Torque Teno Virus Plasma DNA Load: A Novel Prognostic Biomarker in CAR-T Therapy

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Abstract

Torque Teno Virus (TTV) is a single-stranded circular DNA virus which has been identified as a surrogate marker of immune competence in transplantation. In this study we investigated the dynamics of plasma TTV DNAemia in 79 adult patients undergoing chimeric antigen receptor T-cell (CAR-T) therapy for relapsed or refractory large B-cell lymphoma, also evaluating the impact of TTV on immunotoxicities, response and survival outcomes. After lymphodepleting therapy, TTV DNA load decreases slightly until reaching nadir around day 10, after which it increased steadily until reaching maximum load around day 90. TTV DNA load < 4.05 log10 copies/ml at immune effector cell-associated neurotoxicity syndrome (ICANS) onset identified patients at risk of severe ICANS (OR 16.68, P = 0.048). Finally, patients who experienced falling or stable TTV DNA load between lymphodepletion and CAR-T infusion had better progression-free survival than those with ascending load (HR 0.31, P = 0.006). These findings suggest that TTV monitoring could serve as a surrogate marker of immune competence, enabling predictions of CAR-T efficacy and toxicity. This could pave the way for the development of TTV-guided therapeutic strategies that modulate clinical patient management based on plasma TTV load, similar to suggested strategies in solid organ transplant recipients.

INTRODUCTION

Chimeric antigen receptor T-cell (CAR-T) therapy has improved outcomes in patients with relapsed/refractory (R/R) hematological malignancies(1). Novel CAR-T constructs are promising strategies for extending use of this therapy to other malignancies, infections, and autoimmune diseases(2–4). However, CAR-T therapy is associated with significant morbidity and failure due to disease-refractory and immune-mediated toxicities(5, 6). Identifying novel biomarkers to predict these complications could be useful for early intervention.

Torque Teno Virus (TTV) was first described in 1997 following a case of post-transfusion hepatitis(7). It belongs to the *Anelloviridae* family, a group of single-stranded, circular DNA viruses that make up 70% of the human virome. TTV has high prevalence in the general population (> 90%) but has not been consistently linked to any specific disease(8). After an early-life infection(9, 10), TTV typically replicates in granulocytes and may use T lymphocytes as host cells(11, 12). It is insensitive to current antiviral drugs, and TTV plasma load is typically stable with little intraindividual variability(8).

Studies in kidney-graft recipients indicate that higher TTV DNA load is associated with infectious complications(13–17), whereas lower levels correlate with acute rejection(15–18). Data in the setting of allogeneic hematopoietic stem cell transplantation (HSCT) is more limited and challenging to interpret due to the interplay between the underlying disease itself, conditioning chemotherapy, and graft-versus-host disease prophylaxis. Early after HSCT, TTV kinetics may act as a marker of immunological reconstitution (19–21). In the long term, high TTV DNA load is associated with an increased risk of infectious complications(22). These findings, along with high prevalence rates, have helped identify TTV as a surrogate marker of immune competence in both solid organ transplantation (SOT) and HSCT. However, TTV DNA load and plasma TTV kinetics after CAR-T therapy remain unknown, as does the impact on CAR-T safety and efficacy, since no previous studies have been conducted in this setting to date.

Our aim in this study was to examine the dynamics of plasma TTV DNAemia in adult patients undergoing CAR-T therapy for relapsed or refractory (R/R) large B-cell lymphoma (LBCL). We also sought to evaluate the potential association of TTV plasma load kinetics with cytokine-release syndrome (CRS), immune effector cell-associated neurotoxicity syndrome (ICANS), response to therapy, and survival outcomes.

METHODS

Study design

Adult patients with R/R LBCL who received either commercial tisagenlecleucel (tisa-cel) or axicabtagene ciloleucel (axi-cel) were consecutively enrolled at three Spanish centers (Hospital Clínico Universitario de Valencia [HCV], Hospital Vall d'Hebrón de Barcelona [HVH] and Hospital Universitari i Politècnic La Fe de Valencia [HLF]) from January 2020 to June 2022. Data regarding patient and disease characteristics, treatment course, and clinical outcomes were prospectively collected. HLF samples were provided by the Biobanco La Fe (B.0000723). Study samples were processed following standard operating procedures with the appropriate approval of the Ethics and Scientific Committees. The study received approval from the Institutional Research Ethics Board before initiation (reference code 2020/179), and all patients provided informed consent to participate according to the Helsinki statement.

The HEMATOTOX score and Endothelial Activation and Stress Index (EASIX) were calculated prior to lymphodepleting chemotherapy, as previously described(23, 24). For TTV analysis, plasma samples were collected at the following time points: prior to lymphodepleting chemotherapy [TTVpreLD], prior to CAR-T infusion (TTVd0), and at different points after CAR-T infusion (days + 1, +3, + 5, +7, + 10, +14, + 21, +28, + 60 and + 90). After each plasma extraction, samples were frozen at -20ºC and analyzed along with the remaining samples at the HCV Microbiology Department. Genetic material was extracted from 200µL of plasma using the QIAsymphony® DSP Virus / Pathogen Kit Automated Extraction System (QIAGEN), following the manufacturer's instructions. Amplification and TTV DNA quantification were performed using TaqMan® real-time PCR assay kit (Invitrogen), which amplifies a highly conserved segment of the untranslated region of the viral genome, as previously reported(25). The assay can quantitate all known genetic variants of TTV.

Patient management

Standard lymphodepleting chemotherapy was administered according to each commercial product's instructions. Patients were hospitalized for CAR-T infusion, and clinically monitored in a daily basis until discharge. In the outpatient clinic, patients were followed weekly until day + 90. CRS and ICANS were graded according to the American Society for Transplantation and Cellular Therapy recommendations(26) and managed following institutional guidelines, based on national recommendations(27).

Definition and endpoints

Overall response rate (ORR) was defined as the proportion of patients who achieved either partial (PR) or complete response (CR) after CAR-T infusion(28). Duration of response (DOR) was defined as the time from ORR to relapse or death from any cause. Overall survival (OS) was the time from CAR-T infusion until death from any cause. Progression-free survival (PFS) was defined as the time from CAR T infusion until relapse, progression, or death from any cause. Non-relapse mortality (NRM) was defined by any death not related to relapse or progression since CAR-T infusion. Disease status before CAR-T therapy was defined as: i) primary refractory, if the patient never achieved end-of-treatment response, or ii) prior chemosensitivity, if the patient responded to previous treatment but experienced R/R prior to CAR-T. TTV DNA plasma kinetics were defined as decreasing (TTVpreLD > TTVd0), stable (TTVpreLD = TTVd0) or ascending (TTVpreLD < TTVd0).

In addition to preinfusion variables, the study assessed the impact of CRS, ICANS, and the use of tocilizumab and corticosteroids when evaluating response and survival outcomes. Similarly, CRS was considered as a risk factor for ICANS. We also evaluated the TTV plasma load before the onset of both CRS (TTVpreCRS) and ICANS (TTVpreICANS), to assess the likelihood of developing severe forms of these immunotoxicities.

Statistical analysis

A descriptive analysis was conducted including the median, range, and interquartile range for continuous variables, and percentages for categorical variables. Chi-squared test with Yates correction was used for comparison of categorical variables. Logistic regression analysis was carried out to estimate the association between ORR, CRS and ICANS and baseline factors. Kaplan-Meier curves were used to estimate the probability of PFS and OS from the time of CAR-T infusion

until last follow-up, and univariate comparisons were made using the log-rank test. Relapse and death were calculated as cumulative incidences using a competitive risk model. Cox proportional hazards regression models were used for univariable and multivariable analysis to include significant covariates. Statistical significance was determined by a two-side P value < 0.05. The ROC (receiver operating characteristic) curve and the relative area under the curve (AUC) of plasma TTV values were calculated. Youden's index was defined for all points of the ROC curve using the maximum value of the index as the criterion to select the best TTV cut-off. AUC values were calculated using the STATGRAPHIC Centurion XVIII statistics package program (Statpoint Technologies, Inc, Warrenton, VA, USA). Statistical analysis was performed with RStudio version 3.3.0+ (The CRAN project).

RESULTS

Patient characteristics

A total of 79 adult patients with R/R LBCL were included in this study (HCV, n = 36; HVH, n = 24; HLF, n = 19). Table 1 summarizes patient characteristics before treatment. Briefly, median age was 61 years (range, 21–81). Forty (51%) and 30 (49%) patients were treated with tisa-cel and axi-cel, respectively. Most patients were primary refractory (51%) and had advanced stage (stage 3–4, 72%). Sixty-two (78%) patients received bridging therapy, but response was achieved in only 8 (13%) patients (RP, n = 3; CR, n = 5). Patient and disease characteristics were well balanced between the two CAR-T commercial products, except for more progressive disease after bridging therapy in the axi-cel group (87% vs. 50%, P < 0.001).

Table 1
Patient and CAR-T therapy characteristics

Characteristics	Entire Cohort (N = 79)
Age in years, median (range)	62 (21–81)
Male sex, no. (%)	46 (58)
Diagnosis, no. (%)	
DLBCL	56 (71)
tFL	18 (23)
PMLBCL	5 (6)
Cell of origin, no. (%) [N/A, n = 1]	
GCB	56 (71)
Non-GCB	22 (28)
Myc rearrangement, no. (%) [N/A, n = 22]	16 (20)
Primary refractory, no. (%)	40 (51)
Previous lines of therapy, median (range)	2 (2–11)
Prior autologous HSCT, no. (%)	15 (19)
Prior allogeneic HSCT, no. (%)	1 (1)
Ann Arbor stage at apheresis, no. (%)	
1–2	22 (28)
3	11 (14)
4	46 (58)
IPI score at apheresis, no. (%) [N/A, n = 5]	
0–1	14 (17)
2	24 (30)
3	24 (30)
4–5	12 (15)
ECOG at apheresis, no. (%) [N/A, n = 1]	
0	36 (46)
1	35 (44)
≥ 2	7 (9)
Bridge therapy, no. (%)	62 (78)
Response to bridge therapy, no. (%)	
Disease progression	54 (68)
Stable disease	15 (19)

Characteristics	Entire Cohort (N = 79)
Partial response	3 (5)
Complete response	5 (6)
Not evaluated	2 (2)
CAR-T product, no. (%)	
Tisa-cel	40 (51)
Axi-cel	39 (49)
HCT-CI score preLD, no. (%) [N/A, n = 24]	
0–2	37 (47)
≥ 3	18 (23)
Low HEMATOTOX score preLD, no. (%)	47 (60)
EASIX score preLD, median (range)	1.84 (0.36–125.59)
TTVpreLD in log10 in copies/ml, median (range) [N/A, n = 35]	5.0850 (0–9.17)
TTVd0 in copies/ml, median (range) [N/A, n = 14]	4.95 (0–32)
$AUC_{TTVpreLD-TTVd0}$ in copies x days x ml^{-1}, median (range) [N/A, n = 45]	5.80 log10 (0–9.63)

Abbreviations: AUC: area under the curve; CAR-T: chimeric antigen receptor T-cell; DLBCL: diffuse large B-cell lymphoma; EASIX: Endothelial Activation and Stress Index; ECOG: Eastern Cooperative Oncology Group; GCB: germinal center B-cell like; HCT-CI: hematopoietic cell transplantation-specific comorbidity index; HSCT: hematopoietic stem cell transplantation; IPI: international prognostic index; N/A: not available; PMLBCL: primary mediastinal large B-cell lymphoma; preLD: pre-lymphodepleting chemotherapy; tFL: transformed from follicular lymphoma; TTV: torque teno virus ; TTVd0: TTV at day 0; TTVpreLD: TTV pre-lymphodepleting chemotherapy.

Dynamics of plasma TTV DNAemia

All 79 patients had at least one plasma specimen with quantifiable levels of TTV DNA. The median number of plasma samples per patient was 8 (range, 1–12). We collected 631 plasma samples at different time points during the study period: TTVpreLD, n = 44; TTVd0, n = 65; day + 1, n = 54; day + 3, n = 47; day + 5, n = 44; day + 7, n = 63; day + 10, n = 54; day + 14, n = 68; day + 21, n = 65; day + 30, n = 68; day + 60, n = 30; day + 90, n = 29. A total of 9 and 10 plasma samples were excluded at day 60 and day 90, respectively, for the ulterior analysis after disease progression and need of salvage therapy. The kinetics of TTV DNA load in plasma over the study period is shown in Fig. 1. TTV DNA load was seen to decrease slightly after lymphodepleting therapy, with a median TTVpreLD 5.08 log10 copies/ml (range, 0–9.17). Nadir was reached around day 10 with a median TTV DNA load of 4.80 log10 copies/ml (range, 0–9.04). Afterwards, TTV DNA load progressively increased until day 90 with a median load of 8.15 log10 copies/ml (range, 1.12–8.32).

Safety

Cytokine release syndrome

Sixty-three (80%) patients developed CRS (grade ≥ 3, 9%). No grade 5 CRS events were reported. The median time to CRS onset following CAR-T infusion was 2 days [range, 0–10] (Table 2). In univariate analysis, patients who experienced falling or stable TTV DNA load between lymphodepletion and CAR-T infusion had a higher risk of CRS than those with ascending

TTV DNA load (94% vs. 79%, P = 0.035), although this difference did not reach statistical significance in multivariate analysis (STable 1).

Table 2
CRS and ICANS characteristics

Characteristics	Entire Cohort (N = 79)
CRS, no. (%)	63 (80)
CRS grade ≥ 3, no. (%)	7 (9)
Time for CRS onset after infusion in days, median (range)	2 (0–10)
TTV DNA load at CRS onset in copies/ml, median (range) [N/A, n = 1]	5.15 log10 (0–9.54)
ICANS, no. (%)	34 (43)
ICANS grade ≥ 3, no. (%)	18 (22)
Time for ICANS onset after infusion in days, median (range)	7 (2–23)
TTV DNA load at ICANS onset in copies/ml, median (range) [N/A, n = 1]	5.66 log10 (0–9.38)
Tocilizumab, no. (%)	32 (40)
Corticosteroids, no. (%)	36 (45)
Cumulative dose of DXM in mg, median (range), [N/A, n = 9]	184 (10–4140)
Abbreviations: CRS: cytokine release syndrome; DXM: dexamethasone; ICANS: immune effector cell-associated neurotoxicity syndrome; mg: milligram; N/A: not available; TTV: torque teno virus.	

TTVpreCRS was available in 62 (98%) patients. Median TTV DNA load at this time was 5.15 log10 copies/ml (range, 0–9.54). No association was found between TTVpreCRS and the probability of developing severe forms of CRS.

Immune effector cell-associated neurotoxicity syndrome

In total, 34 (43%) patients developed ICANS (grade ≥ 3, 22%). One patient with unresponsive cerebral oedema died at day + 7, which was considered a grade 5 ICANS event. The median time to ICANS onset following CAR-T infusion was 7 days [range, 2–23] (Table 2). Preinfusion TTV DNA load was not identified as a risk factor for ICANS in statistical analysis (STable 2).

TTVpreICANS was available in 33 (97%) patients. Median TTV DNA load at this time was 5.66 log10 copies/ml (range, 0–9.38). Patients with TTVpreICANS < 4.05 log10 copies/ml had a higher risk of progressing to severe forms of ICANS than those with higher TTV DNA load (89% vs. 38%, P 0.025). Multivariate analysis confirmed this association (OR 16.68, 95% CI, 1.38–201, P = 0.048). To avoid biases that could arise in case of worse baseline characteristics in patients with lower TTVpreICANS, a comparative analysis of preinfusion variables was also performed. Both groups were well balanced, without significant differences observed. Using ROC analysis, cut-off points of TTVpreICANS < 4.09 log10 copies/ml and < 3.795 log10 copies/ml had a specificity and sensitivity of 93.8% and 47%, and 100% and 35.3%, respectively (Fig. 2).

Efficacy

Disease response

Fifty-three (67%) patients responded (CR, n = 39 [49%]; PR, n = 14 [18%]) at a median follow-up of 39 days (95% CI, 25–351) after CAR-T infusion. Cumulative incidence of response (CR + PR) at 100 and 180 days was 65% (95% CI, 52–77) and 74%

(95% CI, 62–87), respectively. Thirty-one (58%) patients sustained response at last follow-up. Median duration of response was 183 days (range, 1–860). The probability of sustaining response at 180 and 365 days from disease evaluation was 63% (95% CI, 48–77%) and 53% (95% CI, 36–69), respectively (Fig. 3).

Lower preinfusion TTV DNA load correlated to ORR in univariate analysis. Patients with $AUC_{TTVpreLD-TTVd0}$ < 5.8 log10 copies x days x ml^{-1} (76% vs. 35%, P = 0.038), TTVpreLD < 5.08 log10 copies/ml (73% vs. 41%, P = 0.068) and TTVd0 < 7.59 log10 copies/ml (76% vs. 38%, P = 0.013) had a greater probability of response than those with higher TTV DNA load. None of these variables reached statistical significance in multivariate analysis (Table 3).

Table 3
Protective factors for disease response

Disease response					
Variables	**Protective factor**	**Univariate analysis**		**Multivariate analysis**	
		%	**P**	**OR (95% CI)**	**P**
ECOG at apheresis	0–1	73 vs. 14	0.004		
BT after apheresis	No	94 vs. 61	0.022		
CAR-T product	Axi-cel	82 vs. 55	0.019	0.05 (0.003–0.95)	0.045
EASIX score preLD	< 1.84	87 vs. 46	< 0.001		
TTVpreLD	< 5.08 log10	73 vs. 41	0.068		
TTVd0	< 7.59 log10	76 vs. 38	0.013		
$AUC_{TTVpreLD-TTVd0}$	< 5.80 log10 copies x days x ml^{-1}	76 vs. 35	0.038		

Abbreviations: AUC: area under the curve; BT: bridge therapy; CAR-T: chimeric antigen receptor T-cell; EASIX: Endothelial Activation and Stress Index; ECOG: Eastern Cooperative Oncology Group; preLD: pre-lymphodepleting chemotherapy; TTV: torque teno virus; TTVd0: torque teno virus before CAR-T infusion.

Survival analysis

Forty-three (54%) patients remained alive at a median follow-up of 377 days (range, 165–892) from CAR-T infusion. The probability of OS at 1 year was 57% (95% CI, 45–68) (Supplementary Fig. 2). Lower preinfusion TTV DNA load correlated to OS in univariate analysis. Patients with $AUC_{TTVpreLD-TTVd0}$ < 5.80 log10 copies x days x ml^{-1} (74% vs. 24%, P = 0.017), TTVpreLD < 8.19 log10 copies/ml (56% vs. 18%, P = 0.051) and TTVd0 < 5.08 log10 copies/ml (77% vs. 41%, P = 0.015) had longer OS than those with higher TTV DNA load. None of these variables reached statistical significance in multivariate analysis (Table 4). Eight (10%) patients died without relapse/refractory disease at a median follow-up of 52 days (range, 7–772) from CAR-T infusion (infection, n = 5; unknown, n = 2; ICANS, n = 1). The probability of NRM at 1 year was 8% (95% CI, 2–14).

Table 4
Protective factors for overall survival

Overall survival					
Variables	**Protective factor**	**Univariate analysis**		**Multivariate analysis**	
		%	**P**	**OR (95% CI)**	**P**
Disease status at apheresis	R/R with prior chemosensitivity	75 vs. 39	0.002	0.43(0.20–0.89)	0.024
Ann Arbor stage at apheresis	I–III	71 vs. 47	0.071		
IPI score	=< 3	61 vs. 25	< 0.001	0.20 (0.08–0.50)	< 0.001
ECOG at apheresis	0–1	63 vs. 43	< 0.001		
HCT-CI score	0	84 vs. 38	0.006		
HEMATOTOX score	Low	65 vs. 30	0.018	0.28 (0.12–0.63)	0.002
EASIX score preLD	< 1.84	78 vs. 35	< 0.001	0.97 (0.96–0.99)	0.004
TTVpreLD	< 8.19 log10	56 vs. 18	0.051		
TTVd0	< 5.08 log10	77 vs. 41	0.015		
$AUC_{TTVpreLD-TTVd0}$	< 5.80 log10 copies x days x ml^{-1}	56 vs. 18	0.017		

Abbreviations: AUC: area under the curve; EASIX: Endothelial Activation and Stress Index; ECOG: Eastern Cooperative Oncology Group; HCT-CI: hematopoietic cell transplantation-specific comorbidity index; IPI: international prognostic index; preLD: pre-lymphodepleting chemotherapy; R/R: relapsed/refractory; TTV: torque teno virus; TTVd0: TTV before CAR-T infusion.

Thirty-one (39%) patients remained alive and progression-free at a median follow-up of 371 days (range, 165–892) from CAR-T infusion. The probability of PFS at 6 months and 1 year was 49% (95% CI, 38–60) and 40% (95% CI, 29–51), respectively (Supplementary Fig. 3). In univariate analysis, patients who experienced decreasing or stable TTV DNA load between lymphodepletion and CAR-T infusion had better PFS than those with ascending TTV DNA load [36% vs. 7%, P = 0.005] (Supplementary Fig. 1). Multivariate analysis confirmed this association [HR 0.31, 95% CI, 0.14–0.71, P = 0.006] (Table 5). To avoid biases that could arise in case of worse baseline characteristics in patients with decreasing TTV DNA load, a comparative analysis of preinfusion variables was also performed. Both groups were well balanced, without any significant differences found.

Table 5
Protective factors for progression-free survival

PFS					
Variables	**Protective factor**	**Univariate analysis**		**Multivariate analysis**	
		%	**P**	**OR (95% CI)**	**P**
Disease status at apheresis	R/R with prior chemosensitivity	50 vs. 30	0.045		
IPI score	0–1	40 vs. 25	0.018		
ECOG at apheresis	0–1	45 vs. 0	< 0.001	0.11 (0.03–0.38)	< 0.001
HCT-CI score	0	57 vs. 24	0.067		
HEMATOTOX score	Low	40 vs. 22	0.018		
EASIX score preLD	< 1.84	55 vs. 25	< 0.001		
Dynamics of plasma TTV DNAemia	Decreasing or stable	36 vs. 7	0.004	0.31 (0.13–0.71)	0.005

Abbreviations: EASIX: Endothelial Activation and Stress Index; ECOG: Eastern Cooperative Oncology Group; HCT-CI: hematopoietic cell transplantation-specific comorbidity index; IPI: international prognostic index; preLD: pre-lymphodepleting chemotherapy; R/R: relapsed/refractory; TTV: torque teno virus.

DISCUSSION

To the best of our knowledge, this is the first study to evaluate the use of TTV DNAemia as a prognostic biomarker in CAR-T therapy. The results suggest a potential association of TTV plasma kinetics and TTV plasma load with PFS and ICANS severity in adult patients with LBCL, respectively. Lower TTV plasma load could be linked to higher immune responses, leading to an improved CAR-T effect, but also an increased risk of immunotoxicities. This suggests that TTV monitoring could serve as a surrogate marker of immune competence, enabling CAR-T efficacy and toxicity to be predicted, which opens the possibility of developing personalized strategies for CAR-T patients similar to those suggested in SOT recipients (https://cordis.europa.eu/project/id/896932 and NCT04198506),.

Lower TTV DNA load was generally detected before lymphodepleting therapy, presumably due to prior antineoplastic schemes, especially bridging therapy. Nonetheless, plasma TTV DNA load continued to decrease slightly until nadir 10 days after CAR-T infusion, after which it increased steadily until day 90. Similar dynamics of plasma TTV DNAemia have also been described in the setting of SOT and HSCT(11, 20, 25, 29). Lymphocytes have been proposed as the major site of TTV replication(11, 12), implying that lymphodepletion therapy before CAR-T therapy or conditioning regimens containing alemtuzumab or anti-thymocyte globulin before SOT or HSCT could reduce the number of infected cells in the bloodstream, leading to lower TTV DNA load. As lymphocytes recover, TTV DNA load would rise in parallel(8).

Patients receiving axi-cel had higher ORR but also a greater risk of immune-related complications (both CRS and ICANS) than tisa-cel receptors, in concurrence with prior real-life data (30–33). Due to these differences in response and toxicity based on CAR-T type, a separate statistical analysis was conducted for each CAR-T construct. However, despite a trend towards significance, no statistically significant results were obtained, presumably due to the small sample size of the

study. As previously observed, there were no differences in baseline characteristics based on CAR-T type except for more progressive disease after bridging therapy in the axi-cel group, prompting the decision to analyze all patients together.

Patients with lower preinfusion TTV DNA load seemed to respond better and exhibit a greater risk of immunotoxicity, although these findings were not confirmed in multivariate analysis. Interestingly, among the 34 patients who developed ICANS, TTV DNA load < 4.05 log10 copies/ml at neurotoxicity onset identified patients at risk of progression to severe grades of ICANS (OR 16.68, P = 0.048). As previously reported, the immune system is strongly implicated in ICANS pathophysiology. Loss of blood-brain barrier (BBB) integrity and increased vascular permeability leads to an accumulation of cytokines, host immune cells and CAR-T lymphocytes in the central nervous system, which also activates resident microglial cells(6, 34). Based on the correlation between immune system competency and TTV, our data suggest that a lower TTV DNA load could lead to more intense immune responses, thereby increasing the risk of immune-related complications. Our findings suggest that TTV DNA load could be used as a surrogate marker of immune competence, enabling the identification of patients at risk of developing severe forms of neurotoxicity. By using TTV DNA load as an early warning sign, clinicians could intervene with earlier and more aggressive therapeutic measures, which could improve patient outcomes(35, 36). This is particularly important given that severe ICANS may negatively impact survival following CAR-T therapy(37, 38). Finally, identifying patients at lower risk of severe forms of ICANS would also be crucial to avoid overtreatment in this subset of patients, since higher cumulative doses of corticosteroids have been associated with significantly shorter overall survival(39).

TTV DNA plasma kinetics were calculated in 34 (43%) patients who had available samples both prior to lymphodepletion therapy and before infusion. Twenty patients who had a decreasing or stable TTV DNA load showed better PFS than those with an ascending TTV DNA load (HR 0.31, P = 0.006). As previously mentioned, TTV plasma load seems to correlate inversely with immune system competency, which could at least partly explain our results. In this regard, it would have been of interest to analyze the CAR-T lymphocyte subtype, given the data suggesting a potential impact following CAR-T therapy. For instance, CD8 + effector memory CAR-T and regulatory CAR-T have been associated with toxicity and lack of response, respectively(40–42). Nonetheless, as TTV load allows us to infer patient immune competence, this finding could be used in high-risk patients to establish consolidation strategies such as immunomodulators or checkpoint inhibitors (43, 44), following the same line as the immunosuppression modifications carried out in kidney transplantation based on TTV load, as previously mentioned.

The results of our study should be interpreted in the context of certain limitations, principally the small number of patients in the series, which limits the possibility of any firm conclusions regarding widespread application of TTV monitoring in this context at present. Secondly, the short follow-up precludes drawing conclusions on survival outcomes. Thirdly, the absence of correlation between absolute lymphocyte count and TTV DNA load could potentially create bias if the different TTV load subgroups are not adequately balanced. Despite these limitations, the reported data provides a basis for prospective studies investigating the potential role of TTV in CAR-T therapy, for predicting toxicities (not only CRS or ICANS, but also cytopenias and infections) and response and survival outcomes. A larger sample size would also enable the study of different CAR-T constructs separately, thereby confirming our results, which could encourage the development of TTV-guided therapeutic strategies modulating clinical patient management based on plasma TTV load. Considering the potential clinical significance of these results, prospective and well-powered studies are warranted to confirm our findings.

Declarations

ACKNOWLEDGMENTS

The *Institut Paoli-Calmettes* (Marseille, France) was also included as a collaborating center. The study was classified as a clinical trial in France (NCT04822974). Unfortunately, implementation of the study in France was prevented by the COVID-

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AUTHOR CONTRIBUTIONS

AB, EG and RH conceived the study and interpreted the data; AB and RH wrote the paper; AB, RH and EA performed the statistical analyses; EG, GI, MG, EA, CC, AB, AP, CSA, MASS, PC, JLP, FB, JM, JCHB, AF, BF, MV, PA, DC, MJT, JS, PB, DN and CS reviewed the paper and contributed to the final draft.

DISCLOSURES

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Figures

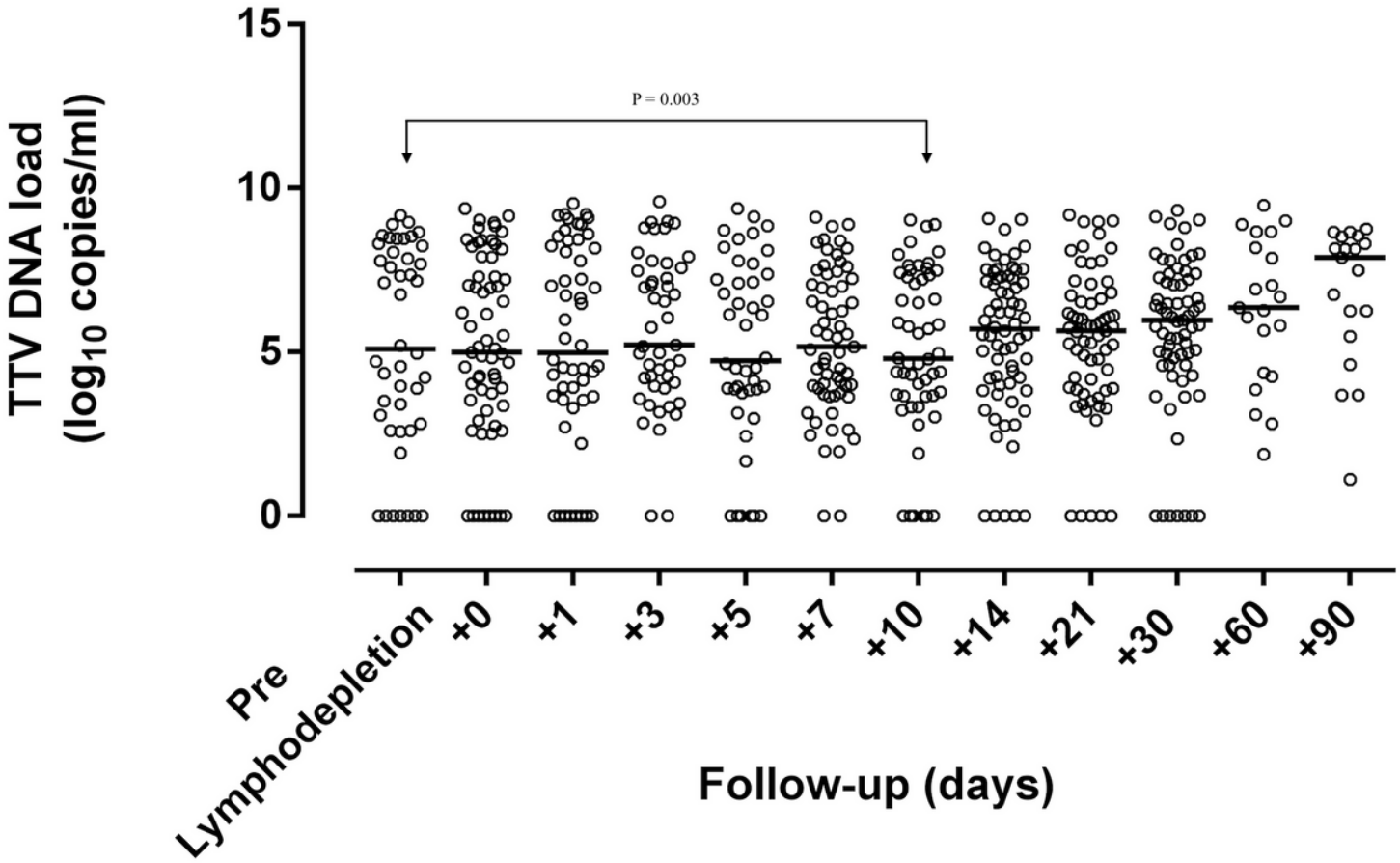

Figure 1

Dynamics of TTV DNAemia in plasma over the study period.

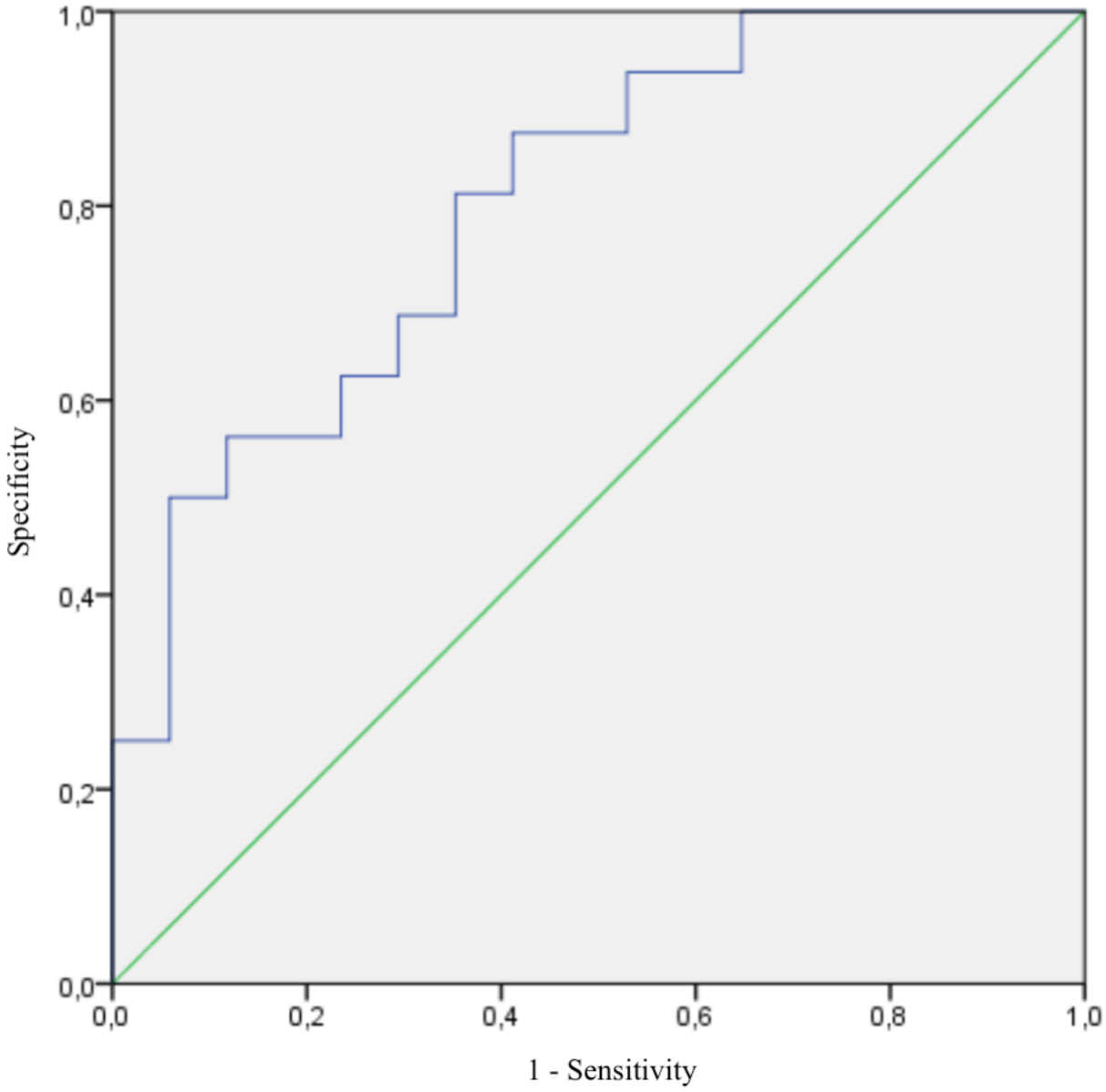

Figure 2

ROC curve analysis according to TTV plasma load at the onset of ICANS.

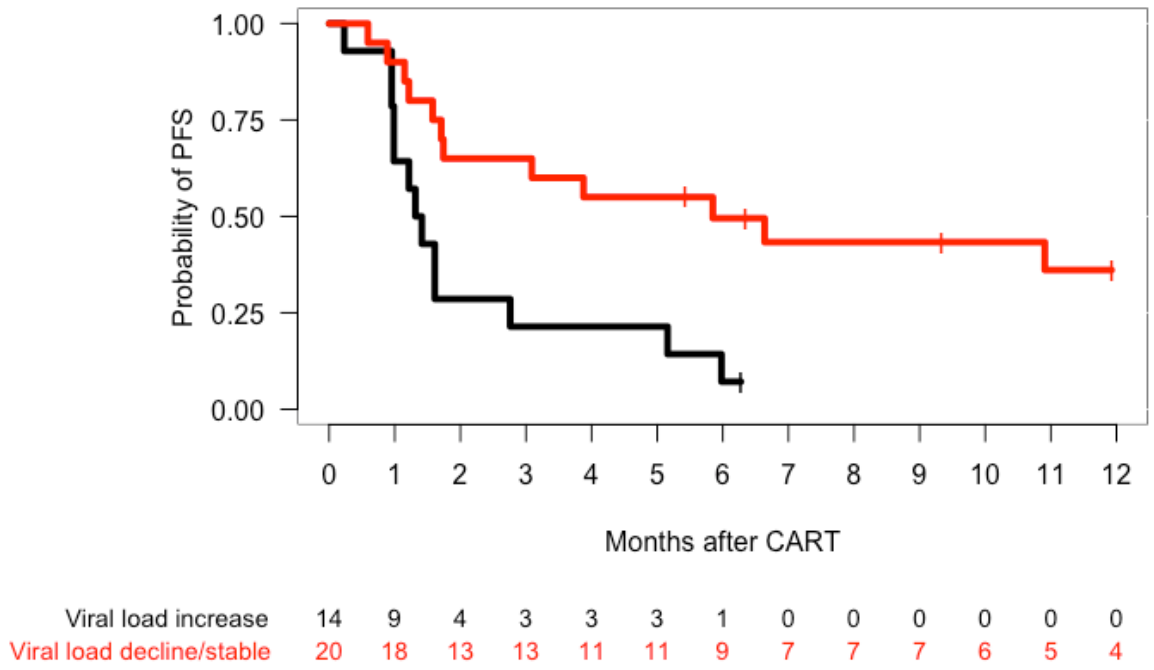

Figure 3

Progression-free survival according to dynamics of plasma TTV DNAemia between pre-lymphodepletion and CAR-T infusion.

Supplementary Files

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