W35: Clonality assessment represents a novel biomarker to predict outcomes on ibrutinib therapy for patients with Waldenström macroglobulinemia carrying CXCR4 S338X nonsense mutations.

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Introduction: Approximately 40% of patients with Waldenström macroglobulinemia (WM) have an activating somatic mutation in CXCR4 (Xu *et al*, BJH 2016). The most common variant is CXCR4 S338X, which represents over half of CXCR4 mutations found in WM patients. CXCR4 S338X mutations are primarily subclonal to mutated MYD88, but show a highly variable clonal distribution. CXCR4 mutations have also been shown to confer *in vitro* and clinical resistance to the BTK inhibitor ibrutinib, particularly nonsense variants such as CXCR4 S338X (Castillo *et al*, EHA 2018). We therefore sought to evaluate the impact of CXCR4 S338X clonality on response and progression-free survival to ibrutinib therapy.

Methods: We identified patients treated at our Institution who met the consensus criteria for WM and received ibrutinib between May 2012 and January 2018. MYD88 and CXCR4 mutation status was determined by AS-PCR and Sanger sequencing methods as before (Xu *et al*, Blood 2013; BJH 2016). Cancer cell fraction (CCF) analysis was performed for patients with CXCR4 S338X mutations as previously described (Xu *et al*, BJH 2016). Time to events was estimated using the Kaplan-Meier method, and comparisons were made using the log-rank test.

Results: One hundred forty-seven patients met eligibility criteria for inclusion in this analysis. The MYD88 L265P and CXCR4 S338X mutations were identified in 147 (100%) and 37 (25%) patients, respectively. The median treatment duration on ibrutinib was 21.1 months (range 0.3-69). Patients with CXCR4 S338X had lower rates of major response (62% vs. 85%; p=0.004) and very good partial response (11% vs. 35%; p=0.006) versus those with wild-type CXCR4. By univariate analysis, CXCR4 S338X was the only variable associated with worse PFS (HR 3.48,

95% CI 1.33-9.08; p=0.001) with a significantly shorter median PFS compared to patients with wild-type CXCR4 (39.8 months vs. not reached [NR]; **Figure 1A**). Age, sex, hemoglobin level, platelet count, serum IgM level, beta-2 microglobulin, bone marrow involvement, IPSSWM score, and prior treatment status did not impact PFS (p>0.05 for all comparisons). Among the 37 patients with CXCR4 S338X, the median clonality was 35.3% (range 0.94-86.2%); 19 patients (51%) had high clonality defined as ≥35%, while 18 (49%) had low clonality defined as <35%. The major response rates for patients with high CXCR4 S338X clonality, low CXCR4 S338X clonality, and wild-type CXCR4 were 63%, 61%, and 85%, respectively (p=0.01), and the very good partial response rates were 5%, 17%, and 35%, respectively (p=0.02). Compared to patients with wild-type CXCR4, high clonality was associated with significantly worse PFS (HR 4.90, 95% CI 1.50-16.1; p<0.001), whereas low clonality did not significantly impact PFS (HR 1.49, 95% CI 0.26-8.44; p=0.70). Patients with high clonality also had a significantly shorter median PFS versus patients with low clonality and wild-type CXCR4 (33.0 months vs. NR vs. NR; p<0.0001; **Figure 1B**).

Conclusion: High CXCR4 S338X clonality is associated with lower major responses, including VGPR attainment as well as shorter PFS in WM patients receiving ibrutinib. Clonality assessment represents a novel biomarker for predicting outcomes on ibrutinib for patients carrying CXCR4 S338X nonsense mutations.

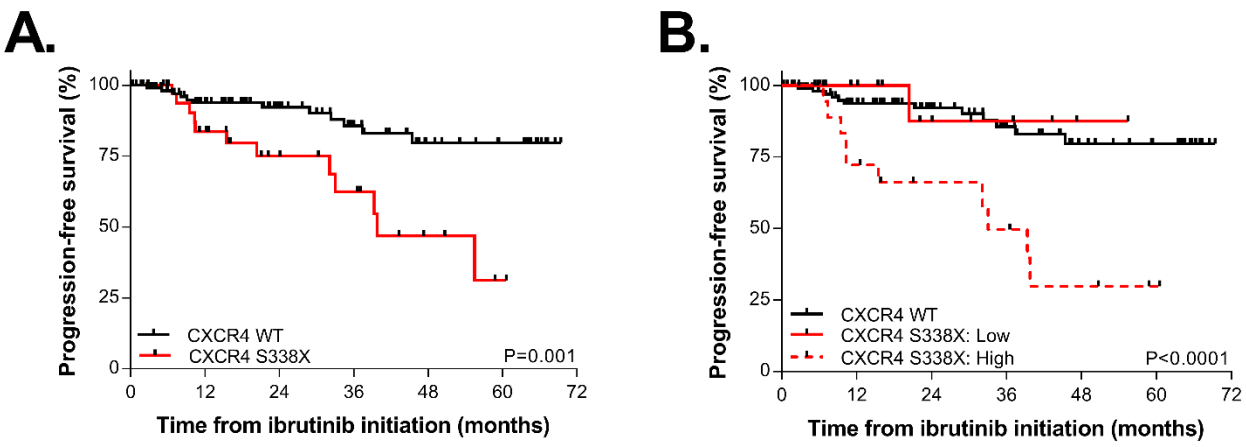

Figure 1. Kaplan-Meier curves for progression-free survival (PFS) stratified by CXCR4 S338X mutational status (A) and CXCR4 S338X clonality (B).