

Mutations at codon 641 of exon 16 of EZH2 in circulating and tumor DNA: a biomarker for relapse in patients with Diffuse Large B-cell Lymphoma treated with the R-CHOP scheme.

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Abstract

Epigenetic regulation through EZH2 constitutes a hallmark in Diffuse large B cell lymphoma (DLBCL). Mutations at 16 exon EZH2 have a prognostic role in this entity. However, the availability of tumor samples may be limited. Therefore, more accessible samples, as circulating DNA in peripheral blood (ctDNA), may be helpful for such purposes.

Background

Diffuse large B cell lymphoma constitutes the most common of all aggressive lymphomas, and has been traditionally classified according with the cell of origin as germinal-center (GC) and non-GC¹. The presences of *EZH2* mutations have been recently implicated in the prognosis of DLBCL.

The mutation at Y646 aminoacid is recurrently in up to 40 % of B-cell lymphomas, and particularly in 13-22 % of DLBCL ².

The circulating tumor DNA (ctDNA) has been used as a marker for identification of tumor biology, and predict long-term outcome in other malignancies, mainly in solid tumors³.

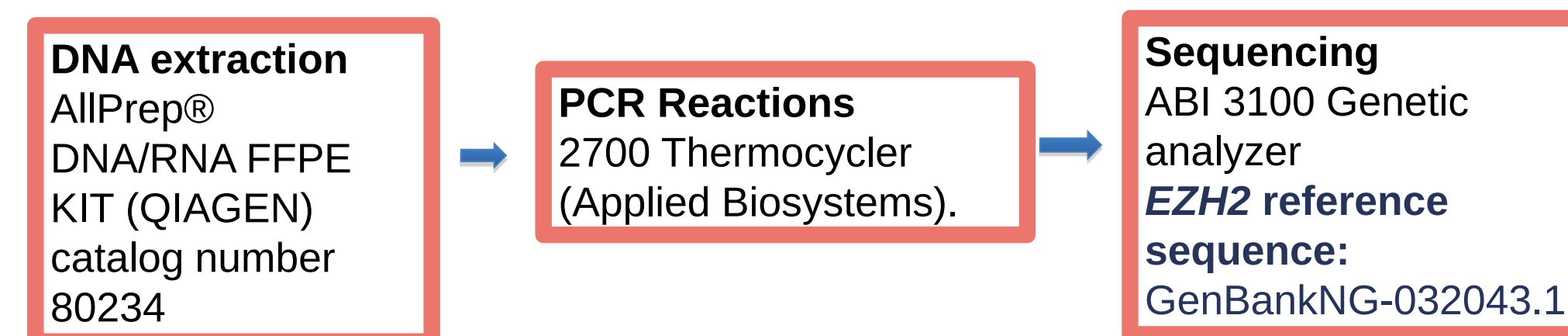
Objective

To evaluate the exon 16 *EZH2* mutations in ctDNA and tumor DNA as a biomarker and its impact on the relapse of patients with DLBCL treated with the R-CHOP regimen. DESIGN: Cohort of previously untreated DLBCL patients between 2016 and 2020.

Materials & Methods

Cohort study of untreated patients with DLBCL, attended at a single national reference centre. Clinical variables: Age, IPI score, bulky disease, clinical stage, serum albumin, LDH and B2-mycroglobuline levels, and ECOG.

Histopathologic variables were GC versus non-GC. All patients were treated with six cycles of standard RCHOP. Clinical response was evaluated by PET-CT.



Protocol was approved by IRB: CEI/966/15

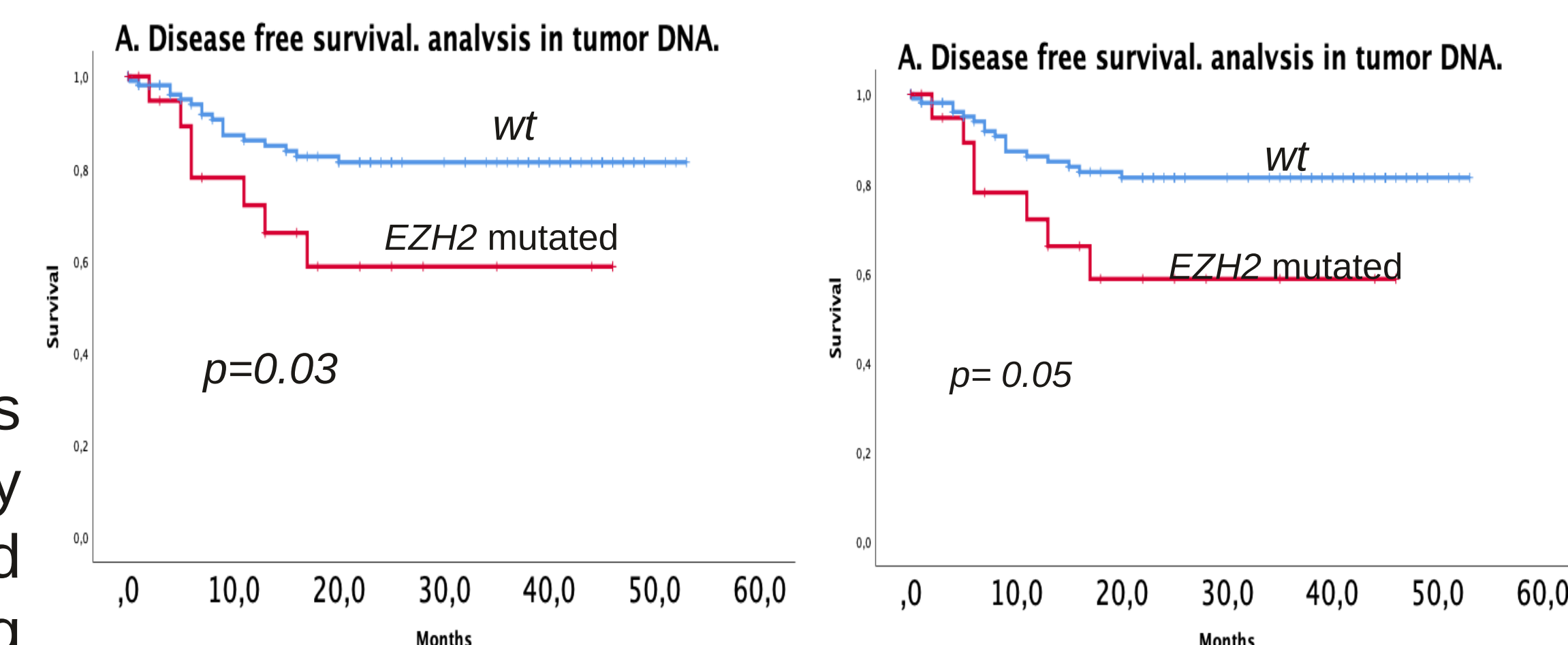
Statistics: Descriptive analysis. Tumor DNA was defined as gold of reference. Sensitivity, specificity of ctDNA was calculated. the Kaplan-Meier method was used to construct survival curves and the Log rank test was used for comparisons.

Results

138 patients were included. Approximately half of the **patients** were male (50.7%), had an ECOG $\geq$ 2: (49.3%), were in the high-risk group, according to the IPI index (43.5%). Most (69.6 %) were in clinical stages III-IV: 69.6%. The mean follow-up was 45 months.

Exon 16 mutations were identified in 20 tumor samples and 11 ctDNA samples. After considering the tumor samples as the reference standard, the sensitivity of ctDNA was 47% and specificity of 99% to diagnose exon 16 *EZH2* mutations.

Mutations had a negative impact on the event-free survival, either if they were analyzed on tumor samples (Median 38.3 vs. 43 months, p= 0.03) or ctDNA (35 vs. 40 months, p=0.05)



Summary

The presence of exon 16 EZH2 mutations may be a biomarker that impacts on the relapse of patients with DLBCL. However, a more sensitive technique, as digital PCR is required to use this biomarker in ctDNA.

Discussion

Circulating DNA is a non-invasive, real-time, tumor-specific biomarker that reliably reflects the comprehensive tumor genetic profile, which is why it is very promising in individualized practice, including for precise diagnosis and prognosis, response monitoring. and detection of relapse in patients with diffuse large B-cell lymphomas.²

The determination of ct-DNA is a biomarker that was evaluated as an impact factor on relapse both in tumor DNA with a p-value of 0.016 and in peripheral blood ct-DNA with a p-value of 0.05, with a sensitivity of 47% and specificity of 99%.

References

- 1.Swerdlow SH, et al. WHO classification of tumors of haematopoietic and lymphoid tissues. 4th ed. Lyon: IARC Press; 2016
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- 3.Roschewski M, et al, Circulating DNA Lancet Oncol. 2015 May;16(5):e199. PMID: 25842160.