W3: Mast Cell Density and Its Clinical Relevance in Waldenström's Macroglobulinemia

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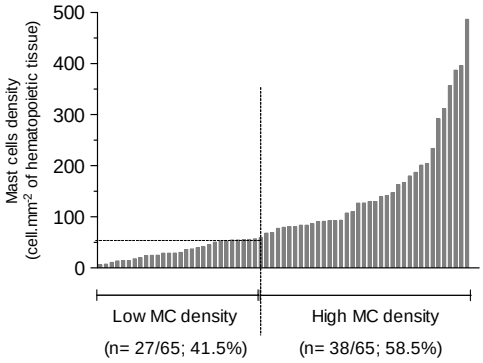
Background. Waldenström's macroglobulinemia (WM) is characterized by a lymphoplasmacytic infiltration of bone marrow (BM) along with a serum monoclonal IgM and includes *MYD88* L265P and *CXCR4* mutations in around 90% and 25% respectively. *CXCR4*^{WHIM} mutations confer worse outcome, and are associated with larger BM involvement and less adenopathies. One hallmark and histological criteria for WM diagnosis is the presence of numerous mast cells (MC) segregating with tumor cells in BM. Even if MC have been shown to support lymphoplasmacytic cell growth, there is so far no demonstration of the prognostic impact of BM MC density in WM.

Aim. We proposed to investigate BM MC density by using sensitive and specific digital quantification, allowing the analysis of large BM tumor cell infiltrated cellular area, and to assess its clinical relevance in WM.

Methods. A total of 65 WM patients were investigated, including 54 pts at diagnosis and 11 at relapse. Allele specific PCR found *MYD88* L265P mutation in 32/35 pts (91%) and deep next-generation analysis found *CXCR4*^{WHIM} mutation in 7/35 (20%) pts. MC density was explored using a digital tool previously used for quantifying immune cells infiltrates on tumor tissues sections (Galon, Science 2006). Tryptase and CD20 immunohistochemisty stainings were done on contiguous sections of deparaffinized BM trephine biopsies. After numerization of each section, BM surface area was manually marked out, excluding bone framework and adipocytes, in order to limit subsequent analyses to hematopoietic tissue. We then defined optimal thresholds for horseradish peroxidase signal detection. MC were counted up with "Immunoscore module" (Definiens Developer XT), on the hematopoietic tissue of whole BM section : MC density was automately recorded as the number of tryptase positive cells per unit of selected bone marrow tissue surface area.

Results. MC density was found to be heterogeneous over BM, with a mean of 106 MC.mm^{-2} of hematopoietic tissue (Figure 1). Higher MC density (> 56 MC.mm^{-2}) was associated with

larger BM involvement by WM cells (BM deep infiltration (p=0.0352) and diffuse tumor pattern (p=0.0132)) and with less frequent hepatosplenic involvement (p=0.023). Higher MC



density was also associated with features of advanced disease such as anemia (<115g.L^{-1} ; p = 0.031) and thrombocytopenia (<100G.L^{-1} ; p=0.037). Furthermore, MC density was significantly correlated with ISSWM score (p=0.0003). Regarding outcome, patients with higher MC density had a shorter median OS (56.5 months *vs* non reached in patients with low MC density, p = 0.0004). In multivariate analysis, OS remains significantly shorter when high MC density, controlling on other predictive variables as age and ISSWM score (Figure 2).
Among the pts scheduled to receive a 1st line treatment for an active disase (n=43) and molecularly characterized (n=20), those with *CXCR4*^{WHIM} mutations had a significantly higher MC density (240.7 MC.mm^{-2} *vs* 90.9 MC.mm^{-2} for *CXCR4* WT pts ; p=0.0221).

Conclusion. By using specific digital tool on well-outlined hematopoietic tissue surface, MC density can be accurately measured in WM patients. High MC density is associated with aggressive features, poor clinical outcome and possibly with *CXCR4*^{WHIM} mutation.

Figure 1. MC density is heterogeneous

Figure 2. MC density impacts on overall survival