

Circulating tumor cells and plasma *MYD88* VAF as Prognostic Markers for Staging and Ibrutinib Response in Waldenström Macroglobulinemia

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Characterization of liquid biopsy in Waldenström's macroglobulinemia (WM) as represented by the presence of circulating tumor cells (CTCs) and cell-free tumor DNA (ctDNA), may provide a powerful tool for disease burden estimation taking advantage of the non-invasive approach and the ability to capture the spatial heterogeneity of the bone marrow (BM) that a single BM biopsy misses.

In this study, we evaluated the feasibility of detecting CTCs in patients with IgM monoclonal gammopathies and quantified the *MYD88*^{L265} mutation allele frequency (MAF) in matched ctDNA. Our aim was to provide a more comprehensive assessment of disease burden and to determine whether these noninvasive markers could offer independent prognostic information beyond current clinical evaluation strategies.

We performed multiparameter flow cytometry to detect CTCs as determined by clonal B cells (CD19⁺ with light chain restriction) at giga per liter (10⁹/L) in the peripheral blood (PB) of patients. All samples were stained with established phenotypic markers of the WM clone including positive selection of CD19, CD20, IgM, CD22, CD25, CD27 and exclusion of CD5 and CD23. Light-chain restriction was evaluated with surface kappa and lambda antibodies. Data were acquired on Accuri™ II cytometer. In parallel, we performed ddPCR assay on Absolute Quantstudio for the detection and quantification of *MYD88*^{L265P} mutation in ctDNA with a detection sensitivity of 5 × 10⁻³.

Among the 196 patients with IgM monoclonal gammopathies, CTCs were detected in 70% of patients. Specifically, CTCs were detected in 22 out of 62 IgM-MGUS (35%, median:0, range: 0- 0.029 x10⁹/L), in 33 out of 43 asymptomatic WM (76%, median:0.0031, range: 0-0.3 x10⁹/L) and 81 out of 91 symptomatic WM (89%, median:0.05 x10⁹/L, range: 0-1.7 x10⁹/L) patients with a highly significant difference in the CTC distributions among the disease status groups (Kruskal-Wallis, *p*=2.187e-08). Concurrently, *MYD88*^{L265P} MAF was evaluated in plasma samples available from the same patient cohort (n = 116). Similarly, *MYD88*^{L265P} MAF (in patients harboring the mutation) was higher in sWM

with a median 4.6% (range: 0.62 – 86.41%) compared to aWM with a median of 1.33% (range: 0.12 – 19.91%) and IGM-MGUS patients with a median of 0.53% (range: 0.1 – 8.59%) indicating its potential use for monitoring tumor burden in WM (Kruskal-Wallis, $p=0.00071$). No significant correlation was observed between CTC count and *MYD88*^{L265P} MAF.

In addition, our study included 37 sequential samples of sWM patients at diagnosis and 6 months post-ibrutinib (IBR) therapy. Patients achieving partial response (PR) or better after 6 months of therapy were defined as responder group (RG, n=24) while patients with less than PR were defined as non-responder group (NRG, n=13). Comparing the CTCs pre- and post-IBR in the RG vs NRG we observed higher numbers of CTCs in the NRG compared to the RG ($p=0.0002$) at the time of diagnosis but also a significant decrease of the CTCs in the RG post therapy ($p=0.025$) which was not observed in the NRG, with significant effects on progression-free survival ($p=0.01$). Concurrently, *MYD88*^{L265P} MAF in ctDNA was significantly decreased in RG ($p=0.0076$), while in the NRG *MYD88*^{L265P} MAF was slightly increased with most cases having a stable MAF pre and post treatment ($p=0.6$).

These findings underscore the potential of CTCs and *MYD88*^{L265P} MAF screening as a valuable tool for assessing disease status and treatment efficacy.

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