Parallel Testing of Liquid Biopsy (ctDNA) and Tissue Biopsy Samples Yields Higher Frequency of *EZH2* Mutations in Follicular Lymphoma

Ákos Nagy[1], Bence Bátai[1], Stefánia Gróf[1], Laura Kiss[1], Alexandra Balogh[2], Gábor Mikala[3], Péter Attila Király[4], Ádám Jóna[5], Judit Demeter[6], Tamás Masszi[2], Tamás Schneider[4], András Masszi[4], Csaba Bödör[1]

[1]HCEMM-SE Molecular Oncohematology Research Group, Department of Pathology and Experimental Cancer Research, Semmelweis University, Budapest, Hungary. [2]Department of Internal Medicine and Hematology, Semmelweis University, Budapest, Hungary. [3]National Institute of Hematology and Infectious Diseases, Central Hospital of Southern Pest, Budapest, Hungary. [4]National Institute of Oncology, Budapest, Hungary. [5]Hematology Division, Department of Internal Medicine, University of Debrecen, Debrecen, Hungary. [6]Department of Internal Medicine and Oncology, Semmelweis University, Budapest, Hungary

Introduction

- The genetic background of follicular lymphoma (FL) is well characterized.
- Almost all cases harbor mutations in the genes of the epigenetic regulatory system.
- The third most common epigenetic mutations in this disease are the gain of function mutations in the *EZH2* gene.
- There are 7 different *EZH2* mutations in FL, located in exon16 (Y646X) and exon18 (A682G, A692V).
- These mutations are present in 25% of FL patients, and emerge as targets for small molecule inhibitors (Figure 1.).
- FL is characterized by a profound morphological and molecular spatial heterogeneity.
- Spatial heterogeneity can be investigated with the minimal invasive analysis of blood plasma derived circulating tumor DNA (liquid biopsy, LB).

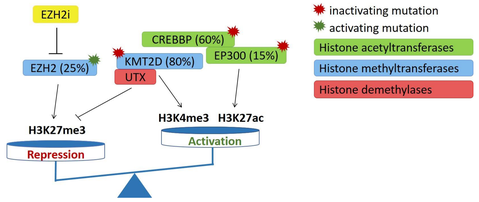


Figure 1. *Function of EZH2 protein in epigenetic regulation. Mutant EZH2 hypermetilates the H3K27 residue causing transcriptional repression in target genes.*

Aims

- Develop a liquid biopsy-based *EZH2* mutation test.
- Test the mutation frequency in a Hungarian FL patient cohort.
- Longitudinally monitor the mutations in patients receiving immunochemotherapy (ICT).

Patients and methods

- Diagnostic and/or relapse tissue biopsy samples (TB) and paired LB samples from 117 FL patients.
- Longitudinal LB samples collected before treatment cycles from 24 patients receiving ICT.
- *EZH2* mutation status was tested with an in-house-designed multiplex droplet digital PCR assay (Bio-Rad Laboratories, USA).

Results I.

- Forty-five patient (39%) proved to be *EZH2* mutant in at least one investigated sample (Figure 2A).
- Six (5%) and nine (8%) patients only harbored an *EZH2* mutation in the paired TB or LB sample, respectively (Figure 2B).
- *EZH2* mutation status class-switch between diagnosis and relapse was observed in 10 out of 50 relapsing patients (Figure 2C).

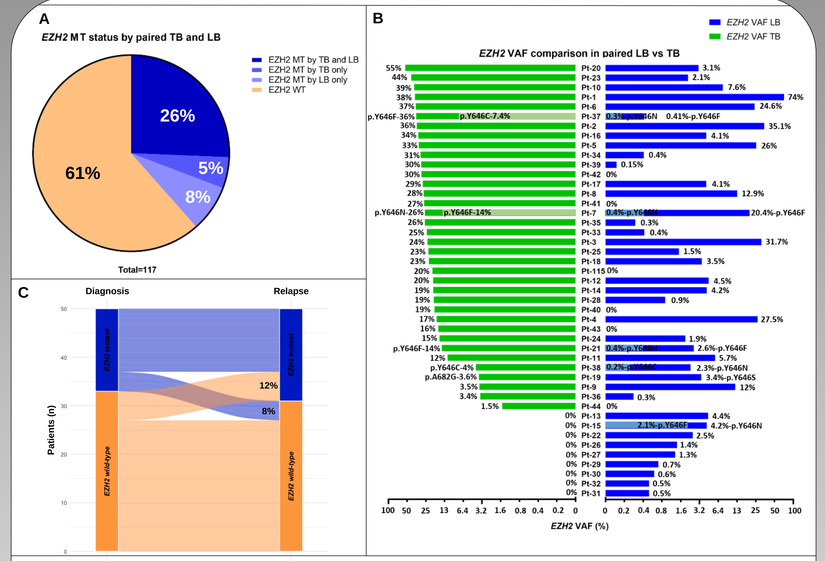


Figure 2. *Spatial (A and B) and temporal (C) heterogeneity in the EZH2 mutation status in FL.*

Results II.

- Grade III/B and transformed FL (tFL) histology was significantly associated with mutant *EZH2* status in TB (Figure 3A).
- Patients with Grade III/B and tFL had significantly higher *EZH2* variant allele frequency (VAF) in LB samples (Figure 3B).
- Bone marrow infiltration was significantly more frequent in *EZH2* wild-type patients (Figure 3C).
- A trend could be observed between LB *EZH2* VAF and clinical stage (Figure 3D).
- *EZH2* mutation status and LB *EZH2* VAF did not correlate with other clinical indexes (FLIPI, bulky disease, SUV max).

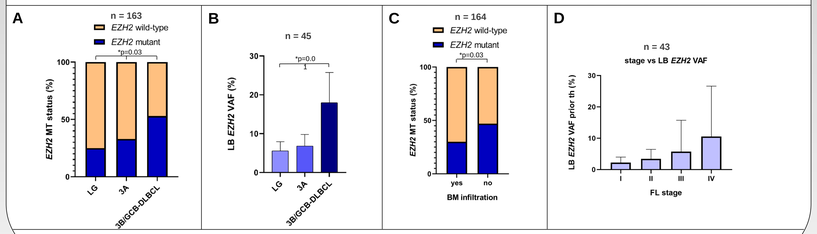


Figure 3. *Correlation between EZH2 mutation status and LB EZH2 VAF with histological and clinical parameters.*

Results III.

- In responding patients LB *EZH2* VAF significantly decreased or eliminated after one or two cycles of ICT (Figure 4A).
- *EZH2* VAF did not change in clinically non-responding patients (Figure 4B).
- Prompt improvement and drop in the LB *EZH2* VAF level was registered in Pt-6 after changing the therapy to R-CHOP.

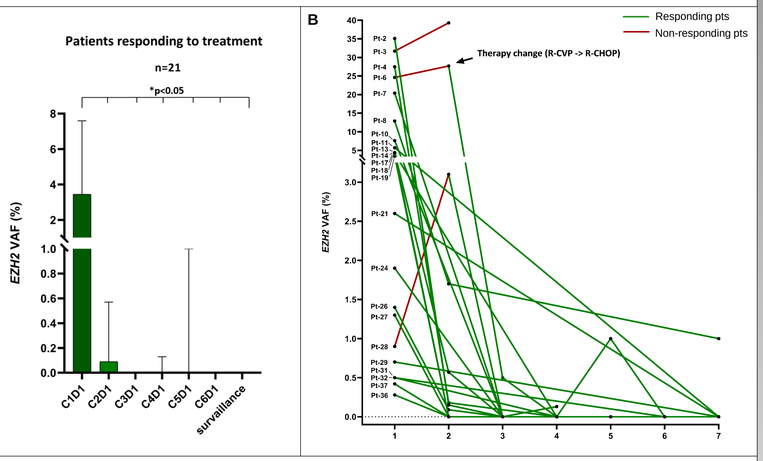


Figure 4. *Therapy monitoring using LB EZH2 mutation analysis.*

Conclusions

- *EZH2* mutations are more frequent in FL compared to historical data (41% vs 25%) if analyzed spatially and temporally.
- Mutant *EZH2* status is associated with higher TB grade and decreased bone marrow infiltration rate.
- Elevated LB *EZH2* VAF levels are associated with aggressive histology and advanced stage disease.
- Rapid elimination of *EZH2* mutations can be detected in the LB specimens in patients responding to therapy.

Acknowledgements

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