INPLASY

Defining the Prognostic Significance of EZH2 Protein Expression in Diffuse Large B-Cell Lymphoma

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ADMINISTRATIVE INFORMATION

Support - None reported.

Review Stage at time of this submission - Data analysis.

Conflicts of interest - None declared.

INPLASY registration number: INPLASY202640031

Amendments - This protocol was registered with the International Platform of Registered Systematic Review and Meta-Analysis Protocols (INPLASY) on 9 April 2026 and was last updated on 9 April 2026.

INTRODUCTION

Review question / Objective This study is a systematic review and meta-analysis (S) designed to evaluate the clinical significance of high EZH2 protein expression (I) compared to low or negative expression (C) in patients with DLBCL (P). The analysis focuses on determining its impact on patient prognosis (OS) and the distribution across molecular subtypes (O), thereby elucidating the biological and clinical role of EZH2 in this malignancy.
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Rationale

The biological complexity of EZH2 is further highlighted by its divergent roles across different tissues; for instance, contrary to its oncogenic role in most cancers, EZH2 expression has been linked to favorable survival outcomes in colorectal cancer. In the context of DLBCL, individual studies have yielded inconsistent results regarding the impact of EZH2 on overall survival (OS) and progression-free survival (PFS). Furthermore, there is a lack of consensus on the correlation between EZH2 protein levels and molecular subtypes (GCB vs. non-GCB), suggesting that protein abundance measured by immunohistochemistry (IHC) may not fully represent the enzyme's functional catalytic activity.
Given the recent clinical focus on EZH2-targeted therapies, a comprehensive systematic review and meta-analysis are necessary to synthesize existing evidence. This study aims to clarify the prognostic significance of IHC-detected EZH2 expression in DLBCL and to explore its relationship with clinicopathological features, ultimately providing a robust evidence base for future precision medicine and patient stratification.

Condition being studied
The patients with Diffuse Large B-cell Lymphoma (DLBCL).

METHODS

Search strategy

PubMed, Embase, Web of Science, Cochrane CENTRAL, and ClinicalTrials.gov.

Participant or population

The patients with Diffuse Large B-cell Lymphoma.

Intervention

The primary intervention evaluated in this review is the high expression level of the EZH2 protein in tumor tissues of patients with Diffuse Large B-cell Lymphoma (DLBCL). EZH2 protein status is determined by immunohistochemistry (IHC) performed on biopsy or surgical specimens. The review will specifically analyze the prognostic impact and clinicopathological associations of 'High EZH2 expression' (as defined by the individual study authors, typically based on staining intensity and the percentage of positive tumor cells) compared to Low or Negative EZH2 expression.

Comparator

The comparative group consists of patients with DLBCL who exhibit low or negative EZH2 protein expression. This group serves as the reference population against which the prognostic outcomes (OS) and clinicopathological characteristics of the high EZH2 expression group are compared. The definition of 'low' or 'negative' expression will be based on the specific scoring systems and cutoff values employed by the individual studies included in this meta-analysis.

Study designs to be included

This study will include observational studies, specifically retrospective cohort studies, that evaluate the prognostic value of EZH2 expression in patients with DLBCL.

Eligibility criteria

1. Data Sufficiency: Studies must provide sufficient data to extract or calculate hazard ratios (HRs) and 95% confidence intervals (CIs) for survival outcomes (OS). Studies using Kaplan-Meier curves from which HRs can be estimated will also be considered.
2. Overlapping Populations: To avoid double-counting, if multiple studies utilize the same patient cohort or overlapping datasets, only the most comprehensive study or the one with the longest follow-up duration will be included.
3. Publication Quality: Only original research published as full-text articles in peer-reviewed journals will be included. We will exclude conference abstracts, case reports, letters to the editor, editorials, and review articles to ensure the reliability of the synthesized data.
4. Diagnostic Confirmation: All included patients must have a histologically confirmed diagnosis of DLBCL according to established international classification systems.
5. Exclusion Criteria: Studies focusing solely on cell lines or animal models (in vitro/in vivo studies) will be excluded. Studies that do not clearly define the cutoff value for high EZH2 expression will also be excluded.

Information sources

To identify relevant studies, we will perform a comprehensive search across the following electronic databases from their inception to October 2025: PubMed, Embase, the Cochrane Library, and Web of Science. The search strategy will utilize a combination of Medical Subject Headings (MeSH) terms and free-text keywords related to 'EZH2', 'Enhancer of zeste homolog 2', 'Diffuse large B-cell lymphoma', and 'DLBCL'.
In addition to database searches, the reference lists of all identified studies, including relevant systematic reviews and meta-analyses, will be manually screened to identify any additional eligible publications. We also intend to search Google Scholar to identify potential grey literature or articles not yet indexed in major databases. If necessary, we will attempt to contact the corresponding authors of original studies to request missing or clarified data required for the meta-analysis.

Main outcome(s) The primary outcomes of this review are the prognostic survival indices of patients with DLBCL, specifically overall survival (OS) and progression-free survival (PFS). These will be measured using Hazard Ratios (HRs) and their corresponding 95% confidence intervals (CIs).
The secondary outcomes include the correlation between EZH2 protein expression and various clinicopathological characteristics, such as molecular subtypes (GCB vs. non-GCB), clinical stage (Ann Arbor stage I/II vs. III/IV), International Prognostic Index (IPI) scores, and age. These categorical associations will be evaluated using pooled Odds Ratios (ORs) or Risk Ratios (RRs) with 95% CIs.
Timing: Survival outcomes and clinical correlations will be assessed based on the total follow-up duration and data reported at the final time point of each individual study included in the meta-analysis.

Additional outcome(s)

Not applicable.

Data management

Literature search results will be managed using EndNote to identify and remove duplicate records. The screening process will be conducted in two stages: title/abstract screening followed by full-text review. To ensure accuracy, two reviewers will

independently perform the screening and data extraction based on a standardized data collection form excel. The extracted data will include study characteristics (author, year, country), patient demographics, EZH2 detection methods (IHC cutoff), and outcome measures (HRs and 95% CIs for OS). Any discrepancies or disagreements between the two reviewers during the screening and extraction phases will be resolved through discussion or by consulting a third senior reviewer. All final datasets and statistical analysis files will be stored in a secure, password-protected cloud-based drive with regular backups to maintain data integrity and prevent loss throughout the study.

Quality assessment / Risk of bias analysis

The methodological quality and risk of bias of the included studies will be independently evaluated by two reviewers using the Newcastle-Ottawa Scale (NOS) for cohort studies. The NOS assigns a maximum of nine stars across three main domains:
Selection of the study groups (0-4 stars)
Comparability of the groups based on the design or analysis (0-2 stars)
Outcome assessment and follow-up duration (0-3 stars)
Studies with a total score of 7 or more stars will be categorized as high quality, while those with scores between 5 and 6 will be considered moderate quality. Any discrepancies in the quality assessment between the two reviewers will be resolved through discussion or by consulting a third senior reviewer. The results of this assessment will be used to conduct sensitivity analyses to ensure the robustness of the pooled prognostic estimates.

Strategy of data synthesis

Statistical analysis will be performed using Comprehensive Meta-Analysis (CMA) v.4. For survival outcomes (OS), pooled Hazard Ratios (HRs) and their corresponding 95% confidence intervals (CIs) will be calculated. For categorical clinicopathological variables (molecular subtypes), pooled Odds Ratios (ORs) and 95% CIs will be utilized.
Statistical heterogeneity among the included studies will be assessed using the Cochran's Q-test and the I-squared (I^2) statistic. A random-effects model will be employed if significant heterogeneity is observed (defined as $I^2 > 50\%$ or $P < 0.1$); otherwise, a fixed-effects model will be used.
Potential publication bias will be assessed qualitatively using funnel plots, and quantitatively evaluated via Egger's linear regression test. A P-value < 0.05 will be considered statistically significant.

Subgroup analysis

Subgroup analyses will be conducted to explore potential sources of statistical heterogeneity and to evaluate the consistency of EZH2's prognostic value across different settings. The predefined subgrouping variables include:
1. IHC-cutoff values: Studies will be categorized based on the specific thresholds used to define high EZH2 expression ($< 50\%$ vs. $\geq 50\%$ positive cells to assess the impact of technical variability on the results.
2. Treatment regimens: Patients will be grouped according to the primary therapy received, specifically comparing those treated with the R-CHOP regimen versus those receiving other or non-standard therapies, to determine if the prognostic significance of EZH2 is influenced by the treatment background.
The differences between subgroups will be assessed using the Q-test for heterogeneity between groups, and a P-value for interaction $p< 0.05$ will be considered statistically significant.

Sensitivity analysis

Sensitivity analysis will be conducted using the 'leave-one-out' method to evaluate the stability and robustness of the combined results.

Language restriction Only English article will be included.

Country(ies) involved

Taiwan

Other relevant information

None

Keywords

Diffuse large B-cell lymphoma; EZH2; Meta-analysis; Prognosis; R-CHOP; Myc.

Dissemination plans

The findings of this systematic review and meta-analysis will be disseminated through publication in a peer-reviewed scientific journal. Additionally, the results may be presented at relevant national or international conferences specializing in hematology and oncology.

Contributions of each author

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