Evaluation of all-cause mortality and cardiovascular safety in patients receiving chimeric antigen receptor T cell therapy: a prospective cohort study

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Summary

Background Assessment of cardiovascular risk is critical for patients with cancer. Previous retrospective studies suggest potential cardiotoxicity of CAR T cell therapies. We aimed to prospectively assess cardiotoxicity and the predictive value of cardiac biomarkers and classical risk factors (age, cardiac function, diabetes, arterial hypertension, smoking) for cardiac events and all-cause mortality (ACM).

Methods In this prospective cohort study, all patients treated with CAR T cell constructs (axi-cel, tisa-cel, brexu-cel, ide-cel, or the 3rd generation CAR HD-CAR-1) from Oct 1, 2018, to Sept 30, 2022 at the University Hospital Heidelberg were included. Surveillance included cardiac assessment with biomarkers (high-sensitive Troponin T (hs-cTnT), N-terminal brain natriuretic peptide (NT-proBNP)), 12-lead-ECG, and 2D echocardiography. ACM was defined as the primary study endpoint, while cardiotoxicity, defined by clinical syndromes of heart failure or decline in ejection fraction, served as a secondary endpoint.

Findings Overall, 137 patients (median age 60, range 20–83, IQR 16), were included in the study. 46 patients died during the follow up period (median 0.75 years, range 0.02–4.33, IQR 0.89) 57 month, with a median survival of 0.57 years (range 0.03–2.38 years, IQR 0.79). A septal wall thickness above 11 mm (HR 2.48, 95%-CI = 1.10–5.67, $p = 0.029$) was associated with an increased risk of ACM, with a trend seen for reduced left ventricular ejection fraction prior to therapy (LVEF <40%; HR 9.17, 95%-CI = 1.30–183.11, $p = 0.051$). Secondary endpoint was reached by 93 patients while no baseline parameter was able to predict an elevated risk. However, hs-cTnT change from baseline of 50% or more during the first 14 days after CAR infusion predicted ACM (HR 3.81, 95%-CI = 1.58–9.45; $p = 0.003$). None of the baseline characteristics was able to predict the incidence of cardiac events.

Interpretation Reduced pre-lymphodepletion ejection fraction and early post-infusion biomarker kinetics may be associated with increased ACM and cardiotoxicity events. These findings may help to identify patients who could benefit from intensified cardio-oncological surveillance.

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Keywords: Cardiotoxicity; CAR T cells; Cardiac biomarkers; Risk stratification

Introduction

Chimeric antigen receptor T cell (CAR T) therapy has been shown to be effective in the treatment of selected B cell malignancies. Six CAR T cell therapeutics are currently approved for clinical use in various subentities (Supplementary Table S1).[1–7] The expansion of CAR T cell therapy into solid tumours is currently the primary focus of ongoing research efforts worldwide, alongside

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Research in context

Evidence before this study

Chimeric antigen receptor (CAR) T cell therapy has shown effectiveness in the treatment of advanced hematological malignancies. We searched PubMed for studies published from Jan 01, 2011, until July 31, 2023, using the search terms 'cardiotoxicity', 'cardiovascular events', 'cardiovascular side effects', 'cardiac events', and 'cardiac side effects' in combination with 'CAR T cells' or 'CAR T cell therapy', with search terms found in abstract, title or MESH headings. Additionally, we also searched references listed in the identified papers. The search displayed 20 publications with direct relation to the topic and that evaluation of cardiotoxicity, a severe complication associated with certain anti-cancer therapeutics, has so far been limited to retrospective analysis of approval trial and registry data or literature reviews, resulting in two major constraints: exclusion of patients with pre-existing cardiovascular disease, and incomplete cardiac biomarkers and surveillance due to focus on symptomatic patients.

Added value of this study

This prospective study expands understanding of cardiac complications during and after CAR T cell infusion. Additionally, it uncovers predictive capabilities using biomarker monitoring for all included patients at various timepoint throughout the trial as well as echocardiographic measurements prior to and at two follow-up timepoints after CAR T cell therapy.

Implications of all the available evidence

This study reports an association of reduced ejection fraction and cardiac hypertrophy with increased all-cause mortality prior lymphodepletion. Additionally, it identifies high-sensitive troponin T change as a potential predictor for all-cause mortality, which allows cost-effective and more comprehensive monitoring of patients at risk who should be considered for intensified surveillance or preemptive intervention.

optimization of currently approved hematological approaches.[8] Furthermore, the use of CAR T cells is also being investigated in individual studies for the treatment of non-cancerous diseases such as cardiac fibrosis, or autoimmune diseases such as lupus erythematodes.[9,10] As CARs continue to be incorporated into therapeutic guidelines, further evaluation of their safety profile is essential.

Cardiotoxicity is a serious complication associated with certain anti-cancer therapeutics. Patients receiving CARs under current FDA- or EMA-approval have often received multiple lines of prior therapy, including agents with potential cardiotoxicity (e.g., anthracyclines or cyclophosphamide). However, there is a paucity of data regarding the impact of cardiac comorbidity on CAR T cell therapy outcomes as well as potential cardiotoxic effects of CAR T therapy.[11–17] Since cardiovascular disease and, in particular, reduced left ventricular ejection fraction (LVEF) is an exclusion criterion in most studies, underrepresentation of patients at high risk of cardiovascular complications cannot be ruled out. Thus, the prevalence of cardiovascular morbidity is likely higher with increasing use of CAR T cell therapy in real-world settings. The cardiac side effects reported in these studies are variable. Arrhythmias and venous thromboembolic events are present, as are hypotension (usually in the setting of a cytokine release syndrome (CRS)), pleural or pericardial disease.[11] Furthermore, some cardiovascular adverse events appear to be typically associated with CRS.[12,17–19]

To address for the aforementioned limitations of retrospective and registry data, our aim was to prospectively investigate the association between cardiac morbidity and CAR T cell therapy.

Methods

Study design and participants

In this prospective cohort study, 137 consecutive patients who received CAR T cell therapy between Oct 1, 2018, and Sept 30, 2022 were enrolled. According to the Declaration of Helsinki, written informed consent was obtained from all patients for biomarker collection and cardiac surveillance. Ethical approval and approvals from the local and federal competent authorities were granted (Ethics Committee of the Medical Faculty, University Heidelberg; October 2017, AFmu-405/2017). The STROBE checklist was utilized for quality assessment.

Axi-cel and brexu-cel are autologous anti-CD19 CAR T cell products containing a second-generation CAR encoded by a retroviral vector with an scFy targeting CD19 with CD3ζ and CD28 intracellular domains that signal T-cell activation, while tisa-cel is generated from autologous T cells transduced with a second-generation lentiviral vector to express an anti-CD19 CAR containing a CD3ζ domain and a 4-1BB (CD137) domain as costimulatory signal.[1,3,4] Ide-cel is generated from autologous T cells transduced with a second-generation lentiviral vector to express an anti-BCMA CAR featuring a CD3ζ signaling domain and a 4-1BB domain as costimulatory signal, whereas for the HD-CAR-1 product, autologous T cells are transduced using a third-generation retroviral CAR vector encoding for anti-CD19 with CD3ζ as signaling domain and both 4-1BB

and CD28 domains as costimulatory signals.[6,20] All patients received a lymphodepleting chemotherapy with fludarabine and cyclophosphamide prior to CAR T cell administration.

Procedures

Each patient was examined for medical history prior to admission with a special emphasis on cardiovascular evaluation, while a physical examination was conducted at admission. 2D echocardiography was performed before lymphodepleting chemotherapy, a first follow-up on day +7, and a second follow-up between day +28 and day +180 (Fig. 1A). 12-lead-electrocardiograms (ECG) were performed before lymphodepleting chemotherapy, at day +7, day +14, day +28, day +56, day +90, and day +180. The cardiac biomarkers high-sensitive Troponin T (hs-cTnT) and N-terminal brain natriuretic peptide (NT-proBNP) were assessed before lymphodepletion, one day prior to CAR T cell application, on day +1, day +3, day +5, day +7, day +14, day +28, day +56, day +90, and day +180. Echocardiographic parameters were assessed at three different timepoints: prior to conditioning chemotherapy, at day 7 after CAR infusion (median day 7, range 6–9, IQR 1) as well as with a second follow up echocardiographic examination after CAR T cell application (median day 90, range 28–180, IQR 90). If patients experienced disease progression during follow-up after CAR T cell infusion, all cardiac assessments from the date of relapse onwards were excluded from the analysis.

The study was designed as an observational cohort study. Cardiotoxicity was defined as worsening LVEF ≥10% or a drop in LVEF below a threshold of ≤50%, any hypo- or hypertensive event requiring medical intervention, or cardiac decompensation (defined as fluid overload with or without pulmonary edema, vena cava inferior >20 mm or not respiratory variable during follow-up-echocardiography). Other cardiac events were defined as worsening of LVEF less than 10%, supraventricular arrhythmia, or unspecific ECG changes. MACE was defined as composite of myocardial infarction (MI), stroke, revascularization, including percutaneous coronary intervention, and coronary artery bypass grafting; ventricular arrhythmias or arrhythmias requiring implantable devices (cardioverter defibrillator or pacemaker).

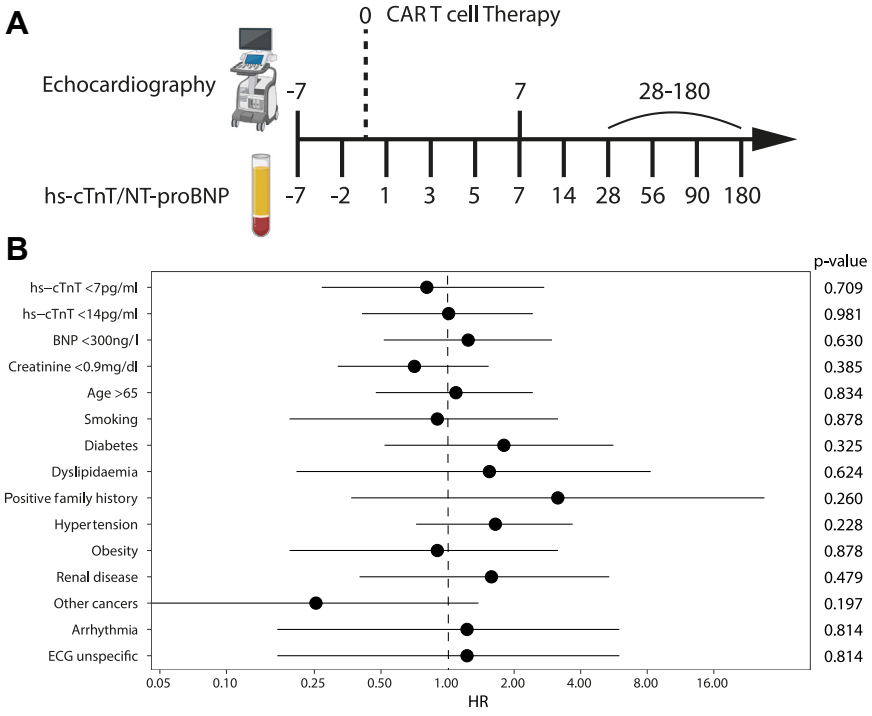

Fig. 1: Study overview & Forest plot of baseline-parameter and their predictive value for all-cause mortality. **A.** Study design and overview. Patients received 2D echocardiography prior to lymphodepleting chemotherapy, with two follow-ups (first at day +7, and a second between day +28 and day +180. Additionally, cardiac biomarkers with high-sensitive Troponin T (hs-cTnT) and N-terminal brain natriuretic peptide (NT-proBNP) were assessed before lymphodepletion, one day prior to CAR T cell infusion, at day +1, day +3, day +5, day +7, day +14, day +28, day +56, day +90, and day +180. **B.** Univariable logistic regression analysis on all-cause mortality (ACM). Hazard ratios (HR) and 95% confidence interval are shown as forest plot. *p*-values as indicated (Wald test). CAR: Chimeric antigen receptor. hs-cTnT: high-sensitive Troponin T. BNP/NT-proBNP: N-terminal brain natriuretic peptide. ECG: electrocardiogram. HR: Hazard ratio.

Data acquisition

Measurement of hs-cTnT in plasma samples was performed using the Elecsys® Troponin T high-sensitive hs-cTnT assay (Roche Diagnostics) on a cobas® e411 immunoassay analyzer in the central laboratory at Heidelberg University Hospital. On Cobas e411, limit of blank (LoB), limit of detection (LoD), 10% coefficient of variation (CV) and 99th percentile cut-off values for the hs-cTnT assay were 3 ng/L, 5 ng/L, 13 ng/L and 14 ng/L. N-terminal pro brain-type natriuretic peptide was measured by the Stratus® CS Acute Care™ NT-proBNP assay (Siemens AG, Berlin and Munich, Germany).

Echocardiography was performed on a General Electrics (GE) Vivid E9 unit. Images were acquired ECG-triggered with a minimum of three beats per frame. LVEF was measured by a physician who is experienced in echocardiography using the biplanar calculation (2- and 4-chamber views).

Statistical analysis

All data were collected and processed in "R" (Version 4.2.1), and a detailed overview of utilized R packages is available in the supplements. Time-to-event was defined as date of CAR T cell administration to date of death or survival by July 1, 2023. The follow-up for all-cause mortality (ACM) was defined as the time difference between the date of CAR T cell administration and the date of death or the date of the last reported status, respectively. The logrank test was used to determine differences in survival, while logistic regression was used as statistical analysis for risk factor analysis. A p-value <0.05 was considered significant. In order to compare continuous variables, we used the Mann–Whitney Test. Univariable logistic regression as well as multivariable logistic regression analyses were also performed in R, as were univariable (cause-specific) Cox regression models. A confidence interval of 95% was denoted as 95%-CI.

Elevated hs-cTnT was defined as levels above the 99th percentile (14 ng/L) and elevated NT-proBNP as levels above the age adjusted rule-out criteria of 450 pg/mL for <50 years, 900 pg/mL for 50–75 years and 1800 pg/mL for >75 years.[21,22] The median laboratory values in our cohort were then used for further analysis in order to predict ACM.

The primary study endpoint was ACM, while cardiotoxicity (clinical symptoms of heart failure or decline in ejection fraction) served as secondary endpoint (Supplementary Table S2).

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. MS and LL had final responsibility for decision of publication.

Results

Patient characteristics

The 137 patients had a median age of 63 years (range 20–83, IQR 16; 71.5% male), and overall were in good condition according to both pre-conditioning Karnofsky index (median 90, range 50–100, IQR 10) and HCT-CI score (median 0, range 0–8, IQR 2, Table 1). Due to administration of five different CAR T cell products, distribution of underlying diagnoses varied: B cell lymphomas were treated the most (76%), followed by B-ALL (14%) and myeloma (10%). 30 of the 137 patients (22%) had a history of cardiac disease, most commonly coronary artery disease (n = 16, 12%). CVRF were present in 68 patients (50%; most common: arterial hypertension, n = 41 (30%); smoking, n = 13 (9%); diabetes, n = 14 (10%); and obesity, n = 13 (9%)). Median creatinine of all patients was 0.87 mg/dl (range 0.40–2.71) prior to lymphodepletion.

Median follow-up was 276 days (9–1581 days, IQR 325 days), the primary endpoint was reached by 46 patients, as they deceased during the follow up period after a median of 0.57 years (range 0.03–2.38 years, IQR 0.79), while 93 patients reached the secondary study endpoint. 87 patients experienced tumour progression after CAR therapy (64%), and the non-relapse mortality was 7% (3 of 46 patients).

Following CAR T cell products were administered: 54 patients with axicabtagene ciloleucel (axi-cel), 17 patients with tisagenlecleucel (tisa-cel), 6 patients with brexucabtagene autoleucel (brexu-cel), 14 patients with idecabtagene vicleucel (ide-cel), and 46 patients within the clinical HD-CAR-1 trial at the Heidelberg University Hospital (EudraCT: 2016-004808-60; NCT: NCT03361748). Axi-cel was administered to 51 r/r DLBCL (diffuse large B cell lymphoma) patients and 3 r/r PMBCL (primary mediastinal B cell lymphoma) patients, and brexu-cel was given to 6 r/r MCL (mantle cell lymphoma) patients. 17 r/r DLBCL patients received tisa-cel. Ide-cel was given to 14 patients with r/r myeloma. In the IIT HD-CAR-1, CAR T cells were administered to 6 r/r DLBCL patients, 7 r/r MCL patients, 9 r/r CLL (chronic lymphocytic leukemia) patients, 5 r/r FL (follicular lymphoma) patients, and 19 ALL (acute lymphoblastic leukemia) patients.

Patients had a median of four therapies (range 2–13, IQR 2) prior to CAR T cell application, with 40 patients (29%) receiving prior autologous and 44 patients (32%) allogeneic stem cell transplantation. 76 patients (55%) required bridging therapy (therapy administered to patients during the manufacturing period). 132 of the 137 patients (96%) received potentially cardiotoxic therapy regimens, the most common being anthracyclines (n = 111, 80%) and cyclophosphamide (n = 129, 94%).

Additional detailed patient characteristics and risk factors are shown in Table 1.

	Patient cohort (n = 137)
Median age at CAR T cell dosage (in years; range, IQR)	60 (20–83, 16)
Gender, No. (%)	
Female	31 (33)
Male	62 (67)
Median Karnofsky at conditioning (range, IQR)	90 (50–100, 10)
HCT-CI score	0 (0–8, 2)
Disease type, No. (%)	
B cell lymphoma	104 (76)
DLBCL	74 (54)
PMBCL	3 (2)
FL	5 (4)
CLL	9 (7)
MCL	13 (9)
ALL	19 (14)
Multiple myeloma	14 (10)
Median number of prior therapy lines (range, IQR)	4 (2–13, 2)
Prior therapies, No. (%)	
Prior autologous stem cell transplantation	40 (29)
Prior allogenous stem cell transplantation	44 (32)
Bridging upfront of CAR T cell therapy	76 (55)
Potential prior cardiotoxic therapy regimens received[a]	132 (96)
Anthracyclines	111 (80)
Cyclophosphamide	129 (94)
Platinum based chemotherapy	50 (36)
Melphalan	40 (29)
Proteasome inhibitors	6 (4)
Checkpoint inhibitors	4 (3)
Kinase inhibitors	6 (4)
CAR T cell product, No. (%)	
Tisagenlecleucel	17 (12)
Axicabtagene ciloleucel	54 (39)
Brexucabtagene autoleucel	6 (4)
Idecabtagene vicleucel	14 (10)
HD-CAR-1	46 (34)
Prior cardiac disease, No. (%)	30 (22)
Coronary artery disease	16 (12)
Valvular heart disease	10 (7)
Atrial fibrillation	7 (5)
ECG abnormalities and other arrythmias	7 (5)
Cardiomyopathy	3 (2)
Pericarditis	3 (2)
Myocarditis	1 (1)
Cardiovascular risk factors, No. (%)	68 (50)
Hypertension	41 (30)
Smoking	13 (9)
Diabetes	14 (10)
Obesity	13 (9)
Dyslipidemia	6 (4)

(Table 1 continues on next column)

	Patient cohort (n = 137)
(Continued from previous column)	
Family history	4 (3)
Other cancer	12 (9)
Renal disease	12 (9)
Median creatinine prior lymphodepletion (mg/dl; range, IQR)	0.87 (0.40–2.71, 0.39)

Abbreviations: CAR, chimeric antigen receptor; IQR, interquartile range; HCT CI, hematopoietic cell transplantation-specific comorbidity index; DLBCL, diffuse large B cell lymphoma; PMBCL, primary mediastinal B cell lymphoma; FL, follicular lymphoma; CLL, chronic lymphocytic leukemia; MCL, mantle cell lymphoma; ALL, acute lymphoblastic leukemia; HD-CAR-1, Heidelberg CAR T cell trial. [a]Patients can have multiple lines of potential cardiotoxic therapy received, number indicates patients with one or more such therapy. Anthracyclines includes doxorubicin (Adriamycin) and daunorubicin. Platinum-based chemotherapy is comprised of cisplatin, oxaliplatin, and carboplatin, while proteasome inhibitors feature bortezomib and carfilzomib, and checkpoint inhibitors include nivolumab and pembrolizumab. Kinase inhibitors feature imatinib, dasatinib, and ponatinib.

Table 1: **Baseline characteristics and risk factors.**

Cardiac and CAR T cell specific adverse events

A total of 37 patients had one or more events classified as cardiotoxicity (Table 2). Collectively, 52 cardiotoxicity events were observed, with the most frequent hypotension (n = 25; 18%), and fluid overload (n = 13; 9%). There were no events classified as MACE. Other cardiac events were new onset of atrial fibrillation (n = 5; 4%) and new unspecific ECG changes (n = 3; 2%; incomplete right bundle branch block, supraventricular contraction, concordant negative T waves).

75 patients developed cytokine release syndrome (CRS) of any grade (55%), while immune effector cell-associated neurotoxicity syndrome (ICANS) was diagnosed in 33 patients (24%) (Table 2). High-grade CRS was observed in 5 patients (4%), and ICANS ≥ grade 3 in 17 patients (12%). Tocilizumab and steroids were applied as part of the treatment for CRS and ICANS according to ASTCT consensus criteria.[23]

Influence of the two major CAR T cell side effect—CRS and neurotoxicity—on all-cause mortality was investigated using univariable Cox regression. Higher grade (3 or higher) CRS significantly associated with survival probability ($p < 0.0001$, Supplementary Figure S1A). Similarly, grade 3 or higher ICANS was identified as risk factor for survival ($p = 0.012$, Supplementary Figure S1B). Additionally, the two most common treatments—tocilizumab (for CRS) and steroids (mainly for ICANS)—were investigated. Both (tocilizumab $p = 0.67$, Supplementary Figure S2A; steroids $p = 0.3$, Supplementary Figure S2B) were not associated with survival probability.

Furthermore, neither CRS or ICANS, nor tocilizumab or steroids had any association with cardiotoxicity.

Cardiac adverse events, No. (%)	
MACE	0
Patients with cardiotoxicity[a]	37 (27)
Hypo- or hypertensive event with necessity of medical intervention	27 (20)
Hypotension	25 (18)
Hypertension	2 (1)
Cardiac decompensation	17 (12)
LV-EF change ≥10% or a drop of LVEF a threshold of 50%	4 (3)
Fluid overload	13 (9)
ECG changes	8 (6)
Atrial fibrillation	5 (4)
Unspecific	3 (2)
CAR T cell specific side effects	
CRS, No. (%)	
No CRS	62 (45)
CRS grade 1-2	70 (51)
CRS grade ≥3	5 (4)
Treatment with tocilizumab	64 (47)
ICANS, No. (%)	
No ICANS	104 (76)
ICANS grade 1-2	16 (12)
ICANS grade ≥3	17 (12)
Treatment with steroids	28 (20)

Abbreviations: CRS, cytokine release syndrome; ICANS, immune effector cell-associated neurotoxicity syndrome. ECG, electrocardiogram; LVEF, left ventricle ejection fraction. MACE, major adverse cardiac events. Impaired LVEF was defined as < 50%. [a]Patients can have multiple different events defined as cardiotoxicity, number indicates patients with one or more such events.

Table 2: **Adverse events.**

Risk factor analysis

On univariable analysis, none of the analyzed cardiovascular risk factors (baseline Troponin T above 7 or 14 ng/L, baseline brain natriuretic peptide above 300 ng/L, baseline creatinine above 0.9 mg/dl, age >65 years, smoking behavior, diabetes, dyslipidemia, positive family history for cardiac events, history of hypertension, obesity, history of renal dysfunction, history of other cancers, arrhythmia, ECG changes) was significant associated with survival (Fig. 1B). Similarly, there was no significant association of the cardiovascular risk factors in multivariable analysis (Supplementary Figure S3).

Additionally, we evaluated the influence of relapse as well as different CAR specific (costimulatory domain) and therapy associated (such as number of prior therapies and potential cardiovascular regiments) parameters using multivariable analysis (Fig. 2). Here, only relapse was significantly associated with ACM (hazard ratio (HR) 8.73, 95%-CI = 2.75–39.14, $p = 0.0009$), while all other parameters failed to predict risk.

Echocardiographic parameters

The results of echocardiographic monitoring at the predefined timepoints are detailed in Supplementary Table S3.

Additionally, the impact of echocardiographic results on ACM were evaluated using univariable logistic regression. There was a statistically significant association between septal wall thickness above 11 mm–measured at baseline prior to lymphodepletion–and ACM (HR 2.48, 95%-CI = 1.10–5.67, $p = 0.029$) (Fig. 3). Trends were seen for baseline LVEF below 40% (HR 9.17, 95%-CI = 1.30–183.11, $p = 0.051$), baseline LVEF lower than 50% (HR 2.27, 95%-CI = 0.89–5.82, $p = 0.084$), and PA pressure >25 mmHg (HR 0.27, 95%-CI = 0.07–1.04, $p = 0.062$). LA diameter at baseline was not significantly associated with increased ACM. Besides the absence of MACE, four patients (2.9%) were found to develop a reduced LVEF (either a drop of >10% or LVEF reduction below 50%) within the follow-up of 21 days ±3.9 days (mean ± SEM) after CAR T cell infusion. Of these four patients, one patient experienced a decline of LVEF of more than 10%, while three had LVEF reduction below 50%. Two patients had no prior cardiac disease, while one patient had known prior atrial fibrillation and hypertension, and another patient had prior coronary artery disease. No higher-grade CRS or ICANS were observed in these patients (CRS: Grade 2 n = 2, Grade 1 n = 1; ICANS: Grade 2 n = 1). Similarly, no dynamic elevation of hs-cTnT (no changes <30%) was observed in any of the four patients. Three patients relapsed and one patient deceased due to disease progression during follow-up.

Cardiac biomarkers

Collectively, both hs-cTnT and nt-proBNP median values increased immediately after CAR T cell infusion before returning back to baseline within 180 days (hs-cTnT (all ng/L): baseline (median 12.30, range 3–118), day 1–day 7 (median 14.55, range 3.5–176), day 14–day 28 (median 13.65, range 3.0–258), day 56–day 180 (median 12.20, range 3.0–189); nt-proBNP (all pg/mL): baseline (median 257, range 20–3306), day 1–day 7 (median 452, range 20–22,308), day 14–day 28 (median 282, range 20–25,833), day 56–day 180 (median 210, range 20–7032), Supplementary Figure S4A and 4B). However, in individual patients there was a significant change of hs-cTnT value over time (Supplementary Figure S5A and 5B).

Hs-cTnT change by percentage of baseline value within the first 14 days after CAR T cell application was evaluated in univariable analysis with regards to survival, revealing a 35% hs-cTnT increase as threshold for prediction of survival (HR 2.26, 95%-CI = 1.00–5.17, $p = 0.049$, Fig. 4). Exploring increasing hs-cTnT cut-offs, a continuous rise of the hazard ratio could be seen, with univariable Cox regression of survival able to confirm this rise in significance (Supplementary Figure S6); from non-significant like 5%-alteration ((HR 0.86, 95%-CI = 0.30–2.70; $p = 0.78$) or 15%-alteration ((HR 1.18, 95%-CI = 0.52–2.73; $p = 0.69$) to significant changes such as 50%-alteration (HR 3.81, 95%-CI = 1.58–9.45;

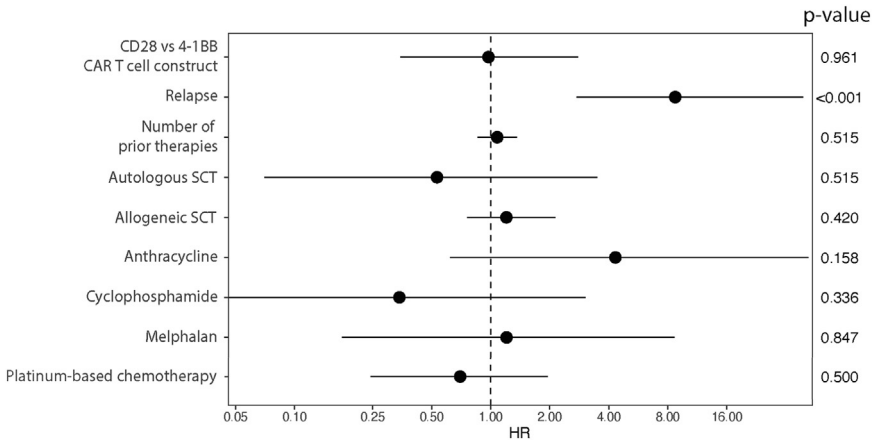

Fig. 2: Forest plot of relapse, different CAR specific as well as therapy associated parameters and their predictive value for all-cause mortality. Multivariable logistic regression analysis on all-cause mortality (ACM). Hazard ratios (HR) and 95% confidence interval are shown as forest plot. *p*-values as indicated (Wald test). All variables reported in below 5% of patients (proteasome inhibitors, checkpoint inhibitors, and tyrosine-kinase inhibitors) were excluded from this graphic (all non-significant) but are featured in Supplementary Table S5. SCT: stem cell transplantation. HR: Hazard ratio. Anthracyclines includes doxorubicin (Adriamycin) and daunorubicin. Platinum-based chemotherapy is comprised of cisplatin, oxaliplatin, and carboplatin, while proteasome inhibitors feature bortezomib and carfilzomib, and checkpoint inhibitors include nivolumab and pembrolizumab. Kinase inhibitors feature imatinib, dasatinib, and ponatinib.

p = 0.003) or 90%-alteration (HR 6.83, 95%-CI = 2.09–26.67, *p = 0.002*).

Overall, hs-cTnT change was comprised of 71 cases of hs-cTnT elevation and 39 cases of hs-cTnT decrease (1.82:1 ratio). When analyzed only for patients with a change significant for association with ACM, 32 patients had an increase in hs-cTnT and 9 had a decline (3.56:1 ratio).

Discussion

CAR T cell therapy is adopted as standard of care in an increasing number of indications in hemato-oncology, making safety and tolerability increasingly important for wider availability and use. Despite some preliminary analysis, there is a paucity of information on the effects of cardiac comorbidity on CAR T therapy outcomes, and on the spectrum, incidence and clinical impact of cardiac side effects of CAR T therapy.[11,24] These retrospective analyses led to both potential underestimation (numbers of patients with reduced cardiac function) and overestimation (number of patients with elevated troponin), as both were mostly assessed only in symptomatic patients within the different study cohorts. Additionally, the composition of the approval study populations provides some additional bias, as real-world analysis for Axi-Cel identified 23% of patients in the analysis who would have been ineligible for the ZUMA-1 trial had a cardiac comorbidity, compared to 0% in the ZUMA-1 eligible subgroup.[25] Similar findings were seen when evaluating a Tisa-Cel real-world setting.[26]

This study provides the first prospective cardiologic evaluation of patients that underwent CAR T cell therapy. In contrast to previous retrospective reports, we did not find severe cardiac events (MACE),[27,28] even not in patients with cardiac comorbidities and cardiac risk factors at baseline. However, four patients (2.9%) developed an impaired LVEF, which affects morbidity and long-term survival. This was less than observed in a comparable retrospective cohort (5.8%) by Alvi et al.,[12] but still relevant regarding future surveillance strategies. Other cardiovascular side effects, such as hypotension, hypertension and cardiac arrhythmias have been reported to occur in 22–38% of patients depending on the grade of CRS,[12,29] which is comparable to what was seen in our cohort. Cardiac arrhythmias have been reported to occur in 5.1%[12] whereas 3.6% occurred in the prospectively studied cohort here. Again, neither arrhythmias nor decline in LVEF could be predicted by baseline cardiac assessment. More importantly, patients with a decline in LVEF did not have high-grade CRS or dynamic elevation of hs-cTnT which is in contrast to previous reports.

However, oncologic patients with pathological cardiac findings (e.g. elevated cardiac biomarkers, reduced left ventricular function) are a high-risk patient cohort per se. This may justify a rigorous cardio-oncologic evaluation at baseline in these patients, as suggested by current guidelines.[30] In particular, early post-treatment echocardiography might be able to identify patients with cardiotoxicity at an early stage.

Beside the occurrence of cardiotoxicity, elevated hs-cTnT in oncology patients appears to predict all-cause mortality in a variety of entities and is associated with the risk of MACE with other therapies, such as immune checkpoint inhibitors.[31–33] In patients who underwent

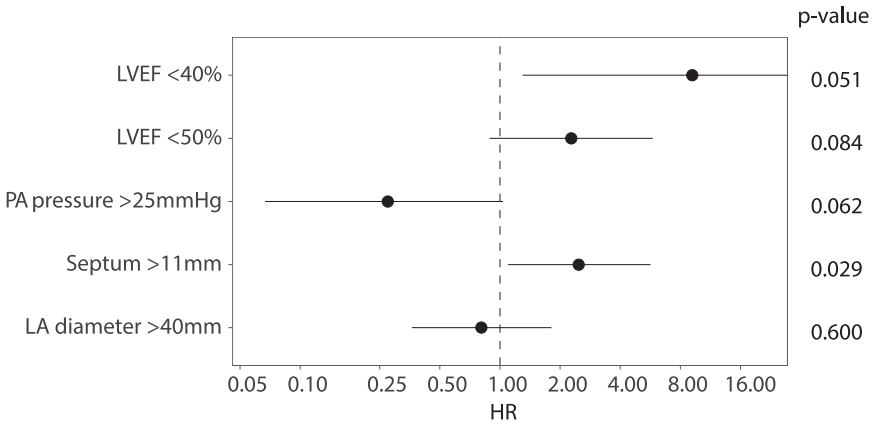

***Fig. 3:* Associations of echocardiographic baseline parameters with all-cause mortality.** Univariable logistic regression analysis of echocardiographic parameter influence on all-cause mortality (ACM). Hazard ratios (HR) and 95% confidence interval are shown as forest plot. Patients with baseline left ventricular ejection fraction (LVEF) below 40% show an increased risk for all-cause mortality. *p*-values as indicated. LVEF: left ventricular ejection fraction. PA: pulmonary artery. LA: left atrium. HR: Hazard ratio.

CAR T cell therapy, we frequently observed a mild increase in hs-cTnT during the early post-dosing phase. This was not associated with angina or ECG changes and never met the criteria for a diagnosis of non-ST elevation myocardial infarction.[34] However, patients with a delta of more than 50% of the baseline troponin had a dramatically increased mortality. This can be explained by a clinically inapparent infection or non-infectious inflammation with subclinical release of cytokines. Additionally, relation with tumour related complication–which often are of cardiovascular or infectious origin and pose a life-threatening risk–is evident, as most of deceased patients (43 of 46, 93%) died after suffering disease relapse after CAR therapy.[35–37] Other factors to significantly predict mortality were relapse of the primary disease and intraventricular wall thickness above 11 mm, both of which has been shown in prior studies (the latter investigating risk for patients with cardiovascular disease).[38–40]

In addition to the oncological use of CAR T cells, preclinical studies have demonstrated the potential use of CAR T cells in the treatment of heart failure by

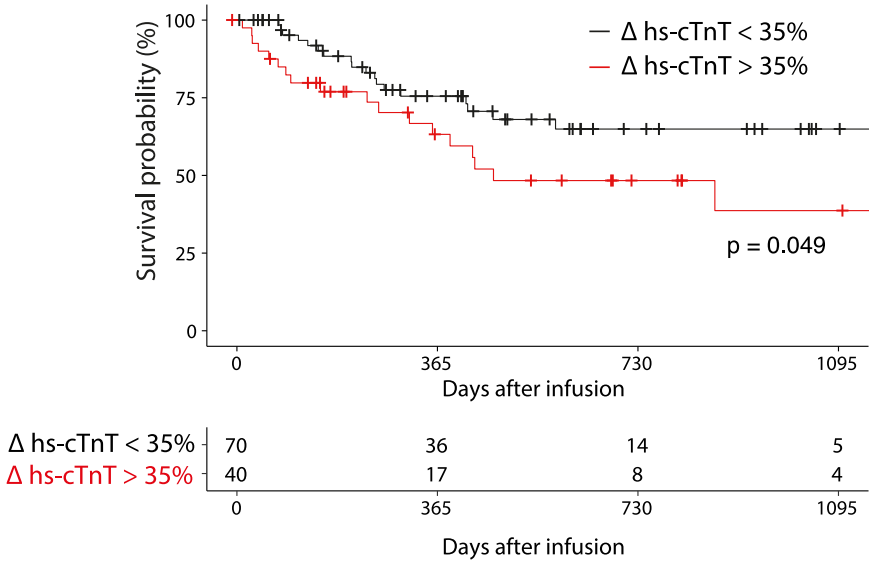

Fig. 4: High-sensitive troponin T change of 35% as threshold for prediction of survival. Kaplan Meier curves on all-cause mortality (ACM) with regards to a 35% hs-cTnT change (red line represents >35% change). *p*-value as indicated (Logrank test). Hs-cTnT: high sensitivity troponin T.

eliminating cardiac cells expressing fibroblast activating protein (FAP).[9,41] For a potential application of CAR T cells in heart failure patients, it will be essential to rule out the potential cardiotoxicity of current CAR T platforms (Supplementary Table S1). Based on the data from our cohort, CAR T platforms appear to be safe for a translational approach in heart disease, but an evaluation of CAR T cell-based therapies in patients with advanced heart failure warrants further data.

The limitations of our study consist in the single centre design of the analysis, which despite the prospective evaluation limits general applicability. Despite the absence of a significant statistical association between cardiovascular events and measured variables in both uni- and multivariable analyses, there is a possibility that potential mediators were underestimated or adjusted for. To account for this, alongside the study potentially being underpowered for rare events/exposures as well as limitations of retrospective power calculation and effect size analysis, these results require evaluation in larger multicentre studies. There is currently no consensus definition of cardiotoxicity caused by CAR T cell therapy, therefore this study based its grading on prior publications and experience from other immunotherapies, with more research needed to further expand understanding of cardiovascular safety. Assessment of LVEF was performed by using 2D echocardiography, which is considered to detect changes of >10%,[42] while 3D echocardiography would be more accurate to discriminate small changes. Also, while this evaluation investigated prior therapies regarding their risk for cardiotoxicity, cumulative dosages of anthracyclines could not be gathered for the whole study cohort. However, according to the standard protocols, no patient received >450 mg/mL, which is associated with a high risk for cardiotoxicity, and anthracycline therapy was ended at least 3 months before CAR T therapy.[43,44]

In conclusion, we found that CAR T cell therapy is safe with respect to major adverse cardiac events. However, patients with reduced baseline ejection fraction have a trend toward higher all-cause mortality. In addition, elevated hs-cTnT during the first two weeks after infusion may be associated with higher mortality and identifies a high-risk patient cohort. These findings need to be confirmed in larger clinical cohorts in multicentre trials.

Contributors

FK, MS and LL designed the research project, FK, LE, SR, AS, EG and LL analyzed, acquired, and discussed the data and the organization of the manuscript. FK and LL wrote the manuscript. All authors critically reviewed the manuscript, CMT, NF, PD, and MS edited the manuscript. LL supervised the work.

FK, LE, MS and LL have access to and verify the underlying study data.

Data sharing statement

The data that support the findings of this study are available with publication upon request from the corresponding author.

Declaration of interests

LHL: served on the advisory board for Daiichi Sankyio, Senaca, Astra Zeneca and Servier, as an external expert for Astra Zeneca and received speakers' honoraria from Novartis and MSD. CMT: research support from Bayer AG. Advisory board member Pfizer, Janssen-Cilag GmbH. Grants and/or provision of investigational medicinal products from Pfizer, Daiichi Sankyo, BiolineRx. MS: research grants from Apogenix, Hexal and Novartis. Travel grants from Hexal and Kite. Financial support for educational activities and conferences from bluebird bio, Kite and Novartis. Advisory board member of MSD. (Co-) PI of clinical trials of MSD, GSK, Kite and BMS. Co-Founder and shareholder of TolerogenixX Ltd. Head of the Cell Therapy Working Group of the German Society of Hematology & Oncology (DGHO). PD: consultancy AbbVie, AstraZeneca, Gilead, Janssen, Novartis, Riemser, Roche; speakers bureau AbbVie, Gilead, Novartis, Riemser, Roche; research support from Neovii and Riemser. AS: Travel grants from Hexal and Jazz Pharmaceuticals. Research grant from Therakos/Mallinckrodt. Consultancy BMS, Janssen-Cilag. Co-founder, shareholder, and part-time employee of TolerogenixX GmBH. EG: Honoraria for lectures by Roche Diagnostics. Member of the ACVC Biomarker Consensus Group. NF: Speaker honoraria/consulting fees from AstraZeneca, Boehringer Ingelheim, Bayer AG/Vital, BMS, Daiichi Sankyo, Edwards Lifesciences, GSK, Novartis, Pfizer, Sciarc, Synlab. Speaker of the Academy of the German Cardiac Society (DGK).

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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.2024.102504.

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