

LETTER



Off-trial outcomes of zanubrutinib in Waldenström macroglobulinemia: the prognostic impact of *CXCR4* and *TP53* alterations

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TO THE EDITOR:

Zanubrutinib is the preferred Bruton tyrosine kinase (BTK) inhibitor for Waldenström macroglobulinemia (WM) based on the Phase III ASPEN trial, which demonstrated safety superiority over ibrutinib [1, 2]. Yet, virtually no off-trial data exist. The molecular impact of zanubrutinib is informed only by a post hoc analysis, which showed superior activity compared with ibrutinib in patients with *CXCR4* mutations and a nonsignificant progression-free survival (PFS) advantage in those with *TP53* mutations (Hazard ratio [HR] 0.66; $p = 0.37$) [3]. Within the zanubrutinib arm, *TP53*-mutated cases had shorter PFS compared with wild-type *TP53*, although the difference was not statistically significant (HR 2.20; $p = 0.12$). These findings are limited by the lack of assessment of the full spectrum of *TP53* alterations (17p deletion and *TP53* mutations, as recommended by current guidelines) and by the known differential impact of *TP53* in previously treated versus untreated populations [4, 5].

To address these gaps, we retrospectively analyzed 236 consecutive patients with WM treated with zanubrutinib at the Dana-Farber Cancer Institute (DFCI), excluding patients who transitioned from ibrutinib due to side effects (Supplementary Fig. S1). This cohort allowed us to evaluate the impact of mutational status on very good partial response (VGPR) according to the 11th International Workshop for Waldenström Macroglobulinemia response criteria [6], and PFS.

Genetic testing for *MYD88*, *CXCR4*, and *TP53* was performed on bone marrow samples. At DFCI, *MYD88* and *CXCR4* were analyzed by allele-specific PCR (AS-PCR) and Sanger sequencing on CD19-selected bone marrow; when CD19 selection was unavailable, *MYD88* was assessed by AS-PCR and *CXCR4* by next-generation sequencing (NGS) on unselected bone marrow. Outside testing included AS-PCR, NGS, or Sanger sequencing on unselected bone marrow for both *MYD88* and *CXCR4*. All *TP53* alterations were assessed by NGS or fluorescence in situ hybridization (FISH).

PFS was estimated using the Kaplan-Meier method and compared using the log-rank test. Logistic and Cox regression models evaluated predictors of VGPR and PFS. Multivariable models included age, sex, prior therapy, zanubrutinib dosage, and *MYD88*, *CXCR4*, and *TP53* status. Median follow-up was calculated using the reverse Kaplan-Meier method. Analyses were performed in Stata 18.0 (College Station, TX, USA).

COHORT CHARACTERISTICS

Patients had a median age of 72 years (range, 23–96), and 103 (44%) had received prior therapy. Most carried *MYD88* mutations

(221/226, 98%), with *CXCR4* mutations in 65/188 (35%) and *TP53* alterations in 19/159 (12%) (Supplementary Table S1).

Response data were available for 225 patients, with 211 responding (overall response rate 94%), including VGPR in 80 (36%) and major response in 188 (84%); no complete responses were observed. With a median follow-up of 27 months (95% CI, 24–30), the 24-month PFS rate was 88% (95% CI, 82.0–91.9).

INFLUENCE OF *CXCR4* MUTATIONS ON TREATMENT OUTCOMES

Patients with *CXCR4* mutations achieved VGPR in 16/62 (26%), compared with 47/118 (40%) in wild-type ($p = 0.06$). In time-to-event analysis, patients with *CXCR4* mutations had a longer time to VGPR than those with wild-type *CXCR4* ($p = 0.04$; Fig. 1A). Consistently, the 6-month VGPR rate was lower in the *CXCR4*-mutated group (10% vs. 25%; $p = 0.03$). However, 24-month PFS rates were similar (81% vs. 88%, $p = 0.78$) (Fig. 1C, D).

When analyzing *CXCR4* subtypes, VGPR was achieved in 4/28 (14%) of patients with frameshift mutations, 12/30 (40%) of patients with nonsense mutations, and 47/118 (40%) of patients with *CXCR4* wild-type disease ($p = 0.04$) (Fig. 1E). In time-to-event analysis, patients with *CXCR4* frameshift mutations experienced a significantly longer time to VGPR compared with those with wild-type *CXCR4* ($p < 0.01$), whereas nonsense mutations had no impact ($p = 0.79$; Fig. 1B). At 6 months, VGPR rates were 4% (frameshift), 15% (nonsense), and 25% (wild-type) ($p = 0.07$). Still, the 24-month PFS rate was not impacted: 80% for frameshift, 81% for nonsense, and 88% for wild-type ($p = 0.93$) (Fig. 1F).

In multivariate analysis, the odds ratio (OR) for achieving VGPR in patients with *CXCR4* mutations was 0.48 (95% Confidence interval [CI] 0.21–0.99, $p = 0.06$). When mutation type was considered, frameshift variants were significantly associated with reduced odds of VGPR (OR 0.18, 95% CI 0.05–0.68, $p = 0.01$), whereas nonsense mutations were not (OR 0.90, 95% CI 0.46–2.26, $p = 0.81$). For PFS, neither overall *CXCR4* status (mutated vs. wild-type; $p = 0.53$) nor frameshift ($p = 0.97$) or nonsense variants ($p = 0.33$) were associated with inferior or superior outcomes.

INFLUENCE OF *TP53* ALTERATIONS ON TREATMENT OUTCOMES

Patients with *TP53* alterations achieved VGPR in 5/18 (28%), compared with 51/134 (38%) in unaltered cases, with no significant difference ($p = 0.40$). There was no difference in time to VGPR by *TP53* status ($p = 0.34$). The 24-month PFS rate was 62% (95% CI 34–81) in *TP53*-altered cases and 89% (95% CI 82–94) in *TP53*-unaltered cases, $p = 0.08$ (Fig. 2A, B).

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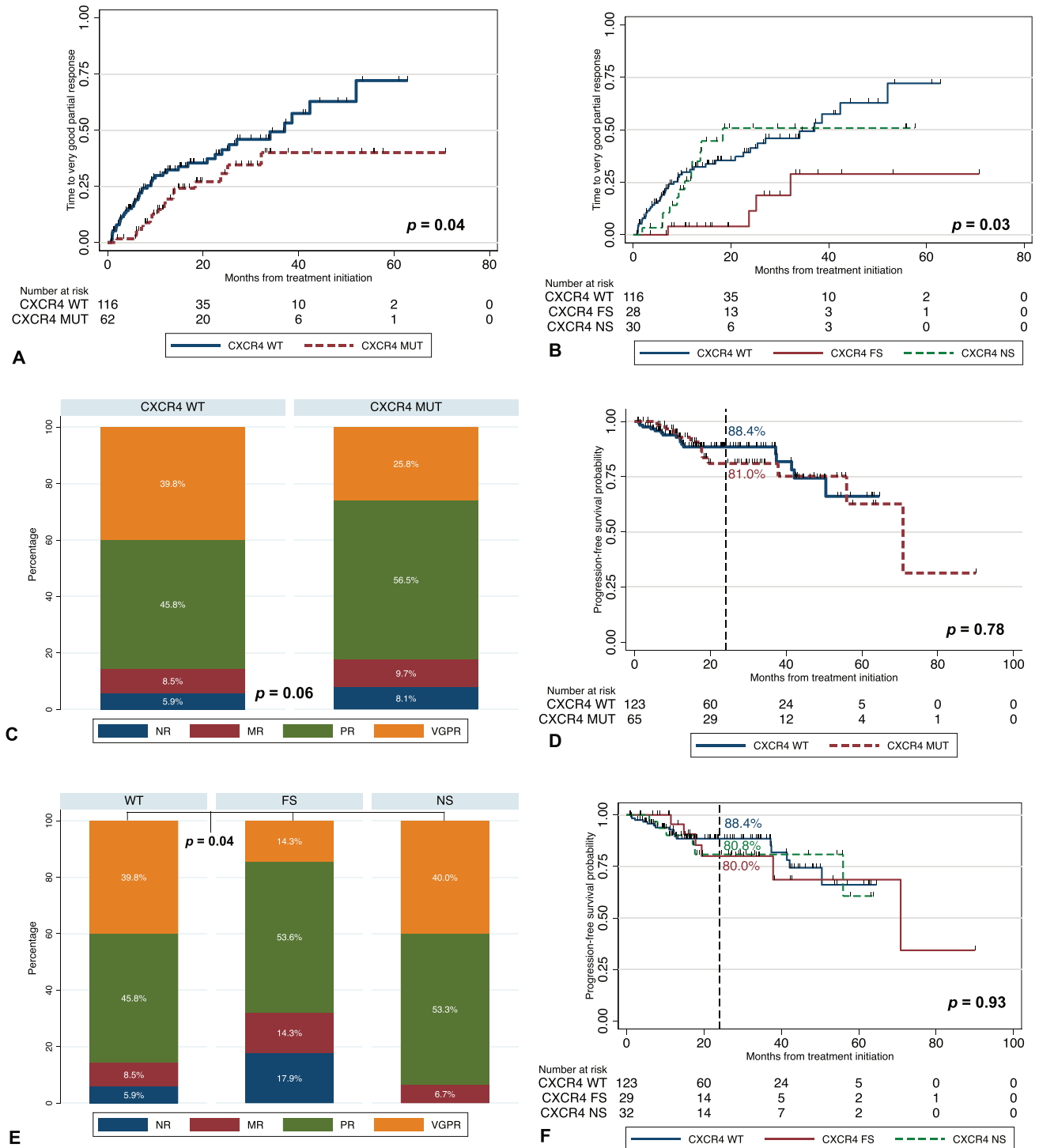


Fig. 1 Treatment outcomes according to CXCR4 mutation status. A Time to very good partial response (VGPR) by CXCR4 mutational status: mutated vs. wild type (WT). **B** Time to VGPR by CXCR4 subgroup: WT, frameshift (FS), and nonsense (NS) mutations. **C** Best response distribution in patients with CXCR4 WT and mutated (MUT). **D** Progression-free survival (PFS) by CXCR4 mutation status. **E** Best response distribution by CXCR4 subgroup: WT, FS, and NS. **F** PFS by CXCR4 subgroup. Time-to-event analyses included the 178 of 188 patients with available time-to-response data and CXCR4 mutational testing; CXCR4 subtyping analyses included the 184 of 188 patients with reported subtypes.

In untreated patients, *TP53* mutational status did not impact outcomes; VGPR rates were 3/9 (33%) vs. 24/80 (30%) ($p = 0.84$), and time to VGPR was not significantly different ($p = 0.53$). The 24-month PFS rate was 95% (95% CI, 86–98) for those without *TP53* alterations and 88% (95% CI, 39–98) for those with *TP53* alterations, with no significant difference ($p = 0.80$) (Fig. 2C, D). In contrast, among previously treated patients, VGPR rates were numerically lower in the

TP53-altered group (2/9, 22%) compared with the *TP53* wild-type group (27/54, 50%) ($p = 0.12$), and time to VGPR was numerically longer in time-to-event analysis ($p = 0.13$). The 24-month PFS rate was 88% (95% CI, 75–94) for those without *TP53* alterations and 50% (95% CI, 18–75) for those with *TP53* alterations ($p = 0.02$) (Fig. 2E, F).

Multivariate analysis showed that *TP53* alterations remained associated with inferior PFS in previously treated patients (HR 3.25,

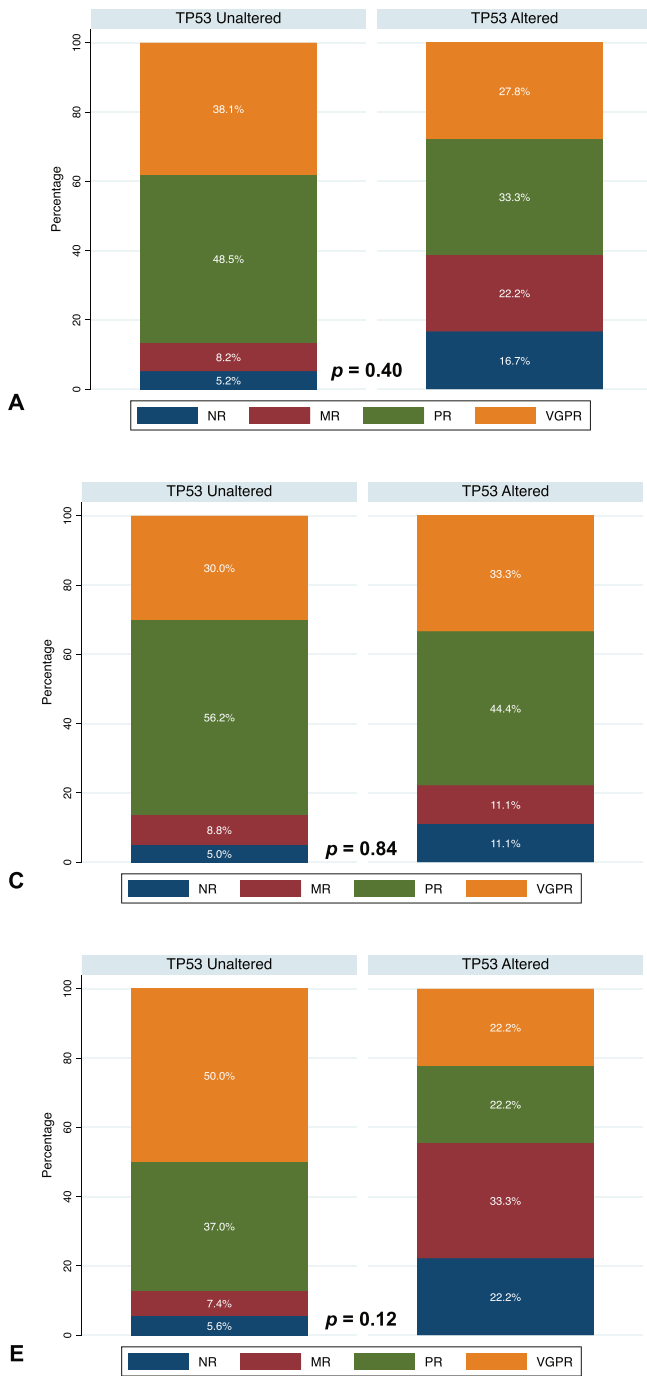


Fig. 2 Treatment outcomes according to TP53 alteration status. A Best response distribution in patients with unaltered and altered TP53. **B** Progression-free survival (PFS) by TP53 alteration status. **C** Best response distribution in untreated patients stratified by TP53 status. **D** PFS in untreated patients. **E** Best response distribution in previously treated patients stratified by TP53 status. **F** PFS in previously treated patients.

95% CI 1.05–10.11, $p = 0.04$), while no effect was observed in untreated patients (HR 1.12, 95% CI 0.10–13.02, $p = 0.93$). No significant impact on VGPR rates was observed in either group.

INFLUENCE OF MYD88 MUTATIONS ON TREATMENT OUTCOMES

Only five patients had MYD88 wild-type disease, none of whom underwent CD19-selected testing. Among these, the best responses were minor response in 2 (40%), partial response in 2 (40%), and VGPR in 1 (20%). None of the five patients with MYD88 wild-type

disease experienced disease progression; however, the median follow-up was short (16.6 months; range, 10.9–41.0 months) (Supplementary Fig. S2).

DISCUSSION

Altogether, our results support the use of zanubrutinib regardless of CXCR4 mutational status. Unlike ibrutinib, zanubrutinib appears largely agnostic to CXCR4 mutations with respect to long-term disease control, with patients achieving durable benefit despite delayed or shallower IgM responses [3, 7]. In ibrutinib-treated patients, nonsense







mutations have been associated with worse outcomes than frame-shift mutations [7], whereas our study showed the opposite pattern in response kinetics. This difference may reflect a true distinction between ibrutinib and zanubrutinib or the higher sensitivity of our PCR-based assays in detecting low-burden nonsense subclones, which are generally linked to better prognosis [8]. Still, neither variant affected 2-year PFS rates, indicating that their influence pertains to IgM kinetics rather than acquired resistance, highlighting the importance of avoiding early discontinuation. Findings from the FILO study [9], which similarly show *CXCR4* agnosticism with bendamustine-rituximab (BR) but a relatively high incidence of secondary malignancies, further supporting zanubrutinib as a first-line option for *CXCR4*-mutated WM.

Moreover, our study suggests a differential impact of *TP53* alterations between treatment-naïve and relapsed or refractory patients, with an adverse effect on zanubrutinib outcomes observed only in the latter group. This pattern parallels observations in chronic lymphocytic leukemia, where therapy selects for *TP53*-deficient clones that may be more difficult to overcome with BTK inhibition in the relapsed setting [10]. Still, the use of unselected bone marrow for *TP53* testing represents an important consideration, as it makes it difficult to determine whether alterations arose within CHIP clones or the WM clone itself [11].

Large retrospective studies suggest similar durability with ibrutinib and chemoimmunotherapy (CIT), while cross-trial comparisons hint at perhaps greater durability with zanubrutinib [1, 12, 13]. A potential concern with CIT is its capacity to induce DNA damage and possibly promote *TP53* alterations [4]. In ASPEN, prior exposure to alkylating agents or nucleoside analogs was very high, and *TP53* mutations were observed in 25% of cases [3], substantially higher than the ~10% typically reported in most series [2, 4, 5]. These observations suggest a possible advantage to the frontline use of zanubrutinib rather than reserving BTK inhibitors for later lines, when *TP53*-mutated disease is more prevalent and outcomes with zanubrutinib are poorer.

While *MYD88* wild-type WM does not benefit from ibrutinib [7], ASPEN reported a median PFS of 41 months in zanubrutinib-treated *MYD88* wild-type disease [14]. However, testing was done without CD19+ selection, possibly misclassifying some cases as wild-type [15]. In our practice, we rarely use zanubrutinib for *MYD88* wild-type disease, and the very small wild-type subgroup in this study ($n = 5$, none tested with CD19 selection) limits the ability to draw conclusions. Still, because CD19+ selection is uncommon outside academic centers, the observed responses support the use of zanubrutinib in presumed wild-type cases tested with less sensitive methods.

Important limitations should be considered when interpreting our data, including its retrospective design, referral-center bias, and heterogeneity in genetic testing methods. Even so, it features a well-characterized, systematically followed, and heavily genotyped cohort that, to the best of our knowledge, represents the largest real-world zanubrutinib WM series reported to date, allowing a meaningful assessment of its high effectiveness across molecular subgroups, including *TP53*-altered cases.

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AUTHOR CONTRIBUTIONS

AG and JJC designed the study. AG, NT, and MK collected the data. JJC, SS, AB, and GVK provided the patients. NT, AK, MLG, ZH, and SPT performed the laboratory studies. AG and JJC performed the statistical analyses. JJC, SS, SPT, and ZH provided overall supervision. AG and JJC drafted the initial version of the manuscript. All authors critically reviewed and approved the final manuscript.

COMPETING INTERESTS

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ETHICS APPROVAL AND CONSENT TO PARTICIPATE

All methods were performed in accordance with the relevant guidelines and regulations governing human subjects research. The study was reviewed and approved by the Dana-Farber Cancer Institute Institutional Review Board (protocol number 25-151). Due to the retrospective nature of the study and the use of previously collected medical record data, the requirement for informed consent was waived.

ADDITIONAL INFORMATION

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