yearly reported SPM cases by the yearly total number of AE cases after CAR-T therapy. This calculation utilized data from both FAERS and VigiBase, covering the study period from 2017 to 2023. Additionally, the annual SPMs incidence rates were calculated for subgroups of solid tumors and HMs.

Signal mining of pharmacovigilance data

In most pharmacovigilance studies, disproportionality analysis has been primarily employed to assess possible associations between specific AEs and drugs, and a further clinical association relationship would be explored for individual cases.[22] In this study, we utilized reporting odds ratio (ROR) to examine the likelihood of an AE of interest for a suspected drug as reported previously.[20,23,24] Firstly, we created a drug adverse reaction contingency table (Supplementary Table S3) and referred it as the basis for subsequent ROR calculations. In this table, a represents the number of cases developed SPMs post CAR-T therapy; b represents the number of cases experienced other non-SPM AEs in CAR-T recipients; c represents the number of cases developed SPMs without CAR-T therapy; d represents the number of cases experienced other non-SPM AEs without CAR-T therapy. Secondly, we calculated the ROR and the 95% confidence intervals (CIs) using the following formulas:

$$ROR = \frac{a * d}{c * b}$$

$$ROR025 = e^{Ln(ROR) - 1.96\sqrt{\frac{1}{a}+\frac{1}{b}+\frac{1}{c}+\frac{1}{d}}}$$

$$ROR075 = e^{Ln(ROR) + 1.96\sqrt{\frac{1}{a}+\frac{1}{b}+\frac{1}{c}+\frac{1}{d}}}$$

A positive signal is defined as the number of an AE no less than three and the lower limit of the 95% CI of the ROR exceeding one as described previously.[20,23,24] In this study, if the assessed SPM meets this criteria as a positive signal for both FAERS and VigiBase, it would be graded as a potentially high-risk SPM after CAR-T therapy. Last but not least, to adjust for the impact of potential confounding factors on SPM incidence, we calculated adjusted reporting odds ratios (aRORs) and 95% CIs via multivariate logistic regression analyses for a more rigorous evaluation of any high-potential SPM signal post CAR-T therapy.

Time-to-onset (TTO) analysis

In FAERS, time to onset is defined as the interval between EVENT_DT (the date of occurrence of AEs) and START_DT (the date when drugs start to be used). We used cumulative distribution curves to present event-to-onset characteristics of SPMs post CAR-T therapy in different subgroups of sexes, ages and cancer types based on the data from FAERS. Additionally, the differences of the SPMs onset time in CAR-T recipients and non-recipients were compared.

Statistics

To compare the baseline characteristics between CAR-T recipients and non-recipients, categorical and numerical variables were analyzed using Chi-square test and Mann–Whitney U test, respectively. Mann–Whitney U test and Kruskal–Wallis test were used to compare the differences of median time to SPMs onset between CAR-T and non-CAR-T cases in further stratified analyses regarding different sexes, age clusters, and cancer subtypes. To visualize the timeline of SPMs incidence post CAR-T therapy, including the SPMs subgroups of solid tumor and HMs, histograms and line charts were plotted to present the yearly number of cases and the corresponding proportions out of the yearly total AEs following CAR-T treatment. A horizontal histogram was employed to present the number of SPMs post CAR-T and the proportion contribution of each CAR-T product. A Sankey diagram was utilized to show the evolutionary relationship between a FPM and the corresponding SPM following CAR-T therapy. Heat maps and forest plots were employed to visualize the ROR of SPMs after CAR-T treatment. Prior to multifactorial logistic regression, missing values of covariates were imputed using the random forest method built on missForest R package.[25] All statistical analyses were performed using R software (https://www.r-project.org/; version 4.2.0) and ggplot2 R package[26] was for data visualization. A two-sided P value less than 0.05 is considered as statistically significant.

Ethics

The FAERS database is publicly accessible, and health professionals can obtain access to VigiBase upon request. Both databases anonymize and de-identify patient records. Consequently, ethical clearance and informed consent are exempted for this study.

Role of the funding source

The funders of the study had no role in study design, data collection, data analysis, data interpretation, or writing of the report. J.Y.S. and R.H. had full access to the data in the study, and the corresponding authors final responsibility for the decision to submit for publication.

Results

The current overview of SPMs profile following CAR-T therapy

We extracted 310 and 297 SPM cases initially diagnosed with HMs from FAERS and VigiBase, respectively (see Fig. 1). In total, 19 and 16 SPMs, each with a minimal of three cases, were identified from FAERS and VigiBase,

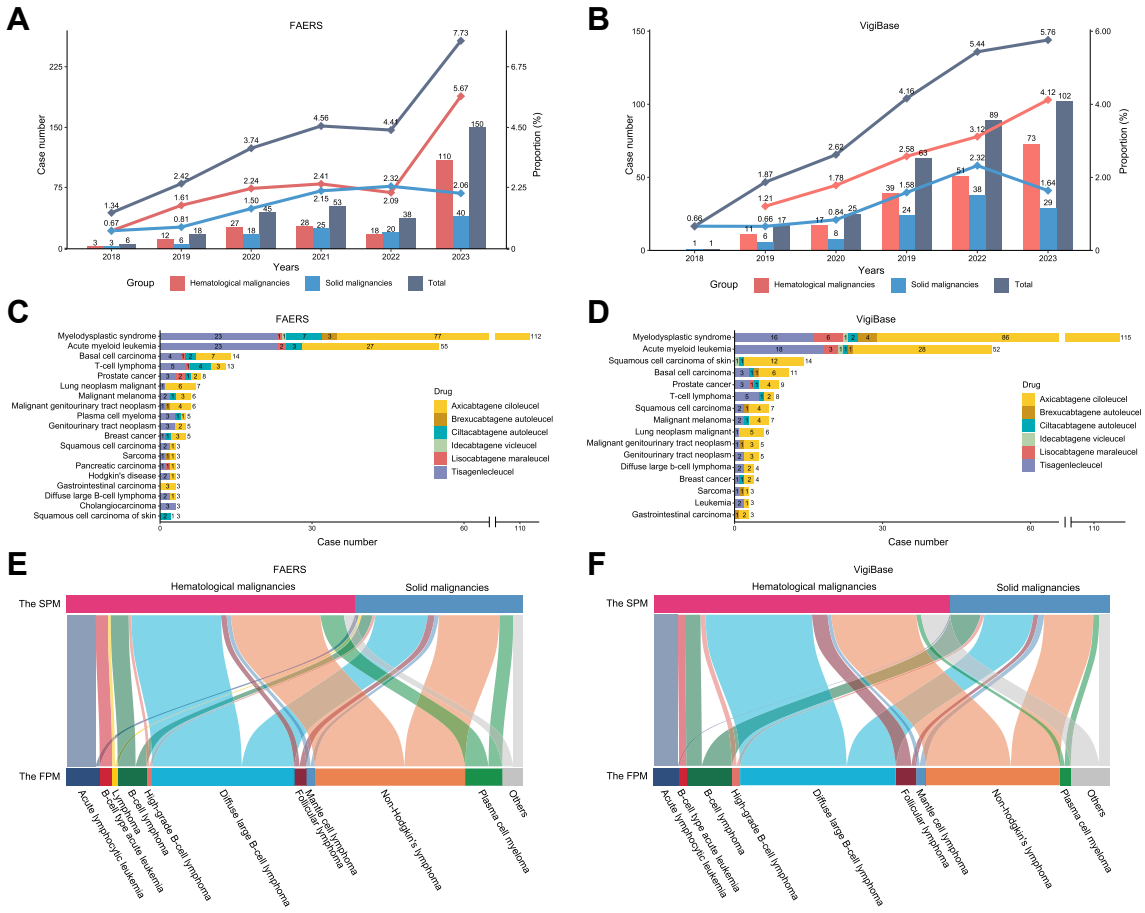

Fig. 2: The overview of SPMs post CAR-T therapy. (A and B) The cumulative histogram illustrates the annual incidence of SPMs post CAR-T therapy in patients with hematological tumors, juxtaposed with corresponding drug-related cases in FAERS and VigiBase. The annual incidence rate from 2017 to 2023 was calculated by dividing the number of reported SPM cases associated with CAR-T therapy each year by the total number of adverse event cases related to CAR-T treatment within the same year. (C and D) Statistical analysis of SPM post CAR-T therapy occurrences in FAERS and VigiBase databases spanning 2017–2023. The histogram showcases the incidence of all malignancies, solid tumors, and hematological malignancies, respectively. The line chart delineates the proportion of SPM cases post CAR-T therapy among all CAR-T-related AEs. (E and F) Examination of the association between the primary malignancies (FPMs) and the corresponding SPMs in CAR-T recipients.

respectively (see Fig. 2A–D). As depicted in the time course plot, there has been an almost yearly increasing trend in both raw counts and SPM percentages of yearly reported total AEs after CAR-T therapy. Meanwhile, the total counts of solid tumors have experienced a slight increase over time, with the annual percentage appearing to plateau around 2% in the past three years (see Fig. 2A and B). Myelodysplastic syndromes (MDS) represented the most commonly reported SPM subtype of HMs (n = 112 and 115, respectively) from FAERS and VigiBase, while basal cell carcinoma and squamous cell carcinoma of skin (each n = 14) were the most commonly reported SPM subtypes of solid tumor from FAERS and VigiBase, respectively. Other SPMs with a high number of cases reported to both FAERS and VigiBase included acute myeloid leukemia (n = 55 and 52, respectively) and T-cell lymphoma (n = 13 and 8, respectively). (see Fig. 2C and D). To provide more detailed insights into T-cell malignancies, we have defined 15 SPMs cases from FAERS, consisting of 13 cases of T-cell lymphoma and two of large granular lymphocytosis. Among these cases, five were associated with death reports. The comprehensive demographic characteristics and clinical information of these 15 patients have been provided in Supplementary Table S4.

Furthermore, our Sankey diagram derived from FAERS visually depicted the evolutionary trajectory between FPMs and the reported SPMs post CAR-T therapy. Analysis of the diagram revealed a notable trend that SPMs originating from leukemia predominantly aligned with HMs rather than solid tumors. However, no other distinct hierarchical patterns were

identified (see Fig. 2E and F and Supplementary Fig. S1A and B).

Clinical characteristics of hematological malignancy patients with and without CAR-T therapy

We compared the demographic and clinical characteristics of HM patients with and without CAR-T therapy (Supplementary Tables S5 and S6). Significant differences existed regarding different age clusters, sexes, reporting regions, reporting years, and FPMs both in FAERS and VigiBase (both $P < 0.001$). In FAERS and VigiBase, the SPMs reports were largely from the United States or region of the Americas, while CAR-T recipients were younger, with a higher proportion of males when compared to non-recipients. Notably, the proportion of lymphoma as the FPM was significantly higher (74.58% vs 17.05% and 76.18% vs 15.32%), whereas myeloma was significantly lower (9.57% vs 55.18% and 7.38% vs 60.12%) in CAR-T recipients than non-recipients in both FAERS and VigiBase. Noteworthy, VigiBase database witnessed a significantly higher proportion of serious outcomes in CAR-T recipients than non-recipients (93.94% vs 57.43%, $P < 0.001$), albeit such difference was non-significant in FAERS. Importantly, the proportion of suspected SPMs reported post CAR-T treatment was significantly higher compared to the non-recipients in both the FAERS (4.87% vs 3.39%, $P < 0.001$) and VigiBase (4.28% vs 2.32%, $P < 0.001$).

Data mining for the SPMs post CAR-T therapy

We continued to conduct disproportionality analyses within HM patients to identify any SPM as a highly suspected safety signal post CAR-T therapy. In FAERS and VigiBase, SMPs post CAR-T therapy were ranked by the ROR [95% CI]. T-cell lymphoma (ROR: 16.49 [9.05–30.05]), gastrointestinal carcinoma (ROR: 8.44 [2.56–27.84]), sarcoma (ROR: 7.60 [2.32–24.90]), MDS (ROR: 5.67 [4.67–6.88]) and acute myeloid leukemia (AML; ROR: 3.21 [2.45–4.21]) have been the positive safety signals post CAR-T therapy identified from FAERS. Data from VigiBase have almost confirmed these results and found an additional SPM signal of squamous cell carcinoma (ROR: 2.07 [1.21–3.52]; see Fig. 3A and B). We further stratified the data into CD-19 and BCMA subgroups according to the target of action of the CAR-T products. The SPMs identified in the CD-19 subgroup were nearly identical to the results from the whole group analysis, whereas in the BCMA subgroup, only T-cell lymphoma and myelodysplastic syndrome emerged as the major SPMs post CAR-T therapy (see Fig. 3A and B).

To further investigate whether there was a higher risk of SPMs following CAR-T treatment compared to chemotherapy alone, we performed a subgroup analysis within FAERS comparing the incidence of SPMs between patients who received CAR-T therapy and those who received chemotherapy. Our findings suggest that CAR-T recipients have higher incidences of certain SPMs, such as T-cell lymphoma (ROR: 5.00 [2.41–10.40]), squamous cell carcinoma of skin (ROR: 3.08 [1.54–6.16]), and MDS (ROR: 1.50 [1.22–1.85]) (see Fig. 3C). However, it's important to note that the positive SPM signals vary across different subgroups of alkylating agents, anti-metabolites, anthracyclines, or alkaloids. Notably, T-cell lymphoma and MDS consistently emerged as positive signals in both the overall analysis and across most subgroup analyses (see Fig. 3C).

To control for the impact of other confounding factors on SPMs incidence, we carried out a multivariable logistic regression analysis using data from FAERS, controlling for patients' sex, age, reporting region, reporting year, and FPM subtypes (leukemia, lymphoma or MM), and calculated aROR and its 95% confidence intervals (CIs). After imputing missing values, we found that positive SPM signals after CAR-T treatment included T-cell lymphoma ($aROR_{FAERS}$: 8.93 [3.58–20.24]; $aROR_{VigiBase}$: 4.76 [1.90–10.99]), MDS ($aROR_{FAERS}$: 3.52 [2.75–4.46]; $aROR_{VigiBase}$: 4.04 [3.23–5.02]), and AML ($aROR_{FAERS}$: 1.93 [1.38–2.61]; $aROR_{VigiBase}$: 1.94 [1.43–2.59]) (see Table 1). When compared to HM patients reported receiving chemotherapy only, SPMs signals of T-cell lymphoma (aROR: 3.42 [1.15–10.06]) has been identified again by multivariable logistic regression analysis after imputing missing values and adjusting for patients' sex, age, reporting region, reporting year, and FPM subtypes (see Supplementary Table S7).

Time to onset analysis of SPMs

In the TTO analysis of SPMs, we noted that CAR-T recipients had earlier median SPM onset time when compared to non-recipients (median [25th–75th percentile] TTO = 282.0 [97.0–574.0] vs 526.0 [183.0–1086.0] days; $P < 0.001$; see Fig. 4A). To further investigate potential factors influencing TTO in the CAR-T population, we performed further analyses controlling for patients' sex, age, and cancer subtypes using data from FAERS. Regarding sex, we did not observe any differences of TTO between female and male CAR-T recipients (see Fig. 4B). However, our analysis revealed that age has been a significant factor affecting TTO among CAR-T recipients (see Fig. 4C and D). Pediatric, adolescent and young adult patients (ages 0–39 years; median [25th–75th percentile] TTO = 35.0 [25.0–64.0] days) have earlier SPMs manifestation compared to patients from 40 to 59 (295.0 [173.0–530.0] days; $P = 0.001$), or over 60 years old (341.0 [165.0–684.0] days; $P < 0.001$). Furthermore, we explored the impact of cancer subtypes on TTO of SPMs and found a trend of delayed manifestation of solid tumors compared to HMs, with a longer median TTO (median after 500 days [25th–75th percentile] TTO = 1070.0 [834.0–1702.0] vs

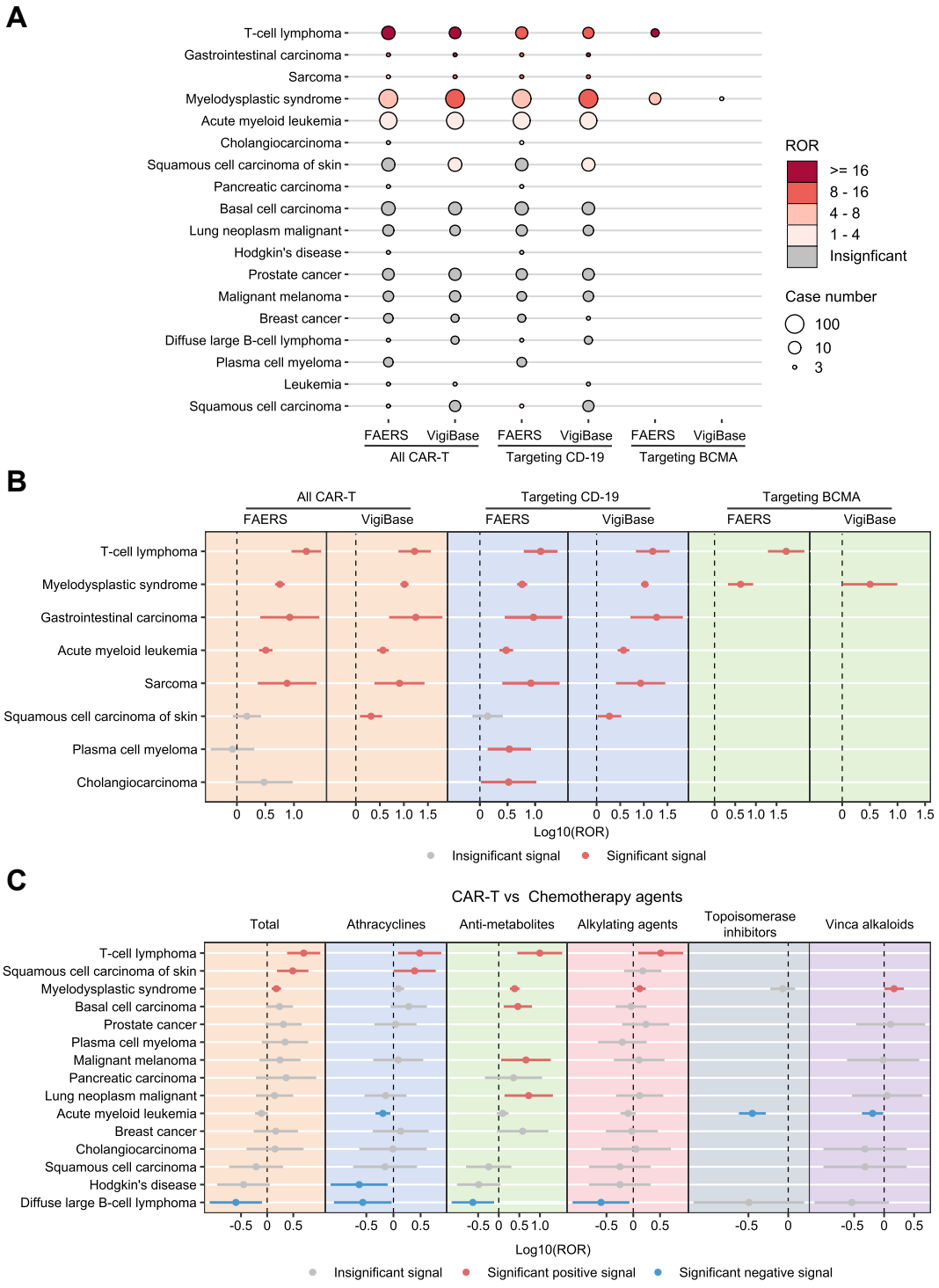

Fig. 3: Identification of high-risk SPMs post CAR-T therapy utilizing FAERS and VigiBase databases. (A) The heatmap displays the ROR of SPMs with cases exceeding three in FAERS and VigiBase, respectively. All CAR-T products and the subgroups targeting CD19-and BCMA-antigens are represented. The size of the point corresponds to the number of cases, while reddish hues indicate higher ROR values. Gray denotes insignificance of the SPM signal. (B) Forest plot presenting ROR values of high-risk SPMs with significant signals across all targets in FAERS or VigiBase. The error bars show the 95% CI of the ROR. Red signifies a significant positive signal, whereas gray denotes insignificance. (C) The

Types of SPMs	FAERS				VigiBase			
	Case number with SPM (Yes/No)		aROR (95% CI)[a]	P-value	Case number with SPM (Yes/No)		aROR (95% CI)	P-value
	With CAR-T	Without CAR-T			With CAR-T	Without CAR-T		
No imputation for missing values								
T-cell lymphoma	8/3377	36/222,919	9.116 (3.552–21.477)	<0.001	3/4073	13/242,574	2.208 (0.476–7.752)	0.249
Myelodysplastic syndrome	54/3331	843/222,112	2.821 (2.076–3.761)	<0.001	88/3988	432/242,155	4.825 (3.667–6.307)	<0.001
Acute myeloid leukemia	29/3356	740/222,215	1.634 (1.086–2.367)	0.013	42/4034	557/242,030	2.622 (1.826–3.684)	<0.001
Random forest imputation[b]								
T-cell lymphoma	13/6357	59/486,416	8.928 (3.584–20.241)	<0.001	8/6934	31/450,364	4.764 (1.898–10.991)	<0.001
Myelodysplastic syndrome	112/6258	1479/484,996	3.518 (2.745–4.455)	<0.001	115/6827	710/449,685	4.036 (3.226–5.015)	<0.001
Acute myeloid leukemia	55/6315	1278/485,197	1.925 (1.384–2.611)	<0.001	52/6890	911/449,484	1.941 (1.426–2.590)	<0.001

[a]The aROR quantifies the differential risk of SPM for CAR-T medications compared to the control group. In the FAERS, the aROR is adjusted for sex, age, reporting region, reporting year, and FPM. In VigiBase, the aROR is adjusted for sex, age, reporting region, reporting year, outcome, and FPM. [b]The "missForest" package in R was used to predict missing values for sex, age, reporting region, and FPM through a random forest trained on the observed values of the data matrix.

Table 1: **Adjusted reporting odds ratios (aROR) for CAR-T associated T-cell lymphoma, myelodysplastic syndrome and acute myeloid leukemia compared to non-CAR-T treatments in the FAERS and VigiBase Database.**

742.0 [673.0–1122.0] days; P = 0.089), although the difference was statistically non-significant (see Fig. 4E).

Discussion

It is worthwhile emphasizing that this is the first overall SPMs profile post CAR-T therapy using FAERS and VigiBase, the two largest pharmacovigilance databases in the world, and our findings indicated that the SPM profile includes HMs and solid tumors after CAR-T treatment. Although we could still not infer a confident causal relationship between CAR-T therapy and the SPMs detected, the positive SPMs signals identified in this pharmacovigilance study are of utmost importance and warrant validation in future CAR-T monitoring efforts.

SPMs of HMs, including MDS and AML, have been identified as the most frequently reported positive signals, which are consistent with the findings from previous independent observational studies.[12–14,27–29] Although scattered evidence has observed the occurrence of these SPMs following CAR-T therapy,[6,13] it remains unclear whether this treatment approach carries a higher risk compared to other therapeutic modalities. Our pharmacovigilance analysis, utilizing big data from a global perspective, highlights the risk of SPM post CAR-T treatment. The results particularly indicated the heightened risks of myeloid and T-cell malignancies in CAR-T recipients. Additionally, our findings have also echoed the FDA updates that T-cell lymphoma is a potential SPM signal post CAR-T therapy. Nevertheless, the causal relationship, besides the finding of CAR transgene in the malignant clone in CAR-T recipients announced by FDA,[6,7] warrants future exploration *in vitro* and/or animal studies.

In the subgroup analyses comparing the SPM profiles of the CD19 and BCMA CAR-T recipients, the CD19 subgroup showed almost identical SPMs profile to the overall group analysis, whereas only T-cell lymphoma and MDS were observed with increased risk post CAR-T therapy in the BCMA subgroup. These discrepancies might be attributed to the substantially later market approval time of Idecabtagene vicleucel and Ciltacabtagene autoleucel, both of which target BCMA and were officially approved by the FDA in 2021 and 2022, respectively.[30,31] The limited follow-up period for these BCMA-targeted CAR-T therapies may have hindered the ability to capture a more comprehensive SPM profile in this subgroup, as the development of SPMs often requires an extended latency period. As more long-term safety data would become available for BCMA-targeted CAR-T therapies in the future, it will be crucial to reassess the SPM profile in this subgroup and compare it with that of CD19-targeted CAR-T therapies to gain a more complete understanding of the potential target-specific differences regarding SPM risk.

Interestingly, we found some age-specific characteristics of SPMs that pediatrics, adolescent and young adult population had earlier SPM manifestation when compared to the older CAR-T recipients. We suspected that the earlier manifestation in pediatrics might be partially associated with the immature immune system in children,[32] potentially resulting in immune evasion. Currently, the age-related characteristics could still not

forest plot illustrates ROR values of SPMs post CAR-T therapy with cases exceeding three in FAERS and VigiBase, using all chemotherapy drugs and specific subgroups as control groups for comparison. The error bars show the 95% CI of the ROR. Red signifies a significant positive signal, blue signifies a significant negative signal, whereas gray denotes insignificance. ROR indicates reporting odds ratio.

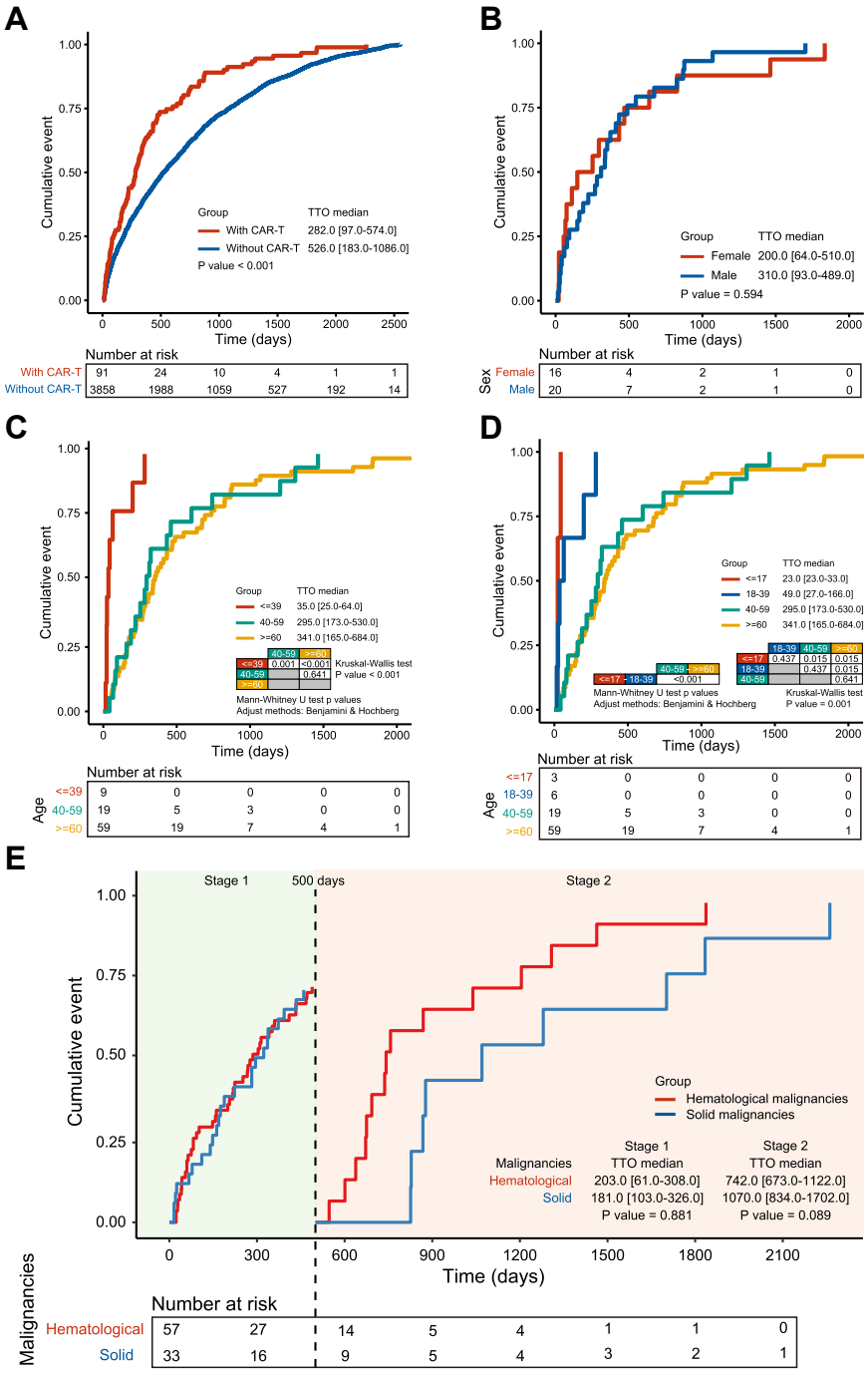

Fig. 4: Time to onset analysis of SPMs. (A) The cumulative distribution curve depicts the onset times of SPMs in patients with hematological malignancies receiving CAR-T vs Non-CAR-T therapy. (B) The cumulative distribution curve depicts the onset time of SPMs post CAR-T therapy across different sexes in FAERS. (C and D) The cumulative distribution curve depicts the onset time of SPMs post CAR-T therapy across different age group in FAERS. In D, the age group of 0–39 years is subdivided into two categories: "0–17 years old" and "18–39 years old". (E) The cumulative distribution curve depicts the onset time of SPMs post CAR-T therapy across different cancer types in FAERS. Utilizing a 500-day cutoff, the landmark method portrays the onset time of various cancer types before and after this threshold. Data with TTO time less than 14 days were excluded. Statistical analyses employed the Mann–Whitney U test and pairwise comparisons were conducted using the Kruskal–Wallis test, with corrections applied using the Benjamini & Hochberg method. TTO indicates time to onset.

be satisfactorily explained as younger patients would generally experience more intensified chemotherapies whereas we could not completely exclude the mixed impact from the neoplastic administered prior to CAR-T treatment. Nonetheless, this age-specific finding of SPMs post CAR-T therapy still highlights the importance and necessity of close monitoring and early detection strategies for younger CAR-T recipients. Currently, we have not observed any sex differences in TTO analysis, temporarily indicating that sex may not play a significant role affecting SPM development following CAR-T therapy. However, we are unsure if any sex difference would exist when more data were available in the future.

We have also noticed an increased risk of solid tumors as the SPMs post CAR-T therapy, albeit with a delayed manifestation compared to the SPMs of HMs, and this is similar to the observations reported by Hsieh et al.[12] in a multi-center retrospective analysis of 420 CAR-T recipients and by Ghilardi et al.[13] in another study of 449 patients receiving CAR-T therapy. All the available evidence at hand suggests that it is reasonable to remain vigilant regarding the risk of solid tumors following CAR-T therapy. Firstly, it has been suggested that the delayed onset of solid tumors appears to be comparable between CAR-T therapy and HSCT.[12] SPMs of HMs post HSCT commonly present within the initial ten-year period, while solid tumors typically exhibit a latency period after ten years post-HSCT.[12] Noteworthy, the risk of solid tumors post HSCT may persistently increase over time without reaching a plateau.[33] Therefore, ongoing monitoring might also be essential to vigilantly assess the potential long-term risks associated with solid tumor development following CAR-T therapy as the major recipients of CAR-T cells are currently within their first ten years after treatment. Secondly, although we have only identified a few subtypes of solid tumors as highly potential SPM signals, the landscape of solid tumors as SPMs may undergo significant evolution in the long-term post CAR-T therapy. To optimize treatment outcomes, CAR-T recipients should be informed not only about the risks of developing T-cell lymphoma and MDS/AML but also about the possibility of developing solid tumors in CAR-T safety monitoring. Specifically, careful considerations should also be given to the timing of solid tumor screening in cancer survivors post CAR-T therapy.

Given the ongoing debate regarding whether SPMs are predominantly linked to prior exposure to chemotherapy or if CAR-T therapy itself contributes to SPM onset,[6,7,12,13,34] we conducted more subgroup analyses within FAERS and found that CAR-T population had a higher risk and earlier onset of SPMs post CAR-T therapy. However, we could not rigorously control the confounding impact from the pre-CAR-T treatment individually as such information is not available in FAERS. Additionally, we should not ignore the fact that CAR-T therapy has substantially extended the survival of HMs patients, especially those classified into high-risk group, allowing us the "survivorship bias" to observe the occurrence of SPMs.[35,36] Therefore, real-world clinical trials with multi-centers and long-term collaborations are expected to allow a more rigorous evaluation of the SPMs after CAR-T therapy.

Beyond our current findings, future research and clinical considerations are urgently needed to address several important aspects of SPMs post CAR-T treatment. As highlighted in the FDA's guidance document for CAR-T products issued in January 2024,[37] retroviral vectors may offer extended transgene expression compared to lentiviral vectors, potentially increasing the risk of SPMs. Currently, we are not able to address this concern with insufficient statistical power, thus further monitoring and future investigations are imperative to substantiate this suspected impact and ascertain whether any other significant contributing factors to SPMs post CAR-T treatment exist. Moreover, despite the identification of certain SPMs post CAR-T therapy, the incomplete availability of treatment history data in both FAERS and VigiBase prohibits the definitive exclusion of the impact from non-CAR-T modalities, especially the pre-CAR-T treatment protocols. Thus, there remains a demand of comprehensive assessment of safety monitoring following CAR-T therapy to effectively screen for and confirm SPMs after this therapeutic modality. Nevertheless, these complex and challenging topics warrant in-depth discussions in the future to better understand the implications and management strategies for patients facing such circumstances.

Overall, SPMs have been identified with an increasing incidence rate after CAR-T therapy and the positive signal of myeloid and T-cell malignancies are consistently emerging in the overall (CAR-T recipients vs non-recipients) and subgroup comparisons (CAR-T recipients vs chemotherapy recipients), and the multivariate analyses adjusting for the confounders available in FAERS and/or VgiBase. According to the current big data mining, CAR-T recipients have earlier SPM onset than non-recipients, while younger patients under 40 are also prone to earlier SPM manifestation, which are previously unknown probably due to the limited sample size of the observational studies. Although CAR-T therapy has revolutionized the landscape of r/r HM treatment, we could not ignore the potentially increased safety signals post CAR-T treatment. Current in-depth analysis of post-marketing data from the two largest world pharmacovigilance databases support the FDA amendment of adding boxed warning to all CAR-T products of increased SPM risk for better patient management.[38]

Although this is the first exploration study discussing SPMs post CAR-T therapy using real-world big data from the two largest world-wide pharmacovigilance databases, it is important to acknowledge the limitations of

this research, and the findings should be interpreted with careful cautions. Firstly, it is crucial to acknowledge that the relatively limited duration of CAR-T products monitoring may imply that we have not comprehensively captured the extent of SPM risks following CAR-T therapy. Additionally, it's essential to recognize that inferring a causal relationship between an AE and a suspected drug is challenging due to the inherent limitations of spontaneous reporting systems like FAERS and VigiBase.[15,16] The FDA's alerts regarding three cases of T-cell lymphoma with confirmed CAR transgene integration in the malignant clone has prompted the reevaluation of the potential risk of SPMs post CAR-T therapy. This cautionary stance underscores the importance of not overlooking the possibility of CAR-T-related SPMs, even when considering a significantly larger cohort of CAR-T recipients. Therefore, the SPMs identified post CAR-T therapy require validation through real-world studies to establish a more definitive understanding of their associations with this treatment modality. Secondly, the etiology of SPMs might be problematic and multifactorial. Demographics, exposure to the environmental carcinogens (e.g., tobacco and alcohol consumption), treatment history (e.g., multiple r/r status and heavily pretreated experience before CAR-T therapy), genetic predispositions or family cancer history, immune suppression or genomic changes induced by the FPM may all contribute to the SPMs incidence following CAR-T therapy. It's important to note that significant public health phenomena should not be disregarded, especially as the primary SPM data in this analysis coincide with the COVID-19 pandemic since 2020. Furthermore, the impact of SARS-CoV-2 on the risk of SPM remains unclear and requires further investigation. However, all these confounding factors mentioned above were largely incomplete in FAERS and VigiBase, suggesting a likelihood of baseline imbalance and introducing potential biases to the current findings. Thirdly, despite our analysis encompassing the largest number of SPM cases post CAR-T therapy to date, the sample size remains limited. Increased public awareness of SPMs following CAR-T therapy, driven by the warnings and regulation updates from FDA, could likely lead to more cases being reported in the future. This influx of data has the potential to enhance the statistical power and facilitate the identification of novel SPMs and/or the contributing factors to SPMs onset.

In summary, our big data analysis of real-world pharmacovigilance databases revealed a higher reporting frequency of SPMs of T-cell lymphoma, myeloid malignancies and solid tumors in HM patients after CAR-T therapy. Long-term safety monitoring is crucial for all CAR-T recipients due to the observed yearly increase in SPMs, which may not be solely attributed to previous non-CAR-T treatments. However, the "survivorship bias" introduced by CAR-T therapy, which has substantially extended the life span of high-risk patients, must be considered before drawing any causal relationship between CAR-T therapy and the increased SPM rates. Thus, further validation through comprehensive clinical observational studies and mechanistic research is necessary. Notably, the distinct age-specific characteristics of SPMs post CAR-T therapy underscore the need for tailored SPM screening guidelines for different age groups of CAR-T recipients, ultimately enhancing early detection, intervention strategies, and patient outcomes.

Contributors

Conceptualization, P.L., J.Z., K.M., and Q.C.; Formal analysis, J.Y.S., R.H., and A.Q.L.; Resources, J.Z., and P.L.; Software, J.Y.S., R.H., A.Q.L., A.M.J., B.F.T., and Z.Q.L.; Supervision, P.L., J.Z., K.M., and Q.C.; Visualization, J.Y.S., R.H., A.M.J., and A.Q.L.; Writing–original draft, J.Y.S., R.H., and A.Q.L.; Writing–review & editing, J.Y.S., R.H., and A.Q.L.; J.Y.S. and R.H. had primary responsibility for final content. All authors read and approved the final version of the manuscript. J.Y.S. and R.H. have verified the underlying data.

Data sharing statement

All the data generated or analyzed during this study are included in this article and/or its files. AE reports after CAR-T therapy can be retrieved form FAERS Public Dashboard (https://www.fda.gov/drugs/questions-and-answers-fdas-adverse-event-reporting-system-faers/fda-adverse-event-reporting-system-faers-public-dashboard) and VigiBase from Uppsala monitoring center (https://who-umc.org/vigibase/vigibase-services/). Notably, VigiBase, the WHO global database of reported potential side effects of medicinal products, developed and maintained by the Uppsala Monitoring Centre, is accessible under specific licensing terms. It is essential to emphasize that the data within VigiBase originates from various sources, and the probability of a suspected adverse effect being linked to a specific drug may vary in different cases. Additionally, we declare that this study does not represent the opinions of the UMC or the World Health Organization.

Declaration of interests

The authors declare that they have no competing interests.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.eclinm.2024.102684.

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