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

David Navarro¹, Carlos de la Asunci´on¹, Estela Giménez¹, Juan Hernández-Boluda¹, María José Terol¹, Eliseo Albert¹, Javier López², Valentín García-Gutiérrez², Rafael Andreu³, MaríA Dolores García Malo⁴, María Laura Fox⁵, María Remigia¹, Paula Amat¹, and Carlos Solano³

¹Hospital Clinico Universitario
²Hospital Universitario Ramon y Cajal
³Hospital Universitari i Politecnic La Fe
⁴Hospital General Universitario Jose M Morales Meseguer
⁵Hospital Universitari Vall d’Hebron

May 3, 2023

Abstract

Background: 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.

Methods: 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 IFN-γ-producing CD8⁺ and CD4⁺ T cells in whole blood was performed by flow cytometry.

Results: 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 (ALC). In ruxolitinib-treated patients, TTV DNA load quantified at baseline was not significantly different from that measured after treatment inception (P>0.12). TTV DNA loads were 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. Conclusion: 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 occurrence of CMV DNAemia or the level of CMV-specific reconstitution.

INTRODUCTION

Torque Teno viruses (TTV), first discovered in 1997¹, are small, non-enveloped, circular, single-stranded negative-sense DNA viruses that belong to the Anelloviridae family². TTV are seemingly apanthetic and represent a major component of the human blood virome²-⁵, which remains relatively stable over time in the immunocompetent host⁶. 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), which largely reflected the crucial role of adaptive T cell immunity in the ultimate control of TTV replication. 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. 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. 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. 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. Both drug types have a deleterious impact on immune system homeostasis, which appears to account for the increased incidence of opportunistic infections. 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, as may be the case in SOT and allo-HSCT recipients.

PATIENTS AND METHODS

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 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.

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. 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, Des Plaines, IL, USA). Viral load monitoring was performed at baseline (prior to 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.

Enumeration of CMV-specific T cells

Enumeration of CMV-specific IFN-γ-producing CD8+ and CD4+ T cells in whole blood was performed by flow cytometry for intracellular cytokine staining (BD FastImmune, BD-Beckton Dickinson and Company-Biosciences, San Jose, CA, USA), as previously reported. 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.

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 value <0.05 statistically significant. Analyses were performed using SPSS version 26.0 (SPSS, Chicago, IL, USA).

RESULTS

Plasma TTV DNA load kinetics

A median of eight plasma specimens (range, 3-10) was available from ibrutinib-treated patients. TTV DNA was detectable in at least one sample from 17/20 patients (85%). The number of patients with detectable TTV DNA increased over time (i.e., there were five undetectable PCR results at baseline and none by day +120). The median TTV DNA load at baseline was 5.76 log10 copies/ml (range, 0-8.98); overall, the viral load increased over the follow-up, the TTV DNA peak being reached by day +120 (median, 7.83 log10 copies/ml; range, 3.13-8.06; P = 0.025 compared with baseline) (Figure 1A).

In turn, a median of 10 samples (range, 3-10) was collected from ruxolitinib-treated patients. All patients had at least one plasma specimen with detectable TTV DNA over the study period. The number of plasma specimens testing positive by PCR at baseline and at different testing times was comparable (P >0.2). The median TTV DNA load at baseline was 3.48 log10 copies/ml (range, 0-6.33) and fluctuated slightly over time (Figure 1B). Overall, the TTV DNA peak was reached by day +75 (median, 6.33; range, 5.19-8.94); nevertheless, the median TTV DNA load quantified at different time points after treatment inception was not significantly different from that measured at baseline (P >0.12).

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

A total of 236 paired specimens from ibrutinib or ruxolitinib-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 moderate inverse correlation (Rho=-0.46; P <0.001) was found between TTV DNA load and ALC in patients treated with ibrutinib (panel A). Nevertheless, a very weak or no correlation was found between TTV DNA levels and total CD4+ (panel B) or CD8+ (panel C) T cell counts. As shown in Figure 3, there was also no correlation between ALC (panel A), CD4+ (panel B) and CD8+ (panel C) T cell counts in ruxolitinib-treated patients.

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. Neither the TTV DNA load quantified at baseline, nor that measured at days +15 or +30 was significantly different (P >0.57) in patients who subsequently developed CMV DNAemia versus those who did not (Table 2). Regarding ruxolitinib-treated patients, 16 out of 21 (76%) had CMV DNAemia, which occurred at a median of 30 days after treatment inception (range, 0-90). CMV DNA peak load was 182 IU/ml (range, 15-182). The TTV DNA load at day +15 but not at baseline or day +30 was associated with the occurrence of subsequent CMV DNAemia (P =0.049).

Ορελια εντεύχεται ο χρόνος της TTV DNA λάδας και της συγκεκριμένης PI 4-γ-προδυσινής Δ8+ του ουρών T γενσ

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

**DISCUSSION**

An increasing body 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. 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 associate with the development of CMV or BK virus DNAemia. 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. Neither TTV DNA load kinetics nor whether TTV DNA load monitoring would be useful to predict the occurrence of infectious events in hematological patients treated with small molecule inhibitors are known. 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. First, TTV DNA levels significantly increased over time in patients treated with ibrutinib, reaching the peak by day +120. This observation can be interpreted as implying a progressive impairment of T cell-mediated immunity, as it is mainly responsible for the control of TTV replication. However, it may also relate to the increase in ALC that commonly occurs during treatment with B cell receptor pathway inhibitors, including ibrutinib, which would promote TTV replication as T cells are the main TTV target cell type.

Supporting the latter hypothesis is the fact that the median duration of lymphocytosis in ibrutinib-treated patients is 12 and 14 weeks in first-line and relapsed/refractory settings, respectively, and that in the current study the TTV DNA peak was measured by day +120 after treatment inception. Moreover, a moderate inverse correlation was seen between TTV DNA load and ALC (but not with CD4+ or CD8+ T cells). As for patients treated with ruxolitinib, a trend toward increasing TTV DNA load levels was observed, with the peak being reached by day +75 after treatment inception, an observation consistent with the known impact of ruxolitinib on dendritic cell differentiation and function that impairs T cell activation. This, to some extent, would impair T cell control of TTV replication; however, overall, the median TTV DNA peak was not significantly different from that measured at baseline. 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, we failed to show a correlation between ALC, CD4+ and CD8+ T cell counts and TTV DNA loads in ruxolitinib-treated patients. Second, the median TTV DNA load at baseline was substantially higher in patients that then underwent ibrutinib therapy compared with those who started with ruxolitinib (5.76 log10 copies/ml vs. 3.48 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.

Third, as a proof-of-concept approach, we sought to determine whether the occurrence of CMV DNAemia could be anticipated via TTV DNA load measurements carried out at different time points after treatment inception. 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. 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 baseline, and days +15 and +30. TTV DNA levels at any of these time points in patients on ibrutinib were not associated with the subsequent development of CMV DNAemia. Regarding patients treated with ruxolitinib, only the TTV DNA load at day +15 was associated with CMV DNAemia (higher viral loads). Nevertheless, the low number of patients developing CMV DNAemia from whom TTV DNA load measurements at day +15 were available (n=3) limits the soundness of our observation. The apparent lack of association between the magnitude of TTV DNA loads and CMV DNAemia risk in both patient groups is consistent with our previously reported data on CMV-specific T cell immunity in these patients, showing no correlation between TTV DNA loads and CMV DNAemia.
pp65/IE-1 IFN-γ-producing CD8+ and CD4+ T cell counts. Of note, peripheral blood levels of both CMV-specific functional T cell subsets were predictive of the risk of CMV DNAemia in SOT29 and allo-HSCT settings22, but not in ibrutinib or ruxolitinib-treated patients19,20.

The main limitation of this study is its relatively small size. 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 substantially differ across ibrutinib and ruxolitinib-treated patients. Neither the occurrence of CMV DNAemia nor the magnitude of CMV-specific T cell responses could be anticipated or inferred, respectively, based on TTV DNA load measurements at different time points 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.

ACKNOWLEDGEMENTS

Estela Giménez (Juan Rodés Contract, JR18/00053) and Eliseo Albert (Juan Rodés Contract; JR20/00011) hold contracts funded by the Carlos III Health Institute (co-financed by the European Regional Development Fund, ERDF/FEDER). This research was supported by a grant from FIS P118/00127 (Fondo de Investigaciones Sanitarias, Ministerio de Sanidad y Consumo, Spain).

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.

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-Jiménez, 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.

REFERENCES


FIGURE LEGENDS

Figure 1. Kinetics of Torque Teno virus (TTV) DNA in plasma in patients treated with Ibrutinib (n=20) or Ruxolitinib (n=21), panels A and B, respectively. 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.

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.

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.

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.

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.
A

TTV DNA viral load (log_{10} copies/ml)

CMV-specific-IFN-γ-producing CD4^+ T-cell count (cells/μl)

\( \rho = 0.17 \)
\( P = 0.11 \)

B

TTV DNA viral load (log_{10} copies/ml)

CMV-specific-IFN-γ-producing CD8^+ T-cell count (cells/μl)

\( \rho = 0.04 \)
\( P = 0.56 \)
Hosted file