# Epigenetic liquid biopsy for diagnosis and surveillance of Waldenström macroglobulinemia

**Ilana Fox-Fisher[1], Omer Weinstein[2], Sheina Piyanzin[1], Daniel Cohen[1], Benjamin Glaser[3], Ruth Shemer[1], Yuval Dor[1], Moshe E Gatt[2]**

[1] Department of Development and Cancer Research, The Hebrew University-Hadassah Medical School, Jerusalem, Israel.

[2]Department of Hematology, Hadassah Medical Center, Faculty of Medicine, The Hebrew University Jerusalem, Israel.

[3]Endocrinology and Metabolism Service, Hadassah Hebrew University Medical Center, Jerusalem, Israel.

## Background:

Waldenström macroglobulinemia (WM) is a rare lymphoproliferative neoplasm characterized by malignant clonal mature lymphocyte cells at different stages of development. Premalignant conditions like IgM MGUS and Smoldering WM (SWM) are closely monitored due to their high risk of progression and the need for early diagnosis and timely treatment.

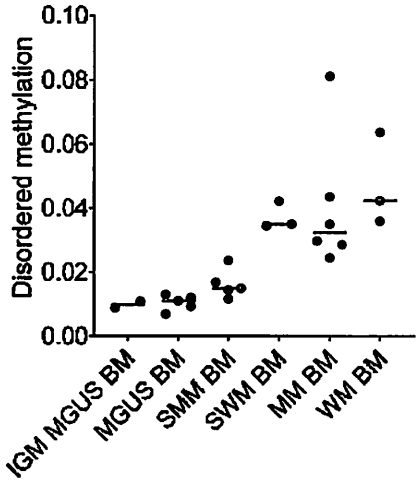
When cells die, they release small fragments of cell-free DNA (cfDNA) into the bloodstream. cfDNA is a hallmark of cell turnover[2], and may be highly informative for cancer detection[3]. Moreover, methylation disruption is a well-documented epigenetic alteration in various cancer types[4]. B-cell differentiation is particularly regulated by epigenetic changes. Each cell type has its unique methylation pattern identity making it an eligible biomarker for cell identification. Previous studies have classified WM patients into two subgroups based on global DNA methylation patterns: plasma cell-like WM and memory B-cell-like WM, with the latter being more prevalent[1].

We developed a PCR-based highly specific methylation pattern analysis for B cells and plasma cells and also recognized specific methylation disruption patterns in the evolution of MGUS to malignant states. We subsequently hypothesized that different B cell/ plasma cell type-specific methylation-based markers and cancer-related methylation disturbances can serve as biomarkers for the surveillance and diagnosis of WM.

**Methods**: Different B-cell states, including naïve B-cells, memory B-cells, and plasma cells, were sorted from blood and BM of healthy individuals and plasma cell disorders. These samples underwent deep whole-genome bisulfite sequencing. Utilizing a comprehensive reference atlas[5] and B-cell type methylomes, we developed a twenty loci simplified and specific PCR-based assay for the sensitive detection of plasma cells, mature B-cells, and lymphoplasmacytic cancer-specific methylation changes in cfDNA. The assay was applied to whole BM samples and cfDNA from plasma samples of patients with IgM MGUS, MGUS, SMM, SWM, Multiple myeloma (MM), and WM.

**Results**: We found that WM, compared to MM, exhibits a more dominant B-cell methylation phenotype in BM, whereas MM shows a dominant plasma cell methylation pattern. Interestingly, cancer-specific methylation disturbance patterns are higher in malignant forms (WM and MM) and lower in pre-malignant forms (Figure). Notably, SWM exhibits high methylation disturbance, indicating its possible proximity to malignant transformation. When applying the assay to cfDNA of patients with WM compared to other malignant and premalignant forms we observed elevated levels of both plasma cell, B-cell methylation-based markers and cancer-specific markers.

**Conclusion:** normal B-cell subtypes, plasma cells and cancer-specific cfDNA methylation patterns are promising biomarkers for diagnosing and surveilling WM patients. This approach can potentially identify patients at risk of progressing to WM from pre-malignant states and may serve as a practical tool for assessing individual patients' risk.



1. Roos-Weil D, Giacopelli B, Armand M, et al. Identification of 2 DNA methylation subtypes of waldenström macroglobulinemia with plasma and memory b-cell features. *Blood*. 2020;136:585–595.

2. Lehmann-Werman R, Neiman D, Zemmour H, et al. Identification of tissue-specific cell death using methylation patterns of circulating DNA. *Proc Natl Acad Sci U S A*. 2016;113:E1826–E1834.

3. Jamshidi A, Liu MC, Klein EA, et al. Evaluation of cell-free DNA approaches for multi-cancer early detection. *Cancer Cell*. 2022;40:1537-1549.e12.

4. Landau DA, Clement K, Ziller MJ, et al. Locally Disordered Methylation Forms the Basis of Intratumor Methylome Variation in Chronic Lymphocytic Leukemia. *Cancer Cell*. 2014;26:813–825.

5. Loyfer N, Magenheim J, Peretz A, et al. A DNA methylation atlas of normal human cell types. *Nat 2023*. 2023;1–10.