**Genomic landscape of IgM MGUS and stable versus progressive asymptomatic Waldenström macroglobulinemia patients**

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The transition from the "pre-malignant" IgM monoclonal gammopathy of undetermined significance (IgM-MGUS) and asymptomatic Waldenström's macroglobulinemia (aWM) to symptomatic WM (sWM) is driven by a multi-step process involving both clonal and microenvironmental changes. The genetic composition of each clone is likely to determine the risk of disease progression, however, novel genetic prognostic markers are yet to be discovered. Although there is data on the genomic aberrations in sWM a detailed genetic landscape of "pre-malignant" lesions is missing. The aim of this study is to describe the genomic alterations that are present in IgM-MGUS and aWM patients and provide reliable genomic features able to identify the patients that will experience progression in WM from the one that will remain stable.

We performed whole exome sequencing (WES) analysis on 166 samples from 88 patients with IgM monoclonal gammopathies including 10 serial samples. This cohort included 45 patients with IgM-MGUS, 21 with stable aWM (aWMst), 9 patients with progressive aWM (aWMpr) and 12 with symptomatic WM (sWM). In all patients with aWM that progressed to sWM (n=10), samples were collected at both the aymptomatic and symptomatic stage of the disease. All patients included in the analysis had matched tumor-normal samples.

We observed an increasing tumor mutation burden (total number of mutations per patient) through the stages of disease evolution: the median number of coding single nucleotide variants (SNVs) in IgM-MGUS patients was 28 (range 10-78), in aWMst was 51 (range (12-168), in aWMpr was 66 (range 52-129) and in sWM was 62 (range 10-137) (asWMst *vs* asWMpr $p<0.05$). The median number of coding Insertions-deletions (indels) in IgM-MGUS patients was 10 (range 3-35), in aWMst was 12 (range 3-27), in aWMpr was 15 (range 7-33) and in sWM was 16 (range 7-29) (asWMst *vs* asWMpr p=NS).

Patients with aWMpr were characterized by higher number of coding mutations in known driver genes as well as genes previously described in WM (n=5.7mutations/patient) with a median mutation density 1.32/Mb compared to aWMst patients (n=4.2 mutations/patient) with a median mutation density 1.02/Mb. Genes such as *KDM6A*, *ARID1B*, *CXCR4*, *TNFAIP3* and *CD79B* were more often mutated in the aWMpr group compared to the aWMst group (36% vs 28%).

Copy number (CN) analysis demonstrated that IgM-MGUS and aWMst patients had less CN aberrations compared to aWMpr and sWM patients (mean CNV events in IgM-MGUS:6.6, aWMst:6.4, aWMpr:8.3, sWM:10.6) which were mainly characterized by recurrent chromosomal abnormalities including del6q, del13, gain18 and gain3q.

The genomic landscape of the entire cohort of patients showed alterations in the nuclear factor-kB pathway (58% with *MYD88* and 8% with *TNFAIP3* mutations), 20% with alterations in the mitogen-activated protein kinase (MAPK) pathway (*MAP3K9*, *MAP3K10*, *NFKB1*), 16% in DNA repair pathway (*BCL2* and *ATM*) and 7% in ERK pathway (*MAPK1* and *EGFR*). *MYD88* was mutated in around 30% of IgM-MGUS, in 80% of aWMst and 100% in aWMpr and sWM patients.

We next analyzed serial samples from 9 patients sampled at the aWM and sWM stage. We observed evidence of clonal heterogeneity with linear evolution in most patients, with a branching evolution in 2 out 9 patients and an increase in the CNV profile in 3 out of 9 patients.

In conclusion, this is first study which focuses on the genomic profiling of IgM-MGUS and aWM patients and suggests that increasing mutation burden and specifically mutations in genes related to WM biology, could represent a potential biomarker for the identification of patients at high risk of progression.