


Assessment of the potential value of plasma Torque Teno virus DNA load monitoring to predict cytomegalovirus DNAemia in patients with hematological malignancies treated with small molecule inhibitors: A proof-of-concept study

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Abstract

It is unknown whether Torque Teno virus (TTV) DNA load monitoring could anticipate the development of infectious events in hematological patients undergoing treatment with small molecular targeting agents. We characterized the kinetics of plasma TTV DNA in patients treated with ibrutinib or ruxolitinib and assessed whether TTV DNA load monitoring could predict the occurrence of Cytomegalovirus (CMV) DNAemia or the magnitude of CMV-specific T-cell responses. Multicenter, retrospective, observational study, recruiting 20 patients treated with ibrutinib and 21 with ruxolitinib. Plasma TTV and CMV DNA loads were quantified by real-time PCR at baseline and days +15, +30, +45, +60, +75, +90, +120, +150, and +180 after treatment inception. Enumeration of CMV-specific interferon- γ (IFN- γ)-producing CD8⁺ and CD4⁺ T-cells in whole blood was performed by flow cytometry. Median TTV DNA load in ibrutinib-treated patients increased significantly ($p = 0.025$) from baseline (median: 5.76 log₁₀ copies/mL) to day +120 (median: 7.83 log₁₀ copies/mL). A moderate inverse correlation ($Rho = -0.46$; $p < 0.001$) was found between TTV DNA load and absolute lymphocyte count. In ruxolitinib-treated patients, TTV DNA load quantified at baseline was not significantly different from that measured after treatment inception ($p \geq 0.12$). TTV DNA load was not predictive of the subsequent occurrence of CMV DNAemia in either patient group. No correlation was observed between TTV DNA loads and CMV-specific IFN- γ -producing CD8⁺ and CD4⁺ T-cell counts in either patient group. The data did not support the hypothesis that TTV DNA load monitoring in hematological patients treated with ibrutinib or ruxolitinib could be useful to predict either the

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occurrence of CMV DNAemia or the level of CMV-specific T-cell reconstitution; nevertheless, due to the small sample size, further studies involving larger cohorts are warranted to elucidate this issue.

KEYWORDS

CMV, CMV DNAemia, CMV-specific T-cells, Ibrutinib, ruxolitinib, TTV DNA load

1 | INTRODUCTION

Torque Teno viruses (TTV), first discovered in 1997,¹ are small, nonenveloped, circular, single-stranded negative-sense DNA viruses that belong to the *Anelloviridae* family.² TTV are seemingly apathogenic and represent a major component of the human blood virome,²⁻⁵ which remains relatively stable over time in the immunocompetent host.⁶ The TTV DNA load in the systemic compartment behaved as a surrogate marker of the net state of immunosuppression in a pivotal study recruiting solid organ transplant recipients (SOT),⁴ which largely reflected the crucial role of adaptive T-cell immunity in the ultimate control of TTV replication.² A large body of experimental evidence gathered in the SOT setting supports the assumption that either high or increasing blood TTV DNA levels after transplantation associate with the occurrence of certain infectious events, whereas the opposite holds true for acute rejection.⁷⁻⁹ In the allogeneic hematopoietic stem cell transplantation setting (allo-HSCT), the plasma TTV DNA load appears to correlate with the degree of T-cell reconstitution early after transplantation (<day 100), whereas it correlates with the level of immunosuppression afterward.¹⁰⁻¹² The potential utility of TTV DNA monitoring in blood to predict the occurrence of a variety of clinical events, including infections, response to vaccination, or mortality, has been suggested in settings other than transplantation, such as COVID-19 or patients with chronic arthritis undergoing biologic therapies.¹³⁻¹⁵ Molecular targeting agents including Bruton Tyrosine Kinase inhibitors (i.e., ibrutinib) and intracellular Janus kinase (JAK) signal transducer and activator of transcription (STAT) pathway inhibitors (i.e., ruxolitinib) are current first-line drugs used to treat several hematological malignancies.¹⁶ Both drug types have a deleterious impact on immune system homeostasis, which appears to account for the increased incidence of opportunistic infections.¹⁷ To our knowledge, there is no published information regarding the potential clinical value of TTV DNA load monitoring to anticipate the development of infectious events in patients treated with molecular targeting agents. Here, we profiled plasma TTV DNA load kinetics in hematological patients treated with ibrutinib or ruxolitinib and assessed whether TTV DNA load monitoring in these patients could predict the occurrence of Cytomegalovirus (CMV) DNAemia, an event frequently developed in these patients,^{18,19} as may be the case in SOT and allo-HSCT recipients.^{12,20}

2 | PATIENTS AND METHODS

2.1 | Patients

In this multicenter, retrospective, observational study, we enrolled 41 adult (≥ 18 years old) patients between January 2019 and January 2022 at Hospital Clínico Universitario of Valencia ($n = 23$), Hospital Universitario y Politécnico La Fe, Valencia ($n = 9$), Hospital Ramón y Cajal, Madrid ($n = 5$), Hospital Vall d'Hebrón, Barcelona ($n = 3$), and Hospital Morales Meseguer, Murcia ($n = 1$), all in Spain. All patients were CMV-seropositive (at baseline) with hematologic malignancies that were treated with ibrutinib ($n = 20$, all with B cell chronic lymphocytic leukemia; median age: 70 years; range: 48–86) or ruxolitinib ($n = 21$; median age: 65 years; range: 22–93) as first-line therapy or after conventional chemotherapy. Relevant patient characteristics were previously reported^{18,19} and are summarized in Table 1. The study period comprised the first 180 days after treatment initiation. This study was approved by the respective Clinical Research Ethics Committees (CEIC) of the participating centers. Informed consent was obtained from all participants.

2.2 | TTV and CMV DNA load monitoring

The plasma TTV DNA load was quantified via an “in-house” TaqMan real-time PCR assay that amplifies a highly conserved segment of the untranslated region of the viral genome, as previously reported.^{11,12} Specimens with undetectable TTV DNA were assigned a value of 0 for analysis purposes. All samples from each patient were assayed simultaneously in singlets. Plasma CMV DNA load monitoring was conducted via RealTime CMV PCR (Abbott Molecular). Viral load monitoring was performed at baseline (before treatment inception) and at days +15, +30, +45, +60, +75, +90, +120, +150, and +180 after initiation of treatment. As per local guidelines, no patient with detectable CMV DNA in plasma was given preemptive antiviral therapy. Moreover, no patient received antiviral prophylaxis against CMV.

2.3 | Enumeration of CMV-specific T cells

Enumeration of CMV-specific interferon- γ (IFN- γ)-producing CD8⁺ and CD4⁺ T-cell responses in whole blood was performed by flow

TABLE 1 Demographic and clinical characteristics of the patients included in the study.

Parameter	No. of patients (%)
Treatment with Ibrutinib	
Gender, male/female (%)	13/7 (65/35)
Underlying disease	
Chronic lymphocytic Leukemia	20 (100)
First-line, <i>n</i> (%)	11 (55)
Prior treatment	9 (45)
Bendamustine ± Obinutuzumab, <i>n</i> (%)	2 (22)
Bendamustine + Rituximab, <i>n</i> (%)	2 (22)
Bendamustine + Rituximab + Chlorambucil <i>n</i> (%)	1 (11)
Cyclophosphamide + Rituximab + Chlorambucil + Prednisone <i>n</i> (%)	2 (22)
Fludarabine + Cyclophosphamide + Rituximab + (Bendamustine or Obinutuzumab), <i>n</i> (%)	2 (22)
Treatment with Ruxolitinib	
Gender, male/female (%)	11/10 (52/48)
Underlying disease	
Primary myelofibrosis, <i>n</i> (%)	16 (76)
Polycythemia vera, <i>n</i> (%)	4 (19)
Chronic myelocytic leukemia, <i>n</i> (%)	1 (5)
First-line, <i>n</i> (%)	15 (71.4)
Prior treatment, <i>n</i> (%)	6 (28.6)
Hydroxyurea, <i>n</i> (%)	4 (66.6)
Hydroxyurea + Danazol, <i>n</i> (%)	2 (33.3)

cytometry for intracellular cytokine staining (BD FastImmune, BD-Beckton Dickinson and Company-Biosciences), as previously reported.²¹ Briefly, two sets of 15-mer overlapping peptides encompassing the entire sequence of CMV pp65 and IE-1 proteins were used in combination for stimulation. A negative control (absence of peptide stimulation) was run in all experiments. The total number of CMV-specific CD8⁺ and CD4⁺ T-cells was calculated by multiplying the corresponding percentage of CMV-specific T-cells (after background subtraction) by the absolute number of CD8⁺ or CD4⁺ T-cells. Responses >0.1% for each population were considered specific (detectable). Immunological monitoring was conducted at baseline and days +30, +60, +90, +120, +150, and +180 after treatment initiation.

2.4 | Statistical analyses

Frequency comparisons for categorical variables were carried out using the chi-square test or Fisher's exact test, as appropriate. Differences between medians (for unpaired data) were compared using the Mann-Whitney *U*-test (two independent variables). Correlation between continuous variables was carried out via Spearman's Rank test. Two-sided exact *p* values are reported,

considering a *p* < 0.05 statistically significant. Analyses were performed using SPSS version 26.0 (SPSS).

3 | RESULTS

3.1 | Plasma TTV DNA load kinetics

A total of 135 plasma samples were available from patients treated with ibrutinib for TTV DNA quantitation, a median of eight plasma specimens (range: 3–10) per patient. TTV DNA was detectable in at least one sample from 17/20 patients (85%). The number of patients with detectable TTV DNA increased from baseline to day +120 (38.4%–100%), and slightly decreased afterwards (83.7% at days +150 and +180) (Figure 1A). The median TTV DNA load at baseline was 5.27 log₁₀ copies/mL (interquartile range [IQR]: 0–6.54); overall, the viral load increased over the follow-up, the TTV DNA peak being reached by day +120 (median, 7.83 log₁₀ copies/mL; IQR: 7.05–7.99; *p* = 0.025 compared with baseline) (Figure 1A).

In turn, 188 plasma specimens were available from ruxolitinib-treated patients, a median of 10 samples (range: 3–10) per patient. All patients had at least one plasma specimen with detectable TTV DNA

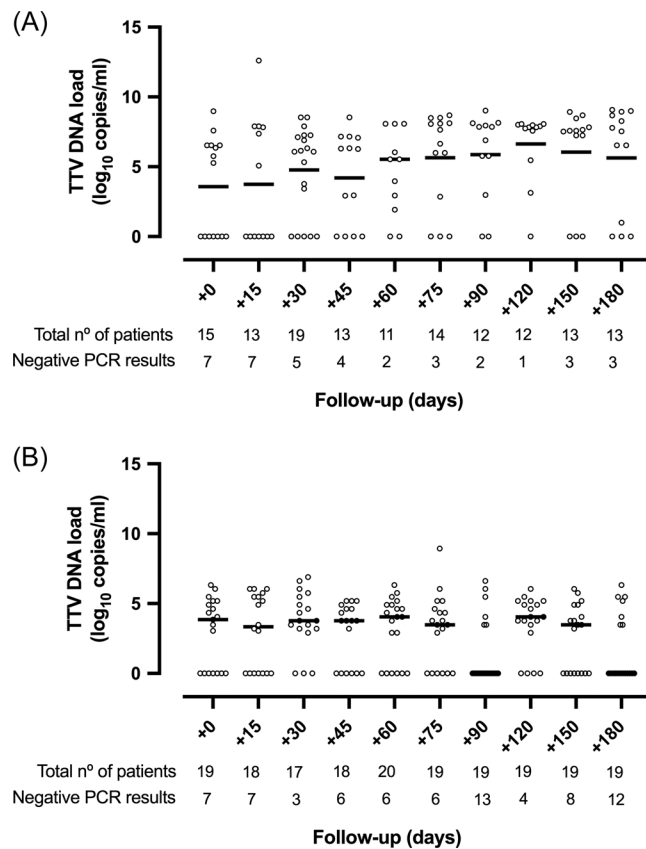


FIGURE 1 Kinetics of Torque Teno virus (TTV) DNA in plasma in patients treated with Ibrutinib ($n = 20$) or Ruxolitinib ($n = 21$), in panels (A) and (B), respectively. Only data from patients with at least one specimen testing positive for TTV DNA over the follow-up are displayed. TTV DNA loads were quantified by PCR at baseline and at different time points after drug administration. The number of samples available for analysis and the number of those yielding negative PCR results (undetectable) at each monitoring time are indicated. Bars represent medians.

over the study period. The number of plasma specimens testing positive by PCR at baseline (63.1%) and at different testing times (maximum at day +120, 78.9%) was not significantly different ($p \geq 0.2$) (Figure 1B). The median TTV DNA load at baseline was 3.86 \log_{10} copies/mL (IQR: 0–5.05) and fluctuated slightly over time (Figure 1B). Overall, the TTV DNA peak was reached by day +60 (median: 4.05 \log_{10} copies/mL; IQR: 0–4.91); nevertheless, the median TTV DNA load quantified at different time points after treatment inception was not significantly different from that measured at baseline ($p \geq 0.12$).

3.2 | Correlation between TTV DNA loads and absolute lymphocyte and total CD4⁺ and CD8⁺ T-cell counts

A total of 236 paired specimens from ibrutinib ($n = 106$) or ruxolitinib ($n = 130$)-treated patients was available for assessment of the overall correlation between TTV DNA loads and absolute lymphocyte (ALC) and total CD4⁺ and CD8⁺ T-cell counts. As shown in Figure 2, a

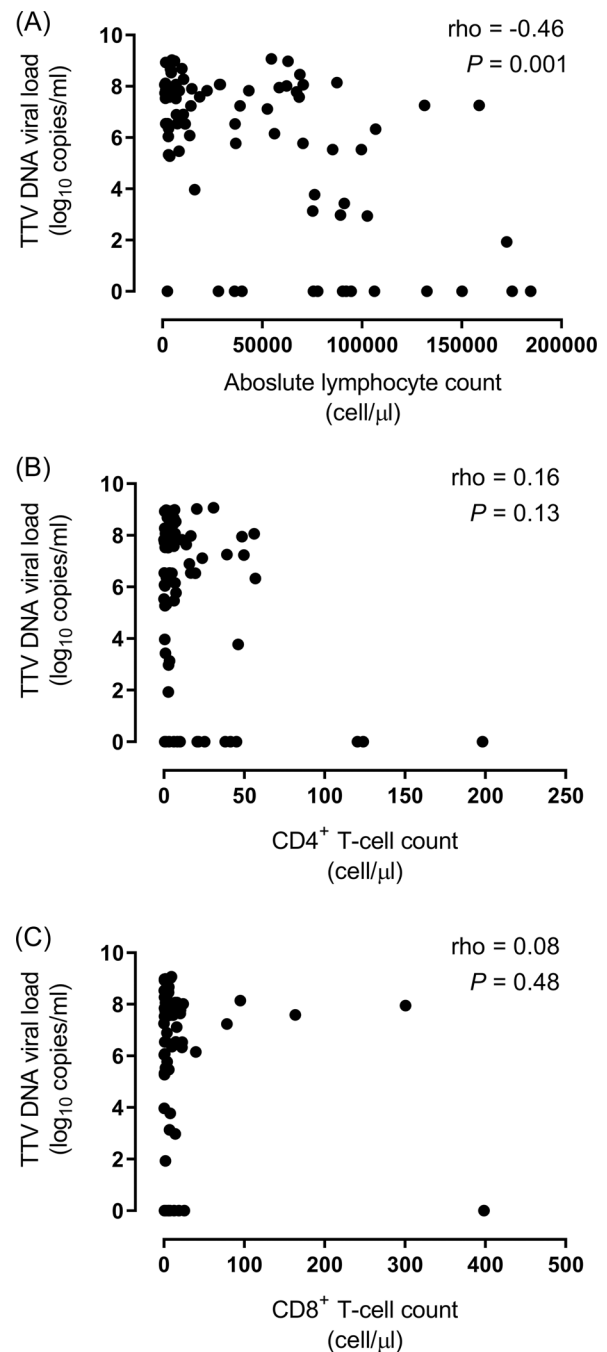


FIGURE 2 Correlation between Torque Teno virus (TTV) DNA in plasma and (A) absolute lymphocyte counts (ALC), (B) total CD4⁺ T-cell counts and (C) total CD8⁺ T-cell counts in patients treated with Ibrutinib ($n = 20$), as determined by Spearman's Rank test. Rho and p values are shown.

moderate inverse correlation ($\rho = -0.46$; $p < 0.001$) was found between TTV DNA load and ALC in patients treated with ibrutinib (Figure 2A). Nevertheless, a very weak or no correlation was found between TTV DNA levels and total CD4⁺ (Figure 2B) OR CD8⁺ (Figure 2C) T-cell counts. As shown in Figure 3, there was also no correlation between ALC (Figure 3A), CD4⁺ (Figure 3B) and CD8⁺ (Figure 3C) T-cell counts in ruxolitinib-treated patients.

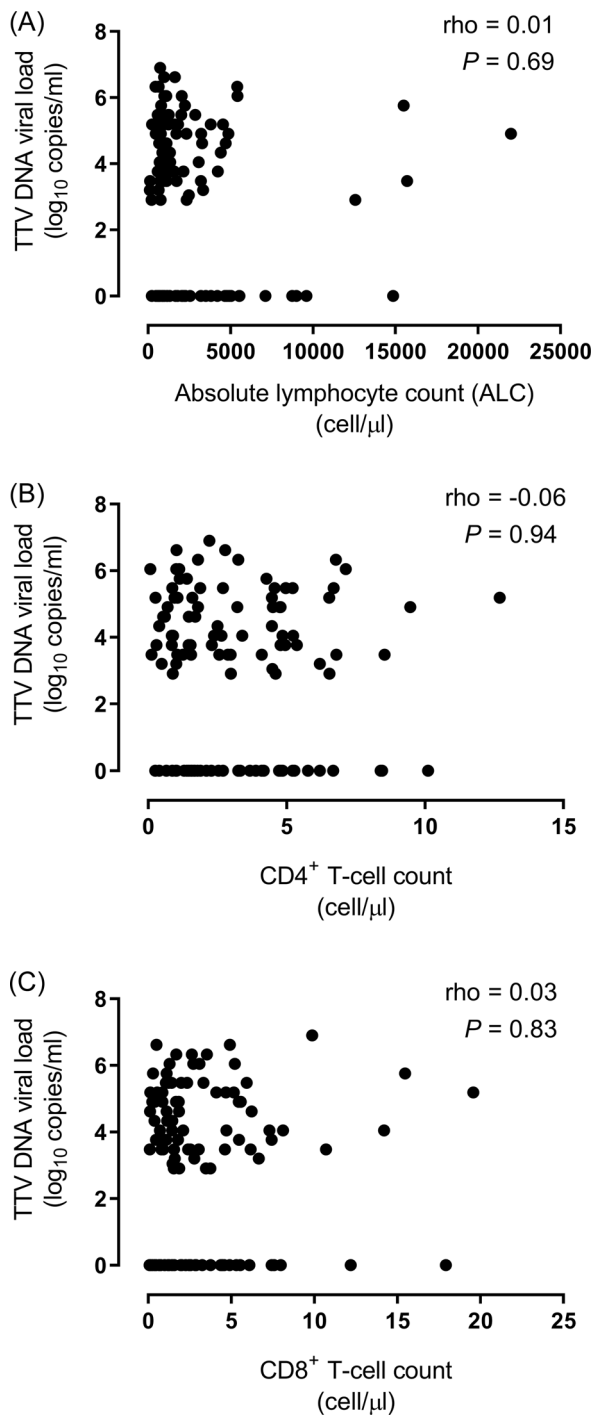


FIGURE 3 Correlation between Torque Teno virus (TTV) DNA in plasma and (A) absolute lymphocyte counts (ALC), (B) total CD4⁺ T-cell counts and (C) total CD8⁺ T-cell counts in patients treated with Ruxolitinib ($n = 21$), as determined by Spearman's Rank test. Rho and p values are shown.

3.3 | Plasma TTV DNA load monitoring for prediction of the occurrence of CMV DNAemia

Of the 20 ibrutinib-treated patients, seven developed low-grade CMV DNAemia (median CMV DNA peak level 106 IU/mL) within a

median of 45 days after treatment inception. We investigated whether TTV DNA load quantified at day +30 was significantly different across patients who did subsequently develop CMV DNAemia ($n = 4$) or not ($n = 12$). We found this not to be the case; in fact, TTV DNA load in patients who went on to develop CMV DNAemia (median: 6.12 log₁₀ copies/mL; IQR: 3.04–6.64) was comparable ($p = 0.77$) to that in patients who did not develop CMV DNAemia (median: 4.55 log₁₀ copies/mL; IQR: 0–6.79). Regarding ruxolitinib-treated patients, 16 out of 21 (76%) had CMV DNAemia, which occurred at a median of 30 days after treatment inception. CMV DNA peak load was 182 IU/mL (range: 15–182). The TTV DNA load at day +30 in patients who developed CMV DNAemia ($n = 7$; median, 3.48 log₁₀ copies/mL; IQR: 3.20–6.05) was comparable ($p = 0.30$) to that in patients who did not ($n = 4$; median: 2.91 log₁₀ copies/mL; IQR: 0–4.91).

3.4 | Correlation between TTV DNA loads and CMV-specific IFN- γ -producing CD8⁺ and CD4⁺ T-cells

A total of 236 paired specimens were available from ibrutinib ($n = 106$) or ruxolitinib ($n = 130$)-treated patients for evaluation of the overall correlation between TTV DNA loads and CMV-specific IFN- γ -producing CD4⁺ and CD8⁺ T-cells. Either a weak or no correlation was observed between these parameters, both in patients treated with ibrutinib (Figure 4A,B for CMV-specific CD4⁺ and CD8⁺ T-cells, respectively) and ruxolitinib (Figure 5A,B for CMV-specific CD4⁺ and CD8⁺ T-cells, respectively).

4 | DISCUSSION

An increasing amount of experimental evidence supports the assumption that the magnitude of the TTV DNA load in blood mirrors the net state of immunosuppression in a variety of clinical settings.^{7–9} In this context, TTV DNA load monitoring may predict the occurrence of virus infectious events in the SOT setting; specifically, it was shown that either high or increasing levels of plasma TTV DNA load early after kidney or liver transplantation is associated with the development of CMV or BK virus DNAemia.^{20,22,23} In the allo-HSCT setting, a subset of patients at high risk of developing high-level CMV DNAemia requiring the inception of pre-emptive antiviral therapy could be identified by analyzing plasma TTV DNA load kinetics early after engraftment.¹² It is currently unknown whether monitoring of the kinetics of TTV DNA load would be useful in predicting the occurrence of infectious events in hematological patients undergoing treatment with small molecule inhibitors. In the current study, we characterized the dynamics of TTV DNA load in patients treated with ibrutinib or ruxolitinib and assessed whether the TTV DNA load measured at different time points after treatment inception could anticipate the development of CMV DNAemia. Our main observations were as follows. Firstly, TTV DNA levels significantly increased over time in patients treated with

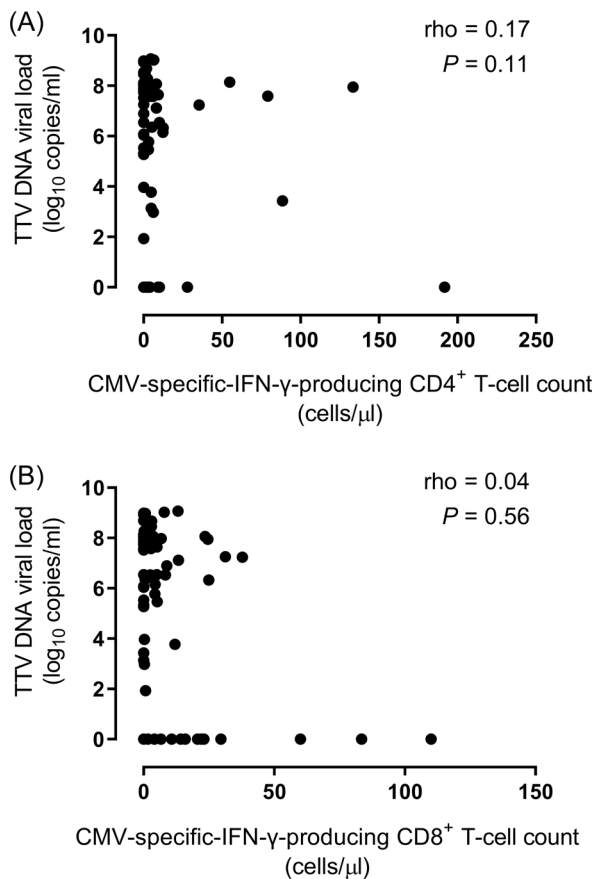


FIGURE 4 Correlation between Torque Teno virus (TTV) DNA in plasma and Cytomegalovirus pp65/IE-1-IFN-γ-producing CD4⁺ (A) and CD8⁺ (B) T-cell counts in whole blood from Ibrutinib-treated patients, as enumerated by flow cytometry for intracellular cytokine staining, and assessed by Spearman's Rank test. Rho and *p* values are shown. IFN, interferon.

ibrutinib, reaching the peak by day +120. This observation may point to a progressive impairment of T-cell mediated immunity, as it is mainly responsible for the control of TTV replication.² However, it may also relate to the increase in ALC that commonly occurs during treatment with B cell receptor pathway inhibitors, including ibrutinib,^{24,25} which would promote TTV replication as T-cells are the main TTV target cell type.²⁶ Supporting the latter hypothesis is the fact that a moderate inverse correlation was seen between TTV DNA load and ALC (although not with CD4⁺ or CD8⁺ T-cells analyzed separately). As for patients treated with ruxolitinib, a trend toward increasing TTV DNA load levels was observed, with the peak being reached by day +60 after treatment inception; yet, the difference between median TTV DNA levels measured at these time points was not statistically significant; this observation is consistent with the known impact of ruxolitinib on dendritic cell differentiation and function that impairs T-cell activation.²⁷ This, to some extent, would impair T-cell control of TTV replication. A non-mutually exclusive explanation for the lesser impact of ruxolitinib compared with ibrutinib on TTV DNA load kinetics might also be related to the relatively stable dynamics of ALC in the former group; in this sense,

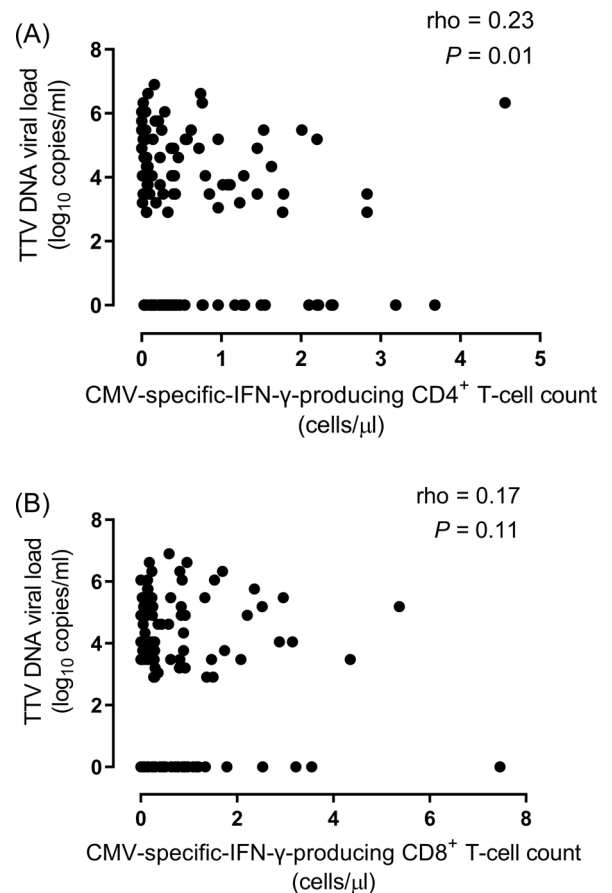


FIGURE 5 Correlation between Torque Teno virus (TTV) DNA in plasma and Cytomegalovirus pp65/IE-1-IFN-γ-producing CD4⁺ (A) and CD8⁺ (B) T-cell counts in whole blood from Ruxolitinib-treated patients, as enumerated by flow cytometry for intracellular cytokine staining, and assessed by Spearman's Rank test. Rho and *P* values are shown. IFN, interferon.

we failed to show a correlation between ALC, CD4⁺ and CD8⁺ T-cell counts and TTV DNA loads in ruxolitinib-treated patients. Moreover, the median TTV DNA load at baseline was substantially higher in patients that then underwent ibrutinib therapy compared with those who started with ruxolitinib (5.27 log₁₀ copies/mL vs. 3.86 log₁₀ copies/mL). Although speculative, the different nature of both the underlying hematological disease and prior chemotherapy drug regimens employed across both patient groups may account for the difference. Furthermore, as a proof-of-concept approach, we sought to determine whether the occurrence of CMV DNAemia could be anticipated via TTV DNA load measurements. In this context, we previously demonstrated that hematological patients undergoing treatment with molecular targeting agents are at increased risk of developing low-level CMV DNAemia.^{18,19} Since CMV DNAemia was detected at a median of +45 and +30 days after ibrutinib and ruxolitinib administration, respectively, we focused on TTV DNA loads quantified at day +30. TTV DNA levels at this time point in patients either treated with ibrutinib or ruxolitinib were not associated with the subsequent development of CMV DNAemia.

The apparent lack of association between the magnitude of TTV DNA loads and the subsequent occurrence of CMV DNAemia in both patient groups is consistent with our observation that no correlation existed between TTV DNA loads and CMV pp65/IE-1 IFN- γ -producing CD8⁺ and CD4⁺ T-cell counts. In this context, it should be noted that peripheral blood levels of both CMV-specific functional T-cell subsets were predictive of the risk of CMV DNAemia in SOT²⁸ and allo-HSCT settings,²¹ but not in ibrutinib or ruxolitinib-treated patients.^{18,19}

The main limitation of this study is the relatively small cohort of available patients. This precluded for example the assessment of whether TTV DNA loads measured earlier than day +30 could predict the occurrence of CMV DNAemia. In addition, the frequency of CMV DNAemia monitoring could have been insufficient, particularly beyond day +90, to capture all episodes of CMV DNAemia that may have occurred. Finally, only monofunctional CMV-specific IFN- γ -producing T-cells were enumerated.

In summary, TTV DNA load kinetics may differ across ibrutinib and ruxolitinib-treated patients. Moreover, our data suggest that neither the occurrence of CMV DNAemia nor the magnitude of CMV-specific T-cell responses might be anticipated or inferred, respectively, based on TTV DNA load measurements following treatment inception. Further studies involving larger cohorts are warranted to precisely gauge the potential utility of serial TTV DNA monitoring in anticipating the development not only of active CMV infection but also other opportunistic infections to which ibrutinib and ruxolitinib-treated patients are at increased risk.

AUTHOR CONTRIBUTIONS

Juan Carlos Hernández-Boluda, María José Terol, Carlos Solano, and David Navarro: conceptualization and funding acquisition. **Carlos Solano de la Asunción, Estela Giménez, Eliseo Albert, María José Remigia, and Paula Amat:** methodology and data curation. **Javier López, Valentín García-Gutiérrez, Rafael Andreu, Dolores García, and Laura Fox:** data curation and project administration. **David Navarro:** wrote the manuscript. All authors reviewed and approved the final version of the manuscript.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

DATA AVAILABILITY STATEMENT

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

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REFERENCES

- Nishizawa T, Okamoto H, Konishi K, Yoshizawa H, Miyakawa Y, Mayumi M. A novel DNA virus (TTV) associated with elevated transaminase levels in posttransfusion hepatitis of unknown etiology. *Biochem Biophys Res Commun.* 1997;241(1):92-97.
- Focosi D, Antonelli G, Pistello M, Maggi F. Torquetenovirus: the human virome from bench to bedside. *Clin Microbiol Infect.* 2016; 22(7):589-593.
- Webb B, Rakibuzzaman A, Ramamoorthy S. Torque teno viruses in health and disease. *Virus Res.* 2020;285:198013.
- De Vlaminck I, Khush KK, Strehl C, et al. Temporal response of the human virome to immunosuppression and antiviral therapy. *Cell.* 2013;155(5):1178-1187.
- Moustafa A, Xie C, Kirkness E, et al. The blood DNA virome in 8,000 humans. *PLoS Pathog.* 2017;13(3):e1006292.
- Focosi D, Spezia PG, Macera L, et al. Assessment of prevalence and load of torquetenovirus viraemia in a large cohort of healthy blood donors. *Clin Microbiol Infect.* 2020;26(10):1406-1410.
- Redondo N, Navarro D, Aguado JM, Fernández-Ruiz M. Viruses, friends, and foes: the case of Torque Teno Virus and the net state of immunosuppression. *Transpl Infect Dis.* 2022;24(2):e13778.
- van Rijn A, Roos R, Dekker F, Rotmans J, Feltkamp M. Torque teno virus load as marker of rejection and infection in solid organ transplantation - A systematic review and meta-analysis. *Rev Med Virol.* 2023;33(1):e2393.
- Jaksch P, Görzer I, Puchhammer-Stöckl E, Bond G. Integrated immunologic monitoring in solid organ transplantation: the road toward torque teno virus-guided immunosuppression. *Transplantation.* 2022; 106(10):1940-1951.
- Albert E, Solano C, Giménez E, et al. Kinetics of Alphatorquevirus plasma DNAemia at late times after allogeneic hematopoietic stem cell transplantation. *Med Microbiol Immunol.* 2019;208(2): 253-258.
- Albert E, Solano C, Pascual T, et al. Dynamics of Torque Teno virus plasma DNAemia in allogeneic stem cell transplant recipients. *J Clin Virol.* 2017;94:22-28.
- Albert E, Solano C, Giménez E, et al. The kinetics of torque teno virus plasma DNA load shortly after engraftment predicts the risk of high-level CMV DNAemia in allogeneic hematopoietic stem cell transplant recipients. *Bone Marrow Transplant.* 2018; 53(2):180-187.
- Roberto P, Cinti L, Napoli A, et al. Torque teno virus (TTV): a gentle spy virus of immune status, predictive marker of seroconversion to COVID-19 vaccine in kidney and lung transplant recipients. *J Med Virol.* 2023;95(2):e28512.
- Martín-López M, Albert E, Fernández-Ruiz M, et al. Torque teno virus viremia in patients with chronic arthritis: influence of biologic therapies. *Semin Arthritis Rheum.* 2020;50(1):166-171.
- Forqué L, Albert E, Giménez E, et al. Monitoring of Torque Teno virus DNAemia in critically ill COVID-19 patients: may it help to predict clinical outcomes? *J Clin Virol.* 2022;148:105082.
- Sochacka-Ćwikła A, Mączyński M, Regiec A. FDA-approved drugs for hematological malignancies—the last decade review. *Cancers.* 2021;14(1):87.
- Ruiz-Camps I, Aguilar-Company J. Risk of infection associated with targeted therapies for solid organ and hematological malignancies. *Ther Adv Infect Dis.* 2021;8:204993612198954.
- Solano de la Asunción C, Terol MJ, Saus A, et al. Cytomegalovirus-specific T-cell immunity and DNAemia in patients with chronic lymphocytic leukaemia undergoing treatment with ibrutinib. *Br J Haematol.* 2021;195(4):637-641.

19. Solano de la Asunción Carlos, Giménez E, Hernández-Boluda JC, et al. Immunobiology of cytomegalovirus infection in patients with haematological malignancies undergoing treatment with small molecule inhibitors. *Br J Haematol.* 2023;200(6):e58-e61.
20. Maggi F, Focosi D, Stazu M, et al. Early post-transplant Torquetenovirus Viremia predicts Cytomegalovirus reactivations in solid organ transplant recipients. *Sci Rep.* 2018;8(1):15490.
21. Solano C, Benet I, Clari MA, et al. Enumeration of cytomegalovirus-specific interferon CD8+ and CD4+ T cells early after allogeneic stem cell transplantation may identify patients at risk of active cytomegalovirus infection. *Haematologica.* 2008;93(9):1434-1436.
22. Fernández-Ruiz M, Albert E, Giménez E, et al. Monitoring of alphatorquevirus DNA levels for the prediction of immunosuppression-related complications after kidney transplantation. *Am J Transplant (AJT).* 2019;19(4):1139-1149.
23. Fernández-Ruiz M, Albert E, Giménez E, et al. Early kinetics of Torque Teno virus DNA load and BK polyomavirus viremia after kidney transplantation. *Transpl Infect Dis.* 2020;22(2):e13240.
24. Herman SEM, Niemann CU, Farooqui M, et al. Ibrutinib-induced lymphocytosis in patients with chronic lymphocytic leukemia: correlative analyses from a phase II study. *Leukemia.* 2014;28(11):2188-2196.
25. Barrientos JC, Burger JA, Byrd JC, et al. Characterizing the kinetics of lymphocytosis in patients with chronic lymphocytic leukemia treated with single-agent ibrutinib. *Leuk Lymphoma.* 2019;60(4):1000-1005.
26. Focosi D, Macera L, Boggi U, Nelli LC, Maggi F. Short-term kinetics of torque teno virus viraemia after induction immunosuppression confirm T lymphocytes as the main replication-competent cells. *J Gen Virol.* 2015;96(Pt 1):115-117.
27. Heine A, Held SAE, Daecke SN, et al. The JAK-inhibitor ruxolitinib impairs dendritic cell function in vitro and in vivo. *Blood.* 2013;122(7):1192-1202.
28. Fernández-Ruiz M, Giménez E, Vinuesa V, et al. Regular monitoring of cytomegalovirus-specific cell-mediated immunity in intermediate-risk kidney transplant recipients: predictive value of the immediate post-transplant assessment. *Clin Microbiol Infect.* 2019;25(3):381.e1-381.e10.

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