MYD88L265P MUTATION DETECTION IN WALDENSTRÖM MACROGLOBULINEMIA BY DROPLET DIGITAL PCR: MINIMAL RESIDUAL DISEASE MONITORING AND CHARACTERIZATION ON CIRCULATING FREE DNA

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Background: MYD88^{L265P} mutation might represent an ideal marker for minimal residual disease (MRD) monitoring. However, allele-specific quantitative PCR (ASqPCR) is not sensitive enough for MRD monitoring on peripheral blood (PB), harboring low concentrations of tumor cells. Besides, cell-free DNA (cfDNA) is increasingly used for mutational studies. We set up a new, highly sensitive, droplet digital PCR (ddPCR) assay for MYD88^{L265P} detection and described: 1) its feasibility for mutation screening and MRD monitoring in bone marrow (BM) and PB; 2) its application for mutational studies on plasmatic cfDNA. **Methods:** BM, PB and plasma from local series of WM, IgG-lymphoplasmacytic lymphoma (LPL) and IgM-MGUS patients (pts) were collected at baseline and during follow-up (FU). 20 healthy subjects were used as negative controls. Genomic DNA (gDNA) and cfDNA were extracted by Maxwell RSC automatic system (Promega). MYD88^{L265P} was assessed on gDNA (100ng) and cfDNA (5µl, from 1ml of plasma) by a custom ddPCR assay on a QX100 System (Bio-Rad). For comparison ASqPCR was assessed on gDNA (100ng), as described [Xu L, 2013]. MYD88^{L265P} cut-off was settled based on the healthy samples background level. IGH-based MRD analysis was performed as described [Ladetto M, 2000]. **Results:** MYD88^{L265P} ddPCR assay sensitivity was compared to ASqPCR on a ten-fold serial dilution standard curve. Whereas ASqPCR confirmed the sensitivity of 1.00E−03, ddPCR reached a sensitivity up to 5.00E−05. Overall, 137 samples from 77 pts (68 WM, 6 LPL, 3 IgM-MGUS), 86 baseline (64 BM, 22 PB) and 51 FU (23 BM and 28 PB), were analyzed. Median values at baseline were: age 67 years (range: 38-88), IgM 2.2 g/l (0.3-10.8), IgG for LPL 1.9 g/l (0.8-3.4), B2M 2.6 mg/l (0.14-7.9), infiltration at BM biopsy 45% (0-90%), by flow cytometry 10% (range: 0-87%). 12 pts had splenomegaly and 15 adenopathies. At diagnosis 63/64 (98.4%) BM and 19/22 (86.4%) PB scored MYD88^{L265P} positive (medians 4.5% and 0.15%, ranges 0.02%-72.6% and 0.01-27.8%,

respectively): all 3 negative PB had a positive BM match. Moreover, 100 samples (60 BM, 40 PB) were tested by both ASqPCR and ddPCR, showing a good concordance ($p<0.0001$), being the majority of discordances in the FU (13/60 ddPCR positive/ASqPCR negative, 11/60 ddPCR negative/ASqPCR positive). However, ddPCR was able to detect at diagnosis a higher number of mutated cases (38 vs 36). Moreover, we compared $MYD88^{L265P}$ ddPCR to the gold standard IGH-ASqPCR for MRD monitoring. From 33/57 (57.9%) pts showing an IGH rearrangement, baseline and FU samples from 4 preliminary pts (18 BM, 5 PB) showed highly superimposable results (Fig.1). Finally, pivotal results on 33 pts showed 1 log higher median levels of $MYD88^{L265P}$ mutation in plasmatic cfDNA (0.7%, range 0-25,7%) compared to PB (0.037%, range: 0.01-20.0%). **Conclusion:** $MYD88^{L265P}$ ddPCR is a feasible and highly sensitive assay for mutational screening and MRD monitoring in WM, particularly in samples harboring low concentrations of circulating tumor cells. Moreover, plasmatic cfDNA represents a promising tissue source and might be an attractive, less invasive alternative to PB or BM for $MYD88^{L265P}$ detection. Methodological validation against IGH-based MRD detection and flow cytometry is ongoing.

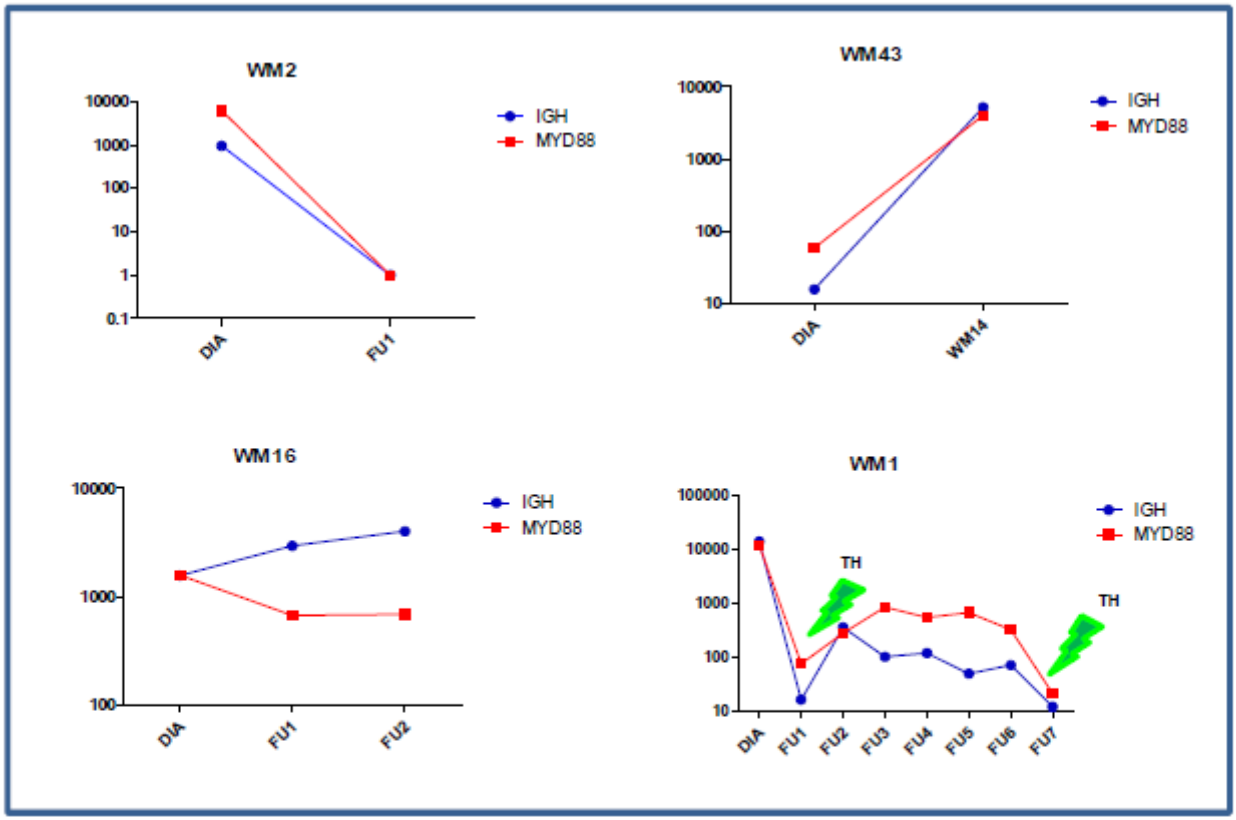

Fig.1 MYD88 mutation (■) and IGH rearrangement (■) detection at baseline and follow up samples from 4 representative WM patients (WM2,WM43,WM16 and WM1).
DIA (diagnosi), FU (follow up), TH (Therapy)