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

Simone Ferrero1, Elisa Genuardi1, Irene Dogliotti1, Ivan Sciascia1, Francesca Guerrini2, Barbara Mantoan1, Milena Gilestro3, Vittorio Muccio3, Paola Ghione1, Paola Omedè3, Sara Galimberti2, Lorella Orsucci4, Federica Cavallo1, Mario Boccadoro1, 3, Marco Ladetto5, Daniela Drandi1

1 Department of Molecular Biotechnologies and Health Sciences, Division of Hematology, University of Torino, Torino, Italy 2 Clinical and Experimental Medicine, University of Pisa, Pisa, Italy 3 Division of Hematology 1, Città della Salute e della Scienza, Torino, Italy 4 Division of Hematology 2, Città della Salute e della Scienza, Torino, Italy 5 Hematology, AON SS. Antonio e Biagio e Cesare Arrigo, Alessandria, Italy

Background: MYD88L265P 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 MYD88L265P 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). MYD88L265P 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]. MYD88L265P cut-off was settled based on the healthy samples background level. IGH-based MRD analysis was performed as described [Ladetto M, 2000]. Results: MYD88L265P 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 MYD88L265P 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.

![Figure 1: MYD88 mutation (■) and IGH rearrangement (□) detection at baseline and follow up samples from 4 representative WM patients (WM2, WM43, WM16 and WM1). DIA (diagnosis), FU (follow up), TH (Therapy).](image)