

Non-invasive *MYD88*^{L256P} detection and MRD analysis by ddPCR in cfDNA in the ECWM2 international, multicentric phase II trial

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Background. Despite the overall indolent disease course, only low complete remission (CR) and minimal residual disease (MRD) negativity rates in bone marrow are induced in Waldenström macroglobulinemia (WM) by conventional immunochemotherapy [Varettoni, Hematol Oncol 2022]. No MRD data exist in the context of prospective clinical trials assessing targeted treatments in WM and very few reports are available on the application of novel, less-invasive, MRD techniques in this disease, so far. [Ferrante, ASH2023] **Aims.** To assess the reliability of plasma cell-free DNA (cfDNA) for screening and tracking *MYD88*^{L265P} mutation for MRD analysis in the context of the ECWM2 trial (NCT03620903), an international, multicenter phase II trial initiated by the European Consortium for Waldenstrom's Macroglobulinemia (ECWM), testing the efficacy of first line bortezomib, rituximab and ibrutinib combination for patients with treatment naive WM. **Methods.** Treatment schedule consisted of: 1) Induction (Cycle 1-6) with rituximab 1400 mg SC day 1, bortezomib 1.6 mg/ m² SC day 1,8,15 and ibrutinib 420 mg p.o. day 1-28; 2) Maintenance I (24 months) with rituximab 1400 mg, every second month and ibrutinib 420 mg daily; 3) Maintenance II with ibrutinib

420 mg, until evidence of progressive disease or unacceptable toxicity (Figure 1). A total of 108 cfDNA plasma samples from 32/53 patients enrolled in the ECWM2 trial were analyzed by ddPCR [Drandi, Haematologica 2018] for *MYD88^{L265P}* mutation: 32 samples at baseline, 29 at mid-induction (after cycle 3), 28 at the end-induction, 12 at Maintenance I and 6 at Maintenance II. **Results.** Overall, only 4/108 samples were excluded from the analysis because of insufficient plasma quality. No difference was recorded in terms of survival in the MRD series vs the overall series enrolled in the trial. 93% of patients (30/32) showed *MYD88^{L265P}* at baseline (median AF: 3.8%; range 50%-0.24%) and were thereafter evaluated for MRD, excluding two patients with *MYD88^{L265P}* WT at baseline and at follow up). High efficacy of the treatment combination was observed already at mid-induction, with 41% (12/29) of samples reaching MRD negativity (Figure 2A) and a considerable (almost 1 log) quantitative shrinkage among patients still MRD positive (median residual AF: 0.63%; range 5.6%-0.1%, Figure 2B). Moreover, further MRD clearance was observed thereafter, with a MRD negativity rate of 65% (17/26) and median residual AF of 0.16% (range 2.34%-0.035%) at end of induction, while only 1/12 patients still scored MRD positive at Maintenance I (AF 0.52%) and 2/6 at Maintenance II (AF 0.3% and 0.064%, respectively). Overall, MRD results correlated with clinical response, with early MRD negativity (at mid-induction) heralding deeper clinical responses at end-induction (1 CR and 3 VGPR among 12 patients MRD negative vs only 2 VGPR among 17 patients still MRD positive). As no patient experienced relapse/progression, so far, longer follow-up is needed for further correlations between MRD response and progression free survival. **Conclusions.** Non-invasive MRD monitoring by *MYD88^{L265P}* in cfDNA from plasma was feasible in the context of a prospective, multicenter trial in WM, correlating with clinical activity of ibrutinib based treatment. Ongoing analyses will demonstrate whether MRD monitoring has the potential to serve as a valuable tool for early prognostication for these patients.

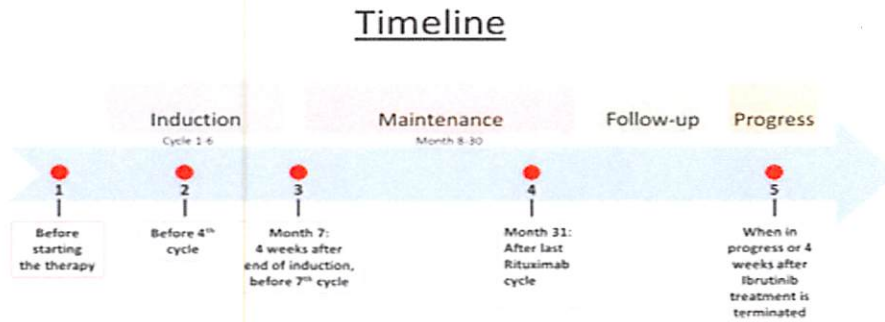


Figure 1. Treatment schedule

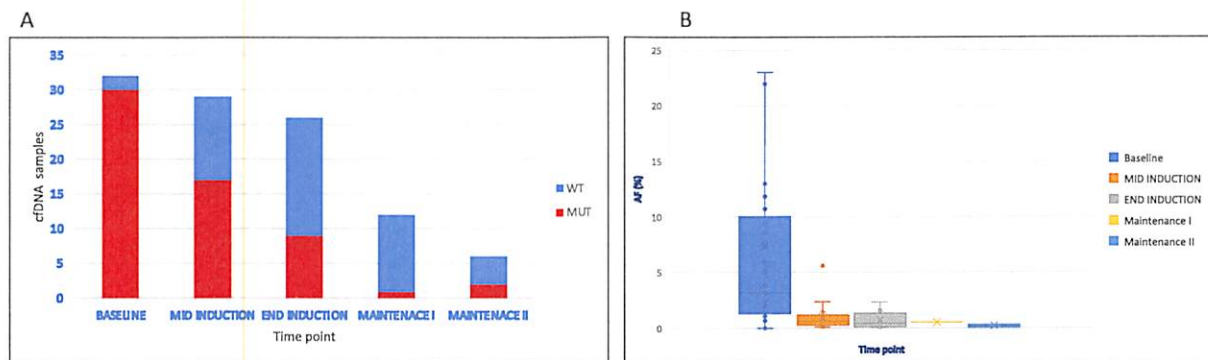


Figure 2. A) MYD88^{L265P} detection on cfDNA in WM patients at different timepoints B) Levels, calculated as allele frequency (AF), of MYD88^{L265P} in cfDNA of WM patients at different timepoints. WT: wild type; MUT: MYD88L265P mutated.