Cukurova Medical Journal
ÇUKUROVA ÜNİVERSİTESİ TIP FAKÜLTESİ

Cukurova Med J 2026;51(3):979-990
DOI: 10.17826/cumj.2001681

RESEARCH

EZH2 expression in diffuse large B-cell lymphoma: clinicopathological associations and relationship with survival

Diffüz büyük B hücreli lenfomada EZH2 ekspresyonu: klinikopatolojik özellikler ve sağkalım ile ilişkisi

Mehmet Mutlu Kıdı[1], Mehmet Türker[2], Sedat Biter[3], Melek Ergin[4], Tuğba Toyran[4], Tolga Köşeci[4], Hatice Asoğlu[4], Yasemin Aydınalp Camadan[5], İsmail Oğuz Kara[4], Berksoy Şahin[4], Ertuğrul Bayram[4]

[1]Seyhan State Hospital, Adana, Türkiye
[2]Adana Training and Research Hospital, Adana, Türkiye
[3]Necip Fazıl City Hospital, Kahramanmaraş, Türkiye
[4]Çukurova University, Adana, Türkiye
[5]Şanlıurfa Training and Research Hospital, Şanlıurfa, Türkiye

Abstract

Purpose: Enhancer of zeste homolog 2 (EZH2) is an epigenetic regulator implicated in the pathogenesis of diffuse large B-cell lymphoma, but the prognostic value of its protein expression is uncertain. We evaluated EZH2 expression and its associations with clinicopathological features and survival.

Materials and Methods: We retrospectively studied 50 patients with diffuse large B-cell lymphoma treated with rituximab-based immunochemotherapy. EZH2 expression was assessed by immunohistochemistry using an H-score and classified as high (≥150) or low (<150). Overall and progression-free survival were analyzed with Kaplan–Meier estimates and Cox regression.

Results: H-scores ranged from 0 to 270 (median 60), and high EZH2 expression was present in 14 of 50 patients (28%). A non-germinal-center cell of origin was more frequent among EZH2-high than EZH2-low tumors (71.4% versus 33.3%; odds ratio 5.00, 95% confidence interval 1.30–19.30); no other clinicopathological variable differed between the groups. After a median follow-up of 73.7 months, 25 patients (50%) had died and 30 (60%) had relapsed. Median overall survival was 96.8 versus 79.5 months for EZH2-high and EZH2-low tumors (hazard ratio 1.14, 95% confidence interval 0.47–2.76), and median progression-free survival 39.9 versus 49.2 months (hazard ratio 1.28, 95% confidence interval 0.58–2.82). Only the International Prognostic Index independently predicted

Öz

Amaç: Enhancer of zeste homolog 2 (EZH2), diffüz büyük B hücreli lenfomanın patogenezinde rol oynadığı düşünülen epigenetik bir düzenleyicidir; ancak protein ekspresyonunun prognostik değeri belirsizdir. Bu çalışmada EZH2 ekspresyonunu ve bunun klinikopatolojik özellikler ile sağkalımla ilişkilerini değerlendirdik.

Gereç ve Yöntem: Rituksimab temelli immünokemoterapi ile tedavi edilen 50 diffüz büyük B hücreli lenfoma hastasını retrospektif olarak inceledik. EZH2 ekspresyonu, immünohistokimya ile H-skoru kullanılarak değerlendirildi ve yüksek (≥150) veya düşük (<150) olarak sınıflandırıldı. Genel sağkalım ve progresyonsuz sağkalım, Kaplan–Meier kestirimleri ve Cox regresyonu kullanılarak analiz edildi.

Bulgular: H-skorları 0 ile 270 arasındaydı (medyan 60) ve yüksek EZH2 ekspresyonu 50 hastanın 14'ünde (%28) saptandı. Germinal merkez dışı köken hücresi EZH2 yüksek tümörlerde daha sıktı (%71,4'e karşı %33,3; olasılık oranı 5,00; %95 güven aralığı 1,30–19,30); diğer klinikopatolojik değişkenler gruplar arasında farklılık göstermedi. Medyan 73,7 aylık takip sonrasında 25 hasta (%50) ölmüş, 30 hastada (%60) nüks gelişmişti. Medyan genel sağkalım EZH2 yüksek ve düşük tümörlerde 96,8'e karşı 79,5 ay (tehlike oranı 1,14; %95 güven aralığı 0,47–2,76), medyan progresyonsuz sağkalım ise 39,9'a karşı 49,2 aydı (tehlike oranı 1,28; %95 güven aralığı 0,58–2,82). Genel sağkalımı bağımsız olarak öngören tek değişken

Address for Correspondence: Mehmet Mutlu Kıdı, Seyhan State Hospital, Department of Medical Oncology, Adana, Türkiye E-mail: mehmetmutlu_01@hotmail.com
Received: 27.07.2026 Accepted: 11.09.2026

overall survival (hazard ratio 2.83, 95% confidence interval 1.11–7.19).
Conclusion: EZH2 overexpression was more frequent in non-germinal-center tumors but was not significantly associated with survival in this rituximab-treated cohort, in which the International Prognostic Index remained the dominant prognostic factor. These findings should be regarded as hypothesis-generating.
Keywords:. Diffuse large B-cell lymphoma, EZH2, immunohistochemistry, prognosis, survival

Uluslararası Prognostik İndeks idi (tehlike oranı 2,83; %95 güven aralığı 1,11–7,19).
Sonuç: EZH2 aşırı ekspresyonu germinal merkez dışı tümörlerde daha sık görüldü; ancak rituksimab ile tedavi edilen bu kohortta sağkalımla anlamlı ilişki göstermedi ve Uluslararası Prognostik İndeks baskın prognostik faktör olmayı sürdürdü. Bu bulgular hipotez oluşturucu nitelikte değerlendirilmelidir.
Anahtar kelimeler: Diffüz büyük B hücreli lenfoma, EZH2, immünohistokimya, prognoz, sağkalım

INTRODUCTION

Diffuse large B-cell lymphoma (DLBCL) is the most common aggressive non-Hodgkin lymphoma and constitutes a clinically and biologically heterogeneous group of neoplasms in the current World Health Organization classification[1]. Gene-expression profiling distinguishes germinal-center B-cell-like (GCB) and activated B-cell-like subtypes with different outcomes[2], and subsequent studies have further refined the genetic landscape of the disease[3]. In routine practice, the cell of origin is approximated by immunohistochemistry-based algorithms[4]. Most patients are treated with rituximab combined with cyclophosphamide, doxorubicin, vincristine, and prednisone (R-CHOP)[5], and clinical risk is estimated with the International Prognostic Index (IPI) and its rituximab-era revision, the NCCN-IPI[6,7]. Nevertheless, a substantial proportion of patients relapse or are refractory to first-line therapy, and additional biomarkers that refine prognosis are needed.

Enhancer of zeste homolog 2 (EZH2) is the catalytic subunit of polycomb repressive complex 2 and mediates trimethylation of histone H3 at lysine 27 (H3K27me3), a repressive chromatin mark[8]. Recurrent gain-of-function mutations at codon Y641 of *EZH2* occur predominantly in GCB-DLBCL and follicular lymphoma[9] and are enriched in *BCL2*-rearranged germinal-center lymphomas[10]. EZH2 is required for germinal-center formation, and its deregulation contributes to lymphomagenesis[11].

Because of this biology, EZH2 has been investigated as a prognostic biomarker in DLBCL, with conflicting results. High EZH2 or H3K27me3 expression has been associated with inferior outcome[12], including when co-expressed with BCL2[13], whereas other studies reported a favorable association in R-CHOP-treated patients[14] or no independent prognostic value[15,16].

Two features of this literature limit its interpretation. First, mutation and protein expression have often been treated as interchangeable, although activating mutations are largely confined to the germinal-center subtype whereas protein abundance can rise through gene amplification, transcriptional upregulation or reduced protein turnover, none of which is subtype-restricted[8,9]. Second, published immunohistochemical studies have used different antibodies, scoring systems and thresholds, most of them derived from the survival data of the cohort in which they were applied, so that a threshold optimized in one series cannot be transferred to another[13–15]. Series in which a threshold is fixed before outcome is examined, in a uniformly treated cohort with mature follow-up, remain scarce.

We therefore evaluated EZH2 protein expression by immunohistochemistry in a single-center cohort of patients with DLBCL treated with rituximab-containing immunochemotherapy, using an H-score threshold defined a priori and not derived from the survival data. The original contribution of this study is threefold: it applies a pre-specified rather than an optimized cut-off; it reports outcome after a median follow-up exceeding six years; and it examines the distribution of EZH2 protein expression across the cell-of-origin subtypes in the same cohort, allowing the relationship between protein abundance and the subtype restriction of EZH2 mutation to be addressed directly. We hypothesized that high EZH2 protein expression identifies a distinct clinicopathological subgroup of DLBCL and is associated with inferior overall and progression-free survival.

MATERIALS AND METHODS

Study design

This was a retrospective, single-center study. Patients diagnosed with DLBCL between 2011 and 2022 were

identified from the archive of the Department of Pathology, Çukurova University Faculty of Medicine, Adana, Türkiye, and were treated at the Department of Medical Oncology of the same institution.

All diagnoses were reviewed on hematoxylin–eosin-stained sections together with immunohistochemical findings and classified according to the 5th edition of the World Health Organization classification of hematolymphoid tumors[1]. This classification defines disease entities by integrating morphology, immunophenotype, cell of origin and, where available, genetic findings; diffuse large B-cell lymphoma, not otherwise specified, remains a diagnosis of exclusion, made once entities with defining genetic or clinicopathological features have been excluded, and cases are further characterized as germinal-center or non-germinal-center B-cell in type.

Sample

Ninety-five consecutive patients were assessed for eligibility. Patients were eligible if they had a histologically confirmed diagnosis of diffuse large B-cell lymphoma made at our institution between 2011 and 2022, an archived formalin-fixed, paraffin-embedded tissue block of adequate quality for immunohistochemistry, first-line treatment with a rituximab-containing regimen given at our center, and clinical follow-up recorded at our center. Forty-five patients were excluded: 30 had received their first-line treatment at another institution and 15 did not have regular follow-up at our center, because treatment details and outcome could not be verified from primary records in either group. No patient was excluded because of inadequate tissue, missing immunohistochemical data or an ineligible diagnosis. Fifty patients met all criteria and were analyzed. No patient was excluded after staining, and no patient was lost to follow-up. EZH2 was assessable in all 50 included patients (Figure 1).

Procedure

The study was approved by the Non-Interventional Clinical Research Ethics Committee of Çukurova University Faculty of Medicine, Adana, Türkiye (meeting no. 162, decision no. 58; 9 January 2026) and conducted in accordance with the Declaration of Helsinki; because of the retrospective design, the requirement for informed consent was waived.

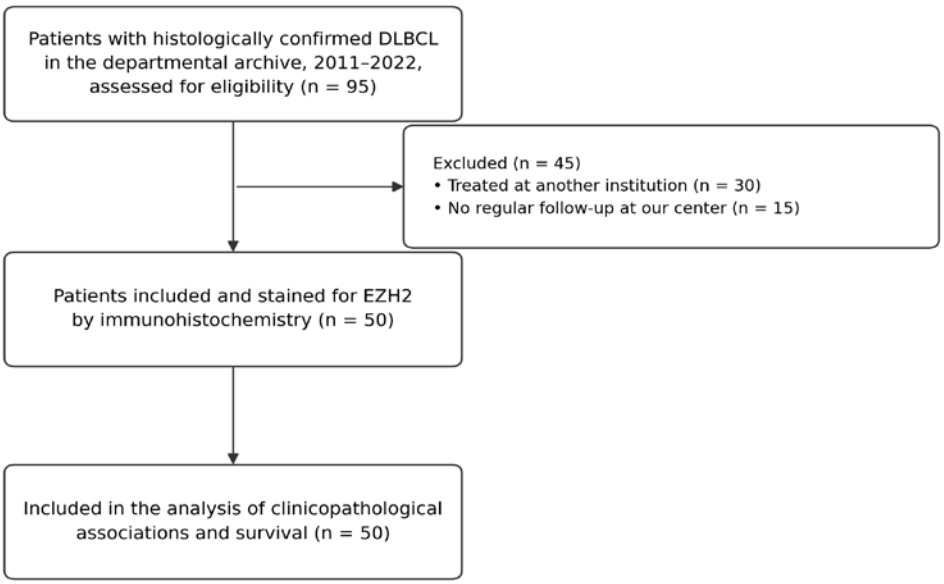


Figure 1. Diagram shows the flow of patients through the study.

Of 95 patients with a histologically confirmed diagnosis of diffuse large B-cell lymphoma identified in the departmental archive, 45 are excluded: 30 had been treated at another institution and 15 did not have regular follow-up at our center. Fifty patients remain for analysis; EZH2 is assessable in all 50 and no patient is excluded after staining.

Immunohistochemistry and EZH2 scoring

All laboratory work was carried out in the Department of Medical Pathology, Çukurova University Faculty of Medicine, a tertiary referral center that serves as the regional diagnostic laboratory for hematolymphoid malignancies and operates under national laboratory accreditation requirements, with routine internal and external quality control of immunohistochemical staining.

Four-micrometer sections were cut from formalin-fixed, paraffin-embedded tissue blocks by the laboratory technical staff. Staining was performed by the pathology laboratory technicians using a mouse monoclonal anti-EZH2 antibody (clone 415 M-15, Cell Marque) on an automated stainer (Ventana BenchMark XT) according to the manufacturer's protocol, with germinal-center cells of reactive lymphoid tissue as the positive control.

EZH2 expression was evaluated on the basis of nuclear staining in tumor cells by two experienced pathologists blinded to the clinical data. Scoring was performed independently by both observers, and discordant scores were resolved by joint review at a multi-headed microscope; the resulting consensus score was used for all analyses.

For each case, the predominant nuclear staining intensity (0, none; 1, weak; 2, moderate; 3, strong) and the percentage of positively stained tumor nuclei (0–100) were recorded. An H-score was calculated as the product of these two values, yielding a range of 0–300, an approach previously applied to EZH2 in DLBCL[15]. Tumors were classified as showing high (H-score ≥150) or low (<150) EZH2 expression. The cut-off was defined a priori as the midpoint of the 0–300 H-score range and was not derived from the survival data; no consensus threshold for EZH2 immunohistochemistry in DLBCL has been established.

Clinicopathological data

Demographic, clinical, laboratory, treatment and outcome data were retrieved from the hospital electronic medical record and from pathology reports by two medical oncologists and entered into a predefined structured database. All entries were taken directly from the primary documents; no variable was imputed, estimated or derived from a secondary source.

Disease stage was recorded according to the Ann Arbor system, in which stage I denotes involvement of a single lymph node region, stage II two or more regions on the same side of the diaphragm, stage III regions on both sides of the diaphragm, and stage IV disseminated involvement of one or more extralymphatic organs; stages III and IV were grouped as advanced disease.

The International Prognostic Index was calculated by assigning one point each for age above 60 years, Ann Arbor stage III or IV, serum lactate dehydrogenase above the upper limit of normal, Eastern Cooperative Oncology Group performance status of 2 or more, and more than one extranodal site; scores of 0–2 were taken as low or low-intermediate risk and scores of 3–5 as high-intermediate or high risk[6,7].

The cell of origin was assigned as GCB or non-GCB using the Hans algorithm (CD10, BCL6, and MUM1/IRF4)[4], applied on an available-case basis. In six patients MUM1/IRF4 was not assessable but the algorithm was nevertheless determinate: five were CD10-positive and were therefore classified as GCB irrespective of MUM1/IRF4, and one was negative for both CD10 and BCL6 and was therefore classified as non-GCB irrespective of MUM1/IRF4. Cell of origin could thus be assigned in all 50 patients. BCL2 and MYC immunostaining, the double-expressor phenotype (MYC ≥40% and BCL2 positivity)[18,19], and the Ki-67 proliferation index were recorded, together with Ann Arbor stage, B symptoms, extranodal, bone-marrow and central nervous system involvement, bulky disease (≥7.5 cm), IPI score, and serum lactate dehydrogenase (LDH). Treatment (rituximab-containing immunochemotherapy, central nervous system prophylaxis, and consolidation radiotherapy) and outcome (positron emission tomography–computed tomography response, primary refractoriness, relapse, and vital status) were also collected.

Statistical analysis

Categorical variables were compared with the chi-square test when all expected cell counts were 5 or greater and with the Fisher exact test otherwise; continuous variables were compared with the Mann–Whitney U test. Immunohistochemical markers were analyzed on an available-case basis without imputation; the denominator for each marker is the number of patients in whom it was assessable and is

given in the table footnotes. Overall survival (OS) was measured from diagnosis to death from any cause, and progression-free survival (PFS) from diagnosis to progression, relapse, or death; patients without an event were censored at the last follow-up (data cut-off, 30 April 2026). Survival was estimated with the Kaplan–Meier method and compared with the log-rank test, and hazard ratios (HR) were obtained by Cox proportional-hazards regression. The multivariable Cox model was specified in advance on clinical grounds rather than by univariable screening: it comprised EZH2 expression, the variable of interest, together with the two established prognostic factors in DLBCL, the IPI and the Hans-defined cell of origin, the number of covariates being restricted to three in view of the 25 observed deaths. No a priori sample size calculation was performed, because the study was a retrospective analysis of a fixed archival series: all 50 patients who met the eligibility criteria over the twelve-year study period were included, and the sample size was therefore determined by the size of the eligible population rather than chosen. To indicate what the resulting sample could detect, a sensitivity analysis was performed rather than a post-hoc power calculation, which is uninformative when computed from an observed effect. With 25 deaths and 14 of 50 patients (28%) in the EZH2-high group, the log-rank test had 80% power at a two-sided alpha of 0.05 to detect a hazard ratio of 3.48 or greater for overall survival, and with 30 events a hazard ratio of 3.12 or greater for progression-free survival. Effects smaller than these could not be reliably excluded, and this is reflected in the width of the reported confidence intervals. Median follow-up was calculated by the reverse Kaplan–Meier method. A two-sided *P* value <0.05 was considered statistically significant. Analyses were performed in SPSS version 25. The study is reported in accordance with the REMARK recommendations for tumor marker prognostic studies[28].

RESULTS

Fifty patients were included (Table 1). The median age was 56 years (interquartile range [IQR], 42–60.8), and 30 patients (60%) were male. Advanced-stage (III–IV) disease was present in 42 patients (84%), extranodal involvement in 41 (82%), and an IPI score of 3–5 in 24 (48%). By the Hans algorithm, 28 tumors (56%) were GCB and 22 (44%) were non-GCB. All patients received rituximab-containing immunochemotherapy: R-CHOP in 34 patients (68%), dose-adjusted R-EPOCH in 13 (26%), R-HyperCVAD in 2 (4%), and another rituximab-containing regimen in 1 (2%). After a median follow-up of 73.7 months (95% CI, 63.7–92.4), 25 patients (50%) had died, 30 (60%) had relapsed, and 15 (30%) had primary refractory disease. Median OS was 79.5 months (95% CI, 39.9–not reached) and median PFS was 47.6 months (95% CI, 7–84.9).

Table 1. Baseline clinicopathological characteristics of the cohort (n = 50).

Characteristic	Value
Age, years — median (IQR)	56 (42–60.8)
Male sex — n (%)	30 (60)
Ann Arbor stage III–IV — n (%)	42 (84)
B symptoms — n (%)	13 (26)
Extranodal involvement — n (%)	41 (82)
Bone-marrow involvement — n (%)	9 (18)
Central nervous system involvement — n (%)	5 (10)
Bulky disease ≥7.5 cm — n (%)	13 (26)
IPI 3–5 — n (%)	24 (48)
LDH, U/L — median (IQR)	303.5 (245–382)
Cell of origin, non-GCB — n (%)	22 (44)
Ki-67 ≥80% — n (%)	28 (56)
BCL2 positive — n (%)	19/42 (45.2)
MYC ≥40% — n (%)	17/50 (34)
Double expressor — n (%)	9/42 (21.4)
High EZH2 expression (H-score ≥150) — n (%)	14 (28)
Rituximab-containing therapy — n (%)	50 (100)
First-line regimen — n (%)	
R-CHOP	34 (68)
Dose-adjusted R-EPOCH	13 (26)
R-HyperCVAD	2 (4)
Other rituximab-containing regimen	1 (2)
Central nervous system prophylaxis — n (%)	17 (34)
Consolidation radiotherapy — n (%)	6 (12)
Death — n (%)	25 (50)
Relapse — n (%)	30 (60)
Primary refractory — n (%)	15 (30)

BCL2, B-cell lymphoma 2; EZH2, enhancer of zeste homolog 2; GCB, germinal-center B-cell; IPI, International Prognostic Index; IQR, interquartile range; LDH, lactate dehydrogenase; R-CHOP, rituximab, cyclophosphamide, doxorubicin, vincristine and prednisone; R-EPOCH, rituximab, etoposide, prednisone, vincristine, cyclophosphamide and doxorubicin; R-HyperCVAD, rituximab with hyperfractionated cyclophosphamide, vincristine, doxorubicin and dexamethasone. The H-score is the product of staining intensity (0–3) and the percentage of positive tumor nuclei (0–100). Marker percentages were calculated on assessed cases; the double-expressor phenotype could be assigned only in the 42 patients in whom both BCL2 and MYC were assessable. Median follow-up was 73.7 months; median overall survival 79.5 months; median progression-free survival 47.6 months.

EZH2 expression and clinicopathological associations

EZH2 showed nuclear staining (Figure 2), with an H-score ranging from 0 to 270 (median, 60); high expression (≥150) was present in 14 patients (28%). High and low EZH2 expression did not differ for most clinicopathological variables (Table 2). The only significant association was with the cell of origin: a non-GCB phenotype was more frequent among EZH2-high than EZH2-low tumors (71.4% versus 33.3%; odds ratio, 5.00; 95% CI, 1.30–19.30; P = 0.034). Because 18 comparisons were made without correction for multiplicity, this association is regarded as exploratory. LDH showed a non-significant trend toward lower values in EZH2-high tumors (median, 253.5 versus 313.5 U/L; P = 0.054). No association was found with stage, IPI, Ki-67, BCL2, MYC, or the double-expressor phenotype.



Figure 2. Photomicrographs show EZH2 immunohistochemical expression in diffuse large B-cell lymphoma. (A) Germinal-center cells of reactive lymphoid tissue serve as the positive control. (B) Strong nuclear staining (intensity 3) is present in approximately 80% of neoplastic nuclei, corresponding to an H-score of 240 (3 × 80) and high EZH2 expression. (C) Moderate nuclear staining (intensity 2) is present in approximately 10% of neoplastic nuclei, corresponding to an H-score of 20 (2 × 10) and low EZH2 expression.

Percentages refer to the illustrated fields. EZH2 immunohistochemistry with hematoxylin counterstain; original magnification ×200. The marker in the lower right corner of each panel is generated by the image acquisition software and spans 200 pixels, corresponding to 100 μm at this magnification.

Table 2. Association of EZH2 expression with clinicopathological characteristics.

Characteristic	EZH2 low (n = 36)	EZH2 high (n = 14)	*P*
Age, years — median (IQR)	58 (39.8–61.2)	53 (48.2–58.8)	0.67 *
Male sex — n (%)	22 (61.1)	8 (57.1)	1.00 †
Ann Arbor stage III–IV — n (%)	29 (80.6)	13 (92.9)	0.41 ‡
B symptoms — n (%)	11 (30.6)	2 (14.3)	0.30 ‡
Extranodal involvement — n (%)	28 (77.8)	13 (92.9)	0.41 ‡
Bone-marrow involvement — n (%)	6 (16.7)	3 (21.4)	0.70 ‡
Central nervous system involvement — n (%)	3 (8.3)	2 (14.3)	0.61 ‡
Bulky disease ≥7.5 cm — n (%)	8 (22.2)	5 (35.7)	0.47 ‡
IPI 3–5 — n (%)	15 (41.7)	9 (64.3)	0.26 †
LDH, U/L — median (IQR)	313.5 (248.8–391.2)	253.5 (245–299.2)	0.054 *
Cell of origin, non-GCB — n (%)	12 (33.3)	10 (71.4)	0.034 †
Ki-67 ≥80% — n (%)	20 (55.6)	8 (57.1)	1.00 †
CD10 positive — n (%)	18 (50)	3 (21.4)	0.13 †
BCL6 positive — n (%)	32/35 (91.4)	11/14 (78.6)	0.33 ‡
MUM1/IRF4 positive — n (%)	17/31 (54.8)	10/13 (76.9)	0.30 †
BCL2 positive — n (%)	15/29 (51.7)	4/13 (30.8)	0.35 †
MYC ≥40% — n (%)	11 (30.6)	6 (42.9)	0.51 ‡
Double expressor — n (%)	7/29 (24.1)	2/13 (15.4)	0.70 ‡

* Mann–Whitney U test; † chi-square test with Yates continuity correction; ‡ Fisher exact test. The chi-square test was applied when all expected cell counts were 5 or greater and the Fisher exact test otherwise. Marker percentages were calculated on assessed cases (CD10, n = 50; BCL6, n = 49; MUM1/IRF4, n = 44; BCL2, n = 42; MYC, n = 50). The double-expressor phenotype could be assigned only in the 42 patients in whom both BCL2 and MYC were assessable, and percentages for that row are calculated on this denominator. BCL2, B-cell lymphoma 2; BCL6, B-cell lymphoma 6; CD10, cluster of differentiation 10; EZH2, enhancer of zeste homolog 2; GCB, germinal-center B-cell; IPI, International Prognostic Index; IQR, interquartile range; LDH, lactate dehydrogenase; MUM1/IRF4, multiple myeloma oncogene 1/interferon regulatory factor 4.

EZH2 expression and survival

EZH2 expression was not significantly associated with survival. Median OS was 96.8 months (95% CI, 6.9–not reached) in the EZH2-high group and 79.5 months (95% CI, 28.5–not reached) in the EZH2-low group (HR, 1.14; 95% CI, 0.47–2.76; log-rank *P* = 0.76; Figure 3), and median PFS was 39.9 months (95% CI, 6.2–84.9) and 49.2 months (95% CI, 6.5–not reached), respectively (HR, 1.28; 95% CI, 0.58–2.82; log-rank *P* = 0.53; Figure 4). In univariable Cox analysis, neither EZH2 expression (HR, 1.14; 95% CI, 0.47–2.76; *P* = 0.767) nor cell of origin (HR, 1.46; 95% CI, 0.66–3.21; *P* = 0.349) was associated with OS, whereas an IPI of 3–5 showed a trend (HR, 2.02; 95% CI, 0.90–4.54; *P* = 0.088). In a multivariable model, the IPI was the only independent predictor of OS (HR, 2.83; 95% CI, 1.11–7.19; *P* = 0.029), while EZH2 expression remained non-significant (HR, 0.65; 95% CI, 0.24–1.76; *P* = 0.398) (Table 3).

Although median OS was numerically longer in the EZH2-high group, the univariable hazard ratio pointed in the opposite direction and reversed after adjustment. These observations are compatible rather than contradictory. The survival curves overlap substantially and cross during follow-up (Figure 3), only 25 deaths were observed, and the confidence intervals are correspondingly wide, each comfortably including unity; under these conditions the median is an unstable summary of the survival distribution and need not track the direction of a single proportional-hazards estimate. The shift in the EZH2 estimate from 1.14 to 0.65, accompanied by a rise in the estimate for non-GCB from 1.46 to 2.17, is most plausibly explained by the association between EZH2 expression and cell of origin (*P* = 0.034) rather than by a protective effect of EZH2, since the two variables carry overlapping information once both are entered. The adjusted value of 0.65 should therefore not be read as evidence of benefit, and neither estimate provides evidence of an association in either direction.

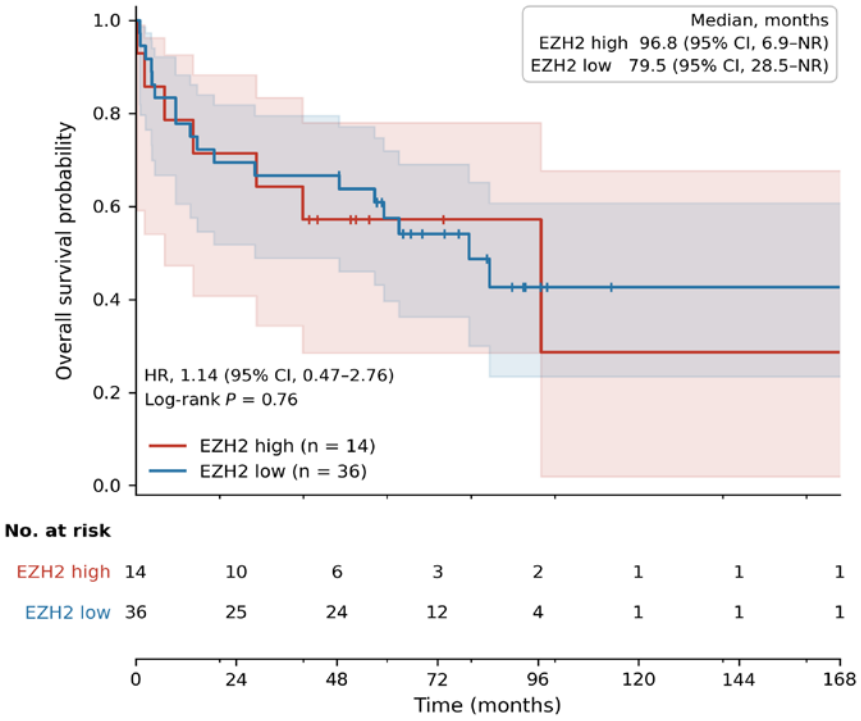


Figure 3. Graph shows Kaplan–Meier estimates of overall survival according to EZH2 expression (high, H-score ≥150; low, <150).

The number of patients at risk appears below the x-axis. Shaded areas denote 95% confidence bands and vertical ticks denote censored observations.

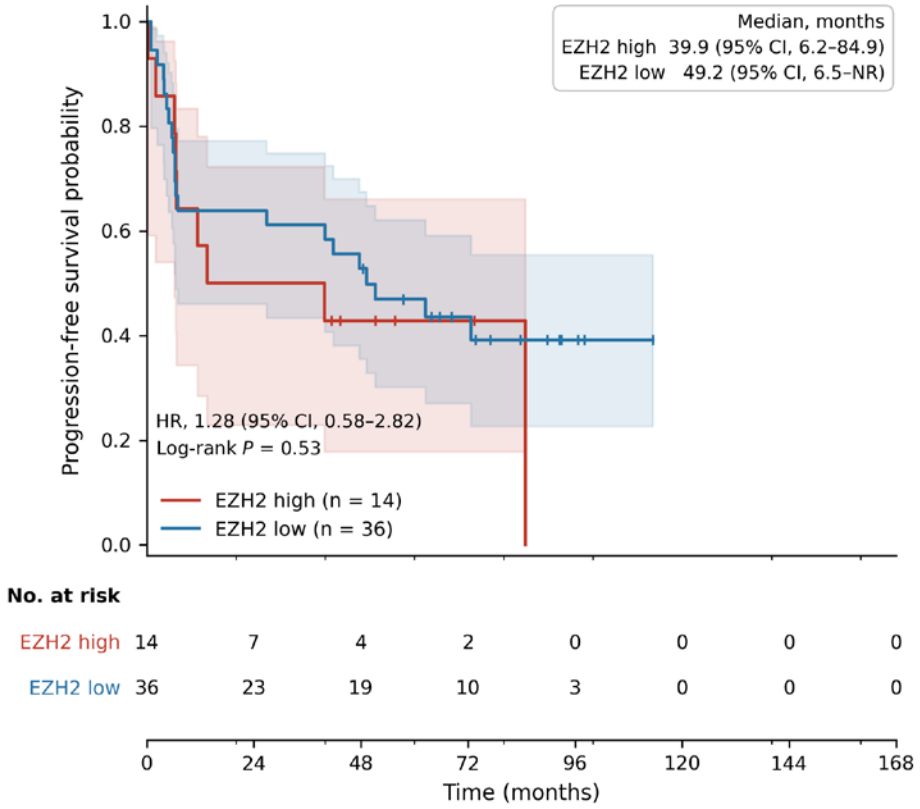


Figure 4. Graph shows Kaplan–Meier estimates of progression-free survival according to EZH2 expression (high, H-score ≥150; low, <150).

The number of patients at risk appears below the x-axis. Shaded areas denote 95% confidence bands and vertical ticks denote censored observations.

Table 3. Univariable and multivariable Cox regression for overall survival.

Variable	Univariable HR (95% CI)	*P*	Multivariable HR (95% CI)	*P*
EZH2 high vs low	1.14 (0.47–2.76)	0.767	0.65 (0.24–1.76)	0.398
Non-GCB vs GCB	1.46 (0.66–3.21)	0.349	2.17 (0.88–5.38)	0.093
IPI 3–5 vs 0–2	2.02 (0.90–4.54)	0.088	2.83 (1.11–7.19)	**0.029**

CI, confidence interval; GCB, germinal-center B-cell; HR, hazard ratio; IPI, International Prognostic Index.

DISCUSSION

In this retrospective single-center cohort of 50 patients with DLBCL treated with rituximab-containing immunochemotherapy, high EZH2 expression was detected in 14 patients (28%) and was associated with a non-GCB cell of origin but not with survival. In multivariable analysis the IPI, rather than EZH2, was the only independent predictor of overall survival.

Our observation of higher EZH2 protein expression in non-GCB tumors deserves comment, because activating *EZH2* mutations at codon Y641 are largely confined to GCB-DLBCL and follicular lymphoma[9,10]. This apparent dissociation between mutation, which is GCB-restricted, and protein overexpression, which we found enriched in non-GCB cases, supports the view that EZH2 protein levels are frequently governed by non-mutational mechanisms, including MYC- and TP53-dependent regulation[20]. It is concordant with reports that strong

EZH2 expression and H3K27me3 accumulation can occur independently of cell of origin and codon 641 status[12,21]. Immunohistochemical EZH2 expression therefore should not be regarded as a surrogate for *EZH2* mutation[11]. Serum LDH was also lower in EZH2-high tumors (median, 253.5 versus 313.5 U/L), although the difference did not reach conventional significance ($P = 0.054$). The direction is counterintuitive, since EZH2-high tumors were enriched for a non-GCB cell of origin, a phenotype usually associated with higher-risk disease. We regard this as an exploratory observation in a subgroup of 14 patients, uncorrected for multiple comparisons and without an evident biological explanation, and it requires confirmation in larger series before any interpretation is placed upon it.

This distinction between enzyme abundance and enzyme activity may also explain the absence of a prognostic signal. EZH2 protein levels and *EZH2* mutational status are not interchangeable, and the prognostically informative variable may be the latter. Activating Y641 mutations alter the catalytic specificity of PRC2 and generate aberrant H3K27 trimethylation[8,9], whereas immunohistochemistry measures how much enzyme is present irrespective of its activity. A tumor with abundant wild-type EZH2 and a tumor with a smaller amount of hyperactive mutant enzyme may therefore look similar by immunohistochemistry while being biologically distinct. In the current molecular classification of DLBCL, which assigns tumors to genetic subtypes on the basis of mutation and copy-number patterns rather than protein levels[3], *EZH2* carries information as a mutation. Because we had neither mutation nor H3K27me3 data, we cannot determine how many of our EZH2-high tumors harbored an activating mutation.

The absence of a prognostic effect of EZH2 expression should be interpreted against a discordant literature. Several studies have linked high EZH2 or H3K27me3 expression to inferior outcome, including as an independent adverse factor[12], in combination with BCL2 co-expression[13], in indolent non-Hodgkin lymphoma[22], and through EZH2-mediated chemoresistance[23,24]. Conversely, one series reported that EZH2 expression was associated with better outcome in R-CHOP-treated DLBCL[14], while other analyses found no independent prognostic value[15,16]. Our findings align with the latter group. The context dependence of EZH2 is not confined to lymphoma: in breast cancer treated with neoadjuvant chemotherapy, EZH2 positivity in the pre-treatment biopsy was associated with a higher rate of pathological complete response, suggesting that EZH2 expression may mark chemosensitivity rather than an intrinsically adverse phenotype in some settings[25]. This heterogeneity likely reflects differences in antibodies, scoring systems and cut-offs, cohort composition, treatment, and the distinction between mutation and protein expression; small sample size and a limited number of events further constrain statistical power in our study.

The choice of an H-score threshold of 150 warrants comment, because no consensus cut-off for EZH2 immunohistochemistry in DLBCL exists and previously published thresholds are neither uniform nor mutually comparable. Lee et al. dichotomized on the proportion of stained tumor cells alone, at 70%[14]. Deng et al. derived an optimal cut-off from their own test cohort using the X-tile algorithm and confirmed it in a validation cohort[13]. Petronilho et al. calculated an H-score over the same 0–300 range but summed the fractions stained at each intensity, and dichotomized at the 75th percentile of their own distribution (204), a value selected because it best separated outcome groups[15]. Each threshold is thus specific to the antibody, scoring formula and cohort in which it was derived, and none transfers directly to another series. We therefore fixed the threshold at the midpoint of the H-score range before examining outcome, accepting a loss of statistical efficiency in exchange for avoiding an optimized cut-off that would itself require independent validation.

Although EZH2 expression did not stratify outcome in our cohort, EZH2 remains a rational therapeutic target. Selective inhibitors such as GSK126 are active against EZH2-mutant lymphoma models[8], and the oral inhibitor tazemetostat has shown clinical activity in relapsed or refractory follicular lymphoma, particularly in EZH2-mutant disease[17]. Dual EZH1/2 inhibition has been proposed for GCB-DLBCL[26], and EZH2 also contributes to immune escape through downregulation of major histocompatibility complex expression, providing a rationale for combining epigenetic and immune-based therapies[27]. Because these effects are driven mainly by enzymatic activity and mutation rather than by protein abundance, mutation testing, rather than immunohistochemistry alone, may be more informative for treatment selection.

Our study has several limitations. It is retrospective and single-center, with a modest sample size.

Although all patients received rituximab-containing immunochemotherapy, the regimen was not uniform: 32% were treated with intensified protocols, which may have been selected on the basis of higher-risk disease features, so the null survival result applies to rituximab-treated DLBCL in general rather than to R-CHOP specifically. EZH2 was scored semi-quantitatively on a single section per case, with a ≥150 cut-off that has not been externally validated, and mutation status, H3K27me3, and gene-expression profiling were not evaluated. Cell of origin was assigned by the Hans algorithm, an imperfect surrogate for molecular classification[4].

The interpretation of the negative survival result is further constrained by the size of the EZH2-high subgroup. With 14 patients in that group and 25 deaths in the whole cohort, the analysis lacked precision rather than validity, and the number of events limited the multivariable model (events per variable <10). This is best conveyed by the confidence interval rather than by a post-hoc power calculation: the interval for overall survival extended from 0.47 to 2.76, so both a halving and a near-tripling of the hazard remain compatible with our data. The proportional hazards assumption was not formally tested; given the small number of events and the crossing survival curves, the reported hazard ratios should be regarded as approximate summary measures rather than as estimates of a constant effect over time. The distribution of EZH2 expression should also be borne in mind: the median H-score was 60 against a pre-specified cut-off of 150, so the threshold lay well above the center of the observed distribution and only 14 tumors (28%) were classified as high; our findings therefore describe a cohort in which strong and widespread EZH2 staining was uncommon. Finally, 18 associations were tested without formal correction and only the non-GCB association reached nominal significance, so that result should be considered hypothesis-generating, and our findings should be read as an absence of a demonstrated association rather than as evidence of no association.

Several directions follow from these findings. First, because immunohistochemistry measures enzyme abundance and not enzyme activity, studies that pair EZH2 protein scoring with mutation testing and with H3K27me3 immunohistochemistry in the same tumors are needed to establish whether any of the three carries prognostic information independently of the others; such a design would also show directly whether protein overexpression in non-GCB tumors arises through a mechanism other than an activating mutation. Second, the absence of a consensus threshold is the main obstacle to comparing published series, and a threshold agreed in advance and tested prospectively in an independent cohort would be more useful than further cut-offs optimized within single datasets. Third, the practical question raised by our results is whether immunohistochemistry has any role in selecting patients for EZH2-directed therapy; given the dissociation we observed between protein expression and the reported distribution of activating mutations, trials of EZH2 inhibitors should continue to select on mutation status, and any proposed immunohistochemical surrogate would need formal validation against sequencing before use. Finally, cohorts substantially larger than ours, ideally assembled across centers, will be required to detect effects of the moderate size that the present study could not exclude.

In conclusion, EZH2 overexpression was enriched in non-GCB DLBCL but showed no association with other clinicopathological variables, and no statistically significant association with overall or progression-free survival was demonstrated in this rituximab-treated cohort, in which the IPI remained the dominant prognostic determinant. Given the small EZH2-high subgroup and the wide confidence intervals, these findings should not be taken as evidence that no association exists. Larger prospective studies that integrate EZH2 protein expression with mutation and H3K27me3 status are needed to clarify its biological and prognostic role in DLBCL.

Author Contributions: Concept/Design : MMK, ME, BS; Data acquisition: MMK, MT, SB, TK, HA, YAC, EB; Data analysis and interpretation: MMK, ME, TT, İOK; Drafting manuscript: MMK; Critical revision of manuscript: MMK, MT, SB, ME, TT, TK, HA, YAC, İOK, BS, EB; Final approval and accountability: MMK, MT, SB, ME, TT, TK, HA, YAC, İOK, BS, EB; Technical or material support: MT, SB, ME, TT, TK, HA, YAC, EB; Supervision: MMK; Securing funding (if available): n/a.

Ethical Approval: Ethical approval was obtained from the Non-Interventional Clinical Research Ethics Committee of Çukurova University Faculty of Medicine, Adana, Türkiye, with the decision numbered 162/58 dated 09.01.2026.

Because the analysis was conducted retrospectively, the requirement for informed consent was waived.

Peer-review: Externally peer-reviewed.

Conflict of Interest: Authors declared no conflict of interest.

Financial Disclosure: This research was supported by the Turkish Society of Medical Oncology (Türk Tıbbi Onkoloji Derneği, TTOD), which funded the EZH2 immunohistochemistry kits. The funder had no role in the design of the study; in the collection, analysis, or interpretation of data; in the writing of the manuscript; or in the decision to publish the results.

REFERENCES

1. Alaggio R, Amador C, Anagnostopoulos I, Attygalle AD, Araujo IBO, Berti E et al. The 5th edition of the World Health Organization Classification of Haematolymphoid Tumours: Lymphoid Neoplasms. Leukemia. 2022;36:1720-48.
2. Alizadeh AA, Eisen MB, Davis RE, Ma C, Lossos IS, Rosenwald A et al. Distinct types of diffuse large B-cell lymphoma identified by gene expression profiling. Nature. 2000;403:503-11.
3. Schmitz R, Wright GW, Huang DW, Johnson CA, Phelan JD, Wang JQ et al. Genetics and pathogenesis of diffuse large B-cell lymphoma. N Engl J Med. 2018;378:1396-407.
4. Hans CP, Weisenburger DD, Greiner TC, Gascoyne RD, Delabie J, Ott G et al. Confirmation of the molecular classification of diffuse large B-cell lymphoma by immunohistochemistry using a tissue microarray. Blood. 2004;103:275-82.
5. Coiffier B, Lepage E, Brière J, Herbrecht R, Tilly H, Bouabdallah R et al. CHOP chemotherapy plus rituximab compared with CHOP alone in elderly patients with diffuse large-B-cell lymphoma. N Engl J Med. 2002;346:235-42.
6. The International Non-Hodgkin's Lymphoma Prognostic Factors Project. A predictive model for aggressive non-Hodgkin's lymphoma. N Engl J Med. 1993;329:987-94.
7. Zhou Z, Sehn LH, Rademaker AW, Gordon LI, LaCasce AS, Crosby-Thompson A et al. An enhanced International Prognostic Index (NCCN-IPI) for patients with diffuse large B-cell lymphoma treated in the rituximab era. Blood. 2014;123:837-42.
8. McCabe MT, Ott HM, Ganji G, Korenchuk S, Thompson C, Van Aller GS et al. EZH2 inhibition as a therapeutic strategy for lymphoma with EZH2-activating mutations. Nature. 2012;492:108-12.
9. Morin RD, Johnson NA, Severson TM, Mungall AJ, An J, Goya R et al. Somatic mutation of EZH2 (Y641) in follicular and diffuse large B-cell lymphomas of germinal-center origin. Nat Genet. 2010;42:181-5.
10. Ryan RJH, Nitta M, Borger D, Zukerberg LR, Ferry JA, Harris NL et al. EZH2 codon 641 mutations are common in BCL2-rearranged germinal center B cell lymphomas. PLoS One. 2011;6:e28585.
11. Béguelin W, Popovic R, Teater M, Jiang Y, Bunting KL, Rosen M et al. EZH2 is required for germinal center formation and somatic EZH2 mutations promote lymphoid transformation. Cancer Cell. 2013;23:677-92.
12. Oh EJ, Yang WI, Cheong JW, Choi SE, Yoon SO. Diffuse large B-cell lymphoma with histone H3 trimethylation at lysine 27: another poor prognostic phenotype independent of c-Myc/Bcl2 coexpression. Hum Pathol. 2014;45:2043-50.
13. Deng Y, Chen X, Huang C, Chen G, Chen F, Lu J et al. EZH2/Bcl-2 coexpression predicts worse survival in diffuse large B-cell lymphomas and demonstrates poor efficacy to rituximab in localized lesions. J Cancer. 2019;10:2006-17.
14. Lee HJ, Shin DH, Kim KB, Shin N, Park WY, Lee JH et al. Polycomb protein EZH2 expression in diffuse large B-cell lymphoma is associated with better prognosis in patients treated with rituximab, cyclophosphamide, doxorubicin, vincristine and prednisone. Leuk Lymphoma. 2014;55:2056-63.
15. Petronilho S, Sequeira JP, Paulino S, Lopes P, Lisboa S, Chacim S et al. Prognostic value of histone modifying enzyme EZH2 in RCHOP-treated diffuse large B-cell lymphoma and high grade B-cell lymphoma. J Pers Med. 2021;11:1384.
16. Lopez-Santillan M, Lopez-Lopez E, Alvarez-Gonzalez P, Martinez G, Arzuaga-Mendez J, Ruiz-Diaz I et al. Prognostic and therapeutic value of somatic mutations in diffuse large B-cell lymphoma: a systematic review. Crit Rev Oncol Hematol. 2021;165:103430.
17. Morschhauser F, Tilly H, Chaidos A, McKay P, Phillips T, Assouline S et al. Tazemetostat for patients with relapsed or refractory follicular lymphoma: an open-label, single-arm, multicentre, phase 2 trial. Lancet Oncol. 2020;21:1433-42.
18. Johnson NA, Slack GW, Savage KJ, Connors JM, Ben-Neriah S, Rogic S et al. Concurrent expression of MYC and BCL2 in diffuse large B-cell lymphoma treated with rituximab plus cyclophosphamide, doxorubicin, vincristine, and prednisone. J Clin Oncol. 2012;30:3452-9.
19. Hu S, Xu-Monette ZY, Tzankov A, Green T, Wu L, Balasubramanyam A et al. MYC/BCL2 protein coexpression contributes to the inferior survival of activated B-cell subtype of diffuse large B-cell lymphoma and demonstrates high-risk gene expression signatures: a report from The International DLBCL Rituximab-CHOP Consortium Program. Blood. 2013;121:4021-31.
20. Neves Filho EH, Hirth CG, Frederico IA, Burbano RM, Carneiro T, Rabenhorst SH. EZH2 expression is dependent on MYC and TP53 regulation in diffuse large B-cell lymphoma. APMIS. 2020;128:308-15.
21. Zhou Z, Gao J, Popovic R, Wolniak K, Parimi V, Winter JN et al. Strong expression of EZH2 and accumulation of trimethylated H3K27 in diffuse large B-cell lymphoma independent of cell of origin and EZH2 codon 641 mutation. Leuk Lymphoma. 2015;56:2895-901.
22. Şener M, Dinçyürek HD, Totik N, Kılıç Bağır E, Paydaş S, Gürkan E. EZH-2 expression and prognostic significance in patients with low-grade non-Hodgkin lymphoma. Sci Rep. 2025;15:19329.
23. Gu J, Chen T, Gao S, Long T. EZH2 suppresses the chemosensitivity of diffuse large B cell lymphoma via regulating TP53INP1. Clin Exp Med. 2025;25:350.
24. Dubois S, Mareschal S, Picquenot JM, Viailly PJ, Bohers E, Cornic M et al. Immunohistochemical and

genomic profiles of diffuse large B-cell lymphomas: implications for targeted EZH2 inhibitor therapy? Oncotarget. 2015;6:16712-24.
25. Yetişir AE, Paydaş S, Büyükşimşek M, Oğul A, Yaprak Ö, Zorludemir S et al. Effects of enhancer of zeste homolog 2 and mucin 1 expressions on treatment response in breast cancer. Rev Assoc Med Bras. 2023;69:153-8.
26. An R, Zhang Z, Zhang D, Li Y, Lin Y, Sun H et al. A novel EZH1/2 dual inhibitor inhibits GCB DLBCL through cell cycle regulation and M2 tumor-associated macrophage polarization. J Biol Chem. 2024;300:107788.
27. Ennishi D, Takata K, Béguelin W, Duns G, Mottok A, Farinha P et al. Molecular and genetic characterization of MHC deficiency identifies EZH2 as therapeutic target for enhancing immune recognition. Cancer Discov. 2019;9:546-63.
28. McShane LM, Altman DG, Sauerbrei W, Taube SE, Gion M, Clark GM. Reporting recommendations for tumor marker prognostic studies (REMARK). J Natl Cancer Inst. 2005;97:1180-4.