Determining the mutational landscape of Waldenström Macroglobulinemia by liquid biopsy: results of the ECWM-2 trial

Philipp Nakov[1], Daniela Drandi[2], Lisa Marie Kaiser[3], D. Kaszynski[3], A. Grunenberg[4], J. Mark[3], E. Runge[3], Meletios-Athanasios C. Dimopoulos[5], Efstathios Kastritis[5], Tina Bagratuni[5], Christian Buske[3,4], Simone Ferrero[2], Christiane Pott[1], Mouhamad Khouja[1] on behalf of the European Consortium for Waldenström's Macroglobulinemia

[1]Second Medical Department, University Hospital Schleswig-Holstein, Campus Kiel, Germany

[2]Department of Molecular Biotechnology and Health Sciences, Hematology Division, University of Torino, 10126 Torino, Italy

[3]Institute of Experimental Cancer Research, University Hospital Ulm, 89081 Ulm, Germany

[4]Department of Hematology and Oncology, University Hospital Ulm, 89081 Ulm, Germany

[5]National and Kapodistrian University of Athens, Athens, Greece

Background

In Waldenström Macroglobulinemia (WM) genetic alterations such as *MYD88, CXCR4* and *TP53* mutations can affect treatment outcome in the era of BTK inhibitors, highlighting the importance of understanding the molecular landscape in this disease. Liquid biopsy and the analysis of cell-free DNA (cfDNA) allows for a minimally invasive approach for the diagnostic assessment and genotyping in lymphomas. Here, we aim to characterize the recurrent mutations in a treatment naïve cohort of patients with WM to determine the suitability of cfDNA for the initial diagnosis and genotyping of WM.

Methods:

A total of 38 plasma samples from a cohort of treatment naïve WM patients from the ECWM-2 study (EudraCT: 2017-004362-95) were analysed. Plasma cfDNA was extracted. The Euroclonality-NGS DNA Capture assay (EC-NDC, Univ8® Genomics, UK) was used for targeted capture-based next-generation sequencing. The data was analysed by ARResT/Interrogate (Bystry, Bioinformatics, 2017) with an adapted pipeline for targeted capture in cfDNA. cfDNA concentration was represented by number of haploid genomic equivalents per mL plasma (hGE/ml).

Single nucleotide variants (SNVs) in 72 genes, structural variants and immunoglobulin and T cell receptor rearrangements were targeted. *MYD88* mutations and *CXCR4* frameshift (FS) or nonsense (NS) mutations from amino acid position 308 to 352 were called with ≥3 unique reads at ≥0.1% variant allele frequency (VAF). Other SNVs were called with ≥3 unique reads at ≥1 % VAF. Benign mutations and germline variants were filtered out.

Results

All patients had detectable cfDNA (median 1813 hGE/ml, range 268- 3668 hGE/ml), however cfDNA levels were significantly lower in comparison with other lymphomas such as DLBCL. *MYD88*^{L265P} was detected in 35/38 samples (92%), with a median *MYD88*^{L265P} VAF of 5.5 % (range 0.1% to 44.2%). No other *MYD88* mutation was observed. *CXCR4* mutations were detected in 13/38 samples (34%) with a median VAF of 1.3% (range 0.1% to 8.2%). *CXCR4*^{S338X} was detected in 7/13 samples, while other FS mutations were found in 6/13 cases. No *MYD88* wildtype samples were *CXCR4* mutated.

Comparison of these data to results in bone marrow samples analysed by routine sequencing in the respective reference pathology labs, revealed a high congruence for *MYD88* detection of 35/38

(92%). Two of the 3 differing samples were positive in pathology and negative in cfDNA, while 1 sample was positive in cfDNA and negative in pathology. For *CXCR4* the congruence was 34/38 (89%), with 2 samples each being positive in one compartment and negative in the other.

Additional putative somatic mutations were identified in 25/38 (66%) patients, the most frequent mutations interestingly comprising CHIP associated mutations with *ARID1A* (16%), *KMT2D* (16%) and *DNMT3A* (13%) (Figure 1). *TP53* was mutated in 3/38 samples.

Summary

Minimally invasive (liquid biopsy-based) diagnostics by targeted capture sequencing using EC-NDC allows for the detection of clinically relevant genetic aberrations in WM and thus might present a powerful tool to monitor dynamic changes of the mutational landscape during treatment and at progression in prospective clinical trials. In addition, this technique identifies potential CHIP variants that warrants further investigation/validation.

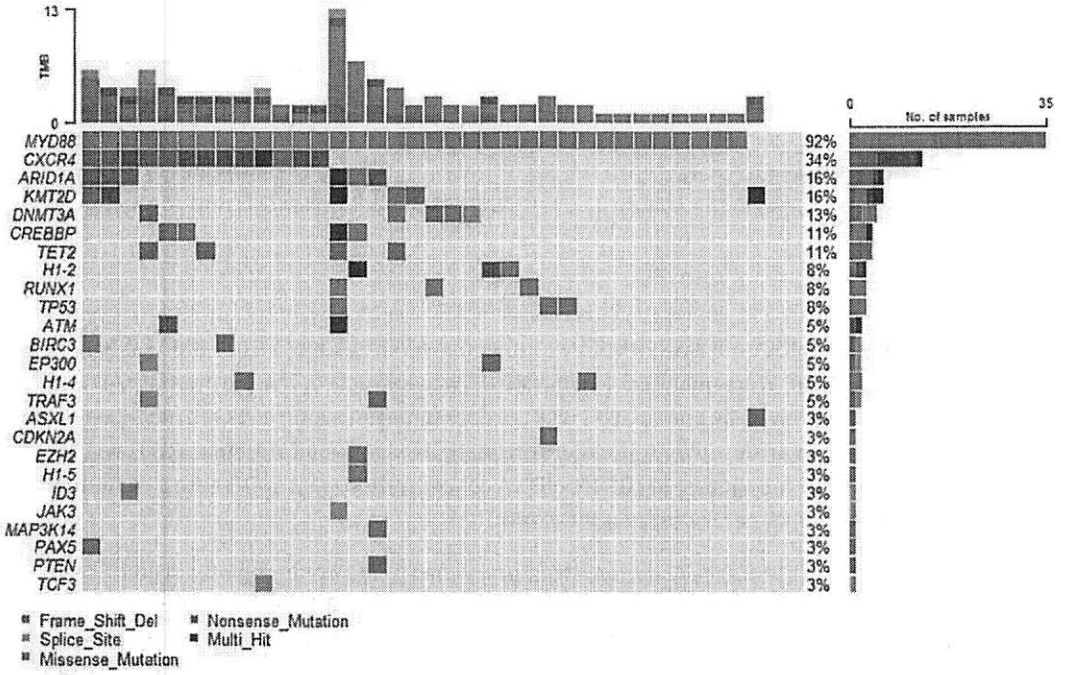

Figure 1 Targeted sequencing in 38 treatment naïve WM cases from cfDNA using the EuroClonality -NGS DNA Capture (EC-NDC) panel. Mutated genes (rows) for each patient (columns) in our cohort. The total amount of mutations as TMB per patient is visualized on top of the figure. On the right is the frequency of each mutation in our cohort.