Title

MURANO: final analysis and retreatment/crossover substudy results of VenR in patients with relapsed/refractory CLL

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The MURANO study: final analysis and retreatment/crossover substudy results of VenR for patients with relapsed/refractory CLL

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Abstract:
Fixed-duration venetoclax-rituximab (VenR) in patients with relapsed/refractory chronic lymphocytic leukemia (CLL) in the phase 3 MURANO trial (NCT02005471) resulted in superior progression-free survival (PFS) and overall survival (OS) vs bendamustine-rituximab (BR). We report the final analyses of MURANO (median 7 years follow-up). Patients were randomized to VenR (venetoclax 400 mg daily for 2 years plus monthly rituximab for 6 months; n = 194) or BR (6 months; n = 195). In a substudy, patients with progressive disease (PD) received VenR as retreatment or crossover from BR. At the final data cut (3 August 2022), median PFS with VenR was 54.7 vs 17.0 months with BR. Seven-year PFS with VenR was 23.0%. Seven-year OS was 69.6% and 51.0%, respectively. Among VenR-treated patients with undetectable (u) minimal residual disease (MRD) and no PD at end of treatment (EOT) (n = 83), median PFS from EOT was 52.5 vs 18.0 months in patients with MRD at EOT (n = 35; P < 0.0001). Fourteen patients had enduring uMRD. Three distinct mutations in BCL2 in four patients were identified. In the substudy, 25 patients were retreated with VenR and nine patients crossed over to VenR; median PFS was 23 and 27 months, and best overall response rate was 72% and 89%, respectively. At the end of combination treatment, following retreatment or crossover, eight and six patients achieved uMRD, respectively. No new safety findings were observed. Overall, these final MURANO analyses support consideration of fixed-duration VenR therapy for patients with relapsed/refractory CLL.

Conflict of interest: COI declared - see note

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Title: MURANO: final analysis and retreatment/crossover substudy results of VenR in patients with relapsed/refractory CLL

Short title: MURANO 7-year follow up and substudy analysis

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Note: these data have been presented as an oral presentation at the EHA 2023 Annual Meeting and ICML 2023 Annual Meeting.

Key points

- In the VenR arm, the 7-year PFS rate was 23% and OS rate was 70%; median time from PD after MRD conversion to next therapy was 5 months.
- In a substudy, 25 patients were retreated with VenR; median PFS was 23 months, ORR was 72%, and 32% achieved uMRD at retreatment EOCT.

Explanation of Novelty

This final MURANO analysis (median follow-up: 7 years) shows clinically meaningful results for VenR in R/R CLL; 7-year PFS rate was 23% (VenR) and none were progression-free with BR; 7-year OS rates were 70% (VenR) and 51% (BR). In a substudy, patients retreated with or crossed over to VenR achieved high ORR; one-third had uMRD at EOCT; median PFS was 23 months in retreated patients. Our data continue to support fixed-duration VenR in R/R CLL and suggest that VenR retreatment is a viable option.

Abstract

Fixed-duration venetoclax–rituximab (VenR) in patients with relapsed/refractory chronic lymphocytic leukemia (CLL) in the phase 3 MURANO trial (NCT02005471) resulted in superior progression-free survival (PFS) and overall survival (OS) vs bendamustine–rituximab (BR). We report the final analyses of MURANO (median 7 years follow-up). Patients were randomized to VenR (venetoclax 400 mg daily for 2 years plus monthly rituximab for 6 months; n = 194) or BR (6 months; n = 195). In a substudy, patients with progressive disease (PD) received VenR as retreatment or crossover from BR. At the final data cut (3 August 2022), median PFS with VenR was 54.7 vs 17.0 months with BR. Seven-year PFS with VenR was 23.0%. Seven-year OS was 69.6% and 51.0%, respectively. Among VenR-treated patients with undetectable (u) minimal residual disease (MRD) and no PD at end of treatment (EOT) (n = 83), median PFS from EOT was 52.5 vs 18.0 months in patients with MRD at EOT (n = 35; $P < 0.0001$). Fourteen patients had enduring uMRD. Three distinct mutations in *BCL2* in four patients were identified. In the substudy, 25 patients were retreated with VenR and nine patients crossed over to VenR; median PFS was 23 and 27 months, and best overall response rate was 72% and 89%, respectively. At the end of combination treatment, following retreatment or crossover, eight and six patients achieved uMRD, respectively. No new safety findings were observed. Overall, these final MURANO analyses support consideration of fixed-duration VenR therapy for patients with relapsed/refractory CLL.

Introduction

Relapsed/refractory chronic lymphocytic leukemia (CLL) remains largely incurable despite developments in targeted therapy, including Bruton's tyrosine kinase inhibitors (BTKi)[1-6], PI3 kinase delta inhibitors (PI3Ki) inhibitors[7-9] and the B-cell lymphoma 2 inhibitor (BCL-2i) venetoclax.[10,11] Patients continue to experience disease progression (PD) and resistance becomes more prevalent over time. Venetoclax induces high response rates in CLL,[12,13] including patients with adverse biologic features, such as unmutated immunoglobulin heavy chain gene (IGHV),[10,14,15] chromosome 17p (del[17p]) deletion,[16,17] and genomic complexity (GC);[18-20] although *TP53* aberrations and high GC retain an adverse impact on progression-free survival (PFS) with venetoclax.[13,21] As combinations of targeted therapies are being used in earlier lines of treatment,[22,23] subsequent treatment options for patients with relapsed/refractory CLL should be considered.

Minimal residual disease (MRD) is utilized in clinical trials as an endpoint, with MRD status at end of treatment (EOT) often predicting long-term clinical outcomes;[24-28] patients with undetectable MRD (uMRD) generally have better PFS and overall survival (OS) than those with residual detectable disease.[25,29] Serial MRD assessment identifies patients with increasing subclinical disease burden months before recurrence,[30] and is increasingly integrated into trials, with the aim of establishing its role in future clinical practice.

The phase 3 MURANO trial (NCT02005471) investigated the efficacy and safety of fixed-duration venetoclax–rituximab (VenR) vs bendamustine–rituximab (BR) in patients with relapsed/refractory CLL.[26,31,32] The primary analysis reported significantly longer PFS with VenR vs BR, with benefits observed in all subgroups

analyzed, including unmutated IGHV or del(17p)/*TP53* mutation.[32] PFS and OS benefits of VenR were sustained at 3, 4, and 5 years.[26,31,33] At 5 years, median PFS was 53.6 vs 17.0 months ($P < 0.0001$) and 5-year OS rates were 82.1% vs 62.2% ($P < 0.0001$) with VenR vs BR.[33] More VenR-treated patients had uMRD in peripheral blood (PB) at end of combination treatment (EOCT) vs BR-treated patients.[31] The genetic risk factors *TP53* and GC (≥3 copy number alterations [CNA]) negatively affected MRD rates and PFS in both arms.[26]

We report the final analyses of MURANO, with 7 years median follow-up and 5 years post-completion of VenR, specifically, updated PFS and OS, MRD evaluation and next-line therapy outcomes. Further, we report outcomes from a substudy where patients were retreated with or crossed over to VenR.

Methods

Study design

Eligibility criteria for MURANO have been published.[32] Patients with relapsed/refractory CLL were randomized to receive VenR (venetoclax 400 mg daily for 2 years plus monthly rituximab for the first 6 months) or BR (for 6 months). Patients with PD were followed for disease response to any subsequent anti-CLL therapy and were assessed for time to second PFS event and OS. In the substudy (2018 onwards), patients initially randomized in the main study, with confirmed PD and in need of therapy (per International Workshop on CLL [iwCLL] 2008 criteria[34]), who had not received any new anti-CLL therapy, were eligible. Minimum time off-therapy was not required. Patients received VenR on the same schedule as the main study, either as retreatment or as crossover from BR (see Supplement). Assessments of next-line therapy, except those conducted in the substudy, were performed outside of the study. Patients who initiated new anti-CLL therapy without an investigator-assessed response were unevaluable.

The trial was conducted in accordance with the Declaration of Helsinki and the International Conference on Harmonisation of Good Clinical Practice guidelines. The protocol was approved by ethics committees at each participating institution; all patients provided written informed consent. Clinical cutoff date for this analysis was 3 August 2022. Eligibility criteria, dosing, prophylactic measures, and monitoring were as previously published.[32]

Endpoints and clinical assessments

The primary efficacy endpoint of the main study was investigator-assessed PFS, defined as time from randomization to PD, relapse, or death. MRD status in PB was a secondary endpoint of the main study, assessed at cycle 4, 2-3 months after EOCT, and every 3-6 months thereafter. MRD was centrally measured in PB using allele-specific oligonucleotide polymerase chain reaction (PCR) and/or flow cytometry.[21,22] Patients were categorized by MRD status: uMRD (<1 CLL cell/10,000 leukocytes [MRD value <0.0001, 10^{-4}]); MRD+ (≥10^{-4}); low-MRD+ (10^{-4} to <10^{-2}); and high-MRD+ (≥10^{-2}).[25] MRD conversion (considered to have occurred at first MRD+ assessment) was defined as two consecutive assays detecting MRD+ or PD by iwCLL criteria[34] in patients previously achieving uMRD.

Other endpoints in the main study (defined in Supplement) included OS, event-free survival (EFS), complete response (CR) and partial response (PR) rates (by iwCLL 2008 criteria[34]), duration of response (DOR), time to next treatment (TTNT), and safety. Safety data collected in the post-treatment period only (not including adverse events [AEs] occurring during treatment) included: pre-specified AEs of concern, serious AEs related to study treatment, and development of a second primary malignancy (SPM). Further details of safety monitoring have been described previously.[31,32]

The main substudy objective was to report outcomes among patients retreated with VenR or crossed over from BR to VenR, and determine if VenR retreatment or crossover is a viable option in pretreated patients. Substudy outcomes were also

compared among patients categorized by genetic profile (e.g., del(17p) and/or *TP53* mutations, unmutated IGHV, or GC).

Molecular assessments

Molecular assessments for analysis of GC were conducted using high-density array comparative genomic hybridization (aCGH), with data processing performed as previously published.[26] Low/intermediate GC was defined as the presence of 3-4 CNAs and high GC as ≥5 CNA.[19,20] High-density aCGH assessed the biomarker-evaluable subset of patients. IGHV mutational status was assessed by PCR and *TP53* status by next-generation sequencing (NGS; whole exome sequencing with a variant allele frequency [VAF] cutoff of 5%). Assessment of *NOTCH1* mutations was performed as previously published.[26]

Deep NGS was performed on MRD samples (without prior cell selection) from a subset of VenR-treated patients who received up to 2 years of VenR and had, at any time, high MRD+ in PB. Further details on the NGS panel and the pre-treatment sample used for comparison have been described previously.[35] Digital droplet PCR (ddPCR) was performed as previously published.[36]

Statistical analysis

There was no alpha spending allocated to the current analysis; all *P* values are therefore descriptive. Kaplan–Meier estimates were used to analyze time-to-event data, including landmark analyses from EOCT and EOT according to MRD status. Survival outcomes are presented as median values with 95% confidence intervals (CIs). Log-rank test and Cox proportional hazards regression model were used to

compare overall PFS and OS across treatment arms. Fisher's exact test was performed to compare MRD status at EOCT and EOT, and clinical and cytogenetic risk factors in VenR-treated patients with and without PD after EOT. Statistical analysis was performed using SAS version 9.04.

Results

Patient characteristics and follow-up

Overall, 389 patients enrolled into the main study; 194 received VenR and 195 received BR. Baseline demographics and disease characteristics are reported in Table 1. Overall, 130 patients completed 2 years of venetoclax without PD; reasons for stopping earlier are detailed in the Supplement.[31] Median (range) duration of follow-up from enrollment for the current analysis was 85.7 months (0.0-99.2); median (range) duration with VenR was 86.8 (0.3-99.2) vs 84.4 months (0.0-95.0) with BR.

Clinical outcomes

Median (95% CI) PFS with VenR was 54.7 (52.3-59.9) vs 17.0 months (15.5-21.7) with BR (hazard ratio [HR]: 0.23; 95% CI: 0.18-0.29; $P < 0.0001$; Figure 1A). Seven-year PFS rate (95% CI) with VenR was 23.0% (16.1-29.9); no BR-treated patients remained progression-free at this timepoint. In VenR-treated patients with high-risk features (del(17p) and/or *TP53* mutation, unmutated IGHV, high GC, or mutated *NOTCH1*), patients with mutated *TP53* and/or del(17p) had the poorest 7-year PFS rate at 5.0% (95% CI: 0.0-13.2; n = 53), followed by mutated *NOTCH1* without mutated *TP53* and/or del(17p) at 11.1% (0.0-25.4; n = 21). The 7-year PFS rate in those with unmutated IGHV was 16.3% (8.8-23.8; n = 123). No patients with high GC were progression-free at 7 years.

Median OS with VenR was not reached (NR) vs 87.8 months (95% CI: 70.1-NR) with BR (HR: 0.53; 95% CI: 0.37-0.74; $P = 0.0002$; Figure 1B). Seven-year OS rates (95% CI) with VenR were 69.6% (62.8-76.5) vs 51.0% (43.3-58.7) with BR. Among

VenR- vs BR-treated patients with high-risk features, 7-year OS rates were 50.6% (95% CI: 35.9-65.4; n = 53) vs 47.0% (95% CI: 31.4-62.6; n = 55) with mutated *TP53* and/or del(17p) (by aCGH); 67.7% (58.9-76.6; n = 123) vs 50.1% (40.1-60.0; n = 123) with unmutated IGHV; 63.5% (95% CI: 37.9-89.1; n = 14) vs 33.3% (95% CI: 9.5-57.2; n = 17) with high GC; and 64.4% (95% CI: 42.9-85.8; n = 21) vs 69.2% (95% CI: 51.2-87.1; n = 32) with mutated *NOTCH1*. Seven-year PFS and OS rates for patients with low-risk genetic features are described in the Supplement. Multivariate Cox analyses in VenR-treated patients showed that independent factors associated with PFS were IGVH mutation and *TP53* mutation or del(17p) (supplemental Table 1). For OS, independent prognostic factors were number of prior therapies (1 vs >1) and *TP53* mutation or del(17p) (supplemental Table 2).

Median EFS with VenR was 53.7 (95% CI: 48.5-59.3) vs 16.4 months (95% CI: 14.2-21.0) with BR (HR: 0.22; 95% CI: 0.17-0.29; $P < 0.0001$; Figure 1C). Median DOR was 53.6 months (95% CI: 49.1-57.0) for the 181/194 (93.3%) responders to VenR and 19.1 months (95% CI: 16.1-23.6) for the 132/195 (67.7%) responders to BR (HR: 0.23; Figure 1D).

MRD status and MRD conversion among VenR-treated patients

As previously reported, 83 (70.3%) VenR-treated patients had uMRD at EOT without PD and 35 (29.7%) were MRD+.[32] Median PFS from EOT for patients with uMRD at EOT was 52.5 (95% CI: 44.5-61.5) vs 18.0 months (95% CI: 8.5-29.3) in those who were MRD+ at EOT ($P < 0.0001$; Figure 2A); 5-year from EOT PFS rates were 40.7% (28.5-52.9) vs 12.5% (0.2-24.9) in patients who were uMRD at EOT vs MRD+ at EOT. Median OS from EOT was NR for patients who were uMRD at EOT and

MRD+ at EOT (Figure 2B); 5-year from EOT OS rates were 85.7% (77.8-93.6) vs 73.6% (58.7-88.5) in patients who were uMRD at EOT vs MRD+ at EOT.

At this 7-year update, 63 (75.9%) had MRD conversion, 14 (16.9%) VenR-treated patients had no PD or confirmed MRD conversion, and 6 (7.2%) had PD or died. Favorable baseline characteristics were over-represented among 14 patients with enduring uMRD: 13/144 (9.0%) with wild-type *TP53* had sustained uMRD vs 1/48 (2.1%) with *TP53* mutation; 7/53 (13.5%) with mutated IGHV had sustained uMRD vs 6/123 (4.9%) with unmutated IGHV. Among 63 patients with MRD conversion, median time from EOT to conversion was 19.4 months (95% CI: 8.7-28.0; Figure 3A); 39 subsequently had PD or died and median time from conversion to PD was 28.3 months (95% CI: 23.2-35.0; Figure 3B). MRD conversion with subsequent PD occurred ~4 years post-EOT. For the 36 patients with PD following MRD conversion, median time from PD to next treatment was 4.5 months (95% CI: 3.3-6.4; Figure 3C).

At 21–42 months post-treatment initiation (prior to progression), 107 PB MRD+ samples from 42 VenR-treated patients were collected to assess the nature/frequency of acquired mutations in *BCL2* family genes and *TP53*. The impact of mutations on TTNT and subsequent response were also analyzed. Responses to next treatment per acquired mutation and impact of *TP53* mutations on TTNT are summarized in the Supplement.

Results previously published using a targeted sequencing panel (limit of detection [LOD] – VAF 1%) showed acquisition of *BAX* and *PMAIP1* mutations (encoding the *BCL2* homology 3-only proteins, BAX and Nova, respectively) in 4/28 and 2/28 patients at the 5-year datacut, respectively, but not *BCL2*.[35] Follow-up analyses using ddPCR (LOD of 0.1%) identified three distinct point mutations in *BCL2* in four

patients (G101V, D103Y, A113G, and G101V and A113G [n = 1 each]). These were observed across two timepoints for two patients; for the remaining two patients, mutations were observed at a single timepoint only (supplemental Table 3). Patient characteristics and responses to treatment are outlined in supplemental Table 4 and supplemental results, respectively.

TTNT, time to second PFS event, and response to next-line therapy

Following cessation of the main study treatment, 95/194 (49.0%) VenR- and 131/195 (67.2%) BR-treated patients received subsequent treatment after PD. In total, 73 (37.6%) in the VenR arm had not received next-line therapy at the final cutoff, and 26 (13.4%) died without subsequent therapy. Median TTNT with VenR was 63.0 (95% CI: 56.1-73.6) vs 24.0 months (95% CI: 20.7-29.5) with BR (HR: 0.30; 95% CI: 0.23-0.39; $P < 0.0001$); median time off-therapy was 28.3 (range: -0.1-68.6) vs 17.9 months (range: 0.7-82.4), respectively.

Two VenR-treated patients received non-CLL therapy for another malignancy, so were excluded from subsequent analysis. Of the remaining 93 VenR-treated patients receiving next-line therapy, 30 (32.3%) received a BTKi (ibrutinib [n = 25], acalabrutinib [n = 4], and zanubrutinib [n = 1]), with 18 ongoing at cutoff; 47 (50.5%) received venetoclax-based therapy (see Supplement), with 12 ongoing at cutoff; 14 (15.1%) received chemoimmunotherapy (supplemental Table 5); and 2 (2.6%) received other novel agents. Of the 131 BR-treated patients receiving next-line therapy, 79 (60.3%) received a BTKi (ibrutinib [n = 69], acalabrutinib [n = 6], and zanubrutinib [n = 4]), with 20 ongoing at cutoff; 17 (13.0%) received venetoclax-based therapy, with 3 ongoing at final cutoff; 24 (18.3%) received

chemoimmunotherapy (supplemental Table 5); and 11 (8.4%) received other novel agents. Median landmark PFS and OS was numerically longer in patients previously randomized to VenR vs BR after initiation of subsequent therapies (supplemental Figure 1). After a median follow-up of 84 months, median time from randomization to second PFS event was NR (95% CI: 87.1 months-NR) in initially VenR-treated patients and 77.8 months (95% CI: 61.1-NR) in initially BR-treated patients ($P < 0.0001$; Figure 4A).

Median time to second PFS event from subsequent therapy in the VenR arm was 42.9 months (95% CI: 27.2-NR) for patients receiving a BTKi, 59.9 months (95% CI: 34.0-NR) for patients receiving venetoclax-based therapy and 12.1 months (95% CI: 7.4-46.7) for patients receiving chemoimmunotherapy (Figure 4B). Median time to second PFS event from subsequent therapy in the BR arm was 44.6 months (95% CI: 32.8-50.3) for patients receiving a BTKi, NR (95% CI: 41.1-NR) for patients receiving venetoclax-based therapy and 14.6 months (95% CI: 9.0-26.9) for patients receiving chemoimmunotherapy (Figure 4B).

Among evaluable patients previously treated with VenR and BR, best overall response rate (ORR) of subsequent venetoclax-based regimens was 76.2% (32/42) and 88.2% (15/17), respectively, while best ORR of subsequent BTKi therapy was 82.6% (19/23) and 78.5% (51/65), respectively (supplemental Figure 2).

Substudy

Overall, 34 patients were enrolled in the substudy; 25 were retreated with VenR and 9 crossed over to VenR from BR (supplemental Figure 3). Baseline demographics

were similar to the main study (Table 1). Of the 25 VenR-retreated patients, 92.0% had ≥1 of the following high-risk features: del(17p) and/or *TP53* mutation, IGHV unmutated disease, or GC (Table 1). CNA and prevalence of high GC increased from the main study baseline to the retreatment baseline (supplemental Figure 4). No crossover patients acquired *TP53* mutations, but a meaningful increase in del(17p) clone size was observed in two patients. Increase in GC was less prevalent than those observed in the retreatment arm (supplemental Table 6).

Median time between last venetoclax dose in the main study and first venetoclax dose in the substudy was 2.3 years (range: 1.2-3.1). Prior to VenR retreatment, patients with ≥2 years off-treatment from the main study had longer PFS vs those with <2 years off-treatment from the main study (supplemental Figure 5). Overall, median follow-up was 33.4 months (range: 2.7-44.0); median follow-up in the retreatment arm was 32.3 (range: 2.7-44.0) vs 36.1 months (range: 24.0-39.8) in the crossover arm.

Median PFS in VenR-retreated patients was 23.3 (95% CI: 15.6-24.3; Figure 5) vs 26.7 months (95% CI: 21.3-NR) in patients who crossed over to VenR (supplemental Figure 6). Median OS was NR for both arms. Estimated OS rates at years 1, 2, and 3 were 96.0% (95% CI: 88.3-100.0), 79.6% (95% CI: 63.6-95.6), and 53.1% (95% CI: 25.1-81.0), respectively, in the retreatment arm, and 100.0% (95% CI: 100.0-100.0) for each year in the crossover arm. In the retreatment arm, six patients died due to

PD and two died due to AEs (COVID-19 and myelodysplastic syndrome [MDS] progression, and sepsis). In the crossover arm, one patient died due to congestive heart failure.

Best ORR to VenR retreatment was 72.0%; six achieved CR and 12 achieved PR. Median DOR was 15.5 months (95% CI: 11.5-NR). Best ORR to crossover VenR was 88.9%; two patients with CR, one with CR with incomplete marrow recovery, two with nodular PR, and three with PR. Median DOR was 22.5 months (95% CI: 12.7-NE).

Among VenR-retreated patients, 13 (52.0%) completed the full treatment course. Eight (32.0%) achieved uMRD at retreatment EOCT (four had CR, three had PR, and one had SD); no patients retained uMRD at retreatment EOT. All eight retreated patients who achieved uMRD at EOCT had unmutated IGHV and six had normal *TP53*. Among crossover patients, six (66.7%) completed the full treatment course. Five (55.6%) had uMRD at crossover EOCT (three had CR, two had PR), and two (22.2%) retained uMRD at crossover EOT.

Safety

An overview of safety in the main study and substudy is provided in Table 2; common AEs (≥2%) in the substudy are shown in supplemental Table 7. With longer follow-up, the safety profiles for VenR and BR remained consistent with the primary manuscript, and no new safety findings were observed beyond the 5-year data cut.

At 7 years, a numerically higher rate of patients with ≥1 SPM was observed with VenR (18.0%) vs BR (13.8%), with a total of 56 events in each arm (supplemental Table 8). The rate of SPMs per 100 patient-years was higher with BR (6.3%) vs VenR (4.9%). Four patients developed MDS (three with VenR, one with BR). One patient in each arm developed acute myeloid leukemia (AML). Since the 5-year data cut, one additional VenR-treated patient developed Richter's transformation, resulting in a total of eight patients (4.1%) in the VenR arm and six patients (3.2%) in the BR arm. The safety profile of VenR in the substudy remained consistent with that in the main study, with no new safety signals observed. Overall, the safety profile of VenR was acceptable and generally consistent with the known safety profiles of venetoclax and rituximab as single agents.

Discussion

This final long-term analysis of the MURANO trial, with a median follow-up of 85.7 months, continues to demonstrate clinically meaningful benefits for fixed-duration VenR over BR in relapsed/refractory CLL. VenR was associated with a clinically meaningful prolongation of PFS, an improvement in OS and a longer TTNT vs BR. Most patients who completed 2 years of VenR had uMRD at EOT and achievement of uMRD was associated with prolonged PFS; generally, MRD conversion with subsequent PD did not occur until ~4 years post-EOT.

While the structural/functional consequences of acquired *BCL2* mutations reported in the literature vary, they are typically associated with reduced binding affinity and/or diminished susceptibility to venetoclax inhibition *in vitro*.[37] However, in some cases these variants remain sensitive to clinically relevant concentrations of venetoclax, especially in combination with CD20 antibodies.[36] In this cohort we showed that the acquisition of *BCL2* mutations was rare and did not preclude attainment of disease response to retreatment. More studies, especially exploring serial changes in disease subpopulations during therapy, are required to evaluate their impact on the durability of these responses.

In the substudy, with a median follow-up of 33.4 months, both retreated patients and those who crossed over to VenR had high ORR, and over one-third achieved uMRD at EOCT. PFS was almost 2 years in retreated patients; as early progressors in the BR arm were not captured, PFS in patients who crossed over to VenR was slightly

higher at just over 2 years. Overall, VenR retreatment is a viable option following initial VenR. Larger cohorts will be required to identify the influence of duration of time off-venetoclax and biologic features that may predict favorable responses to VenR retreatment, but it is biologically plausible that retreatment may be optimal in patients with longer (such as ≥2 years) time off-venetoclax, or in patients achieving uMRD at EOT with initial VenR.

Safety data were consistent with those reported previously, with no new safety signals observed. The safety profile of VenR continues to be manageable, predictable, and consistent with the known safety profile of both agents. There were no clinically meaningful differences in rates of SPM, including myeloid malignancies or Richter's transformation, between treatment arms.

Although patients in our study had relapsed/refractory CLL, they had not previously received novel agents; only five (VenR) and two (BR) patients received B-cell receptor inhibitors (BCRi) prior to enrollment,[32] so outcomes in this study may not be applicable to the current relapsed/refractory CLL population. Of note, the VENICE-1 study demonstrated deep and durable responses with venetoclax monotherapy for both BCRi-naïve and BCRi-pretreated patients.[11] Due to the frequent use of fixed-duration regimens and the combination of targeted therapies, more relapsed patients will have been exposed to venetoclax. It was hypothesized that among previously exposed patients, BTKis at the time of relapse would result in longer PFS vs retreatment with venetoclax-based therapies. However, this was not observed in

MURANO; median time to second PFS event was 42.9 and 59.9 months, respectively. Of note, only a few BR-treated patients received venetoclax-based next therapy, because these were not approved/available when patients needed next therapy (the Food and Drug Administration granted approval for use in relapsed/refractory CLL in June 2018). Another feature of the study population that reflects the era of conduct was that some patients received further chemoimmunotherapy at PD (17.2% post-VenR and 18.3% post-BR), which is no longer recommended[38] and may have compromised OS outcomes; however, these rates were similar between treatment arms.

Current literature on venetoclax retreatment is limited. Among nine patients retreated with VenR in a phase 1b study, all achieved PR or better.[39] Similarly, a retrospective study of venetoclax retreatment in heavily pretreated patients (n = 46) demonstrated high ORR (79.5%) and a median PFS of 25 months.[40] Our substudy results are consistent with these: retreated patients had high ORR and a median PFS of almost 2 years. Although PFS was longer in crossover vs retreatment patients, the increased toxicity from numerous chemoimmunotherapies may increase the risk of secondary MDS/AML and drive the development of unfavorable prognostic clones compared with targeted combination therapy.

Despite limited data on BTKis following venetoclax-based therapy in first-line or relapsed/refractory CLL, some studies suggest that BTKis could be a good option for salvage therapy.[41-43] A large retrospective study demonstrated higher ORR (84%) in BTKi-naïve patients receiving BTKis following venetoclax vs those who had previously received BTKis (54%).[41] BTKis provided durable disease control after PD on venetoclax in a retrospective evaluation of 23 patients with relapsed/refractory

CLL.[42] In the CAPTIVATE study, 21/22 patients retreated with ibrutinib after PD following ibrutinib-venetoclax were evaluable: all responded except for one SD and one PD.[44] Further, pirtobrutinib demonstrated high ORR (73.3%) in patients with heavily pretreated CLL or small lymphocytic lymphoma (247 had previously received a BTKi and 100 had also received a BCL-2i such as venetoclax).[6] Sustained benefits after venetoclax cessation may provide an opportunity for patients to experience a treatment-free interval prior to BTKi initiation.

In a retrospective analysis of heavily pre-treated patients with CLL, ORR was 79% in patients who received venetoclax after ibrutinib discontinuation vs those who received idelalisib (46%).[45] Similarly, in a study of 144 ibrutinib-treated CLL patients, salvage therapy with venetoclax-based treatments resulted in longer OS and treatment-free survival vs PI3Ki-based treatment, chemoimmunotherapy and anti-CD20 treatment.[46] In a real-world study, switching from a BTKi to venetoclax-based therapy resulted in higher ORR (84%) vs a different BTKi (63%).[6] Altogether, these indicate the positive potential for venetoclax regimens following progression on BTKis.

Conclusions

This final long-term analysis of the MURANO trial continues to demonstrate clinically meaningful results for fixed-duration VenR in patients with relapsed/refractory CLL. Based on the substudy, retreatment with VenR is a viable option following initial VenR treatment; its implementation in routine clinical practice deserves further investigation. Overall, these data continue to support the use of fixed-duration VenR in patients with relapsed/refractory CLL.

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Conflict-of-interest disclosure

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Tables

Table 1. Baseline demographics and disease characteristics of patients in the main study and the substudy

	Main study (N = 389)		Substudy (n = 34)	
	VenR (n = 194)	**BR (n = 195)**	**Retreatment with VenR (n = 25)**	**Crossed over to VenR (n = 9)**
Mean age (SD), years	63.9 (10.5)	64.4 (9.6)	65.8 (8.3)	62.3 (16.8)
Sex, n (%)				
Male	136 (70.1)	151 (77.4)	19 (76.0)	9 (100.0)
Female	58 (29.9)	44 (22.6)	6 (24.0)	0
Number of prior cancer therapies, n (%)				
1	111 (57.2)	117 (60.0)	20 (80.0)	7 (77.8)
2	58 (29.9)	43 (22.1)	4 (16.0)	0
≥3	25 (12.9)	35 (17.9)	1 (4.0)	2 (22.2)
del(17p) and/or *TP53* mutation, n (%)				
Yes	72 (37.1)	75 (38.5)	9 (36.0)	0
No	106 (54.6)	95 (48.7)	12 (48.0)	8 (88.9)
Unknown	16 (8.2)	25 (12.8)	4 (16.0)	1 (11.1)
del(17p), n (%)				
Yes	46 (23.7)	46 (23.6)	4 (16.0)	0
No	127 (65.5)	123 (63.1)	16 (64.0)	8 (88.9)
Unknown	21 (10.8)	26 (13.3)	5 (20.0)	1 (11.1)
TP53 mutation, n (%)				
Yes	48 (24.7)	51 (26.2)	7 (28.0)	0

No	144 (74.2)	132 (67.7)	18 (72.0)	8 (88.9)
Unknown	2 (1.0)	12 (6.2)	0	1 (11.1)
IGHV, n (%)				
Mutated	53 (29.4)	51 (28.3)	1 (4.0)	3 (33.3)
Unmutated	123 (68.3)	123 (68.3)	22 (88.0)	5 (55.6)
Unknown	4 (2.2)	6 (3.3)	2 (8.0)	1 (11.1)
GC, n (%)				
0–2	106 (54.4)	114 (58.5)	11 (44.0)	7 (77.8)
3–4	34 (17.4)	29 (14.9)	9 (36.0)	1 (11.1)
≥5	14 (7.2)	17 (8.7)	2 (8.0)	0
Unknown	40 (20.5)	35 (17.9)	3 (12.0)	1 (11.1)

BR, bendamustine–rituximab; del(17p), deletion in chromosome 17p; GC, genomic complexity; IGHV, immunoglobulin heavy chain gene; SD, standard deviation; *TP53*, tumor protein P53; VenR, venetoclax–rituximab.

Table 2. Safety summary for the safety-evaluable patients in the main study and substudy

	Main study (N = 382)		Substudy (n = 34)	
	VenR (n = 194)	BR (n = 188)	Retreatment with VenR (n = 25)	Crossed over to VenR (n = 9)
Total no. of patients with ≥1 AE	194 (100.0)	185 (98.4)	19 (76.0)	9 (100.0)
Total no. of AEs	2368	1877	60	26
Total no. of deaths	60 (30.9)	84 (44.7)	8 (32.0)	1 (11.1)
Total no. of patients withdrawn from study due to an AE	1 (0.5)	0	0	0
Total no. of patients with ≥1 AE with fatal outcome	18 (9.3)	17 (9.0)	1 (4.0)	0
Total no. of patients with ≥1 serious AE	101 (52.1)	84 (44.7)	13 (52.0)	5 (55.6)
Total no. of patients with ≥1 related serious AE	44 (22.7)	51 (27.1)	4 (16.0)	4 (44.4)
Total no. of patients with ≥1 AE leading to withdrawal from any treatment	37 (19.1)	18 (9.6)	2 (8.0)	1 (11.1)
Total no. of patients with ≥1 AE leading to dose interruption	136 (70.1)	76 (40.4)	10 (40.0)	8 (88.9)
Total no. of patients with ≥1 AE leading to	30 (15.5)	28 (14.9)	2 (8.0)	0

dose reduction				
Total no. of patients with ≥1 related AE	170 (87.6)	170 (90.4)	10 (40.0)	7 (77.8)
Total no. of patients with ≥1 grade 3/4 AE	150 (77.3)	121 (64.4)	16 (64.0)	8 (88.9)

AE, adverse event; BR, bendamustine–rituximab; VenR, venetoclax–rituximab

Figure Legends

Figure 1. Kaplan–Meier estimates of (A) investigator-assessed PFS, (B) OS, (C) EFS, and (D) DOR in the overall intent-to-treat population

Log-rank test and Cox proportional hazards regression model were used to compare overall PFS and OS across treatment arms.

CI, confidence interval; DOR, duration of response; EFS, event-free survival; NR, not reached; OS, overall survival; PFS, progression-free survival.

Figure 2. Kaplan–Meier estimates of (A) PFS and (B) OS by MRD* response status at EOT in the overall intent-to-treat population

Fisher's exact test was performed to compare MRD status at EOCT and EOT.

*MRD was categorized as uMRD (<1 CLL cell/10,000 leukocytes [MRD value <0.0001, 10^{-4}] and MRD+ (≥10^{-4}).

EOT, end of treatment; MRD, minimal residual disease; OS, overall survival; PFS, progression-free survival.

Figure 3. Kaplan–Meier estimates of (A) time to MRD* conversion, (B) time from conversion to PD, and (C) time from PD to next treatment in the overall intent-to-treat population

*MRD was categorized as uMRD (<1 CLL cell/10,000 leukocytes [MRD value <0.0001, 10^{-4}] and MRD+ (≥10^{-4}).

MRD, minimal residual disease; PD, progressive disease.

Figure 4. Kaplan–Meier estimate of investigator-assessed time to second PFS event for (A) patients receiving subsequent therapy previously randomized to VenR and BR and (B) patients previously randomized to VenR and BR receiving subsequent therapy by treatment type

BR, bendamustine–rituximab; BTKi, Bruton tyrosine kinase inhibitor; PFS, progression-free survival; VenR, venetoclax–rituximab.

Figure 5. Kaplan–Meier estimate of investigator-assessed PFS in the VenR retreatment population

CI, confidence interval; PFS, progression-free survival; VenR, venetoclax–rituximab.

Final Analysis of the MURANO Trial: Venetoclax-Rituximab (VenR) vs Bendamustine-Rituximab (BR) in Patients With Relapsed/Refractory (R/R) Chronic Lymphocytic Leukemia (CLL)

Context of Research

- In the **phase 3 MURANO trial** (**NCT02005471**), fixed-duration VenR resulted in superior progression-free survival (PFS) and overall survival (OS) vs BR
- We report the **final analyses** of MURANO (median follow-up: 7 years), including results of a **retreatment/crossover substudy**

Methods

Main study

Randomized to VenR (n=194)

Randomized to BR (n=195)

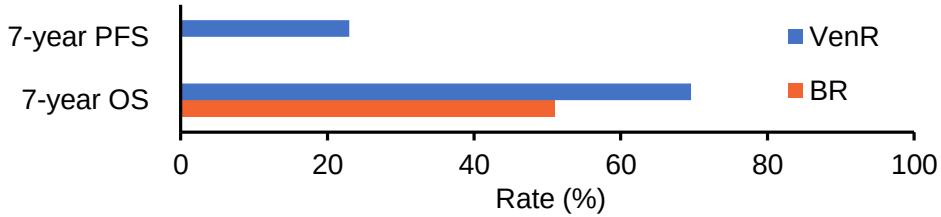
Substudy

Progression after treatment cessation and **retreated** with VenR (n=25)

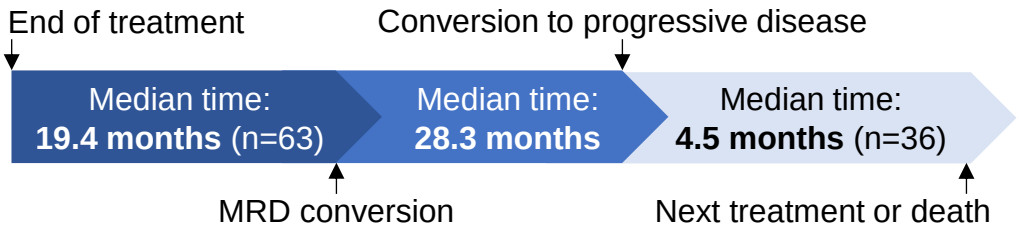
Progression after treatment cessation and **crossed over** to receive VenR (n=9)

Main Findings

Survival benefits with VenR vs BR were **sustained**



VenR-treated patients who achieved **undetectable minimal residual disease** (MRD) (n=83):



Substudy Results

VenR retreatment (n=25)
Median PFS: 23.3 months
Best overall response rate: 72.0%

VenR crossover (n=9)
Median PFS: 26.7 months
Best overall response rate: 88.9%

Conclusions: This final long-term analysis of the MURANO trial continues to demonstrate clinically meaningful benefits for fixed-duration VenR over BR in patients with R/R CLL. Retreatment with VenR is a viable option in pretreated patients.

*Kater et al. DOI: 10.xxxx/**blood**.2024xxxxxx*

blood Visual Abstract

Figure 1

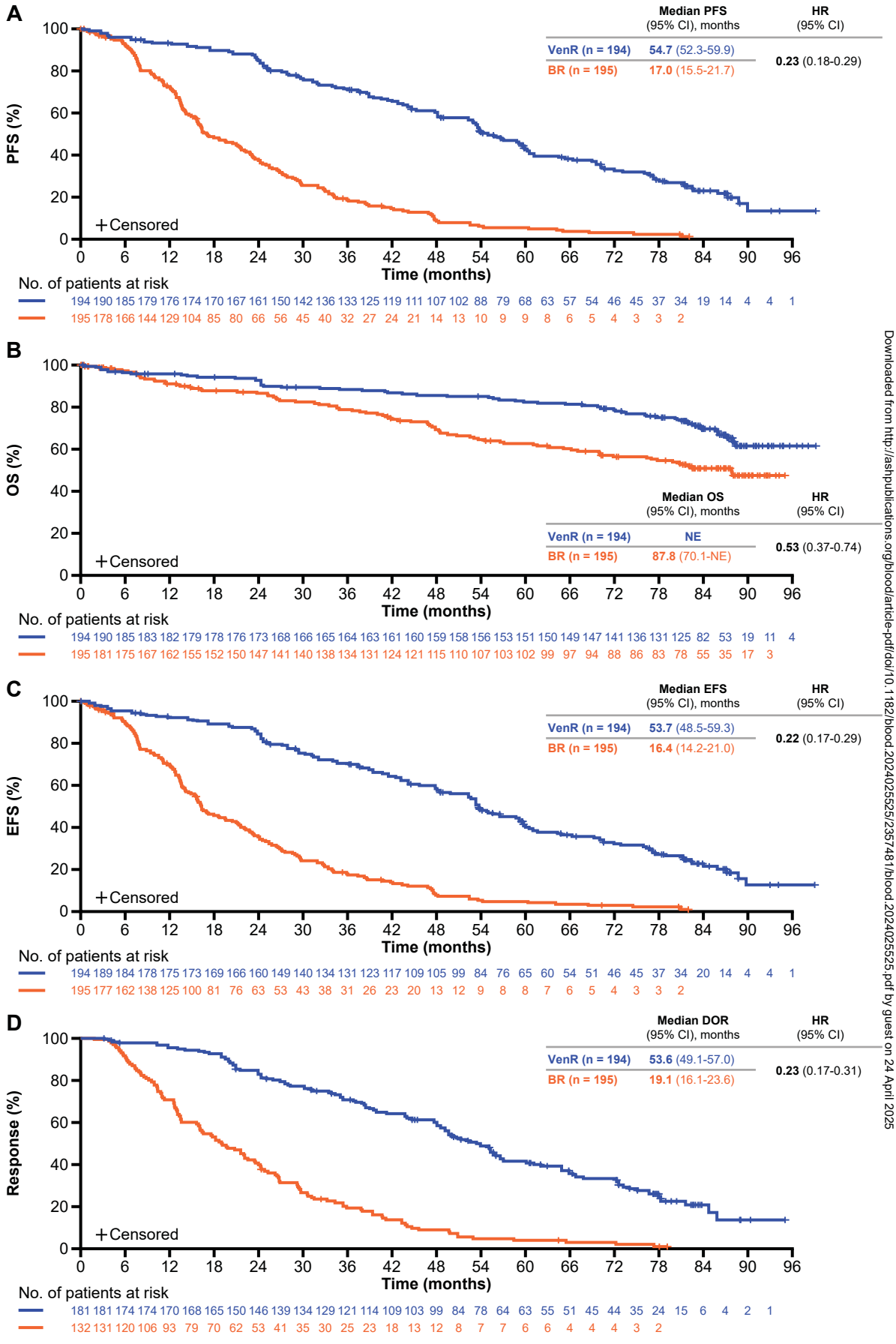


Figure 2

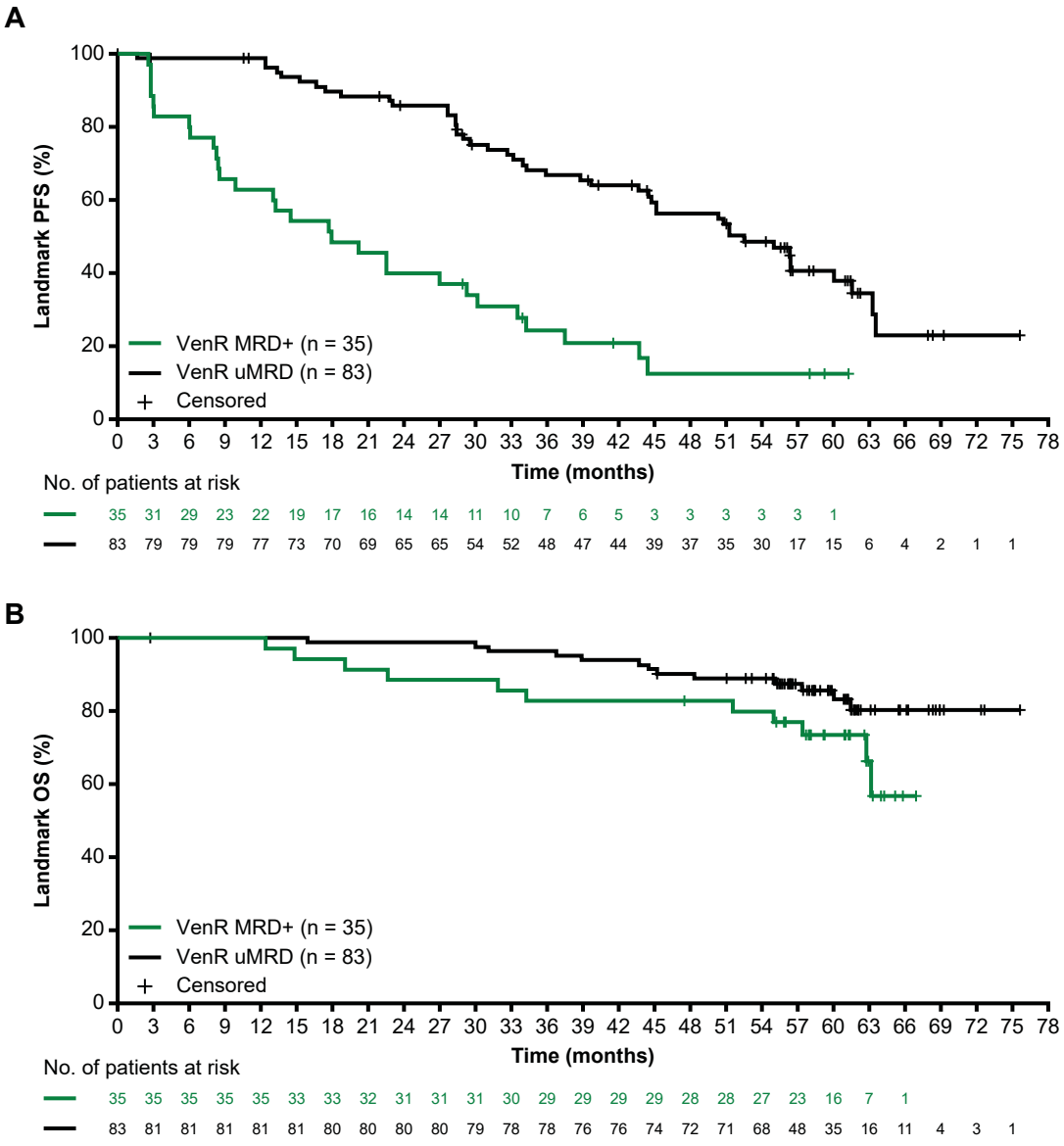


Figure 3

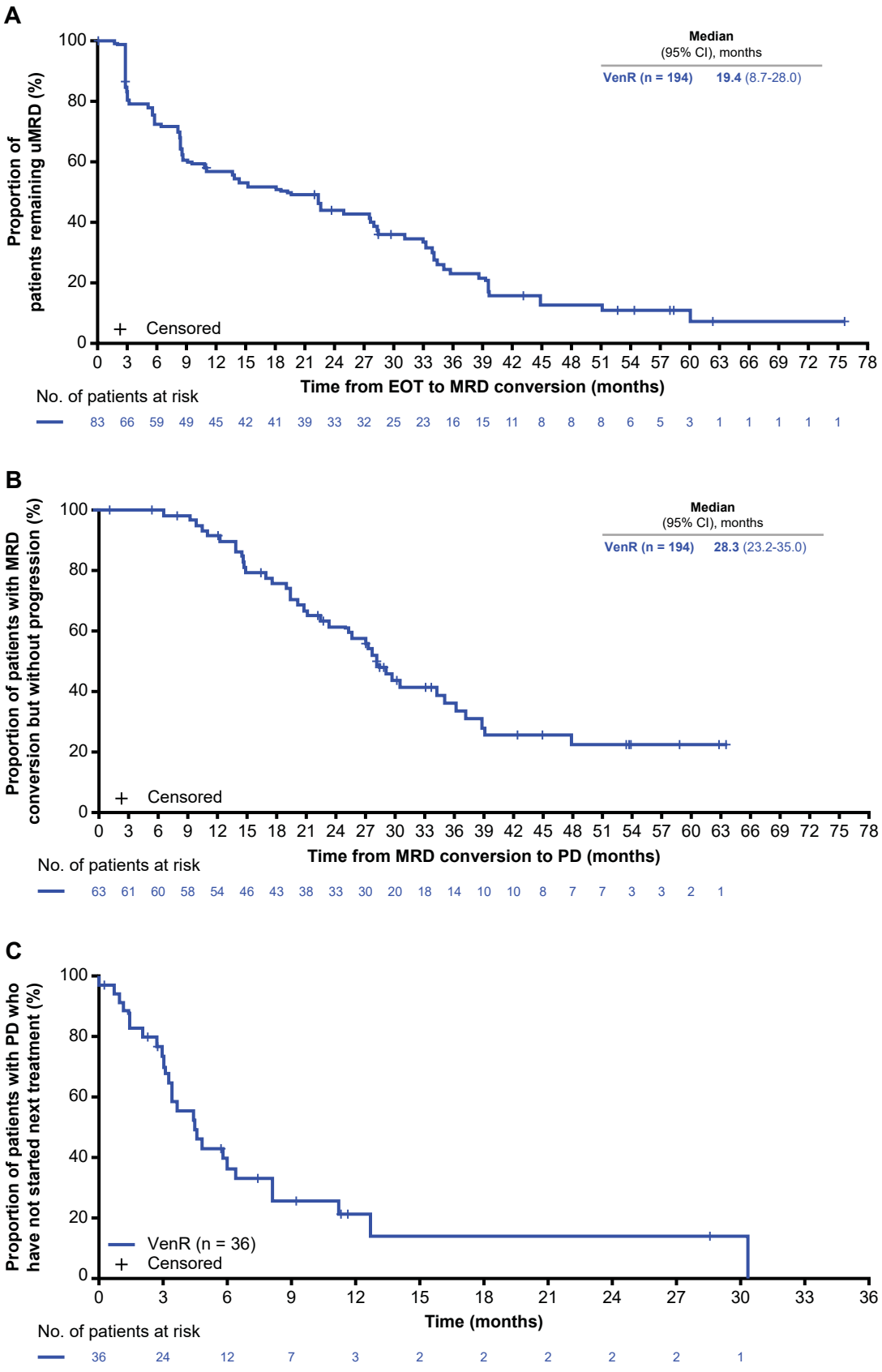


Figure 4

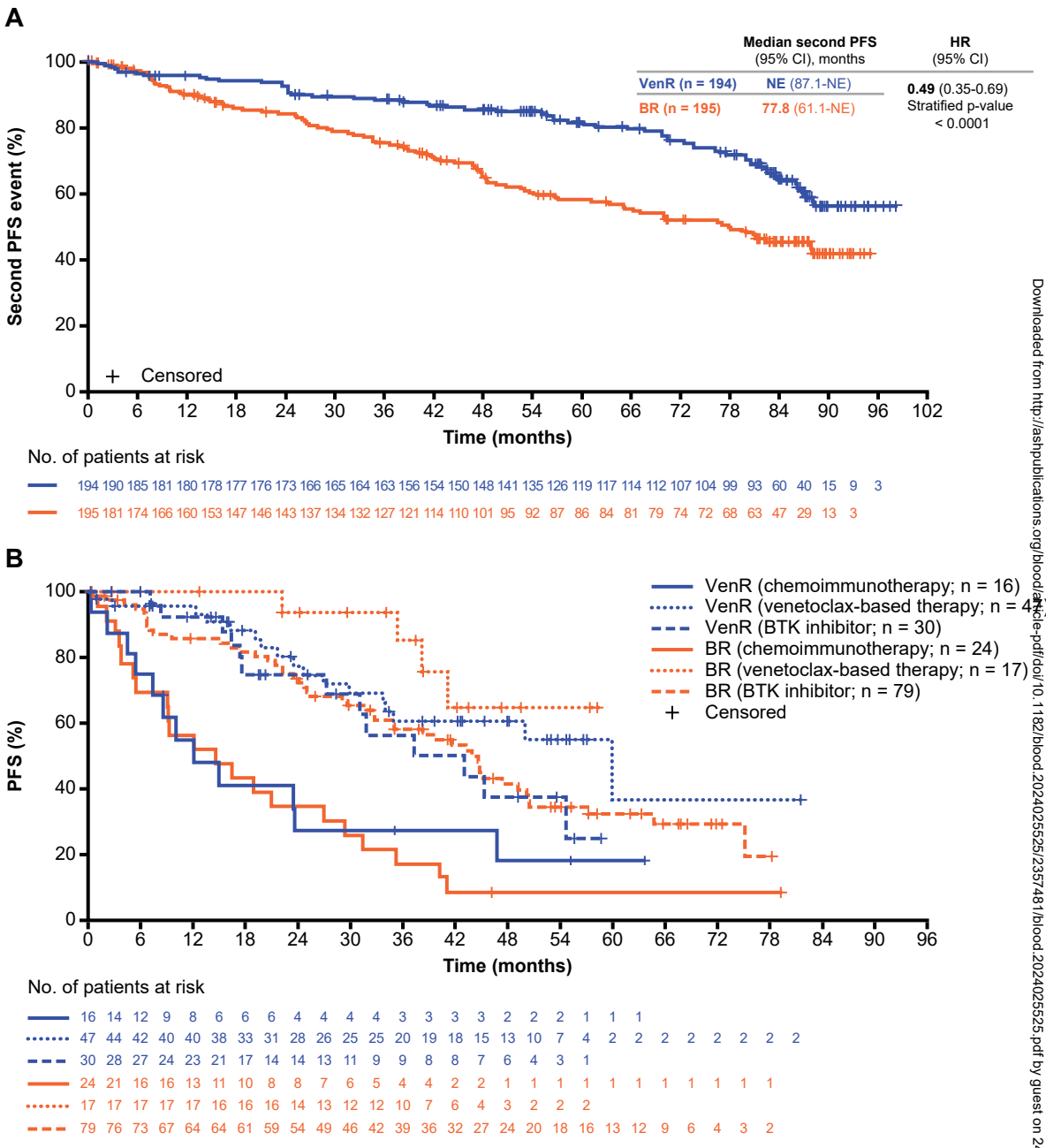


Figure 5