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ORIGINAL RESEARCH

Peripheral Blood Glucocorticoid Receptor α/β (GRα/GRβ) Ratio Predicts Response to Pemetrexed-Based Chemotherapy in Non-Squamous NSCLC: A Prospective Cohort Study

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Video Abstract

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Background: This study evaluated whether the glucocorticoid receptor α/β (GRα/GRβ) mRNA expression ratio in peripheral blood mononuclear cells (PBMCs) can serve as a predictive biomarker for treatment response and survival in patients with non-squamous non-small cell lung cancer (NSCLC) receiving pemetrexed-based chemotherapy.

Methods: Thirty-five patients with confirmed non-squamous NSCLC were prospectively enrolled and received platinum–pemetrexed chemotherapy with standard dexamethasone premedication. Quantitative PCR was used to measure GRα and GRβ mRNA levels in PBMCs, and patients were categorized into high- and low-ratio groups based on the median GRα/GRβ value. Tumor response was assessed per RECIST 1.1 criteria, and progression-free survival (PFS) and overall survival (OS) were analyzed using Kaplan–Meier and Cox regression methods.

Results: Compared with the high-ratio group, patients in the low GRα/GRβ group had a higher response rate (82.4% vs 44.4%, $p = 0.035$), greater tumor shrinkage (55% vs 42%, $p = 0.027$), and more pronounced lymph node regression ($p = 0.039$). Median PFS was longer in the low-ratio group (5.5 vs 3.5 months; log-rank $p = 0.031$; adjusted HR = 0.72, 95% CI: 0.53–0.91), whereas the OS benefit seen in unadjusted analysis (14.0 vs 11.6 months; log-rank $p = 0.042$) was not significant after adjustment.

Conclusion: A lower GRα/GRβ ratio in PBMCs was suggestively associated with improved tumor response and PFS in this small, exploratory cohort. However, the limited sample size, lack of an independent validation cohort, reliance on PBMC-derived measurements, potential confounding factors, and absence of multiple comparisons adjustment warrant cautious interpretation. These results should be considered hypothesis-generating, and validation in larger, multicenter, and adequately powered studies—including paired PBMC–tumor analyses—is essential before clinical implementation.

Plain Language Summary: Lung cancer is one of the most common and serious cancers worldwide. A type called non-small cell lung cancer (NSCLC) is often treated with a chemotherapy drug named pemetrexed, but not all patients respond equally to this treatment.

This study explored whether a blood-based biological marker could help predict how patients respond to pemetrexed. We focused on a protein called the glucocorticoid receptor (GR), which exists in two forms—GR-alpha (GRα) and GR-beta (GRβ). These forms influence how the body responds to dexamethasone, a medicine commonly given before chemotherapy.

We measured GRα and GRβ levels in immune cells from patients' blood, known as peripheral blood mononuclear cells (PBMCs), and calculated their ratio. Patients with a lower GRα/GRβ ratio had better treatment responses, greater tumor shrinkage, and longer progression-free survival than those with a higher ratio.

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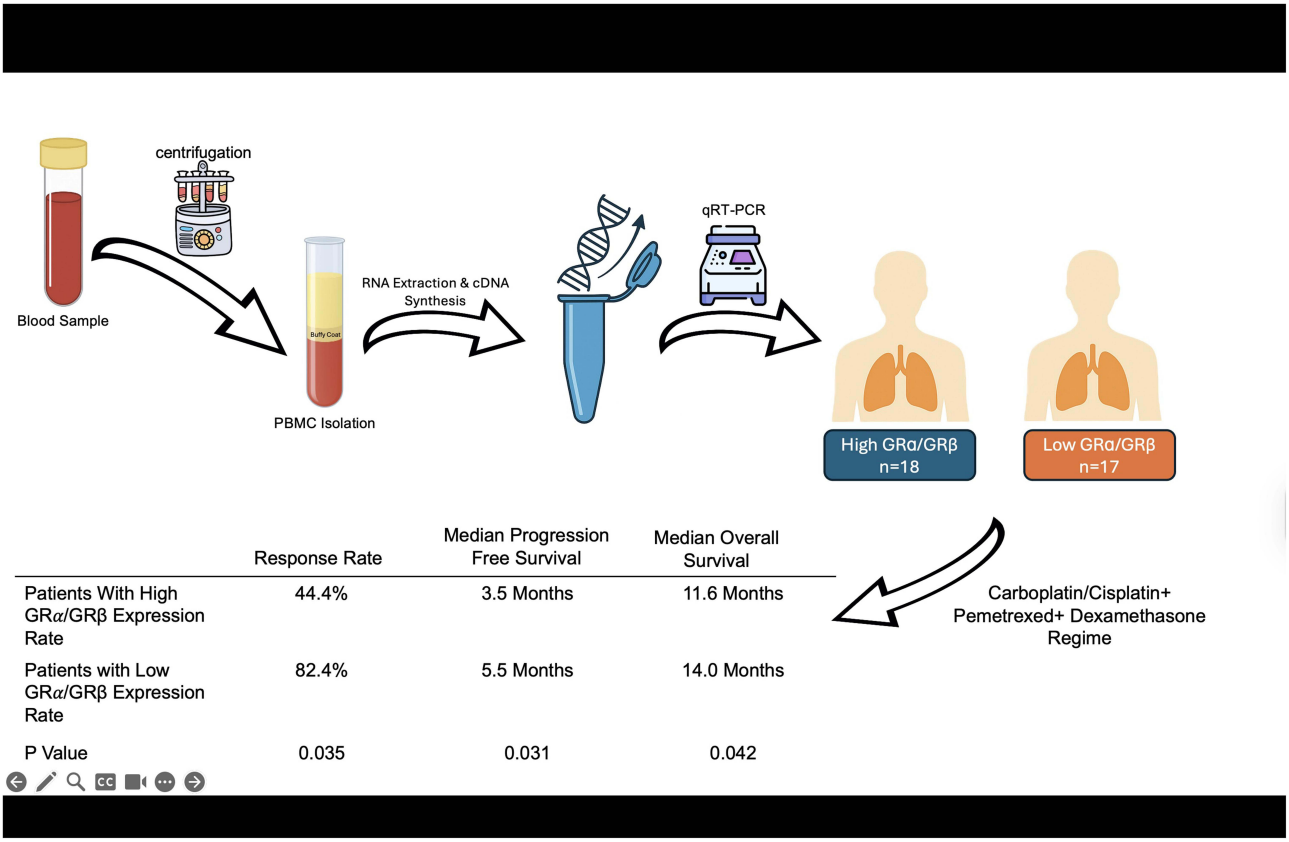
However, this was a small study with only 35 patients, conducted at a single medical center, and without an independent validation group. Measuring GR in blood cells may not perfectly reflect tumor biology, and other factors such as medications, dexamethasone dose differences, or health conditions could have influenced the results.

These findings are preliminary but suggest that the GRα/GRβ ratio in blood could become a simple, noninvasive tool to help guide treatment choices for NSCLC patients. Larger, multi-center studies that also compare blood and tumor measurements are needed to confirm these results before clinical use.

Keywords: chemotherapy resistance, biomarker stratification, dexamethasone premedication, thymidylate synthase, personalized oncology

Graphical Abstract



	Response Rate	Median Progression Free Survival	Median Overall Survival
Patients With High GRα/GRβ Expression Rate	44.4%	3.5 Months	11.6 Months
Patients with Low GRα/GRβ Expression Rate	82.4%	5.5 Months	14.0 Months
P Value	0.035	0.031	0.042

Introduction

Lung cancer is one of the most common malignancies globally, responsible for 11.6% of cancer cases and 18.4% of cancer-related deaths.[1] Non-small cell lung cancer (NSCLC) accounts for approximately 80% of lung cancer cases.[2] Chemotherapy, particularly pemetrexed-based regimens, remains a standard first-line treatment for advanced NSCLC, supported by multiple clinical trials and regulatory approval by the US Food and Drug Administration (FDA).[3,4] Despite its efficacy, the aggressive nature of NSCLC necessitates predictive biomarkers to optimize treatment outcomes.[5,6] Pemetrexed acts by inhibiting thymidylate synthase (TS) and dihydrofolate reductase (DHFR), critical enzymes in folate metabolism and DNA synthesis.[7,8] The drug is transported into cells via the proton-coupled folate transporter (PCFT) and the reduced folate carrier (RFC), and subsequently polyglutamated to enhance intracellular retention.[9,10]

Prophylactic dexamethasone is commonly administered to reduce pemetrexed-induced toxicities, such as rash and nausea.[11–13] As a synthetic glucocorticoid, dexamethasone exerts its effects through activation of the glucocorticoid receptor (GR), a transcription factor regulating immune response, apoptosis, and inflammation.[14,15]

Emerging evidence suggests that dexamethasone-mediated pathways may attenuate pemetrexed efficacy by down-regulating its transporters (PCFT, RFC) and target enzymes (TS, DHFR), potentially through glucocorticoid receptor signaling mechanisms.[16,17] Moreover, variability in GR expression among NSCLC tumors suggests that GR status could influence therapeutic response. GRα is the transcriptionally active isoform mediating glucocorticoid responses, whereas GRβ acts as a dominant-negative regulator.[18]

This study investigates the association between GR expression and clinical outcomes in NSCLC patients treated with pemetrexed-based chemotherapy. We hypothesized that a lower GRα/GRβ ratio would be associated with improved treatment response, progression-free survival (PFS), and overall survival (OS) in these patients. Because the study measured GR expression in peripheral blood mononuclear cells (PBMCs) rather than tumor tissue, this approach provides a minimally invasive means of assessing systemic glucocorticoid signaling.

Materials and Methods

Patients

This prospective cohort study was conducted at the Cancer Research Center, Shiraz University of Medical Sciences (SUMS), Shiraz, Iran, between June 2022 and December 2023. Thirty-five patients were enrolled based on the following criteria: (1) histologically confirmed stage IV non-small cell lung cancer (NSCLC); (2) adenocarcinoma histology; (3) no prior chemotherapy; (4) absence of actionable mutations (eg, EGFR, ALK, ROS1); (5) eligibility for platinum-based, pemetrexed-containing first-line therapy; (6) measurable disease per Response Evaluation Criteria in Solid Tumors (RECIST) version 1.1[19]; (7) adequate bone marrow, hepatic, and renal function; and (8) provision of written informed consent.

The study protocol was approved by the Ethics Committee of SUMS (IR.SUMS.REC.1401.275) and conducted in accordance with the Declaration of Helsinki. Patients were followed for a median of 18 months or until death or loss to follow-up. Demographic and clinical variables, including age at diagnosis, sex, smoking status (never vs smoker), T stage, N stage, and M stage, were recorded at baseline.

Treatment

Patients received four cycles of platinum-based chemotherapy combined with pemetrexed (cisplatin 75 mg/m² and pemetrexed 500 mg/m²). Maintenance therapy with pemetrexed monotherapy was administered in cases of treatment response; otherwise, treatment was switched to alternative regimens. Standard premedication protocols, including dexamethasone administration (8 mg orally twice daily on the day before, the day of, and the day after pemetrexed chemotherapy) were followed. This regimen was implemented to minimize pemetrexed-related dermatologic toxicity and to ensure uniform corticosteroid exposure across participants.

Blood Sampling and Buffy Coat Isolation

During routine follow-up, 5 mL of peripheral blood was collected in EDTA tubes. Peripheral blood was drawn prior to the first chemotherapy cycle and before dexamethasone premedication.

Leukocyte-rich buffy coats were isolated using Ficoll density gradient centrifugation (400 g, 20 minutes, room temperature) and stored at −80°C for subsequent analyses.

RNA Extraction

Total RNA was extracted from 100 µL of buffy coat using the RNeasy Midi Kit (Qiagen, Cat# 74104, Germany) following the manufacturer's instructions. RNA concentration and purity were assessed with a NanoDrop spectrophotometer (Thermo Scientific, USA) by measuring A260, A260/A280, and A260/A230 ratios. RNA integrity was evaluated by agarose gel electrophoresis (1% agarose gel in 1X TAE buffer, stained with SYBR Green II RNA Gel Stain) by visualizing 28S and 18S rRNA bands.

cDNA Synthesis and qRT-PCR

First-strand cDNA was synthesized using the RevertAid First Strand cDNA Synthesis Kit (Thermo Fisher Scientific, Cat# K1622, USA). Quantitative real-time PCR (qRT-PCR) was performed using the LightCycler 96 system (Roche, Germany) and Maxima SYBR Green/ROX qPCR Master Mix (Thermo Fisher Scientific, Cat# K0221). Beta-actin (ACTB) served as the housekeeping gene. Gene expression levels of GRα and GRβ were normalized to ACTB using the 2^−ΔCT method. Primer sequences used for GR isoform quantification were as follows: GRα: forward 5′-CATAGACTCCAGTAGCCTCAGTG-3′ and reverse 5′-GTCACATCGGAGCCATTGCCAA-3′; GRβ: forward 5′-GAAGGAAACTCCAGCCAGAA-3′ and reverse 5′-CCACATAACATTTTCATGCATAGA-3′. All primers were designed based on the NCBI reference sequence for the human *NR3C1* gene (RefSeq ID: NM_001018077.2).

The detailed protocol describing sample processing, RNA extraction, and qRT-PCR analysis is available at: DOI: dx.doi.org/10.17504/protocols.io.5jyl889p7l2w/v1

Statistical Analysis

Data were analyzed using SPSS version 16.0 (SPSS Inc., Chicago, IL, USA). Continuous variables were reported as mean ± SD or median (IQR) based on the Shapiro–Wilk normality test. Independent-samples t-tests or Mann–Whitney *U*-tests were used for comparisons, as appropriate. Categorical variables were compared using the Chi-square or Fisher's exact test. No a priori power calculation was performed because this was a single-center exploratory study constrained by the limited number of eligible patients during the recruitment period. The sample size was therefore determined by feasibility rather than formal statistical power considerations, and all analyses should be regarded as hypothesis-generating.

Tumor response was assessed according to the RECIST 1.1 criteria by an assessor blinded to GR status, categorizing responses as complete response (CR), partial response (PR), stable disease (SD), or progressive disease (PD). Imaging (CT or MRI) was performed at baseline and after treatment.

Patients were categorized into high or low GRα/GRβ ratio groups based on the median value. Survival outcomes, including progression-free survival (PFS) and overall survival (OS), were estimated using the Kaplan–Meier method and compared with the Log rank test. For each survival plot, an at-risk table was included to display the number of patients remaining at risk at pre-specified time points (0, 6, 12, and 18 months), thereby providing additional context for event timing and censoring patterns. Associations between GR expression and survival were further assessed using Cox proportional hazards models adjusted for age, sex, smoking status, and tumor stage. Hazard ratios (HRs) with 95% confidence intervals (CIs) were reported. Both unadjusted log-rank p-values from Kaplan–Meier analyses and adjusted hazard ratios from Cox proportional hazards models are presented; discrepancies may arise due to covariate adjustment.

Given the exploratory nature of this study and the limited number of planned comparisons, p-values were not adjusted for multiple testing. All p-values are presented as raw (unadjusted) values, and findings should be interpreted with caution due to the potential for type I error inflation.

Results

Demographic and Clinical Characteristics

A total of 35 patients with confirmed stage IV NSCLC (adenocarcinoma) were included (13 females and 22 males), with a median age of 53 years (range: 38–77 years). Twenty-three patients (66%) were smokers, and 12 patients (34%) were non-smokers. T staging distribution included 7 patients (20%) in T1, 11 patients (31%) in T2, and the remainder in T3 and T4. The sample size (n=35) was constrained by strict inclusion criteria and patient availability.

Expression Levels of Glucocorticoid Receptor Gene

GR gene expression in peripheral blood mononuclear cells (PBMCs) was assessed using qRT-PCR. Patients were divided into low and high GRα/GRβ ratio groups based on the median GRα/GRβ ratio of 1.50. Baseline characteristics, including tumor size and lymph node involvement, were similar between the two groups. The mean baseline tumor size was 6.8 ± 0.5 cm in the Low GRα/GRβ group and 6.5 ± 0.4 cm in the High GRα/GRβ group, without significant differences in lymph node involvement. Baseline demographic and clinical characteristics stratified by GRα/GRβ ratio levels are summarized in Table 1.

Tumor Size Reduction

Tumor size significantly decreased in both groups following treatment. In the low GRα/GRβ ratio group, the mean tumor size reduced from 6.8 ± 0.5 cm at baseline to 3.1 ± 0.6 cm after treatment, corresponding to a 55% reduction (95% CI: 50.2–59.8%). In the high-ratio group, the mean tumor size decreased from 6.5 ± 0.4 cm to 3.8 ± 0.5 cm, a 42% reduction (95% CI: 38.1–45.9%). The between-group difference in mean tumor size reduction had a statistically significant trend (p = 0.027, independent-samples *t*-test), indicating greater tumor shrinkage in the low-ratio group (Figure 1).

Lymph Node Involvement

At baseline, the mean number of involved lymph nodes was 3.5 ± 0.4 in the low GRα/GRβ ratio group and 4.2 ± 0.5 in the high-ratio group. Following treatment, the mean number decreased to 2.2 ± 0.2 in the low-ratio group (37% reduction; 95% CI: 32.5–41.5%) and to 2.8 ± 0.3 in the high-ratio group (33% reduction; 95% CI: 29.2–37.4%). The difference in lymph node reduction between groups had nominally significant association in this exploratory analysis (p = 0.039, independent-samples *t*-test), with a moderate-to-large effect size (Cohen's d = 0.71) (Figure 2).

Treatment Response Based on GR Expression Levels

Patients with a low GRα/GRβ ratio exhibited significantly higher response rates to pemetrexed-based chemotherapy compared to those with a high ratio. The response rate (RR) in the low-ratio group was 82.4% (95% CI: 64.2–97.5%) versus 44.4% (95% CI: 21.5–67.4%) in the high-ratio group (p = 0.035, Chi-square test). The estimated odds ratio from logistic regression was 5.83, indicating that patients in the low-ratio group were substantially more likely to respond to treatment (Figure 3).

Progression-Free Survival (PFS) and Overall Survival (OS)

In Kaplan–Meier analysis, the median PFS was 5.5 months (95% CI: 1.73–9.27) in the low GRα/GRβ ratio group and 3.5 months (95% CI: 1.33–6.67) in the high-ratio group (log-rank p = 0.031). At 12 months, approximately 38% of patients in the low-ratio group remained progression-free, compared to 28% in the high-ratio group. In the multivariable Cox

Table 1 Baseline Demographic and Clinical Characteristics According to GRα/GRβ Ratio Status

Variable	Category	Low GRα/GRβ (%)	High GRα/GRβ (%)	P-value
Sex	Female	5 (29.4)	8 (44.4)	0.5687
Sex	Male	12 (70.6)	10 (55.6)	
Smoking Status	Non-smoker	4 (23.5)	8 (44.4)	0.3438
Smoking Status	Smoker	13 (76.5)	10 (55.6)	
T stage	T1	4 (23.5)	3 (16.7)	0.8454
T stage	T2	6 (35.3)	5 (27.8)	
T stage	T3	4 (23.5)	5 (27.8)	
T stage	T4	3 (17.6)	5 (27.8)	
N Stage	N1	10 (58.8)	5 (27.8)	0.1774
N Stage	N2	3 (17.6)	6 (33.3)	
N Stage	N3	4 (23.5)	7 (38.9)	
M Stage	M1a	8 (47.1)	10 (55.6)	0.8695
M Stage	M1b	9 (52.9)	8 (44.4)	

Abbreviation: GR, glucocorticoid receptor.

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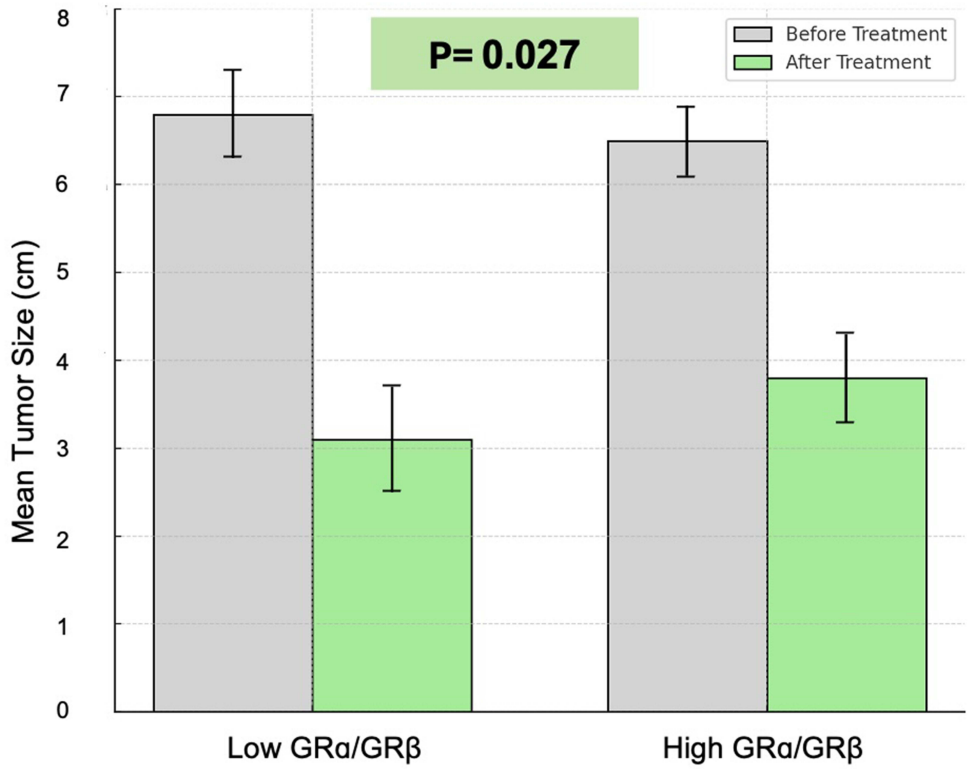


Figure 1 Tumor size before and after treatment by GRα/GRβ ratio status. Data are presented as means; error bars indicate standard deviation of tumor size in each group.
Abbreviation: GR, glucocorticoid receptor.

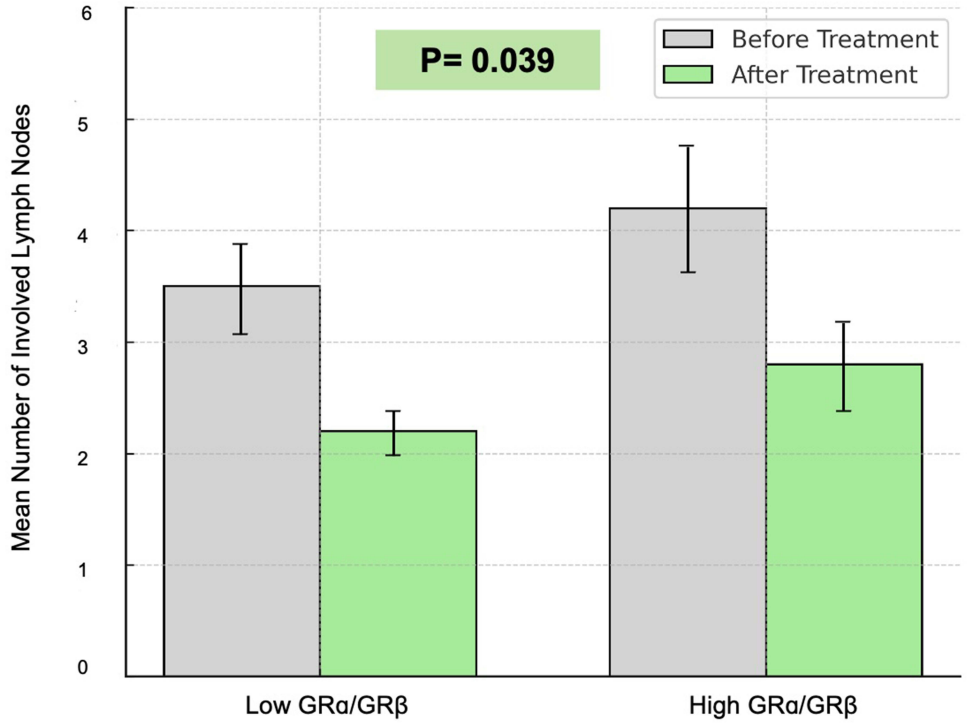


Figure 2 Lymph node involvement before and after treatment according to GRα/GRβ ratio. Data are presented as means; error bars indicate standard deviation of the involved lymph nodes in each group.
Abbreviation: GR, glucocorticoid receptor.

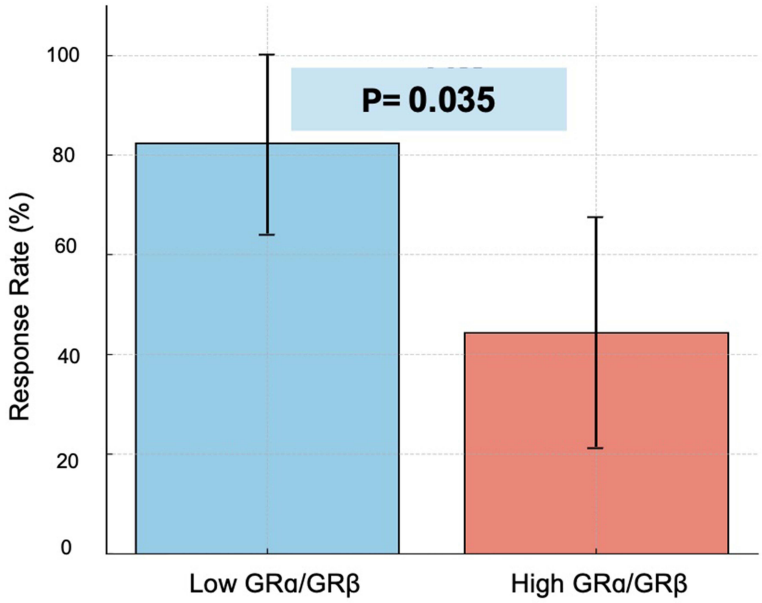


Figure 3 Treatment response rates based on GRα/GRβ ratio status. Error bars indicate the 95% confidence intervals of the observed response rates, demonstrating the precision of the estimates.
Abbreviations: GR, glucocorticoid receptor; NSCLC, non-small cell lung cancer; RR, response rate.

proportional hazards model adjusted for age, sex, smoking status, and tumor stage, the association remained statistically significant (HR = 0.72; 95% CI: 0.53–0.91; p = 0.038).

For OS, the median was 14.0 months (95% CI: 4.40–23.60) in the low GRα/GRβ ratio group and 11.6 months (95% CI: 3.87–19.33) in the high-ratio group (log-rank p = 0.042). At 18 months, 22% of patients in the low-ratio group were alive compared to 12% in the high-ratio group (p = 0.021). In the multivariable Cox proportional hazards model with the same adjustments, the difference was not statistically significant (HR = 0.95; 95% CI: 0.75–1.15; p = 0.421).

These findings are illustrated in Figure 4. The log-rank p-values correspond to the Kaplan–Meier curves, while the p-values from the Cox proportional hazards model are adjusted for baseline covariates.

Discussion

Summary of Findings

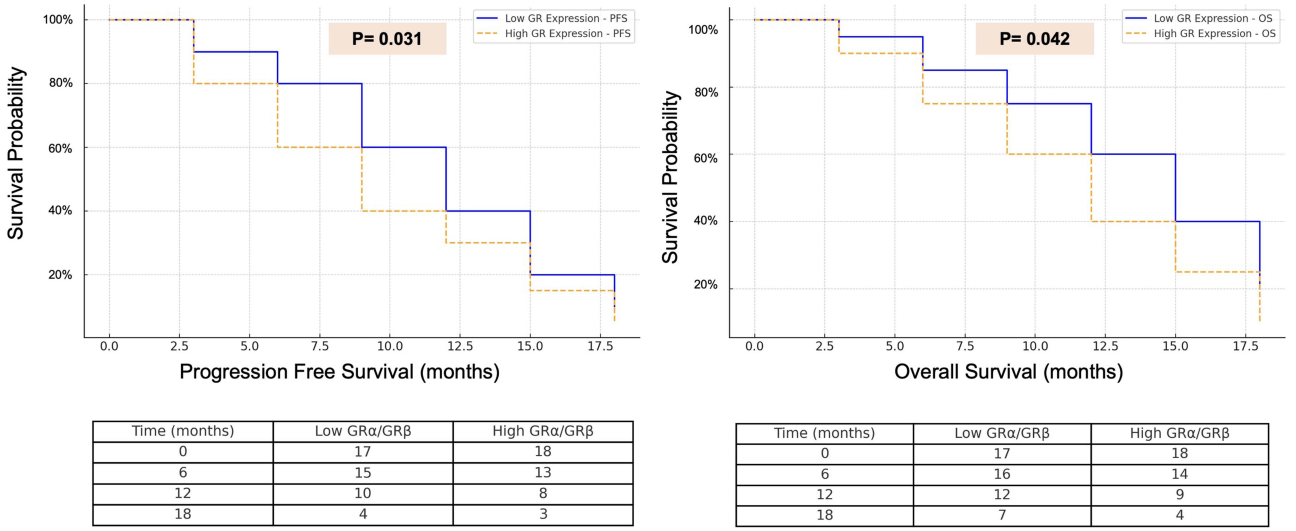
This study demonstrates that a lower GRα/GRβ ratio in peripheral blood mononuclear cells (PBMCs) is associated with better clinical outcomes in patients with advanced non-squamous NSCLC treated with pemetrexed-based chemotherapy. Patients in the low-ratio group achieved a higher response rate (82.4% vs 44.4%, $p = 0.035$, chi-square test), greater tumor size reduction (mean reduction: 55% vs 42%, $p = 0.027$, independent-samples t-test), and greater lymph node regression (37% vs 33%, $p = 0.039$, independent-samples t-test).

In survival analyses, the median progression-free survival (PFS) was 5.5 vs 3.5 months (log-rank $p = 0.031$; Cox-adjusted HR = 0.72; 95% CI: 0.53–0.91; $p = 0.038$), favoring the low-ratio group. Median overall survival (OS) was 14.0 vs 11.6 months (log-rank $p = 0.042$), but this difference was not statistically significant in the multivariable Cox proportional hazards model (HR = 0.95; 95% CI: 0.75–1.15; $p = 0.421$). These results indicate that while a lower GRα/GRβ ratio is associated with longer PFS, its apparent effect on OS may be influenced by other clinical or molecular factors.

Although the absolute difference in median PFS was modest (approximately 2 months), this improvement is clinically relevant in the context of advanced NSCLC, particularly considering the limited treatment options and comparable effect sizes reported for other validated biomarkers.

Comparison with Previous Studies

Previous research indicates that a higher GRα/GRβ ratio (or heightened GR signaling) is associated with reduced sensitivity to pemetrexed-based chemotherapy in NSCLC, whereas a lower ratio tends to align with improved treatment response. Patki



Time (months)	Low GRα/GRβ	High GRα/GRβ
0	17	18
6	15	13
12	10	8
18	4	3

Time (months)	Low GRα/GRβ	High GRα/GRβ
0	17	18
6	16	14
12	12	9
18	7	4

Figure 4 Kaplan–Meier survival curves for progression-free survival (PFS) and overall survival (OS) by GRα/GRβ ratio status. At-risk tables are shown below each plot, indicating the number of patients remaining at risk at 0, 6, 12, and 18 months. Log-rank p-values are displayed on the plots; Cox-adjusted hazard ratios and p-values are reported in the text.

Abbreviations: GR, glucocorticoid receptor; NSCLC, non-small cell lung cancer; PFS, progression-free survival; OS, overall survival.

et al[20] initially demonstrated GR-mediated modulation of pemetrexed-relevant transporters and targets in NSCLC models. Zhao et al[21] subsequently reported in clinical samples that a lower GRα/GRβ ratio in PBMCs correlated with better response rates and survival outcomes, although their analysis emphasized static endpoints rather than dynamic tumor metrics.

The present prospective cohort study extends these findings by relating GR status not only to response and survival (PFS and OS) but also to dynamic measures—tumor size reduction and lymph node response—thereby enhancing the clinical relevance of GR signaling in NSCLC management. A comparative summary of Patki et al, Zhao et al, and the present study is provided in Table 2.

Mechanistic studies further suggest that GR activation can promote cellular dormancy via CDKN1C (p57) and IGF-1R-linked survival pathways, potentially contributing to pemetrexed resistance.[22] In addition, elevated levels of GR-regulated proteins (eg, SGK1, NDRG1) have been associated with greater recurrence and worse survival in lung adenocarcinoma.[23] These observations are consistent with our results and reinforce the role of GR signaling in chemoresistance.

Mechanistic Insights and Clinical Implications

The detrimental impact of higher GRα/GRβ ratio on pemetrexed efficacy may, in part, result from GR-mediated down-regulation of key pharmacologic targets. Dexamethasone—routinely administered to mitigate pemetrexed toxicities—can suppress enzymes and transporters such as TS, DHFR, RFC, and PCFT, with effects more pronounced in tumors exhibiting elevated GR activity, thereby reducing intracellular pemetrexed accumulation and therapeutic effectiveness. In addition, the more pronounced lymph node regression in patients with lower GR expression suggests a role for GR signaling in metastatic dissemination and immune evasion, motivating further mechanistic work beyond drug-resistance pathways alone.

Using PBMCs as a surrogate for tumor GR expression is supported by biological rationale and clinical feasibility: PBMCs reflect systemic immune context influenced by tumor-derived glucocorticoid signaling and share downstream targets with tumor cells. Prior translational work has shown concordance between peripheral GR activity and tissue-level signaling in glucocorticoid-regulated drug resistance. PBMC analysis also enables serial monitoring during treatment, which is often impractical with repeat biopsies, thereby enhancing the potential utility of PBMC-based GRα/GRβ assessment in personalized therapy.

Table 2 Comparative Summary of Key Studies

Study	Study Type	Sample Type	GR Analysis	Outcome Measures	Key Findings
Patki et al (2014)[20]	In vitro study	NSCLC cell lines (A549, H460)	GR expression (overall)	Cell viability assays	High GRα/GRβ ratio reduces pemetrexed sensitivity by downregulating transporters (PCFT, RFC) and target enzymes (TS, DHFR).
Zhao et al (2017)[21]	Retrospective clinical study	Peripheral blood mononuclear cells (PBMCs)	GRα/GRβ ratio	Response rate (RR), PFS, OS	Lower GRα/GRβ ratio associated with better chemotherapy response and survival outcomes.
Present Study	Prospective clinical cohort	Peripheral blood mononuclear cells (PBMCs)	GRα/GRβ ratio	Tumor size reduction, lymph node response, RR, PFS, OS	Lower GRα/GRβ ratio associated with greater tumor reduction, reduced lymph node involvement, improved PFS, and favorable OS trend.

Abbreviations: GR, glucocorticoid receptor; NSCLC, non-small cell lung cancer; PBMCs, peripheral blood mononuclear cells; PFS, progression-free survival; OS, overall survival; RR, response rate.

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Limitations and Strengths

The small sample size (n = 35) limits the precision of effect estimates and increases the risk of both type I and type II errors, reducing statistical power and generalizability. Consequently, the observed associations—especially the response-rate differences—should be interpreted cautiously and validated in larger, adequately powered studies. Although the observed differences in tumor reduction, lymph node regression, and PFS were consistent and statistically significant in this exploratory analysis, sampling variability may partially account for these findings. The notably high response rate in the low GRα/GRβ group compared with large pemetrexed trials could reflect genuine biological sensitivity, but confirmation in larger, independent cohorts is required.

The analysis relied exclusively on PBMC-derived GRα/GRβ measurements. While PBMCs are minimally invasive and clinically practical, they may not fully capture the intratumoral heterogeneity of GR expression. Evidence from NSCLC and other contexts suggests that peripheral GR activity can mirror tissue-level signaling, but direct, paired PBMC–tumor isoform comparisons remain scarce; integrating tumor tissue analyses in future studies will be essential to confirm concordance and refine clinical thresholds.

Potential confounders (concomitant medications, variation in dexamethasone dosing, and comorbidities) were not systematically controlled and could influence GRα/GRβ ratio and the observed associations. For example, inter-patient differences in dexamethasone compliance or metabolism might alter GR isoform balance and downstream glucocorticoid signaling. Future studies should standardize dexamethasone regimens, collect detailed medication histories, and adjust for key comorbidities to minimize residual confounding.

Several statistical tests were conducted without formal adjustment for multiple comparisons (eg, Bonferroni or false discovery rate methods). This choice reflects the exploratory design and a limited number of pre-specified endpoints; nonetheless, the absence of adjustment increases type I error risk, and some significant results may represent false positives. The consistency of associations across related endpoints partially mitigates this concern.

Discrepancies between unadjusted Log rank tests and covariate-adjusted Cox proportional hazards models—particularly for OS, where significance was lost after adjustment—likely reflect methodological differences: Log rank tests ignore baseline covariates, whereas Cox proportional hazards models adjust for age, sex, smoking, and stage. In small cohorts, adjustment widens confidence intervals and reduces power, attenuating significance even when effect direction is consistent. Clinically, this suggests that the apparent OS advantage with a lower GRα/GRβ ratio may be partly attributable to baseline imbalances, warranting cautious interpretation.

Finally, the lack of an independent validation cohort limits robustness and reproducibility; results may reflect center-specific characteristics. Future work should incorporate independent cohorts from diverse settings to assess generalizability and strengthen the evidence base.

Despite these limitations, the study has notable strengths: a prospective design; standardized RECIST-based assessments; evaluation of dynamic endpoints (tumor size and lymph node changes) alongside survival; and rigorous, reproducible GRα/GRβ quantification. These strengths enhance the potential clinical relevance of GRα/GRβ assessment in optimizing pemetrexed-based treatment for non-squamous NSCLC.

Clinical Translation and Future Directions

The current findings suggest that PBMC-based GRα/GRβ assessment could serve as an exploratory biomarker to inform therapeutic decision-making in non-small cell lung cancer (NSCLC). Stratifying patients by GR status may enable more personalized pemetrexed-based regimens, potentially improving outcomes while minimizing unnecessary toxicities. From a workflow standpoint, PBMC GRα/GRβ testing could be integrated as a pre-chemotherapy triage step alongside routine labs and imaging. Implementation will require assay harmonization (platform, primer set, reporting units) and a clinically validated decision threshold, ideally derived from multicenter cohorts. Given the role of glucocorticoids across multiple regimens, GRα/GRβ profiling may have relevance beyond pemetrexed—for example, in other chemotherapy protocols or combined modalities where steroid premedication is standard.

Future research should focus on:

(i) delineating molecular pathways linking GR signaling to chemotherapy resistance;
(ii) investigating differential roles of GRα versus GRβ;
(iii) developing strategies to modulate GR activity; and
(iv) validating these findings in larger, multicenter prospective cohorts, ideally including randomized trials.

Incorporating GR evaluation into routine care holds promise for refining patient stratification and advancing precision oncology in lung cancer.

Conclusion

In this prospective cohort study, a lower glucocorticoid receptor (GR) α/β ratio in PBMCs was associated with improved treatment response, greater tumor size reduction, reduced lymph node involvement, and prolonged progression-free survival in non-squamous non-small cell lung cancer (NSCLC) treated with pemetrexed-based chemotherapy. These results suggest that the GRα/GRβ ratio may serve as a minimally invasive biomarker reflecting treatment outcomes and informing personalized strategies.

However, the small sample size, absence of an independent validation cohort, and exploratory design limit statistical power and generalizability. Reliance on PBMC-derived GRα/GRβ measurements may not fully capture intratumoral heterogeneity, and potential confounders (concomitant medications, dexamethasone variability, comorbidities) were not systematically controlled. In addition, multiple tests were performed without formal adjustment, and discrepancies between unadjusted and adjusted survival analyses suggest baseline imbalances may partly explain observed associations.

Consequently, these findings should be considered hypothesis-generating and interpreted with caution. Future studies should validate the results in larger, multicenter, adequately powered cohorts, ideally incorporating paired PBMC–tumor analyses and external validation to confirm concordance, refine clinical thresholds, and clarify the role of GRα/GRβ testing in non-small cell lung cancer (NSCLC) decision-making.

Abbreviations

ACTB, beta-actin; CI, confidence interval; DHFR, dihydrofolate reductase; EGFR, epidermal growth factor receptor; GR, glucocorticoid receptor; HR, hazard ratio; IQR, interquartile range; NSCLC, non-small cell lung cancer; OS, overall survival; PBMCs, peripheral blood mononuclear cells; PCFT, proton-coupled folate transporter; PFS, progression-free survival; qRT-PCR, quantitative real-time polymerase chain reaction; RECIST, Response Evaluation Criteria in Solid Tumors; RFC, reduced folate carrier; ROS1, ROS proto-oncogene 1; SD, standard deviation; TS, thymidylate synthase.

Data Sharing Statement

The datasets used and analyzed during the current study are available from the corresponding author upon reasonable request.

Ethics Approval and Informed Consent

This study was conducted in accordance with the Declaration of Helsinki and was approved by the Ethics Committee of Shiraz University of Medical Sciences (approval number: IR.SUMS.REC.1401.275). Written informed consent was obtained from all participants prior to sample collection and data analysis.

Consent for Publication

Not applicable. This study does not include any identifiable personal data, images, or recordings requiring consent for publication.

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Author Contributions

All authors made a significant contribution to the work reported, including the conception, study design, execution, data acquisition, analysis, and interpretation.

Dr. Bahareh Forouzani-Haghighi was responsible for study design, statistical analysis, and manuscript preparation.

Dr. Afsaneh Vazin supervised the research, critically revised the manuscript, and approved the final version.

Dr. Alireza Rezvani served as the clinical lead of the study, coordinated patient recruitment, and jointly supervised the work and manuscript correspondence with Dr. Vazin.

Dr. Bita Geramizadeh and Elaheh Esfandiari provided laboratory and pathological expertise.

Dr. Mehdi Ghasemian assisted in patient recruitment and clinical data collection.

All authors reviewed and approved the final manuscript, agreed on the journal to which the article has been submitted, and are accountable for all aspects of the work.

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