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T cell lymphoma and secondary primary malignancy risk after commercial CAR T cell therapy

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We report a T cell lymphoma (TCL) occurring 3 months after anti-CD19 chimeric antigen receptor (CAR) T cell immunotherapy for non-Hodgkin B cell lymphoma. The TCL was diagnosed from a thoracic lymph node upon surgery for lung cancer. The TCL exhibited $CD8^+$ cytotoxic phenotype and a *JAK3* variant, while the CAR transgene was very low. The T cell clone was identified at low levels in the blood before CAR T infusion and in lung cancer. To assess the overall risk of secondary primary malignancy after commercial CAR T (CD19, BCMA), we analyzed 449 patients treated at the University of Pennsylvania. At a median follow-up of 10.3 months, 16 patients (3.6%) had a secondary primary malignancy. The median onset time was 26.4 and 9.7 months for solid and hematological malignancies, respectively. The projected 5-year cumulative incidence is 15.2% for solid and 2.3% for hematological malignancies. Overall, one case of TCL was observed, suggesting a low risk of TCL after CAR T.

A recent warning from the US Food and Drug Administration (FDA) reports a 'Serious Risk of T cell Malignancy Following BCMA-Directed or CD19-Directed Autologous Chimeric Antigen Receptor (CAR) T Cell Immunotherapies'[1]. Notably, the report suggests that some of the cases of TCL after CAR T were 'CAR-positive', highlighting a potential association between CAR transduction and transformation.

The occurrence of secondary primary malignancies (SPMs), including TCL, in patients with a previous diagnosis of hematological malignancies has been reported[2–4]. Further, simultaneous or sequential occurrence of two different B cell lymphomas[5] or two hematological cancers have been described[6]. For example, patients with B cell non-Hodgkin lymphoma (NHL) have an approximately fivefold higher

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incidence of second primary TCL over time compared to the general population[7] and patients with Hodgkin lymphoma have a relative risk 11–17-fold higher than untreated patients to develop second primary NHL[8]. Notably, DNA-damaging treatments such as chemotherapy and radiation therapy cause genomic alterations also in non-neoplastic cells, leading to an increased risk of SPMs, in particular myelodysplastic syndromes (MDS) and acute myeloid leukemia (AML) characterized by complex karyotype and poor prognosis[9,10]. The risk of SPMs is even higher when considering patients after autologous or allogeneic stem cell transplantation (SCT)[11–13].

After CAR T immunotherapy, data from long-term follow-up studies show that SPMs occur in approximately 15% of patients[14–18]. One study showed that SPMs occurred in 15% of patients treated with CD19-directed CAR T (CART-19) and the reported malignancies included MDS, non-melanoma skin cancers, melanoma, bladder cancer and multiple myeloma (MM)[16]. Our previous findings indicate that among patients with NHL treated with CD19-targeted CTL019 (now known as tisagenlecleucel), there was a 16% incidence of SPMs observed over a 5-year follow-up period. SPMs included cases of non-small cell lung cancer (NSCLC; 5%), AML (3%), MDS (3%), prostate cancer (3%) and melanoma (3%)[14]. Of note, another study by Steffin et al. showed no CAR transgene in all 11 post-CAR T SPMs analyzed[17].

Thus far, after over a decade of clinical use, CAR T products have not shown a direct role in causing malignant transformation. However, in 2018, we described a case of a pediatric patient with B cell acute lymphoblastic leukemia (ALL) who relapsed 9 months after CTL019 with CD19-negative ALL that was demonstrated to aberrantly express the CAR. The CAR transgene was inadvertently introduced during CART-19 manufacturing[19]. This case showed a clear role of CAR-induced resistance but did not suggest a direct role for insertional mutagenesis in inducing malignant transformation. In 2021, two cases of CAR$^+$ TCL following infusion of piggy-Bac transposon-engineered CD19-directed CAR T were described. Analysis of these cases showed a CAR-expressing CD4$^+$ TCL with extremely high transgene and altered genomic copy numbers and point mutations. In both cases, the post-CAR T TCL progressed and resulted in the death of one of the patients, whereas the other patient successfully received an allogeneic transplant[20,21]. Furthermore, we previously described a patient with chronic lymphocytic leukemia treated with CTL019, who experienced abnormal CAR T expansion that included 94% T cell clonality. Clonal CAR T cells were characterized by lentiviral insertion of the CAR transgene in the *TET2* gene, resulting in its disruption and consequent promotion of CAR T proliferation[22]. In another report, a pediatric patient with ALL treated with CD22-directed CAR T showed notable clonal expansion of CAR T cells due to lentiviral insertion of the CAR in the *CBL-B* locus[23]. In both cases, while the CAR T cells expanded in an abnormal and clonal pattern, they still led to disease remission without malignant transformation of T cells. Indeed, the safety of gammaretroviral vector-engineered T cells has also been reported in patients with over a decade of follow-up[24].

Other than in the cases treated with the piggy-Bac transposon-engineered CAR T cells[20], none of the previously discussed SPMs was directly caused by CAR transduction; however, a case of CAR$^+$ TCL occurring after infusion of the BCMA-directed CAR T (CART-BCMA) ciltacabtagene autoleucel (cilta-cel), likely due to lentiviral unintentional transduction during manufacturing, was recently described[25]. Genetic mutations that may have been present before CAR T manufacturing were identified in the CAR$^+$ TCL (*TET2*, *NFKB2*, *PTPRB* and a germline-activating mutation of *JAK3*). The *CAR* insertion in *PBX2* represented a possible contributor to the TCL development[26].

We describe here a single case of TCL after commercial CAR T that occurred 3 months after the infusion of a retrovirally transduced, CD28-co-stimulated CART-19 axicabtagene ciloleucel (axi-cel). As shown in Fig. 1a, the patient was a 64-year-old man who had a diagnosis of B cell lymphoma, unclassifiable, with features intermediate between diffuse large B cell lymphoma and classic Hodgkin lymphoma ('gray-zone' lymphoma; GZL) (Extended Data Fig. 1). The GZL had no relevant mutations at next-generation sequencing (NGS) and was positive for loss of *MAF* at fluorescence in situ hybridization (FISH). The patient was treated first-line with chemoimmunotherapy (six cycles of DA-EPOCH-R) but progressed soon after completion. He was then treated with seven cycles of pembrolizumab, reaching a partial response that was complicated by grade 4 autoimmune colitis. The patient was then treated with three cycles of brentuximab vedotin as a bridging therapy to third-line axi-cel CART-19 therapy. Before CART-19, he received lymphodepletion with fludarabine/cyclophosphamide (dose reduced by 25%; day −5 to −3) and was then infused with 2×10^6 CAR T cells per kg on day 0. The time from apheresis to CAR T infusion was 28 days. He tolerated the treatment well with mild toxicity, including grade 1 cytokine-release syndrome and neurotoxicity. Of note, the infused axi-cel CAR$^+$ cells expanded to high levels (44,261.06 CAR copies per µg of gDNA) at day 7 after infusion compared to other patients with large B cell lymphoma (LBCL) treated with commercial CART-19 (Fig. 1b); however, at day 14, CAR T cells were already decreasing (3,542.52 CAR copies per µg of gDNA), as expected. At 3 months after axi-cel, the patient was in complete metabolic remission of the GZL, but a persistent right lower lobe localization was observed on a positron emission tomography/computed tomography (PT/CT) scan. The lesion and three adjacent lymph nodes were surgically removed and histological analysis showed a poorly differentiated squamous NSCLC (Extended Data Fig. 2). Of note, the patient was a former heavy smoker (more than 30 pack-years). Unexpectedly, one of the three lymph nodes showed a peripheral TCL, not otherwise specified (NOS) without infiltration of the NSCLC. After NSCLC surgery, the PT/CT scan was negative and the patient received four additional cycles of brentuximab vedotin for the TCL without evidence of relapse of either of the lymphomas. Unfortunately, approximately 5 months after surgery, the NSCLC relapsed and, despite palliative treatment with gemcitabine/cisplatin and docetaxel, the patient rapidly progressed and died 18 months after surgery.

Microscopic analysis of the TCL nodal mass by hematoxylin and eosin (H&E) staining showed focal deposits of anthracotic pigment and an architecture that was effaced by a dense infiltrate of predominantly large lymphoid cells with oval to irregular nuclei, vesicular chromatin, conspicuous nucleoli, abundant cytoplasm and numerous mitotic figures. Scattered small, mature lymphocytes, rare eosinophils and a few plasma cells were also present. No B cells were seen, highlighted by negative staining for CD19 and PAX5 with background anthracotic pigment. CD79a and CD138 highlighted scattered plasma cells that seem polytypic by kappa and lambda staining. Immunohistochemistry analysis showed large, atypical cells that were CD45$^+$, CD30$^+$, CD2$^+$, CD3$^+$, CD5 (partial weak)$^+$, CD4 (partial weak)$^+$, CD8$^+$ and perforin$^+$ (Fig. 1c). In-situ hybridization for Epstein-Barr encoding region (EBER-ISH) was negative for and the Ki-67 proliferation index was approximately 70%. The TCL was therefore diagnosed as a CD8$^+$ peripheral TCL, NOS with a cytotoxic phenotype. Targeted PCR-based NGS of the TCL demonstrated the presence of a *JAK3* variant of uncertain significance, (p.D640Efs*30) with a variant allele fraction (VAF) of 11%. Sequencing of the initial GZL sample did not detect the *JAK3* variant, whereas sequencing of the NSCLC case detected a *TP53* p.Y205C c.614A>G mutation (VAF 64%) that was not present in the TCL or the GZL. Notably, the *JAK3* variant was assessed with different sequencing strategies in the TCL (PCR-based NGS) and in the blood (capture-based NGS) and was not covered by the sequencing panel used in the lung specimen. We then performed immunoglobulin heavy chain (IGH) and T cell receptor gamma gene (TRG) rearrangement studies (PCR amplification followed by size separation using capillary electrophoresis at the time of diagnosis, but later repeated by sequencing for this study for TRG) in both the initial GZL and the subsequent TCL tissues. A clonal TRG rearrangement was identified in the TCL but not

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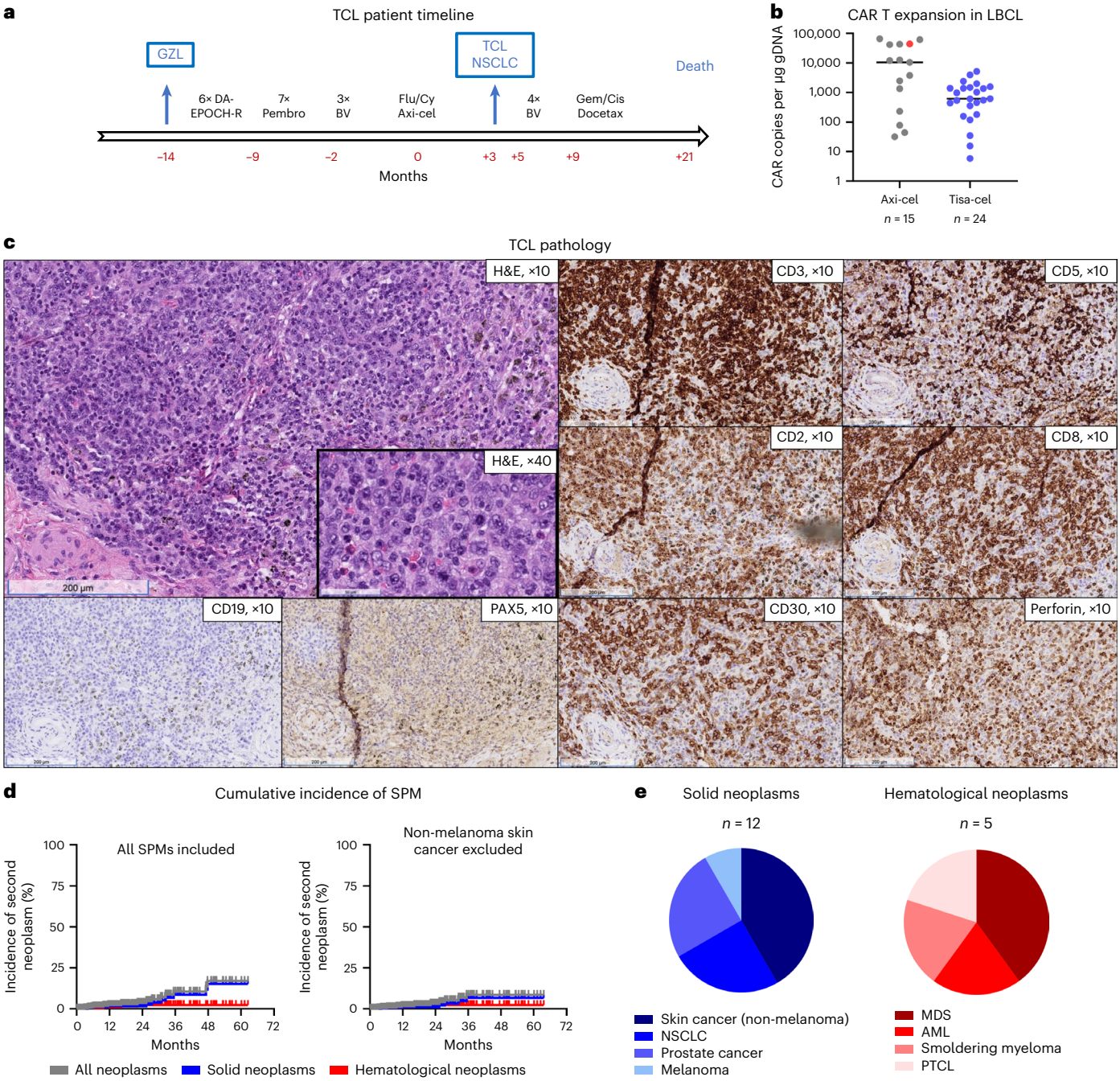

Fig. 1 | Risk of TCL and SPM after commercial CAR T. a, Patient's treatment timeline from diagnosis to progression. **b**, Peak of CAR T expansion in the blood of commercially treated CART-19 patients with LBCL (axi-cel and tisa-cel). The red dot indicates the patient with secondary TCL after CART-19. **c**, Histological and immunohistochemical evaluation of the TCL. H&E immunostaining (×10 magnification with ×40 inset). Immunohistochemistry analysis of the TCL biopsy, staining for CD3, CD5, CD2, CD8, CD19, PAX5, CD30 and perforin. **d**, Cumulative incidence of SPMs. All SPMs (left) and excluding non-melanoma skin cancers (right). The gray line highlights any SPM, the blue line shows secondary solid neoplasms and the red line shows hematological neoplasms. **e**, Specific cancers within the recorded solid (left) and hematological (right) SPMs. The analyses were conducted during the diagnostic assessment of the patients or as correlative exploratory analyses (qPCR) and as such were performed only once. BV, brentuximab vedotin; DA-EPOCH-R, dose-adjusted etoposide, vincristine, cyclophosphamide, doxorubicin, rituximab and prednisone chemoimmunotherapy scheme; Docetax, docetaxel chemotherapeutic scheme; Flu/Cy, fludarabine and cyclophosphamide lymphodepletion; Gem/Cis, gemcitabine/cisplatin chemotherapeutic scheme; Pembro, pembrolizumab.

the GZL. The TRG clone was characterized by peaks of 234 and 238 bp using Vγ 1–8 primers. Conversely, a clonal *IGH* gene rearrangement (125-bp peak using framework 3 primers) was identified in the GZL but not in the TCL biopsy. FISH studies for *DUSP22-IRF4* rearrangement and *TBL1XR1:TP63* and *TP63* rearrangements were negative in the TCL biopsy.

To test whether the TCL was carrying the axi-cel CAR transgene, we performed quantitative PCR (qPCR) on DNA obtained from the tumor biopsy. The qPCR revealed very low levels of CAR transgene copies (eight copies per µg of gDNA), which would correspond to approximately 0.005% of the cells. Based on this finding, it is most likely that the *CAR* signal was due to infiltrating CAR T cells rather than the TCL

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harboring the CAR transgene, given the low transgene copy number and the relatively recent axi-cel infusion (3 months before), although we cannot exclude CAR positivity in a subset of the TCL cells. Given these results, the TCL was defined as CAR negative. To understand whether the T cell clone was present in the patient's blood before CAR T infusion, we performed NGS of the TRG in the TCL tissue to identify the specific neoplastic TRG clonal rearrangement and then sequenced the blood before axi-cel (pre-lymphodepletion; day −5) and 14 d after infusion to look for the presence of that specific TRG clonotype sequence. In the diagnostic TCL tissue, a TRG clonotype, representing approximately 20% of total TRG sequencing reads met criteria for clonality (with a CDR3 sequence of GCCACCTGGGACTCTAATTATTATAAGAAACTC). This clonotype was detected reproducibly at a very low copy number (less than 0.01% of total TRG sequencing reads) in the pre-CAR T blood (before lymphodepletion) but was absent in day 14 post-CAR T lymphodepleted blood (confidence >99% at 1×10^{-3}); however, when we evaluated the presence of the specific TRG sequence in the NSCLC tissue collected 3 months later, it was identified in approximately 1% of the total TRG sequencing reads. With bulk sequencing, we cannot discriminate whether the T cell clone in the NSCLC was a resident population or circulating in the blood in the tissue. Therefore, we concluded that the T cell population from which the neoplastic T cell clone arose was already circulating at the time of axi-cel infusion and, likely, at the time of apheresis (day −28). Given the absence of the clone on day 14, we speculate that the clone was not abundant in the axi-cel product and that disappeared or was below the limit of detection upon lymphodepletion. Unfortunately, we have no access to the axi-cel product and cannot perform additional analyses. The specific TRG clone then re-expanded, leading to overt TCL at month 3. We then evaluated whether the *JAK3* variant identified in the TCL was detectable in the pre-CAR T blood by using a new capture-based NGS method and it was not detected; however, this could be due to the different analytic sensitivity of the technique used or the NGS platform itself.

The timing of disease development and the co-occurrence and proximity of the TCL with a new squamous NSCLC diagnosis makes this case particularly notable as we cannot exclude a possible correlation with CAR T manufacturing, CAR T- and NSCLC-related inflammation leading to the potential stimulation of a pre-existent TCL clone. Indeed, the presence of a variant of *JAK3*, as in the only reported CAR$^+$ TCL[25], suggests its role in TCL development, including post-CAR T cases. The nature and role of this variant is indeterminate and more studies are needed to clearly define its role. Of note, a previous preclinical report showed that retroviral activation of JAK kinases might lead to transformation[27]. Despite the absence of high levels of a CAR transgene in the TCL, the timing and the fact that it displayed an uncommon cytotoxic immunophenotype might suggest a contribution of T cell activation during CAR T manufacturing or post-CAR T inflammation. Furthermore, the fact that the TCL was adjacent to a newly diagnosed NSCLC might hint at the possibility of reactivity against the NSCLC as a stimulating factor. More studies are needed to determine whether T cell activation and expansion during manufacturing could drive the transformation of *JAK3*-mutated T cells that also receive T cell receptor stimulation[27]. A possible additional risk factor for transformation could be underlying clonal hemopoiesis related to older age and previous exposure to chemotherapy. Indeed, NHL and MM have overall high rates of clonal hemopoiesis, including up to 20–60% of patients receiving CAR T[28–30]. Moreover, previous exposure to seven cycles of pembrolizumab and the consequent development of autoimmunity might have played a role in stimulating a possible pre-existing TCL clone[31].

Therefore, to more broadly assess the risk and nature of SPMs, particularly TCL, after CAR T immunotherapy, we analyzed a large cohort of patients treated at the University of Pennsylvania. We studied all adult patients (n = 449) treated with commercial CAR T for NHL, MM and ALL between January 2018 and November 2023 at our institution (Table 1). Of these, 259 patients had LBCL, 28 mantle cell lymphomas (MCL), 30 follicular lymphomas (FL), 125 MM and 7 ALL. Sixty-nine patients received axi-cel, 189 received tisagenlecleucel, 32 received lisocabtagene maraleucel, 34 received brexucabtagene autoleucel, 67 received idecabtagene vicleucel and 58 received cilta-cel. We first analyzed the cumulative incidence of SPMs in this cohort of patients treated with commercial CAR T by retrospectively reviewing the electronic medical records (Fig. 1d). Overall, 16 of 449 (3.6%) patients were diagnosed with SPMs. At the median follow-up time of 10.3 months, the predicted incidence of SPMs was 1.9%. The predicted 5-year incidence of SPMs was 17.0%. The most frequent SPMs were solid malignancies (12 of 449, 2.7%), including five non-melanoma skin cancers, three prostate cancers, three NSCLC and one melanoma. The estimated 5-year incidence of second solid neoplasms was 15.2%. Hematological cancers occurred in 5 of 449 (1.1%) patients, including two cases of MDS, one case of AML, one case of smoldering MM and a single case of TCL.

Table 1 | Patients' characteristics

Characteristics	All cases 449 (100%)	No second cancer 433 (96.4%)	Second cancer 16 (3.6%)	p
Sex				
Female	162 (36.1%)	159 (36.7%)	3 (18.8%)	0.142
Male	287 (63.9%)	274 (63.3%)	13 (81.2%)	
Age at CART infusion				
≤65	270 (60.1%)	263 (60.7%)	7 (43.8%)	0.173
>65	179 (39.9%)	170 (39.3%)	9 (56.2%)	
Diagnosis				
NHL	317 (70.6%)	304 (70.2%)	13 (81.2%)	0.601
MM	125 (27.8%)	122 (28.2%)	3 (18.8%)	
ALL	7 (1.6%)	7 (1.6%)	0 (0.0%)	
# of previous lines of therapies				
≤3	206 (45.9%)	201 (46.4%)	5 (31.2%)	0.232
>3	243 (54.1%)	232 (53.6%)	11 (68.8%)	
Previous autologous SCT				
No	291 (64.8%)	280 (64.7%)	11 (68.8%)	0.737
Yes	158 (35.2%)	153 (35.3%)	5 (31.2%)	
Product infused				
Axi-cel	69 (15.4%)	65 (15.0%)	4 (25.0%)	0.412
Tisa-cel	189 (42.1%)	182 (42.0%)	7 (43.7%)	
Liso-cel	32 (7.1%)	32 (7.4%)	0 (0.0%)	
Brexu-cel	34 (7.6%)	32 (7.4%)	2 (12.5%)	
Ide-cel	67 (14.9%)	64 (14.8%)	3 (18.8%)	
Cilta-cel	58 (12.9%)	58 (13.4%)	0 (0.0%)	
Previous neoplasm				
No	374 (83.3%)	363 (83.8%)	11 (68.8%)	0.112
Yes	75 (16.7%)	70 (16.2%)	5 (31.2%)	
Second solid neoplasm				
No	436 (97.3%)	433 (100%)	4 (25.0%)	-
Yes	12 (2.7%)	0 (0.0%)	12 (75.0%)	
Second hematological neoplasm				
No	444 (98.9%)	433 (100%)	11 (68.8%)	-
Yes	5 (0.1%)	0 (0.0%)	5 (31.2%)	

Brexu-cel: brexucabtagene autoleucel; Ide-cel: idecabtagene vicleucel; Liso-cel: lisocabtagene maraleucel. Categorical variables were evaluated with the chi-squared test or Fisher's exact test as appropriate. All tests were two-sided and significance was defined as $P < 0.05$.

The 5-year incidence of second hematological neoplasms was 2.3% (Fig. 1e). If the five cases of non-melanoma skin cancers were excluded, the predicted 5-year incidence of SPMs decreased to 9.5% (6.6% for solid neoplasms; Fig. 1d). The characteristics of the SPMs are shown in Extended Data Table 1. The median SPM onset was 26.4 months for solid SPMs and 9.7 months for hematological SPMs. When we analyzed possible risk factors (univariate analysis; Extended Data Table 2), we found a trend toward age ≥65 years at CAR T infusion positively correlating with a higher risk of developing an SPM. We then performed a multivariate analysis, including sex, age, number of previous lines of therapies, CAR T product, co-stimulatory domain and previous autologous SCT, confirming that age ≥65 years at CAR T infusion was the only factor independently correlating with the incidence of SPM. No association with a specific histology or CAR T product was observed, although the numbers are limited (Extended Data Table 2).

Our retrospective SPM results are reinforced by the analysis of the US FDA Adverse Events Reporting System database, which shows approximately 20 cases of TCL among roughly 8,000 patients treated with CAR T therapy. Given that the voluntary reporting nature of this database may introduce certain limitations, including a propensity to report cases of TCL SPMs compared to cases with no adverse events, the TCL incidence might be overestimated. Of note, the incidence of TCL after CAR T seems to be lower than what is typically observed following treatment, for example, with checkpoint inhibitors using the same database[31]. Given the estimated ~35,000 patients treated with commercial CAR T in the United States, gathering more data on the other ~27,000 patients who were not reported into the US FDA Adverse Events Reporting System is imperative. It is important to state that, for our study, we did not call all patients to inquire specifically about a diagnosis of a new SPM that could not have been included in the charts for any reason; therefore, it is possible that the results underestimate the actual incidence of some SPMs. Overall, we observed a 17.0% estimated risk for SPMs over 5 years after commercial CAR T, which is consistent with previously reported CAR T cohorts and what was observed in patients treated with chemotherapy and/or radiation treatment and overall lower than that observed after SCT[32–34]. We observed a single case of TCL after axi-cel treatment with a *JAK3* variant and a $CD8^+$ cytotoxic phenotype with no clear evidence of CAR transgene integration.

Nevertheless, strategies to reduce SPMs and TCL are warranted and include treatment of patients earlier in the course of their disease to avoid repetitive exposure to chemotherapy and/or radiation, surveillance for clonal hematopoiesis, reduction of actionable risk factors (for example, smoking) and close clinical monitoring for possible occurrence[33]. For example, strategies to reduce the intensity of lymphodepleting chemotherapy might be beneficial also in reducing the risk of SPMs, although there are no data available[34]. While SPMs are generally aggressive and refractory to standard treatments, we previously speculated that the rare event of CAR expression by neoplastic cells could offer a possible therapeutic target, for example, using a CAR T product that specifically recognizes and kills FMC63-CAR-19^+ cells[35]. Last, this study highlights the need to improve the mechanisms of reporting adverse events after the commercialization of new agents, ensuring timely notification and high quality. Of note, prospective biobanking was essential for this study to allow our ability to perform mechanistic studies.

In conclusion, the observed very low incidence of secondary T cell lymphomas should provide reassurance to the scientific community regarding the safety of commercially available CAR T products[36,37]. This aligns with the FDA's assertion that '…the overall benefits of these products continue to outweigh their potential risks for their approved uses…'[1].

Online content

Any methods, additional references, Nature Portfolio reporting summaries, source data, extended data, supplementary information, acknowledgements, peer review information; details of author contributions and competing interests; and statements of data and code availability are available at https://doi.org/10.1038/s41591-024-02826-w.

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Methods

Patient characteristics and clinical outcomes

This research was conducted according to the Declaration of Helsinki. The study protocol was approved by the University of Pennsylvania Institutional Review Board. Patients did not receive compensation for participating in this study. The patient provided written consent to evaluate their medical history and perform research analysis on their collected biospecimens.

The treatment administered for GZL included six cycles of first-line R-EPOCH[38] administered in another institution; seven cycles of pembrolizumab (dose, 200 mg intravenously every 3 weeks (q3w)); three cycles of brentuximab vedotin (dose, 1.8 mg kg^{-1} q3w) before axi-cel infusion and four additional cycles after TCL diagnosis; pre-CAR T lymphodepletion day −5 to −3 before axi-cel infusion with fludarabine (dose, 22.5 mg m^{-2}) and cyclophosphamide (dose, 375 mg m^{-2}) (dose reduced by 25% compared to standard dose recommended for axi-cel[39]). Axi-cel was infused at the dose of 2×10^6 per kg CAR T cells on day 0. For NSCLC, the patient received six cycles of gemcitabine 1,000 mg m^{-2} (days 1 and 8 q3w) and cisplatin (70 mg m^{-2} q3w); six cycles of docetaxel 60 mg m^{-2} q3w. Disease response after treatment for GZL and TCL were defined according to Lugano 2014 criteria[40]. Disease response after treatment for the NSCLC was defined according to RECIST 1.1 criteria[41]. CAR T-related toxicities were defined according to the American Society for Transplantation and Cellular Therapy criteria[42]. Autoimmune colitis grade was defined according to the Common Terminology Criteria for Adverse Events v.5.0.

We also retrospectively evaluated the clinical outcomes of 449 patients with relapsed or refractory NHL, MM and ALL treated consecutively with commercial CAR T at the University of Pennsylvania between January 2018 and October 2023. No eligible patients were excluded from this analysis. The data collection cutoff date was 30 November 2023. Patient demographics and outcomes were obtained from the electronic medical records. Time to second tumor was calculated as the time within CAR T cell infusion and date of second malignancy diagnosis (event) or last follow-up (censor).

Histopathological evaluation

Histopathological analysis of the biopsies as well as lung lobectomy was performed by hematopathologists and surgical pathologists at the Department of Pathology and Laboratory Medicine of the Hospital of the University of Pennsylvania. H&E-stained slides and immunohistochemical stains of the inguinal and mediastinal lymph node biopsies as well as the right lower lobe mass lobectomy were performed at the time of diagnostic work-up for the patient at the Laboratory of Immunohistochemistry, Anatomic Pathology at the Hospital of the University of Pennsylvania. For GZL the following antigens were evaluated: CD79a, PAX5, BCL2, MUM1, CD30, CD15, CD138, κ and λ light chain, CD3, CD5, CD20, CD10, BCL6, CD45, CD56, OCT2, BOB1, EBER(ish), HHV8, ALK, Ki-67 and MYC. For TCL the following antigens were evaluated: CD45, CD30, CD2, CD3, CD5, CD4, CD8, perforin, TIA1, CD43, BCL2, MUM1, MYC, GATA3, PD1, CD57, CD25, OCT2, TCRβF1, TCRδ, granzymeB, lysozyme, CD7, CD56, CD19, CD20, PAX5, CD79a, Bob1, CD15, CD10, BCL6, CD21, CD23, ALK, CD138, EMA, panCK, AE1-3, TdT, EBER(ISH), Ki-67, CD21, CD23, CD138 and κ/λ staining. For NSCLC the following antigens were evaluated: panCK, p63, TTF-1, Napsin A, P40, CAM 5.2 and PD-L1. Histopathological slides of cervical, inguinal and mediastinal biopsies as well as the right lower lobe mass lobectomy, were re-evaluated during preparation of the manuscript with representative microphotographs collected for publication.

FISH

FISH for GZL was performed on interphase nuclei from the paraffin-embedded left inguinal lymph node excision specimen. This test was developed, and its performance characteristics determined, by the University of Pennsylvania Cytogenetics Laboratory as required by the CLIA 88 regulations. Probes were used to detect 1p32/1q21/gain of 1q21, involving *CDKN2C* and *CKS1B*; t(4;14), involving *FGFR3* (4p16) and IGH (14q32); *CCND1*; TP53/monosomy 17; *MYC* (8q24) dual color/break-apart probe set; t(14;16), involving *IGH* and *MAF* (16q23); and *NF1*. Copy number changes detected in less than 20% of cells examined are not reported here as they may represent cut artifacts.

FISH for TCL was performed by NeoGenomics according to their protocols. Probes were used to detect *DUSP22-IRF4* and *TBL1XR1:TP63* and *TP63*.

Targeted NGS

Genomic DNA was extracted, with the DNA sequenced through one of the following assays at the Center for Personalized Diagnostics at the University of Pennsylvania. Samples were sequenced on Illumina sequencers, which included MiSeq, HiSeq and NovaSeq instruments. Mutations were reported as disease-associated or variants of uncertain significance (VUS), which includes those variants internally categorized as probably disease-associated VUS and likely benign, based on the review of the literature and publicly available databases. These assays were validated to a VAF of 4% for single-nucleotide variants and indels and 1% for FLT3-ITDs. We used three different panels as described below:

Lymphoma sequencing panel. DNA was derived from paraffin-embedded tissue (PET) from TCL and GZL, and was subjected to targeted PCR-based NGS using a commercially available lymphoma-focused panel targeting 40 relevant genes[43]: *ATM*, *B2M*, *BIRC3*, *BRAF*, *BTK*, *CARD11*, *CD79A*, *CD79B*, *CIITA*, *CREBBP*, *CXCR4*, *EGR2*, *EZH2*, *GNA13*, *ID3*, *IDH2*, *JAK3*, *KLF2*, *MAP2K1*, *MYD88*, *NFKBIE*, *NOTCH1*, *NOTCH2*, *PLCG1*, *PLCG2*, *POT1*, *RHOA*, *RPS15*, *RRAGC*, *SF3B1*, *SOCS1*, *STAT3*, *STAT5B*, *TCF3*, *TET2*, *TNFAIP3*, *TNFRSF14*, *TP53*, *TRAF3* and *XPO1*.

PennSeq panel. The PennSeq panel later substituted the lymphoma sequencing panel at our institution. This is a hybrid-capture-based NGS panel based on probe design from the GOAL consortium (www.goalabs.org)[44]. DNA was derived from peripheral blood mononuclear cells collected before lymphodepletion started. A total of 116 genes are reported from the PennSeq-hem panel, with targets, including the full gene sequence of the genes and the assay validated to detect single-nucleotide variants, indels and limited copy number calls from the following genes[45]: *ABL1*, *ASXL1*, *ATM*, *B2M*, *BCL2*, *BCOR*, *BCORL1*, *BIRC3*, *BRAF*, *BRCA1*, *BRCA2*, *BRIP1*, *BRINP3*, *BTK*, *CALR*, *CARD11*, *CBL*, *CD79A*, *CD79B*, *CDKN2A*, *CEBPA*, *CIITA*, *CREBBP*, *CSF1R*, *CSF3R*, *CXCR4*, *DDX3X*, *DDX41*, *DICER1*, *DNMT3A*, *EGR2*, *ERCC4*, *ETV6*, *EZH2*, *FANCA*, *FANCC*, *FANCD2*, *FANCE*, *FANCF*, *FANCG*, *FANCL*, *FANCM*, *FBXW7*, *FLT3*, *GATA2*, *GNA13*, *GNAS*, *HNRNPK*, *ID3*, *IDH1*, *IDH2*, *IKZF1*, *IL7R*, *JAK2*, *JAK3*, *KIT*, *KLF2*, *KLHL6*, *KRAS*, *MAP2K1*, *PAK1*, *RIP142*, *MPL*, *MYC*, *MYCN*, *MYD88*, *NF1*, *NFKBIE*, *NOTCH1*, *NOTCH2*, *NPM1*, *NRAS*, *PALB2*, *PDGFRA*, *PHF6*, *PLCG1*, *PLCG2*, *POT1*, *PRPF40B*, *PTEN*, *PTPN11*, *RAD21*, *RAD51*, *RAD51C*, *RHOA*, *RIT1*, *RPS15*, *RRAGC*, *RUNX1*, *SETBP1*, *SF1*, *SF3A1*, *SF3B1*, *SLX4*, *SMC1A*, *SOCS1*, *SRSF2*, *STAG2*, *STAT3*, *STAT5B*, *TBL1XR1*, *TCF3*, *TERT*, *TET2*, *TNFAIP3*, *TNFRSF14*, *TP53*, *TPMT*, *TRAF3*, *U2AF1*, *U2AF2*, *WT1*, *XPO1*, *XRCC2*, *ZMYM3* and *ZRSR2*.

Penn precision panel NGS panel. DNA was derived from PET from the pleural mass of NSCLC and was subjected to targeted NGS using a commercially designed solid-tumor-focused panel, utilized for low input and highly degraded DNA 59 relevant genes: *ABL1*, *AKT1*, *ALK*, *APC*, *ATM*, *BRAF*, *CDH1*, *CDKN2A*, *CSF1R*, *CTNNB1*, *DDR2*, *DNMT3A*, *EGFR*, *EIF1AX*, *ERBB2*, *ERBB4*, *ESR1*, *EZH2*, *FBXW7*, *FGFR1*, *FGFR2*, *FGFR3*, *FLT3*, *FOXL2*, *GNA11*, *GNAQ*, *GNAS*, *HNF1A*, *HRAS*, *IDH1*, *IDH2*, *JAK2*, *JAK3*, *KDR*, *KIT*, *KRAS*, *MAP2K1*, *MET*, *MPL*, *MSH6*, *NOTCH1*, *NPM1*, *NRAS*, *PDGFRA*, *PIK3CA*, *PTEN*, *PTPN11*, *RB1*, *RET*, *ROS1*, *STK11*, *SMAD4*, *SMARCB1*, *SMO*, *SRC*, *TP53*, *TSC1*, *TSHR* and *VHL*.

TRG and IGH rearrangement analysis

DNA was extracted from PET (TCL and NSCLC) and from pre-lymphodepletion and day 14 after infusion of lymphodepleted blood cells using QIAGEN reagents, according to the manufacturer's recommendations. IGH PCR amplification was performed using two sets of fluorescently labeled primers (IGH Gene Rearrangement Assay, InVivoScribe) that hybridize to a conserved V-framework (FR2 or FR3) region and the conserved J-region of IGH.

Assessment of TRG clonality was conducted by PCR amplification followed by either capillary electrophoresis (PCR-CE) or massively parallel sequence analysis (TRG NGS)[46]. PCR-CE was performed using two PCR reactions, one with Vγ 1–8 primers and the second with Vγ 9–11 primers, both combined with consensus J-region primers as described[46]. The PCR products were separated by size with a 3500 genetic analyzer (Thermo Fisher). TRG NGS was performed using the LymphoTrack TRG Assay Panel-MiSeq (Invivoscribe) according to the manufacturer's recommendations. Briefly, sequencing of PCR-enriched libraries was performed on the MiSeqDx platform using multiplexed paired-end reads. Run analysis was performed using LymphoTrack Software-MiSeq (Invivoscribe) in combination with an internally developed computational pipeline. The analysis provides the sequence of each V–J rearrangement corresponding to a unique TRG clonotype and its read count. Interpretation of clonality for each clonotype is based on the frequency of unmerged reads (minimum 2.5% of total reads) and additional metrics to assess the significance of the clonotype frequency in the context of the overall distribution of clonotype frequencies detected in the sample. All samples that were initially tested by PCR-CE were retested by TRG NGS. Lymphotrack MRD software (Invivoscribe) was used for evaluation of the identified neoplastic TRG clone (clonotype sequence: GGAGTCAGTCCAGGGAAGTATTATACTTACGCAAGCACAAGGAACAACTTGAGATTGATACTGCAAAATCTAATTGAAAATGACTCTGGGGTCTATTACTGT**GCCACCTGGGACTCTAATTATTATAAGAAACTC**TTTGGCAGTG with CDR3 sequence highlighted in bold) in subsequent biosamples at a low level.

CAR-19 quantitative PCR

Day 7 and 14 peripheral blood mononuclear cells or DNA obtained from tissue slides from the TCL biopsy were collected. The blood was separated from EDTA-preserved whole blood using FICOLL (Sigma-Aldrich; 26873-85-8) gradient purification, followed by direct isolation of genomic DNA. In a subset of 15 patients with LBCL treated with axi-cel and 24 patients with LBCL receiving tisa-cel, sufficient material allowed for DNA isolation and subsequent quantitative PCR to assess CAR levels per µg of genomic DNA. For qPCR analysis, transgene-specific primers targeting the integrated anti-CD19 CAR transgene sequence (FMC63 scFv; amplicon: GCCATTTACTACTGTGCCAAACATTATTACTACGGTGGTAGCTATGCTATGGACTACTGGGGCCAAGG) were used. The qPCR was performed using TaqMan technology (Applied Biosystems), with each reaction including triplicates of 200 ng genomic DNA per time point sourced from peripheral blood samples[47,48]. To ensure the quality of the assay, a parallel amplification was conducted with 20 ng genomic DNA using a primer/probe combination specific for a non-transcribed sequence upstream of the cyclin-dependent kinase inhibitor 1A (p21) gene. This parallel amplification generated a correction factor, which was applied to adjust for the differences between the calculated and actual DNA input. The lower limit of quantification for this method was established at 25 copies per µg of genomic DNA.

Statistical analysis

Categorical variables were evaluated with the chi-squared test or Fisher's exact test as appropriate. Univariate analysis of the factors associated with the risk of SPMs was performed using the Kaplan–Meier method and compared using a log-rank test; the results were expressed as hazard ratio (95% CI). Multivariate analysis was performed using the Cox regression method and the results were expressed as hazard ratio (95% CI). All tests were two-sided and significance was defined as $P < 0.05$. Analyses were performed with the Statistical Package for the Social Sciences software v.22.0. Figures were generated using GraphPad Prism software v.9.5.0.

Reporting summary

Further information on research design is available in the Nature Portfolio Reporting Summary linked to this article.

Data availability

All requests for raw and analyzed data and materials are promptly reviewed by the University of Pennsylvania and the corresponding author, to determine whether they are subject to intellectual property or confidentiality obligations. Patient-related data not included in the paper may be subject to patient confidentiality. The email address for the corresponding author is mruella@upenn.edu. Any data and materials that can be shared will be released via a material transfer agreement.

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Author contributions

G.G. and M.R. conceptualized the project, interpreted the results and drafted the manuscript. G.G., L.P., E.F., E.R.C. and V.P. collected the clinical data. G.G., L.P., O.H.U. and R.P. collected and processed the biospecimens. J.A.F. and I.K. performed CAR qPCR on biospecimens and interpreted the results. J.C.H. and G.C.C. evaluated histopathological samples, integrated morphological, immunophenotypical and genomic data, edited the manuscript and prepared pathology microphotographs. J.J.D.M., V.M.V. and D.B.L. performed, analyzed and interpreted sequencing analysis and TRG/IGH clonality analysis, respectively. J.N.G. managed the patients with TCL. E.A.C., S.P.S.A., J.S., S.D.N., D.J.L., A.J.W., A.D.C., E.A.S., N.V.F., D.T.V., E.O.H., S.K.B., D.L.P., A.L.G. and S.J.S. treated the other patients analyzed in the study. B.L.L. and C.H.J. reviewed, provided conceptual feedback and edited the manuscript. All authors reviewed and approved the manuscript.

Competing interests

G.G. served as a scientific consultant for viTToria Biotherapeutics. M.R. holds patents related to CD19 CAR T cells, served as a consultant for NanoString, Bristol Myers Squibb, GlaxoSmithKline, Scaylite, Bayer and AbClon; and receives research funding from AbClon, NanoString, Oxford NanoImaging, viTToria Biotherapeutics, CURIOX and Beckman Coulter. M.R. is the scientific founder of viTToria Biotherapeutics. J.S. served as a consultant for Genmab, Adaptive, AstraZeneca, BMS, ADCT, Atara, Pharmacyclics and Seattle Genetics and received research funding from AstraZeneca, BMS, Incyte, Merck, Seattle Genetics, Pharmacyclics and TG Therapeutics. G.C.C. served as a consultant for DAVA Oncology and GLG consulting. E.A.C. served as a consultant for AstraZeneca, Novartis, Beigene, KITE and Juno/BMS and received research funding from Abbvie and Genentech/Roche. S.K.B. served as a consultant to Acrotech, Affimed, Kyowa Kirin, Daiichi Sankyo, Janssen and Seagen. S.D.N. received research funding from Pharmacyclics, Roche, Rafael and FortySeven/Gilead. J.D.L. received research funding from Curis, Takeda and Triphase and served on the Board of Directors, advisory committees and data and safety monitoring board for Incyte, ADCT, Karyopharm and Morphosis. S.J.S. served as a consultant to AstraZeneca, BeiGene, Celgene, Genentech, Genmab, Fate Therapeutics, Roche, Incyte, Juno Therapeutics, Legend Biotech, Loxo Oncology, MorphoSys, Mustang Biotech, Nordic Nanovector, Novartis and Regeneron; received research funding from AbbVie, Adaptive Biotechnologies, Celgene, DTRM, Genentech, Roche, Juno Therapeutics, Merck, Novartis, Incyte, Pharmacyclics and TG Therapeutics; received honoraria from Celgene and Novartis; and holds patents related to CD19 CAR T cells and autologous co-stimulated T cells. D.L.P. declares funding from the National Marrow Donor Program; membership on an entity's Board of Directors or advisory committees of Kite/Gilead, Janssen, Genentech, DeCart, Sana Biotechnology, Verisimo and Novartis; is a current equity holder of the American Society for Transplantation and Cellular Therapy and Verismo; declares honoraria for Incyte; patents and royalties in Tmunity and Wiley and Sons Publishing. A.L.G. declares research support from Janssen, Novartis, Tmunity and CRISPR Therapeutics; consultancies/honoraria from Janssen, Novartis, BMS, GSK and Legend Bio; and DSMB membership for Janssen. C.H.J. and the University of Pennsylvania have patents pending or issued related to the use of gene modification in T cells for adoptive T cell therapy. C.H.J. is a co-founder of Tmunity, is a scientific co-founder and holds equity in Capstan Therapeutics, Dispatch Biotherapeutics and Bluewhale Bio. C.H.J. serves on the board of AC Immune and is a scientific advisor to BluesphereBio, Cabaletta, Carisma, Cartography, Cellares, Cellcarta, Celldex, Danaher, Decheng, ImmuneSensor, Kite, Poseida, Verismo, Viracta, Vittoria Bio and WIRB-Copernicus group. C.H.J. is an inventor on patents and/or patent applications licensed to Novartis Institutes of Biomedical Research and Kite and may receive license revenue from such licenses. J.A.F. is a member of the Scientific Advisory Boards of Cartography Bio and Shennon Biotechnologies and has patents, royalties and other intellectual property. B.L.L. reports personal fees from Akron, Avectas, Immuneel, Immusoft, In8bio, Ori Biotech, Oxford Biomedica, Thermo Fisher Pharma Services, UTC Therapeutics and Vycellix and is a co-founder and equity holder in Tmunity Therapeutics (acquired by Kite Pharma) and Capstan Therapeutics. N.V.F. declares an affiliation with Sana Biotechnology; consultancy roles for Novartis and Syndax Pharmaceuticals; and research funding from Kite Pharma. E.A.S. declares an affiliation with Oncopeptides; consultancy for Amgen, BMS Celgene, G.S.K., Janssen and AbbVie. E.O.H. declares an affiliation with Tmunity Therapeutics and research funding from Blueprint Medicines and PharmaEssentia; membership on an entity's Board of Directors or advisory committees for Blueprint Medicines and PharmaEssentia. D.T.V. has received research funding from Takeda and Active Biotech and consulting fees from Takeda, Karyopharm, GSK, Genentech and Sanofi. A.D.C. is a scientific advisor for Janssen and BMS and has received research support and royalties from Novartis. The other authors declare no competing interests.

Additional information

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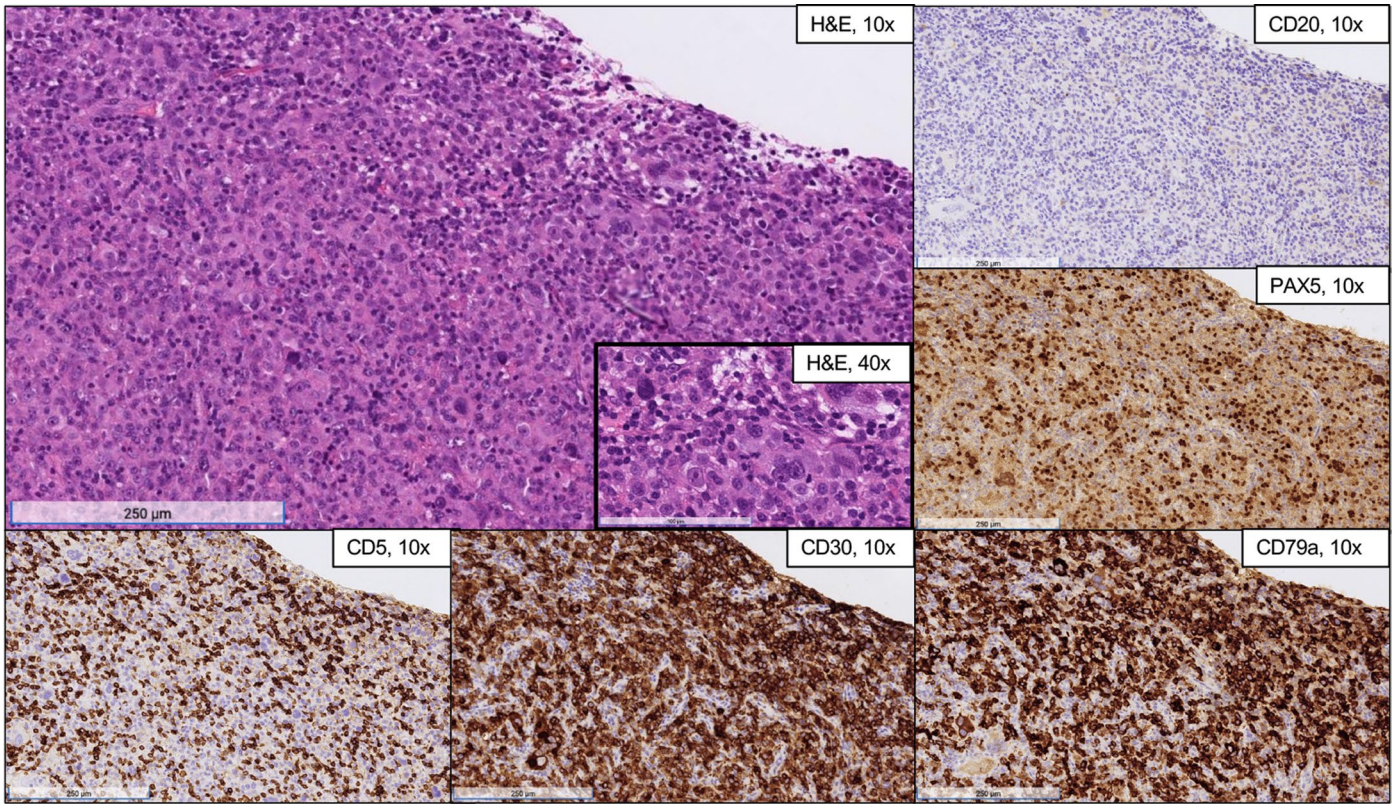

Extended Data Fig. 1 | Histopathological evaluation of the recurrent B cell lymphoma, unclassifiable, with features intermediate between diffuse large B-cell lymphoma and classic Hodgkin lymphoma ('grey zone' lymphoma). H&E staining and immunohistochemistry were performed for CD5, CD20, CD30, CD79a, and PAX5, highlighting B cell origin. Histopathologic evaluation reveals aggressive B-lineage neoplasm, with a large CD30+ B lineage neoplasm for which the combined features favor a B cell lymphoma, unclassifiable (BCL-U), with features intermediate between diffuse large B cell lymphoma and classic Hodgkin lymphoma. H&E shows architectural effacement by sheets of mitotically active large cells with oval nuclei, condensed chromatin, prominent nucleoli, and ample eosinophilic cytoplasm. Occasional multinucleate and bizarre forms are noted. Immunohistochemical stains show CD20 negativity consistent with clinical history of rituximab. B cell lineage confirmed by CD79a and Pax5. CD5 highlights scattered T cells. 10x magnification, expect inlet 40X. The analyses were conducted during the diagnostic assessment of the patients or as correlative exploratory analyses (qPCR) and as such were performed once. *Abbreviations: H&E: hematoxylin and eosin.*

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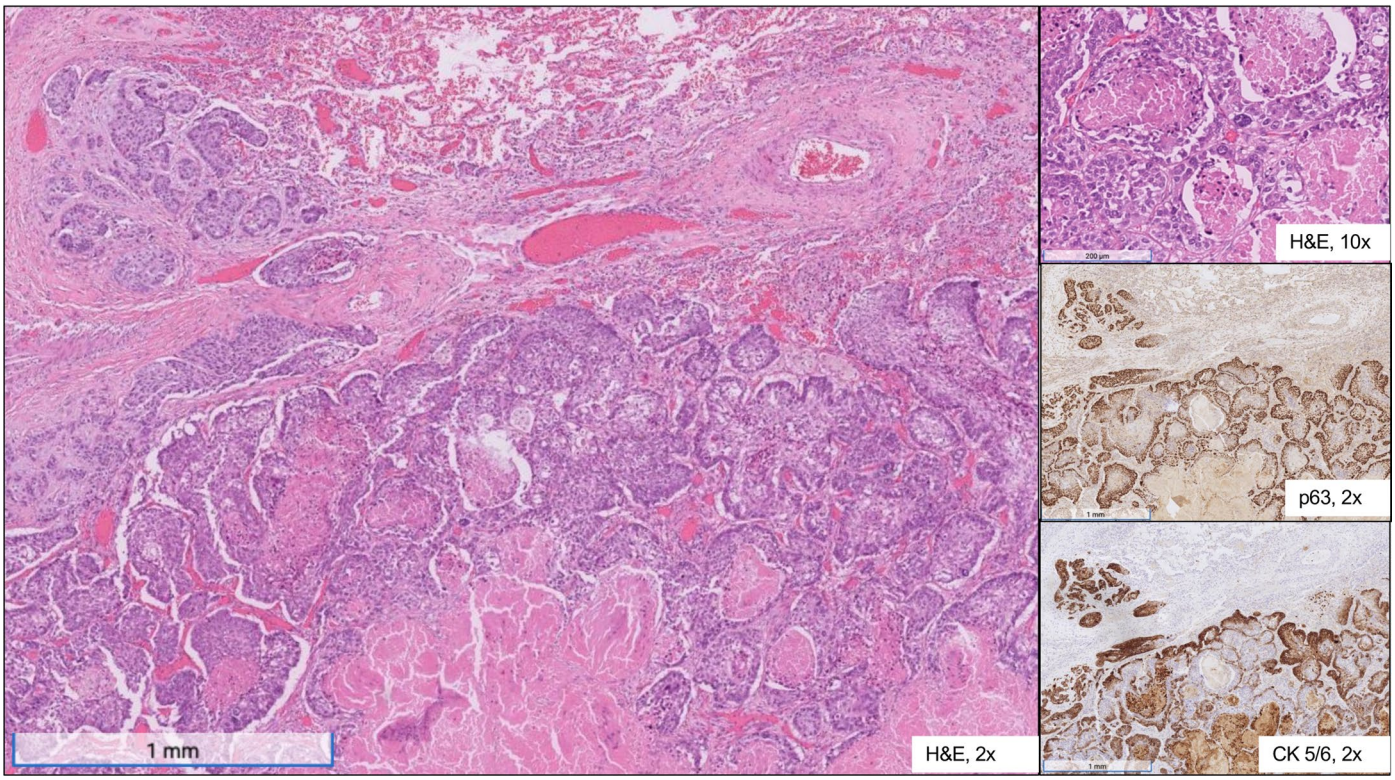

Extended Data Fig. 2 | Histopathological evaluation of the non-small cell lung cancer. H&E staining; 2x and 10x magnification. Immunohistochemistry for p63 and CK5/6; 2x magnification. H&E sections show invasive poorly differentiated carcinoma with marked nuclear enlargement and atypia (10x image) infiltrating into the lung parenchyma and with large areas of necrosis. Immunohistochemical stains showed tumor cells staining strongly positive for CK5/6 and p63 and supported the diagnosis of invasive poorly differentiated squamous cell carcinoma. The analyses were conducted during the diagnostic assessment of the patients or as correlative exploratory analyses (qPCR) and as such were performed once. *Abbreviations: H&E: hematoxylin and eosin staining. NSCLC: non-small cell lung cancer.*

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Extended Data Table 1 | Abbreviations: Axi-cel: axicabtagene ciloleucel; Brexu-cel: brexucabtagene autoleucel; Ide-cel: idecabtagene vicleucel; LBCL: large B cell lymphoma; MCL: mantle cell lymphoma; MDS: myelodysplastic syndrome; MM: multiple myeloma; NSCLC: non-small cell lung cancer; TCL: T cell lymphoma; Tisa-cel: tisagenlecleucel

ID	Disease	Product	# of previous lines of therapies	Age at second tumor	Second tumor	Months to second tumor
1	LBCL	Axi-cel	2	61	Skin cancer - not melanoma	47.7
2	LBCL	Axi-cel	2	67	Smoldering Myeloma	16.2
3	LBCL	Axi-cel	6	68	Prostate Cancer	32.5
4	LBCL	Tisa-cel	3	54	NSCLC	35.4
5	LBCL	Tisa-cel	4	69	Prostate Cancer	11.5
6	MCL	Brexu-cel	3	76	NSCLC	25.4
7	LBCL	Tisa-cel	6	71	Skin cancer - not melanoma	47.2
8	LBCL	Tisa-cel	4	75	MDS	2.8
9	LBCL	Axi-cel	2	64	TCL and NSCLC	4.0
10	LBCL	Tisa-cel	4	53	AML	9.7
11	MCL	Brexu-cel	4	70	Skin cancer - not melanoma	5.5
12	LBCL	Tisa-cel	4	61	Prostate Cancer	27.4
13	LBCL	Tisa-cel	5	75	Skin cancer - not melanoma	30.7
14	MM	Ide-cel	9	75	MDS	22.2
15	MM	Ide-cel	5	74	Skin cancer - not melanoma	4.2
16	MM	Ide-cel	5	65	Melanoma	6.2

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Extended Data Table 2 | Statistical considerations: Univariate analysis was performed using the Kaplan Meier method and compared using the Log-rank test. Multivariate analysis was performed using the Cox regression method. All tests were two-sided, and significance was defined as p < 0.05

	Univariate			Multivariate		
Characteristics	**HR**	**95% CI**	**p**	**HR**	**95% CI**	**p**
Sex						
Female	-	-	0.124	-	-	0.180
Male	2.216	0.8041-6.110		2.384	0.669-8.504	
Age at CART infusion						
≤65 years	-	-	0.051	-	-	0.033
>65 years	2.842	0.995-8.115		3.284	1.102-9.791	
# of previous lines of therapies						
≤3	-	-	0.116	-	-	0.170
>3	2.196	0.821-5.873		2.223	0.709-6.965	
Previous autologous SCT						
No	-	-	0.781	-	-	0.822
Yes	1.168	0.391-3.494		0.872	0.265-2.876	
CAR T cell target						
CD19	-	-	0.374	-	-	0.444
BCMA	2.063	0.418-10.180		1.943	0.354-10.662	
Costimulatory domain						
4-1BB	-	-	0.377	-	-	0.084
CD28	0.610	0.203-1.827		0.348	0.105-1.153	

Abbreviations: CAR: chimeric antigen receptor; CI: confidence interval; HR: hazard risk.

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For all statistical analyses, confirm that the following items are present in the figure legend, table legend, main text, or Methods section.

n/a	Confirmed
☐	☒ The exact sample size (*n*) for each experimental group/condition, given as a discrete number and unit of measurement
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☐	☒ The statistical test(s) used AND whether they are one- or two-sided *Only common tests should be described solely by name; describe more complex techniques in the Methods section.*
☐	☒ A description of all covariates tested
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☐	☒ For null hypothesis testing, the test statistic (e.g. *F*, *t*, *r*) with confidence intervals, effect sizes, degrees of freedom and *P* value noted *Give P values as exact values whenever suitable.*
☒	☐ For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings
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☒	☐ Estimates of effect sizes (e.g. Cohen's *d*, Pearson's *r*), indicating how they were calculated

Our web collection on statistics for biologists contains articles on many of the points above.

Software and code

Policy information about availability of computer code

Data collection	TaqMan technology (Applied Biosystems), Illumina sequencers (San Diego, CA) which included MiSeq, HiSeq, and NovaSeq instruments, 3500 genetic analyzer (ThermoFisher), LymphoTrack TRG Assay Panel-MiSeq (Invivoscribe, Inc.), MiSeqDx platform (Invivoscribe, Inc.), NeoGenomics, Inc. (Fort Myers, FL),
Data analysis	Statistical Package for the Social Sciences software v.22.0 (Chicago, IL, USA). GraphPad Prism software 9.5.0 (730).

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All manuscripts must include a data availability statement. This statement should provide the following information, where applicable:

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All requests for raw and analyzed data and materials are promptly reviewed by the University of Pennsylvania, and the corresponding author, to determine if they are subject to intellectual property or confidentiality obligations. Patient-related data not included in the paper may be subject to patient confidentiality. The email

addresses for the corresponding author is mruella@upenn.edu. Any data and materials that can be shared will be released via a material transfer agreement. Data include patient's information, and in particular, the description of a second T cell lymphoma in a single individual. Over the manuscript we performed several studies on this specific individual, including genetic tests. Therefore, given the extremely sensitive nature of the information, we prefere to share all the information on request.

Research involving human participants, their data, or biological material

Policy information about studies with human participants or human data. See also policy information about sex, gender (identity/presentation), and sexual orientation and race, ethnicity and racism.

Reporting on sex and gender	We evaluated biological sex of patient population as a co-variate to study incidence of second malignancies post CART
Reporting on race, ethnicity, or other socially relevant groupings	Not applicable
Population characteristics	We evaluated sex, age at CART infusion, specific disease, oncological history and exposure to previous stem cell transplantation, CART product infused and type of costimulatory domain. We evaluated also history of previous previous neoplasm, history of newly diagnosed second neoplasm after CART.
Recruitment	We retrospectivelly evalauted patient information of all the patient receiving commercial CART at the university of pennsylvania hospital between January 2018 and November 2023.
Ethics oversight	The study was approved by University of Pennsylvania Institutional Review Board.

Note that full information on the approval of the study protocol must also be provided in the manuscript.

Field-specific reporting

Please select the one below that is the best fit for your research. If you are not sure, read the appropriate sections before making your selection.

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Life sciences study design

All studies must disclose on these points even when the disclosure is negative.

Sample size	449 patients treated with commercial CART. We included all the patients treated with commercial CART immunotherapy at the university of Pennsylvania between 2028 and November 2023. No patients were excluded from the analysis.
Data exclusions	No data were excluded from this analysis.
Replication	Not applicable. The analyses were performed during the clinical-diagnostic investigations and as such were performed once.
Randomization	This is a retrospective analysis evaluating the incidence of second primary malignancies of commercial CART treated patients at the University of Pennsylvania. Therefore randomization was not applicable.
Blinding	This is a retrospective analysis evaluating the incidence of second primary malignancies of commercial CART treated patients at the University of Pennsylvania. Therefore blinding was not applicable.

Reporting for specific materials, systems and methods

We require information from authors about some types of materials, experimental systems and methods used in many studies. Here, indicate whether each material, system or method listed is relevant to your study. If you are not sure if a list item applies to your research, read the appropriate section before selecting a response.

Materials & experimental systems

n/a	Involved in the study
☒	☐ Antibodies
☒	☐ Eukaryotic cell lines
☒	☐ Palaeontology and archaeology
☒	☐ Animals and other organisms
☒	☐ Clinical data
☒	☐ Dual use research of concern
☒	☐ Plants

Methods

n/a	Involved in the study
☒	☐ ChIP-seq
☒	☐ Flow cytometry
☒	☐ MRI-based neuroimaging

Plants

Seed stocks: N/A

Novel plant genotypes: *Describe the methods by which all novel plant genotypes were produced. This includes those generated by transgenic approaches, gene editing, chemical/radiation-based mutagenesis and hybridization. For transgenic lines, describe the transformation method, the number of independent lines analyzed and the generation upon which experiments were performed. For gene-edited lines, describe the editor used, the endogenous sequence targeted for editing, the targeting guide RNA sequence (if applicable) and how the editor was applied.*

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