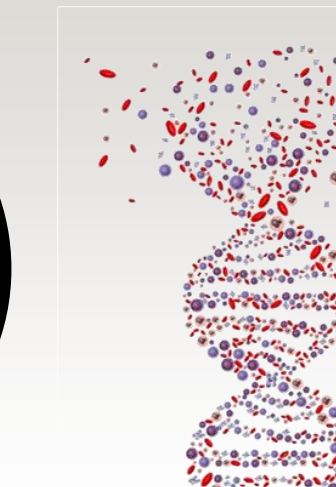




Non-Invasive Quantitative Analysis of *EZH2* Mutations in Follicular Lymphoma

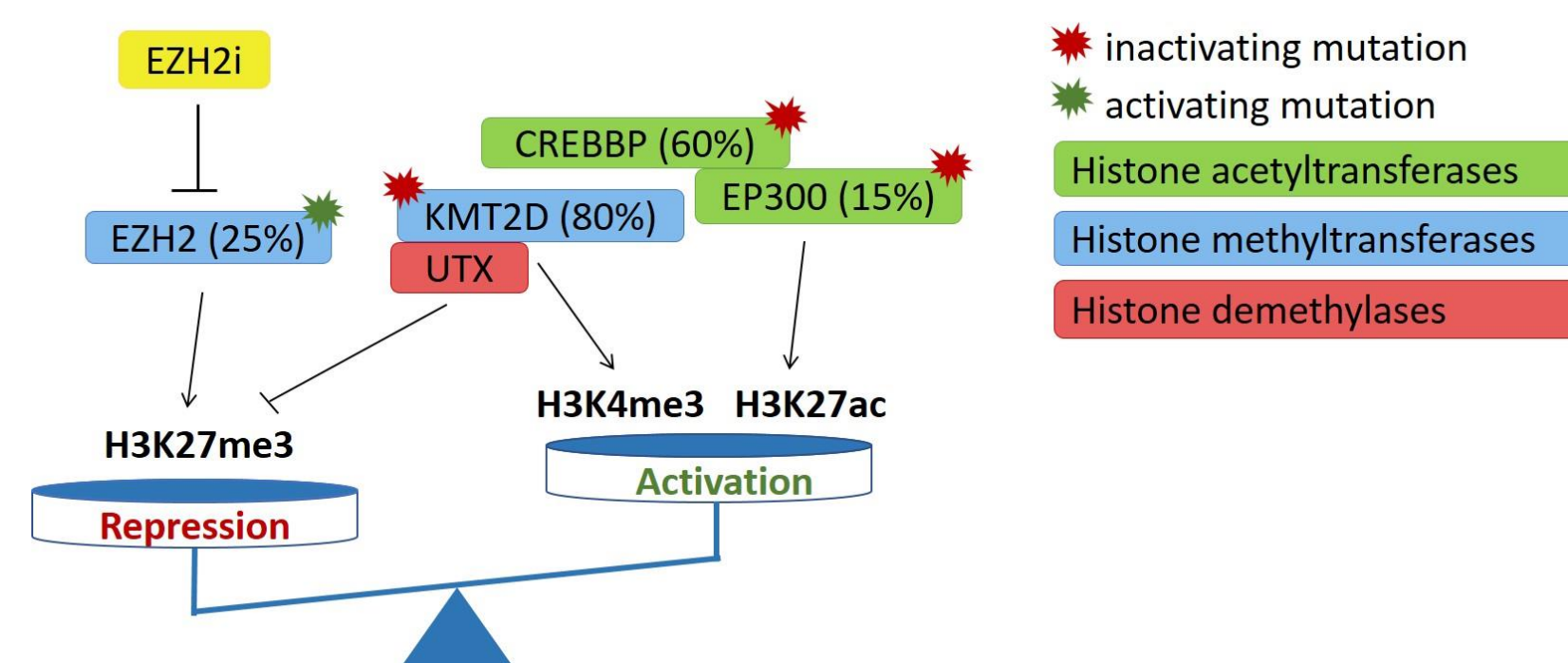
Ákos Nagy¹, Bence Báta¹, Alexandra Balogh², Sarolta Illés², Gábor Mikala³, Bálint Várnai⁴, Tamás Schneider⁴, András Masszi⁴, Ádám Jóna⁵, Tamás Masszi², Csaba Bödör¹

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



Introduction

- Follicular lymphoma (FL) has an **overall survival** of approximately **15 years**, and is characterized by a **remitting, relapsing** disease course.
- Routine **PET-CT** based monitoring has **limited** specificity.
- Molecular analysis of tumor derived **circulating DNA (ctDNA)**, is a promising **minimally invasive, radiation-free** disease monitoring tool for patients suffering from malignancies.
- Gain of function mutations in the *EZH2* gene are present in 25% of FL patients**, and emerge as targets for **small molecule inhibitors** (Figure 1.).
- There are 7 different *EZH2* mutations in FL, located in exon16 (Y646X) and exon18 (A682G, A692V).



Aims

- Develop a **liquid biopsy-based *EZH2* mutation test**.
- Test the feasibility of ***EZH2* mutation analysis in ctDNA** of FL patients.
- Monitor the mutations** in patients receiving immunochemotherapy.

Patients and methods

- Longitudinal plasma samples (n=98) from **46 *EZH2* mutant FL patients** (at the time of sample collection 32 patients presented with active FL (diagnosis or relapse), meanwhile 14 patients were at remission).
- ctDNA isolation** using the QIAamp Circulating Nucleic Acid Kit (Qiagen, Germany) and **digital droplet PCR** (ddPCR, BioRad, USA) analysis of *EZH2* mutation hotspots previously identified in tumour tissue samples using an in-house designed **multiplex *EZH2* detection assay**.

Results I.

- Among the 46 patients, 39 responded to treatment (85%); meanwhile, 7 patients did not (15%).
- We detected an ***EZH2* mutation in 91% of the samples collected prior to treatment (21/23)**.
- Samples collected during therapy from **patients not responding to treatment proved to be *EZH2* mutated in 75% (6/8)**; meanwhile **21% of the samples were positive from responding patients (8/39)**.
- Only one sample (1/28) was *EZH2* mutant among samples collected during remission.
- The median **sensitivity** of our ddPCR approach was **0.14%**, with ***EZH2* variant allele frequencies (VAF)** ranging between **0.14 and 74%**.

Results II.

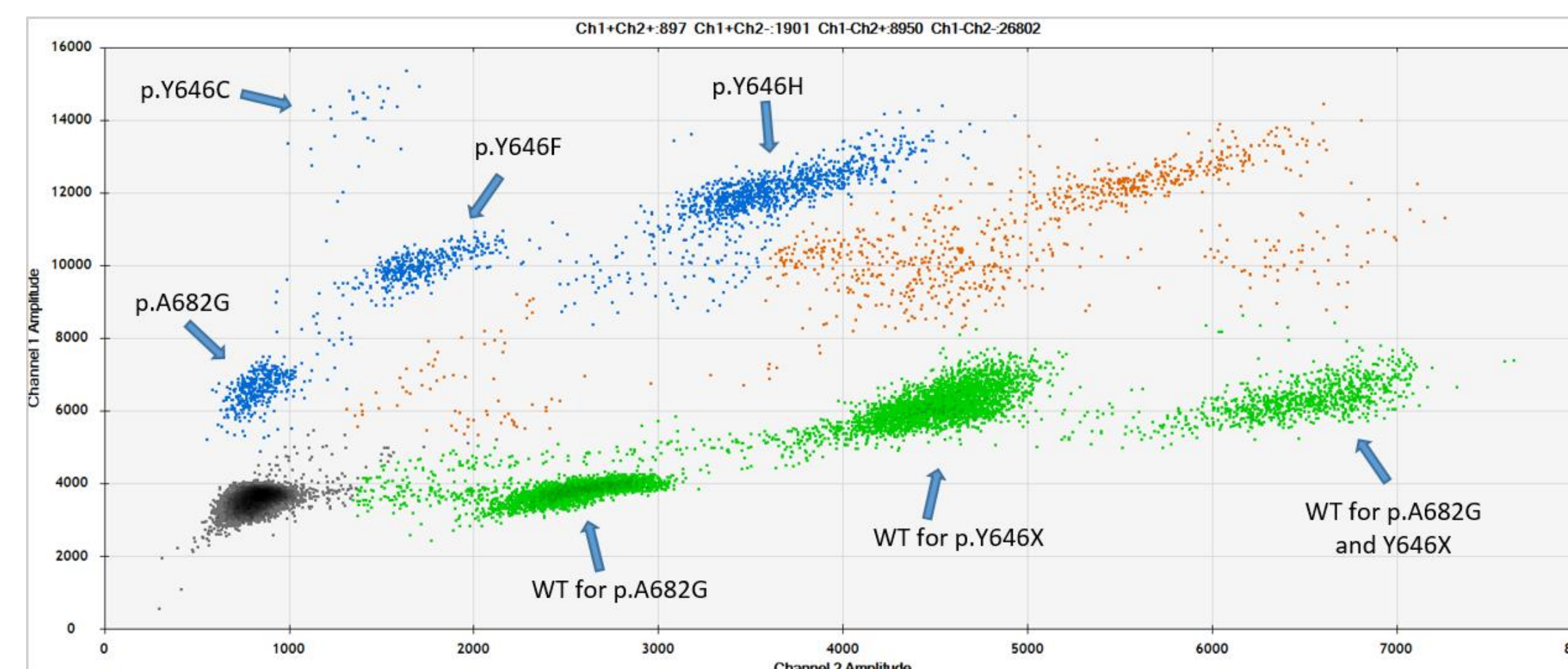


Figure 2. The in-house designed multiplex *EZH2* mutation detection assay can detect and differentiate up to four distinct *EZH2* mutations using only one PCR reaction, therefore all *EZH2* mutations can be detected in two PCR reactions. The multiplexing approach requires less DNA template, hence it is applicable for testing liquid biopsy samples with low DNA amount.

Results III.

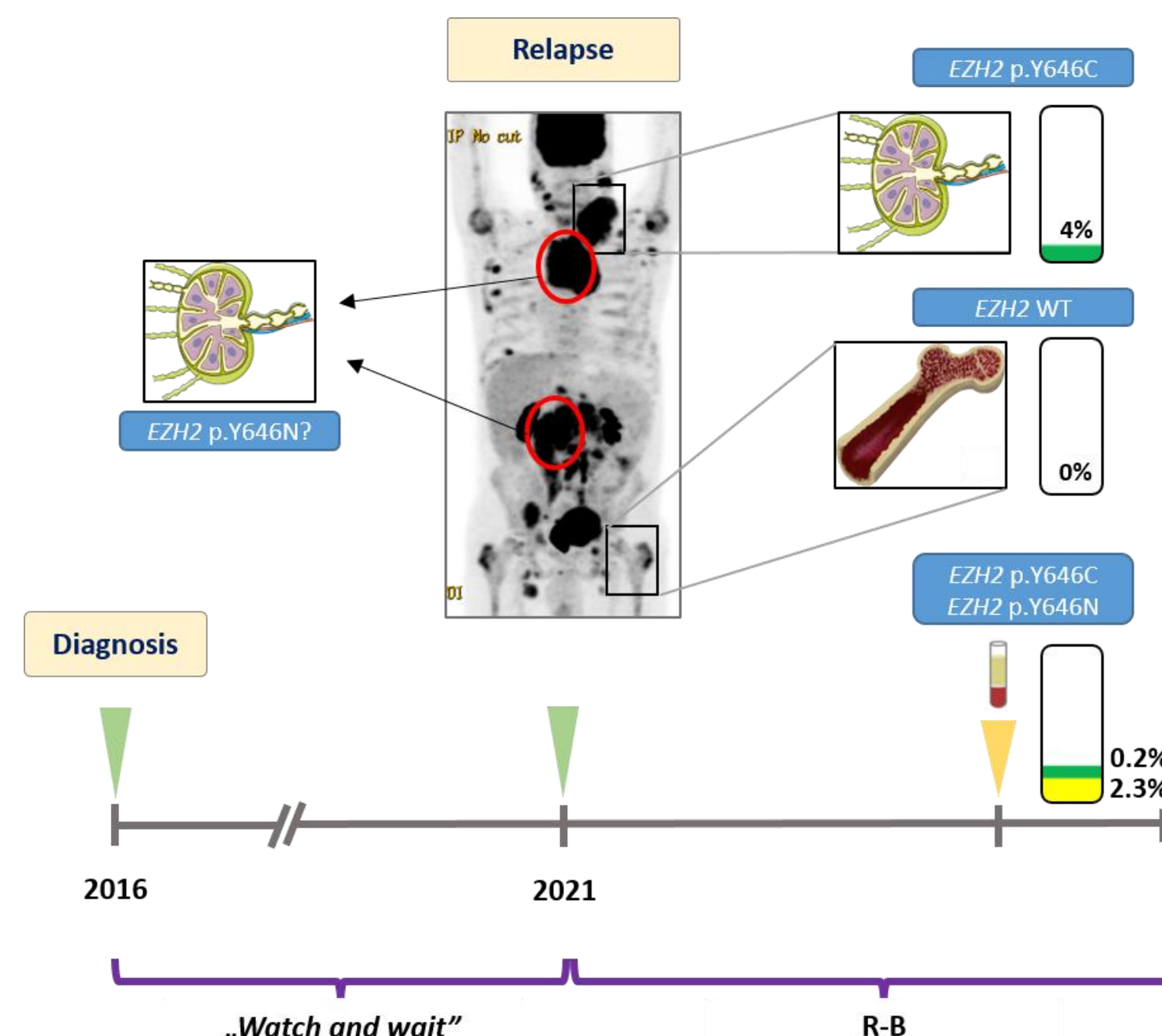


Figure 3. Case #1 was diagnosed with *EZH2* wild-type FL in 2016. The patient relapsed in 2021 and an *EZH2* mutant (p.Y646C) low-grade FL was diagnosed in a cervical lymph node. Interestingly, in the pre-treatment plasma sample two *EZH2* mutations were found (p.Y646C and p.Y646N). The Y646N mutation was not present in the cervical lymph node, therefore it originated from an unbiopsied area (e.g. from the retroperitoneal region). This case shows that liquid biopsy based mutation testing can capture spatial heterogeneity in FL.

Results IV.

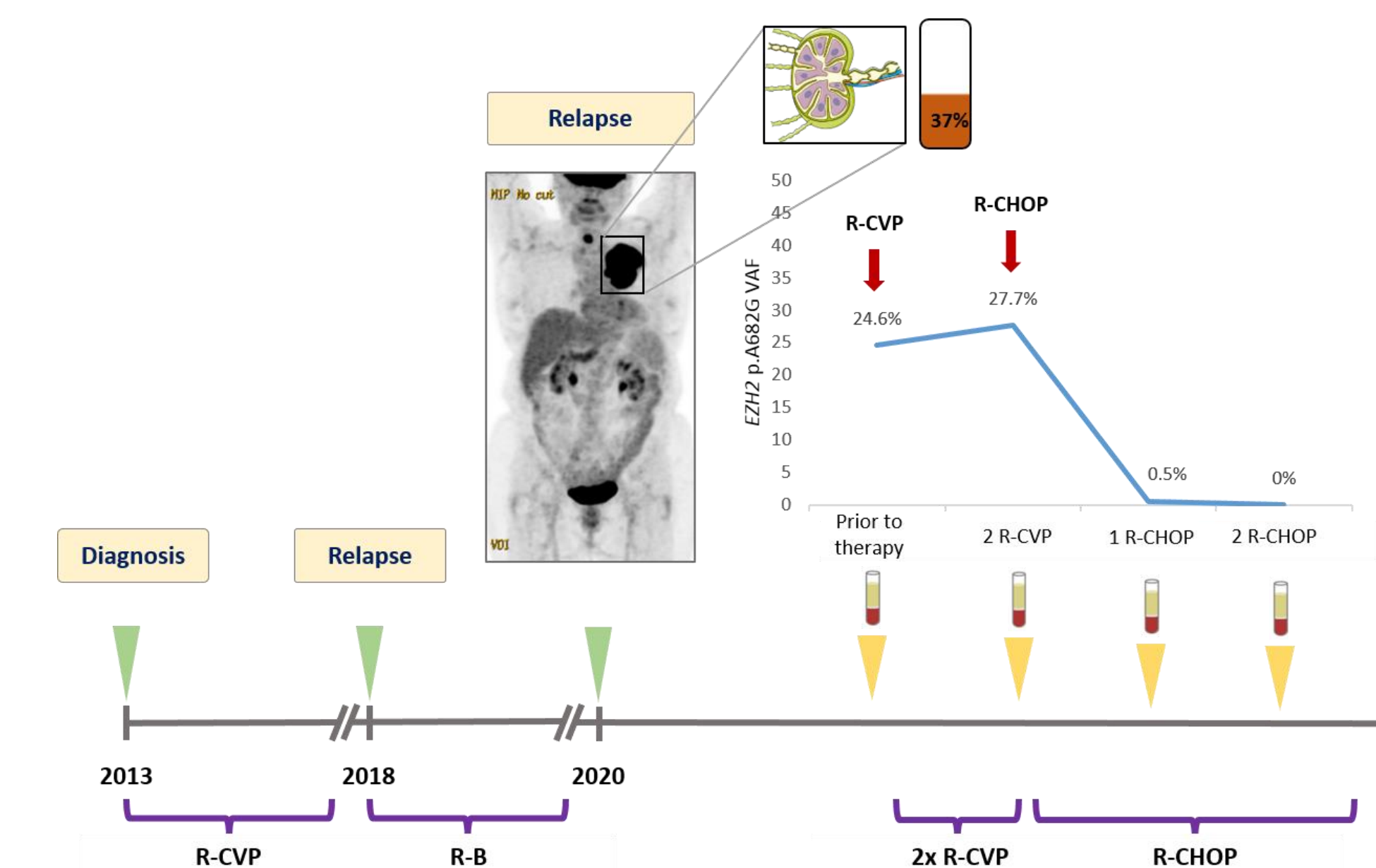


Figure 4. Case #2 was diagnosed in 2013. At the time of second relapse an *EZH2* (p.A682G) mutation was found in an enlarged mediastinal lymph node. The *EZH2* mutation was also present in the pre-treatment plasma sample. The patient did not respond to two cycles of R-CVP and paralelly, the *EZH2* mutation persisted in the plasma. Upgrading the therapy to the more potent R-CHOP regimen resulted in rapid decrease of *EZH2* VAF after one cyclce and complete elimination after two cycles. This case shows that our method is feasible to monitor treatment efficacy in *EZH2* mutant FL.

Conclusions

- EZH2* mutations can be identified in the plasma** of patients with FL using a multiplex ddPCR assay.
- Liquid biopsy offers a **sensitive, radiation-free, minimally invasive** monitoring method for *EZH2*-mutant FL patients.
- The variant allele frequency of mutations can be used in the **monitoring of treatment response**.
- ctDNA analysis is also useful for **detecting spatial heterogeneity**.

Acknowledgements

Prepared with the support of the H2020 (SGA No. 739593) grant of the European Union, the Doctoral Student Scholarship (NKFIH KDP-1022882) of the Co-operative Doctoral Program of the Ministry of Innovation and Technology, the Semmelweis 250+ Excellence PhD Scholarship from the EFOP-3.6.3-VEKOP-16-2017-00009 grant, the Semmelweis Scientific and Innovation fund (STIA-KFI 618306 69322), the Hungarian National Research, Development and Innovation Office (NKFIH K21-137948), and the Higher Education Institutional Excellence Program of the Ministry of Human Capacities in Hungary.