ORIGINAL ARTICLE

Impact of Prophylactic Use of PEG-rhG-CSF on First-Line Immunochemotherapy in Advanced NSCLC: A Cohort Study

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ABSTRACT

Introduction: This study aimed to assess the impact of prophylactic use of PEG-rhG-CSF on first-line immunochemotherapy in advanced NSCLC.

Methods: A cohort of patients with advanced NSCLC who received first-line immunochemotherapy at Shengjing Hospital of China Medical University between January 2019 and July 2024 was selected for this study. Patients were divided into the following two groups: a treatment group that received prophylactic PEG-rhG-CSF (≥1 cycle) 48 hours after immunochemotherapy and a control group that did not receive PEG-rhG-CSF. The primary end points were progression-free survival (PFS), overall survival (OS), overall response rate, and safety. A propensity score-matched analysis was performed to reduce potential confounders.

Results: A total of 220 patients were enrolled, with 87 in the treatment group and 133 in the control group. Median PFS was 10.5 months in both the treatment and control groups ($p = 0.86$), and median OS was 33.9 months in the treatment group versus not reached in the control group ($p = 0.71$). The overall response rate was 64.4% in the treatment group and 58.6% in the control group ($p = 0.40$). After propensity score-matched analysis (each group included 78 patients), median PFS was 12.6 months in the treatment group versus 10.5 months in the control group ($p = 0.99$), and median OS remained 30.3 months in the treatment group versus not reached in the control group ($p = 0.85$). The treatment group had a reduced incidence of chemotherapy interruptions, any grade of leukopenia, any grade of neutropenia, and grades 3 to 5 neutropenia, without an increase in immune-related adverse events.

Conclusions: The prophylactic use of PEG-rhG-CSF in patients with advanced NSCLC undergoing first-line immunochemotherapy did not compromise efficacy and safety. It reduced chemotherapy interruptions and neutropenia, without increasing immune-related adverse events, thus supporting safe and uninterrupted treatment.

Keywords: Immunochemotherapy; Non–small cell lung cancer; PEG-rhG-CSF; Safety

Introduction

Lung cancer stands as one of the most common malignant tumors globally. It remains the primary cause of cancer-related mortality, with NSCLC accounting for approximately 85% of all lung cancers.[1] On initial diagnosis, more than 70% of NSCLC cases are already in an advanced stage, forfeiting the opportunity for radical surgery and often necessitating comprehensive treatment with systemic therapy as the mainstay.[2] Specifically, for advanced NSCLC lacking actionable genomic

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alterations, the combination of immune checkpoint inhibitors (ICIs) with chemotherapy, termed immunochemotherapy or chemoimmunotherapy, has emerged as the first-line standard of care due to the substantial improvement in patient survival in combination therapy compared with chemotherapy alone. Nevertheless, chemotherapy-related hematological toxicities, particularly leukopenia and neutropenia, are prevalent in these immunochemotherapy regimens, affecting approximately 78.0% to 83.8% of patients.[3–5] These toxicities sometimes prompt delays and dose reductions in chemotherapy, thereby impeding the full therapeutic potential of these combinations.[6]

Granulocyte colony-stimulating factor (G-CSF), particularly in its PEGylated recombinant human form (PEG-rhG-CSF), plays a crucial role in supportive care in oncology. It is primarily administered to mitigate or prevent chemotherapy-induced neutropenia.[7] PEG-rhG-CSF is a long-acting formulation with an extended half-life of approximately 48 to 60 hours, which reduces the administration frequency while maintaining a favorable safety profile compared with standard rhG-CSF.[8] PEG-rhG-CSF has become a guideline-recommended prophylactic measure against high-risk febrile neutropenia in patients with solid tumors.[7]

Studies have further illuminated the potential of PEG-rhG-CSF in modulating the immune response. It stimulates dendritic cell maturation in vitro and enhances T cell proliferation.[9] In patients with SCLC, prophylactic use of PEG-rhG-CSF has been associated with elevated levels of CD3+ and CD4+ T cells and increased diversity of T cell receptors in the peripheral blood.[10] Furthermore, it seems to increase the CD4+/CD8+ T cell ratio and increase natural killer (NK) cell counts more effectively than rhG-CSF.[11] Nevertheless, the impact of prophylactic PEG-rhG-CSF administration after immunochemotherapy on the immune status of patients with advanced NSCLC, and its potential synergy with immunotherapy, remains unexplored.

The administration of antibiotics alongside immunotherapy may disrupt the balance of the gut microbiome, negatively influencing the efficacy and outcomes of programmed cell death protein 1 (PD-1) and programmed death-ligand 1 (PD-L1) monoclonal antibody therapies.[12,13] Notably, PEG-rhG-CSF reportedly decreases antibiotic use during chemotherapy cycles.[14] Nevertheless, whether this effect extends to immunochemotherapy regimens and could potentially improve their efficacy by reducing reliance on antibiotics is still unclear.

Despite its well-established role in the chemotherapy era, the specific impact of PEG-rhG-CSF on the outcomes and safety profile of contemporary immunochemotherapy regimens remains under investigation. This study aimed to bridge this knowledge gap by evaluating the influence of prophylactic PEG-rhG-CSF use on the efficacy and safety of first-line immunochemotherapy in patients with advanced NSCLC.

Materials and Methods

This retrospective cohort study included patients diagnosed with having advanced NSCLC who underwent first-line immunochemotherapy at the Shengjing Hospital of China Medical University from January 2019 to July 2024. Patients were categorized into treatment and control groups based on whether they received prophylactic PEG-rhG-CSF after chemotherapy. Both cohorts underwent four to six cycles of a regimen combining PD-1 inhibitors with standard platinum-based chemotherapy. Patients were allowed to continue ICIs as maintenance therapy after the initial treatment if no disease progression or intolerable toxicity emerged.

In the treatment group, each chemotherapy cycle was accompanied by prophylactic PEG-rhG-CSF at a fixed dose of 6 mg, administered 48 hours after chemotherapy. In contrast, the control group did not receive prophylactic PEG-rhG-CSF as part of their treatment regimen. Owing to the retrospective nature of the study, informed consent was waived. The study was approved by the ethics committee of Shengjing Hospital of China Medical University (Ethics Approval Number: 2022PS013T).

Study Participants

The inclusion criteria were as follows: patients diagnosed with having advanced, inoperable, or recurrent NSCLC after surgery, as confirmed by cytologic or histopathologic examination; an Eastern Cooperative Oncology Group performance status (ECOG PS) score of 2 or less; age 18 years or older; presence of at least one measurable lesion; completion of a minimum of two cycles of immunotherapy alongside standard chemotherapy regimens; and imaging evaluations of both target and non-target lesions every 6 weeks during the treatment period. Patients were selected to receive prophylactic PEG-rhG-CSF primarily based on clinical guidelines and the judgment of the treating physician, particularly for high-risk patients such as those with advanced age or a history of severe myelosuppression in previous chemotherapy cycles. If a patient proceeded to maintenance chemotherapy, the use of PEG-rhG-CSF was discontinued. Patients who received standard G-CSF for the treatment of neutropenia during the study period were not excluded from the study.

The excluded criteria were as follows: patients who underwent localized therapy (such as interventional procedures or radiotherapy) targeting either target or

non-target lesions during the study; those without assessable lesions; patients with a follow-up duration of less than 3 months; and cases with significant amounts of missing study data.

Collection of Clinical Data

Comprehensive clinicopathologic data were collected from enrolled patients who met the inclusion criteria. This included details such as sex, age, smoking history, Charlson's Comorbidity Index (CCI), histopathologic diagnosis, American Joint Committee on Cancer eighth edition staging, specifics of the clinical therapeutic regimen (including the types of platinum doublets and immunotherapies), and the median relative dose intensity, defined as the ratio of the actual dose administered to the planned dose. This also encompassed imaging-based efficacy assessments, laboratory test results obtained during each treatment cycle, and hematological parameters, notably the neutrophil-to-lymphocyte ratio (NLR), where NLR is calculated as the ratio of peripheral blood neutrophil count to lymphocyte count. The pretreatment NLR is designated as NLR0, whereas the NLR measured at the time of the first imaging evaluation is referred to as NLR1. In addition, counts of lymphocyte subsets were documented at baseline (before the start of treatment) and at specific follow-up time points (e.g., after each cycle of chemotherapy). These subsets included T suppressor cells (Ts), T helper cells (Th), the Th/Ts ratio, NK cells, and total T cells and B cells. Nevertheless, it is worth noting that not all patients underwent regular lymphocyte subset testing during the data collection process. Only patients with complete lymphocyte subset data were included in the analysis of lymphocyte subset changes.

Survival data, follow-up information, and detailed records of any adverse events experienced during the combination therapy were also documented. For each adverse reaction, the timing and severity grade were meticulously recorded, along with any antibiotic administration as part of the management strategy.

Study End Points

The primary study end points of this study were progression-free survival (PFS), overall survival (OS), objective response rate (ORR), disease control rate (DCR), and safety in both the treatment and control cohorts. Efficacy was determined based on the Response Evaluation Criteria in Solid Tumors version 1.1. Efficacy measures included categorizing responses as complete response (CR), partial response (PR), stable disease, and progressive disease (PD). ORR is defined as the percentage of patients whose tumors have a PR or CR to treatment. DCR is defined as the percentage of patients whose tumors have a CR, PR, or stable disease. Both ORR and DCR were assessed at the first evaluation (typically 6–8 wk after the start of treatment) and confirmed at subsequent evaluations. PFS was defined as the duration from the initiation of first-line treatment to disease progression or death without previous progression. OS was defined as the time from the start of first-line treatment to death or the cutoff date of follow-up, with patients still alive at the cutoff considered censored for survival analysis. Safety was evaluated by grading adverse events according to the Common Terminology Criteria for Adverse Events version 5.0.

Statistical Analysis

Statistical analyses were performed using SPSS software version 25.0 (IBM Corporation, Armonk, NY). To address potential confounding factors and ensure comparable patient characteristics across groups, we used the propensity score-matched (PSM) technique with a 1:1 nearest-neighbor algorithm and a caliper width of 0.05. Binary categorical variables were compared between cohorts using chi-square tests or Fisher's exact tests, as appropriate. Continuous data were analyzed using independent-sample *t* tests, with data expressed as means ± SDs. Receiver operating characteristic curves were generated for continuous variables to identify optimal cutoff values, determined by maximizing the Youden index. These values were then used to convert the continuous variables into binary classifiers. Kaplan-Meier estimation and Cox multivariate regression analysis were used to assess and validate categorical variables. Survival analyses were performed using the Kaplan-Meier method, with differences evaluated through log-rank tests. Cox proportional hazards regression modelling was used for both univariate and multivariate analyses. The follow-up time was calculated using reverse Kaplan-Meier method. Variables statistically significant at p less than 0.05 in univariate analyses were included in the multivariate models. A significance level of p less than 0.05 was considered statistically significant for all analyses.

Results

Basic Information

This study included 220 patients with advanced NSCLC who met the enrollment criteria. The median age of the patients was 65.5 (range: 36–75) years. Histologically, 170 cases (77.3%) were squamous cell carcinoma and 50 cases (22.7%) were adenocarcinoma, with no *EGFR* mutations or *ALK* rearrangements. Among these, 150 cases (68.2%) were classified as stage IV. Of the total, 187 patients (85.0%) were male and 211 patients (95.9%) had an ECOG PS score of 0 to 1. The

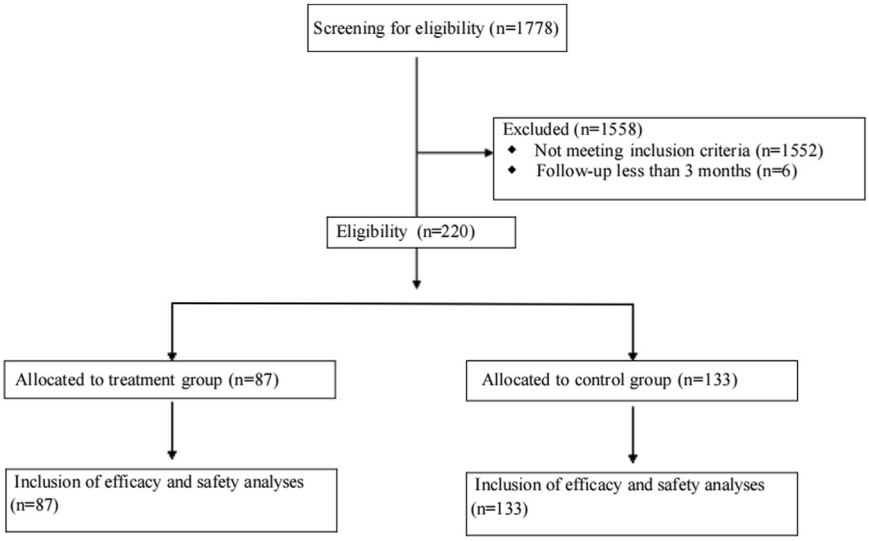


Figure 1. The CONSORT flow diagram of the study.

cohort was divided into a treatment group consisting of 87 patients (39.5%) and a control group comprising 133 patients (60.5%) (Fig. 1). A detailed presentation of the distribution of the baseline characteristics between the two groups is provided in Table 1.

Regarding treatment adherence, the completion rates for four and six cycles of first-line immunochemotherapy were 81.6% versus 87.2% and 42.5% versus 51.1% for the treatment and control groups, respectively, with no statistically significant differences between the groups ($p > 0.05$). The distribution of chemotherapy regimens in the treatment and control groups was generally balanced. Gemcitabine-based platinum-containing chemotherapy accounted for 8.7% versus 8.3%, pemetrexed-based chemotherapy for 13.8% versus 18.8%, and paclitaxel-based chemotherapy for 79.3% versus 72.9%, respectively ($p > 0.05$). The median relative dose intensity of chemotherapy was 98.7% in the treatment group and 98.3% in the control group ($p = 0.64$). After the initial immunochemotherapy, 45.8% of patients in the treatment group and 48.2% of patients in the control group proceeded to maintenance therapy with PD-1 inhibitors.

Results of the Primary Study End Point

The median follow-up for the entire cohort was 16.3 (range: 3.2–48.4) months, with the final follow-up date being September 19, 2024. The ORR across the population was 60.9%. The median PFS was 10.5 months (95% confidence interval [CI]: 8.5–12.4), and the median OS had not been reached during the analysis.

When comparing the treatment and control groups, the ORR was 64.4% in the treatment group and 58.6% in the control group ($p = 0.40$), whereas the DCR was 93.1% versus 88.7% ($p = 0.75$), respectively. The median PFS was 10.5 months (95% CI: 8.8–16.3) in the treatment group and 10.5 months (95% CI: 9.0–13.4) in the control group, with no statistically significant difference (hazard ratio [HR] = 0.97, 95% CI: 0.66–1.42; $p = 0.86$) (Fig. 2). The median OS was 33.9momths (95% CI: 21.5–NR) in the treatment group compared with NR (95% CI: 33.2–NR) in the control group (HR = 1.11, 95% CI: 0.65–1.89; $p = 0.71$) (Fig. 3). The 1- and 2-year survival rates were 87.3% versus 85.7% and 57.5% versus 64.7%, respectively, in the treatment and control groups ($p > 0.05$) (Table 2).

Subgroup analyses of PFS and OS across categories such as age, pathology, stage, presence of liver and bone metastases, antibiotic use, and NLR trends echoed the overall findings. These analyses revealed no significant impact of the use of PEG-rhG-CSF on the outcome ($p > 0.05$) (Fig. 4*A* and 4*B*).

To mitigate intergroup differences, we conducted a PSM analysis with a caliper value of 0.05. Variables including age, sex, ECOG PS score, smoking history and index, clinical stage, histologic type, liver metastasis, and bone metastasis were matched. This resulted in 78 matched pairs in each group, ensuring a balanced representation of clinical features (Table 1). After matching, the ORR was 66.7% in the treatment group compared with 67.9% in the control group ($p = 0.84$), and the median PFS was 12.6 months (95% CI: 9.5–16.8) versus 10.5 months (95% CI: 8.1–23.2), respectively (HR = 1.00, 95% CI: 0.63–1.60; $p = 0.99$) (Fig. 5). Although the median OS was 30.3 months (95% CI: 23.9–NR) for the treatment group compared with NR (21.1–NR) for the control group (Fig. 6), the 1-year and 2-year survival rates were 85.5% versus 80.4% and 55.2% versus 60.4%, respectively, in the treatment and control groups

Table 1. Baseline Characteristics

	Before PSM					After PSM				
Variable	Total (n = 220, %)	Control Group (n = 133, %)	Treatment Group (n = 87, %)	Statistic	*p*	Total (n = 156, %)	Control Group (n = 78, %)	Treatment Group (n = 78, %)	Statistic	*p*
Age, n (%)				$\chi^2 = 0.458$	0.498				$\chi^2 = 0.026$	0.873
≥65	125 (56.82)	78 (58.65)	47 (54.02)			83 (53.21)	42 (53.85)	41 (52.56)		
<65	95 (43.18)	55 (41.35)	40 (45.98)			73 (46.79)	36 (46.15)	37 (47.44)		
Sex, n (%)				$\chi^2 = 0.567$	0.451				$\chi^2 = 0.048$	0.827
Male	187 (85.00)	115 (86.47)	72 (82.76)			131 (83.97)	66 (84.62)	65 (83.33)		
Female	33 (15.00)	18 (13.53)	15 (17.24)			25 (16.03)	12 (15.38)	13 (16.67)		
ECOG, n (%)				$\chi^2 = 1.178$	0.278				$\chi^2 = 0.693$	0.405
0–1	211 (95.90)	126 (94.70)	85 (97.70)			150 (96.20)	74 (94.9)	76 (94.4)		
2	9 (4.10)	7 (5.30)	2 (2.30)			6 (3.80)	4 (5.10)	2 (2.60)		
Smoke history, n (%)				$\chi^2 = 7.387$	0.007				$\chi^2 = 0.000$	1.000
Never	68 (30.91)	32 (24.06)	36 (41.38)			54 (34.62)	27 (34.62)	27 (34.62)		
Yes	152 (69.09)	101 (75.94)	51 (58.62)			102 (65.38)	51 (65.38)	51 (65.38)		
Smoke index, n (%)				$\chi^2 = 7.408$	0.025				$\chi^2 = 0.088$	0.957
<400 Cigarette-y	20 (9.09)	13 (9.77)	7 (8.05)			13 (8.33)	6 (7.69)	7 (8.97)		
≥400 Cigarette-y	132 (60.00)	88 (66.17)	44 (50.57)			89 (57.05)	45 (57.69)	44 (56.41)		
Never	68 (30.91)	32 (24.06)	36 (41.38)			54 (34.62)	27 (34.62)	27 (34.62)		
Pathology, n (%)				$\chi^2 = 0.340$	0.560				$\chi = 0.174$	0.676
Squamous carcinoma	170 (77.27)	101 (75.94)	69 (79.31)			128 (82.05)	63 (80.77)	65 (83.33)		
Adenocarcinoma	50 (22.73)	32 (24.06)	18 (20.69)			28 (17.95)	15 (19.23)	13 (16.67)		
Stage, n (%)				$\chi^2 = 0.041$	0.840				$\chi^2 = 0.281$	0.596
IIIB/C	70 (31.82)	43 (32.33)	27 (31.03)			45 (28.85)	24 (30.77)	21 (26.92)		
IV	150 (68.18)	90 (67.67)	60 (68.97)			111 (71.15)	54 (69.23)	57 (73.08)		
Liver metastasis, n (%)				$\chi^2 = 0.000$	1.000				$\chi^2 = 0.000$	1.000
No	210 (95.45)	127 (95.49)	83 (95.40)			147 (94.23)	73 (93.59)	74 (94.87)		
Yes	10 (4.55)	6 (4.51)	4 (4.60)			9 (5.77)	5 (6.41)	4 (5.13)		
Bone metastasis, n (%)				$\chi^2 = 0.950$	0.330				$\chi^2 = 0.000$	1.000
No	194 (88.18)	115 (86.47)	79 (90.80)			142 (91.03)	71 (91.03)	71 (91.03)		
Yes	26 (11.82)	18 (13.53)	8 (9.20)			14 (8.97)	7 (8.97)	7 (8.97)		

Note: Data are presented as no. (%) unless otherwise specified.
ECOG, Eastern Cooperative Oncology Group; PSM, propensity score matching.

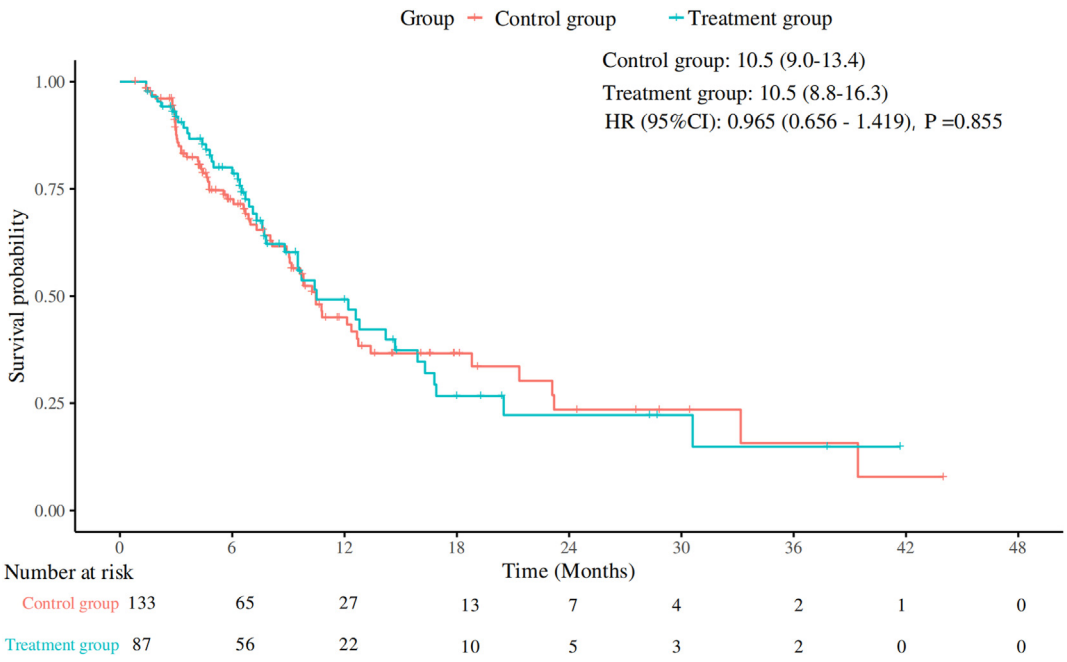


Figure 2. Kaplan-Meier analysis of PFS. Control group median PFS: 10.5 months (95% CI: 9.0–13.4); treatment group median PFS: 10.5 months (95% CI: 8.8–16.4); HR = 0.97 (95% CI: 0.67–1.42), $p = 0.86$. CI, confidence interval; HR, hazard ratio; PFS, progression-free survival.

($p > 0.05$). The results of survival and efficacy analyses before and after PSM were generally consistent.

Safety Analysis

As of September 19, 2024, the safety analysis encompassed 220 patients, with 87 in the treatment group and 133 in the control group. The occurrence of any-grade treatment-related adverse events (TRAEs) and severe TRAEs (grade $\geq$ 3) was similar in both groups (96.6% versus 91.7%, $p = 0.15$; 27.6% versus 30.8%, $p = 0.61$). There was a statistically significant difference in the incidence of chemotherapy

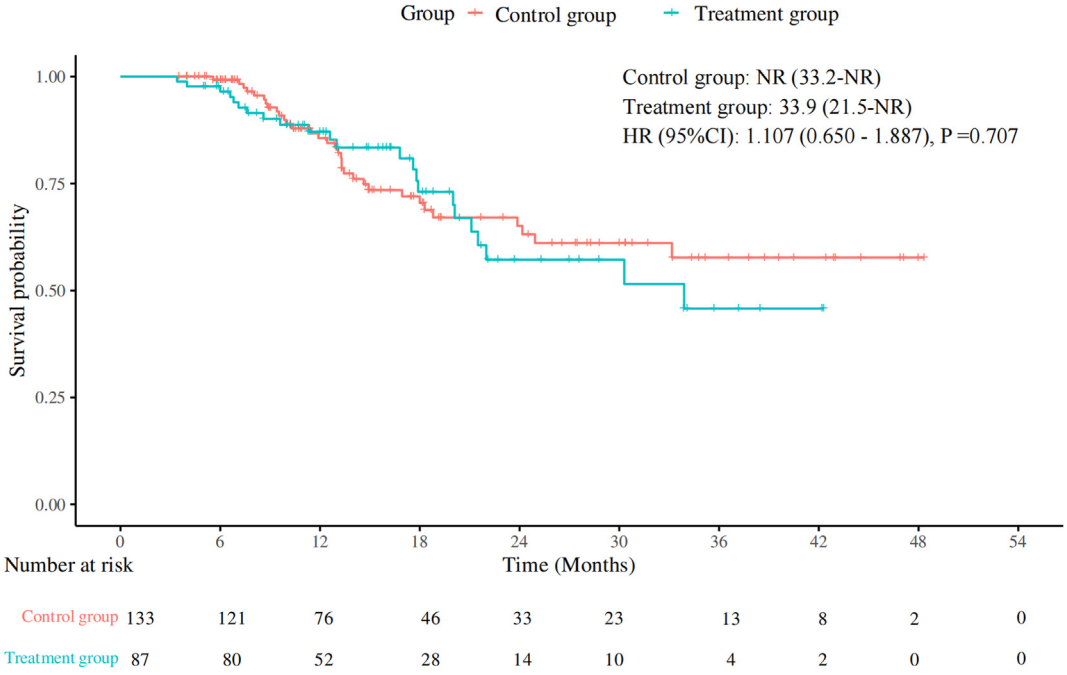


Figure 3. Kaplan-Meier analysis of OS. Control group median OS: NR (33.2–NR); treatment group median 33.9 (21.5–NR); HR = 1.107 (95% CI: 0.65–1.89), $p = 0.71$. CI, confidence interval; HR, hazard ratio; NR, not reached; OS, overall survival.

Table 2. The Main Study Results

End Points	Treatment Group (N = 87)	Control Group (N = 133)	*p*
ORR	56/87 (64.4)	78/133 (58.6)	0.40
DCR	81/87 (93.1)	118/133 (88.7)	0.75
1-y OS rate	76/87 (87.3)	114/133 (85.7)	0.55
2-y OS rate	50/87 (57.5)	86/133 (64.7)	0.37
The antibiotic utilization rate	8/87 (9.2)	23/133 (17.3)	0.09
1-y OS rate using antibiotics	6/8 (75.0)	17/23 (73.9)	0.47
1-y OS rate not using antibiotics	70/79 (88.6)	98/110 (89.1)	0.95
2-y OS rate using antibiotics	4/8 (50.0)	9/23 (39.1)	0.59
2-y OS rate not using antibiotics	48/79 (60.8)	77/110 (70.0)	0.14

Note: Data are n/N (%) unless otherwise indicated.
DCR, disease control rate; ORR, objective response rate; OS, overall survival.

interruptions between the treatment group and the control group (2.3% versus 10.5%, $p = 0.022$). The incidence of chemotherapy-induced dose reductions and withdrawals was similar in the two groups (4.6% versus 7.5%, 2.3% versus 6.0%, $p > 0.05$). The prevalence of immune-related adverse events (irAEs) was comparable between the groups (36.8% versus 32.3%, $p = 0.50$). Detailed results are presented in Table 3.

Common adverse reactions experienced in both groups included leukopenia (36.8% versus 42.1%), neutropenia (31.3% versus 45.9%), anemia (82.8% versus 91.2%), thrombocytopenia (55.2% versus 40.1%), elevated alanine transaminase level (25.3% versus 32.3%), and elevated aspartate transaminase level (28.7% versus 34.6%). The incidence of any grade of leukopenia and grades 3 to 5 neutropenia (4.6% versus 17.3%) was significantly less frequent in the treatment group ($p < 0.05$). Furthermore, antibiotic use was less frequent in the treatment group (9.2% versus 17.3%). No statistically significant differences were observed between the groups for all grades of irAEs, including immune-related pneumonia (4.6% versus 9.0%), hypothyroidism (5.7% versus 6.9%), and rash (28.7% versus 20.3%). The main grade 3 or higher irAEs were immune-related pneumonia (4.6% versus 8.3%), which were lower in the treatment group, although without statistical significance. No adverse events attributable to PEG-rhG-CSF, such as thrombosis or bone pain, were found in this study (Table 3).

Univariate and Multivariate Analyses

Regarding PFS, the univariate analysis indicated that general clinical factors such as age, sex, smoking history, CCI, pathology type, clinical stage, antibiotic use, and NLR trends did not significantly affect PFS (all *p* values > 0.05). Nevertheless, regarding OS, the univariate analysis indicated that although age, sex, smoking history, CCI, pathology type, and clinical stage did not significantly influence OS, antibiotic use and changes in NLR trends were significantly associated with OS ($p < 0.05$).

Progressing to multivariate Cox regression analysis, the absence of antibiotic use and a decreasing NLR trend emerged as independent predictors of improved OS, with HRs of 0.51 (95% CI: 0.28–0.94, $p = 0.030$) and 0.57 (95% CI: 0.33–0.98, $p = 0.041$), respectively. In the subgroup receiving antibiotics, the treatment group had a numerically longer OS compared with the control group. Nevertheless, this did not reach statistical significance (23.8 versus 20.0 mo, HR = 0.96, 95% CI: 0.30–3.1, $p = 0.95$). The 2-year OS rate was higher in the treatment group using antibiotics, albeit without statistical significance (50.0% versus 39.1%, $p = 0.59$). None of the groups in the no-antibiotics subgroup reached median OS, with no statistically significant difference; the 2-year OS rates were comparable at 60.8% and 70.9%, respectively ($p = 0.14$). Detailed data are presented in Table 2.

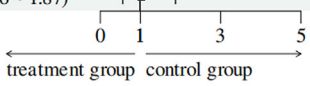
Regarding the NLR, prophylactic use of PEG-rhG-CSF did not significantly elevate NLR. NLRs from baseline (NLR0) to the first imaging evaluation (NLR1) were 3.58 ± 2.75 and 3.95 ± 2.78 versus 3.56 ± 2.52 and 3.39 ± 2.56 in the treatment and control groups, respectively. The percentage change was slightly more pronounced in the treatment group, but this difference was not statistically significant (10.3% versus 4.8%, $p = 0.71$). Notably, OS was superior in the subgroup with a declining NLR compared with the subgroup with an increasing NLR, with a statistically significant difference (38.3 versus 28.6 mo, $p = 0.043$).

Effects of PEG-rhG-CSF on Lymphocyte Subpopulations in Immunochemotherapy

To investigate the influence of PEG-rhG-CSF on lymphocyte subsets during immunochemotherapy, we performed pre- and post-treatment measurements of peripheral blood lymphocyte subsets from a subset of patients. A total of 36 paired data sets were collected,

A

Variables	n (%)	control group	treatment group	HR (95% CI)	P	P for interaction
		No. of events/ No. of total				
All patients	220 (100.00)	63/133	44/87	0.96 (0.66 ~ 1.42)	0.855	
Age						0.678
<65	95 (43.18)	28/55	20/40	0.88 (0.50 ~ 1.57)	0.676	
≥ 65	125 (56.82)	35/78	24/47	1.01 (0.60 ~ 1.71)	0.959	
Pathology						0.565
Squamous carcinoma	170 (77.27)	46/101	35/69	1.03 (0.66 ~ 1.60)	0.912	
Adenocarcinoma	50 (22.73)	17/32	9/18	0.82 (0.36 ~ 1.87)	0.642	
Stage						0.359
IIIB/C	70 (31.82)	19/43	15/27	1.24 (0.63 ~ 2.45)	0.529	
IV	150 (68.18)	44/90	29/60	0.87 (0.55 ~ 1.40)	0.574	
Liver metastasis						0.719
Yes	10 (4.55)	3/6	4/4	0.82 (0.16 ~ 4.16)	0.815	
No	210 (95.45)	60/127	40/83	0.94 (0.63 ~ 1.40)	0.750	
Bone metastasis						0.841
Yes	26 (11.82)	11/18	6/8	0.96 (0.33 ~ 2.77)	0.934	
No	194 (88.18)	52/115	38/79	0.97 (0.64 ~ 1.48)	0.896	
Antibiotic						0.867
Yes	31 (14.09)	49/110	39/79	0.94 (0.61 ~ 1.43)	0.757	
No	189 (85.91)	14/23	5/8	1.12 (0.40 ~ 3.14)	0.826	
NLRtendency						0.964
Increase NLR	120 (54.55)	40/79	24/41	1.00 (0.60 ~ 1.66)	0.992	
Decrease NLR	100 (45.45)	23/54	20/46	1.02 (0.56 ~ 1.87)	0.938	



B

Variables	n (%)	control group	treatment group	HR (95% CI)	P	P for interaction
		No. of events/ No. of total				
All patients	220 (100.00)	33/133	23/87	1.11 (0.65 ~ 1.89)	0.708	
Age						0.279
<65	95 (43.18)	14/55	8/40	0.79 (0.33 ~ 1.88)	0.593	
≥ 65	125 (56.82)	19/78	15/47	1.43 (0.72 ~ 2.82)	0.304	
Pathology						0.269
Squamous carcinoma	170 (77.27)	24/101	19/69	1.34 (0.73 ~ 2.46)	0.346	
Adenocarcinoma	50 (22.73)	9/32	4/18	0.68 (0.21 ~ 2.21)	0.521	
Stage						0.954
IIIB/C	70 (31.82)	10/43	7/27	1.07 (0.41 ~ 2.80)	0.896	
IV	150 (68.18)	23/90	16/60	1.11 (0.59 ~ 2.11)	0.746	
Liver metastasis						0.634
Yes	10 (4.55)	1/6	2/4	2.00 (0.18 ~ 22.06)	0.571	
No	210 (95.45)	32/127	21/83	1.06 (0.61 ~ 1.84)	0.835	
Bone metastasis						0.590
Yes	26 (11.82)	7/18	2/8	0.74 (0.15 ~ 3.59)	0.707	
No	194 (88.18)	26/115	21/79	1.20 (0.68 ~ 2.14)	0.531	
Antibiotic						0.677
Yes	31 (14.09)	10/23	4/8	1.03 (0.32 ~ 3.34)	0.954	
No	189 (85.91)	23/110	19/79	1.25 (0.68 ~ 2.30)	0.477	
NLR tendency						0.239
Increase NLR	120 (54.55)	21/79	16/41	1.52 (0.79 ~ 2.93)	0.214	
Decrease NLR	100 (45.45)	12/54	7/46	0.76 (0.30 ~ 1.94)	0.572	

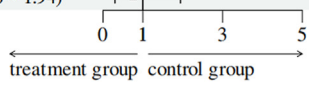


Figure 4. Subgroup analyses of PFS (*A*) and OS (*B*). CI, confidence interval; HR, hazard ratio; NLR, neutrophil-to-lymphocyte ratio; PFS, progression-free survival; OS overall survival.

comprising 17 from the treatment group and 19 from the control group. Our analysis revealed no statistically significant differences in the Th/Ts ratios and the counts of Ts, Th, NK, and B cells in the peripheral blood of patients between the treatment and control groups, either before or after treatment (all p values > 0.05).

Although the counts of Ts, Th and NK cells tended to increase after treatment compared with their

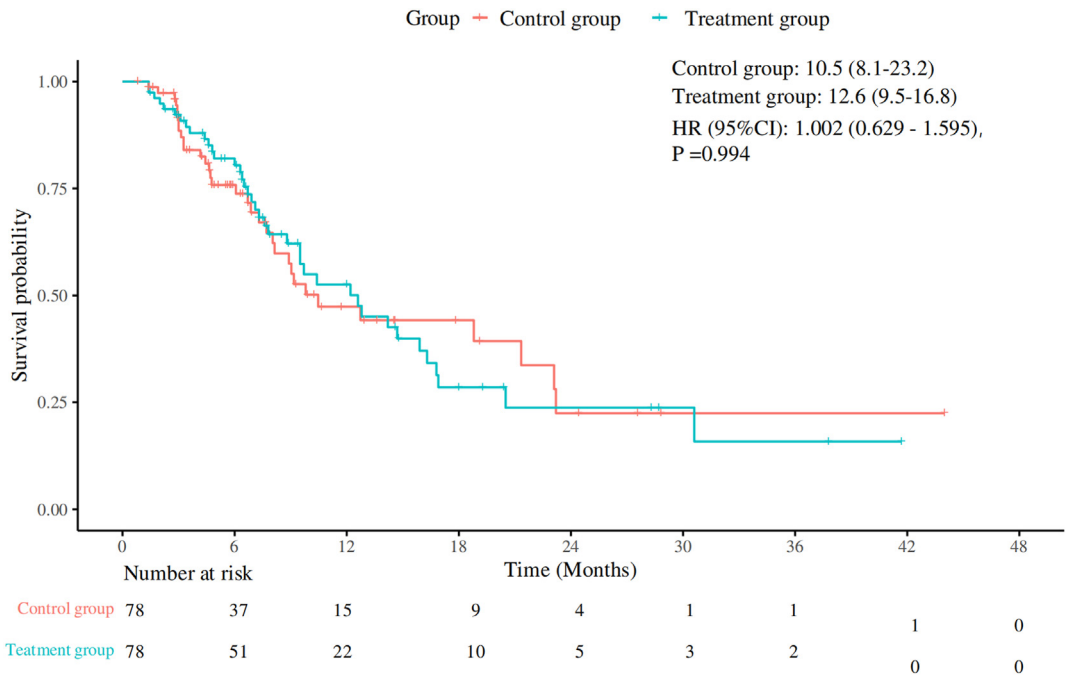


Figure 5. Kaplan-Meier analysis of PFS after propensity score matching. Control group median PFS: 10.5 months (95% CI: 8.1-23.2); treatment group median PFS: 12.6 months (95% CI: 9.5-16.8); HR = 0.95 (95% CI: 0.63-1.60), $p = 0.99$. CI, confidence interval; HR, hazard ratio; PFS, progression-free survival.

pretreatment counts in both groups, these increases did not reach statistical significance (p values > 0.05). Detailed findings are provided in Supplementary Table 1.

Discussion

The urgent clinical challenge is to ensure the efficacy and safety of patients with advanced NSCLC undergoing

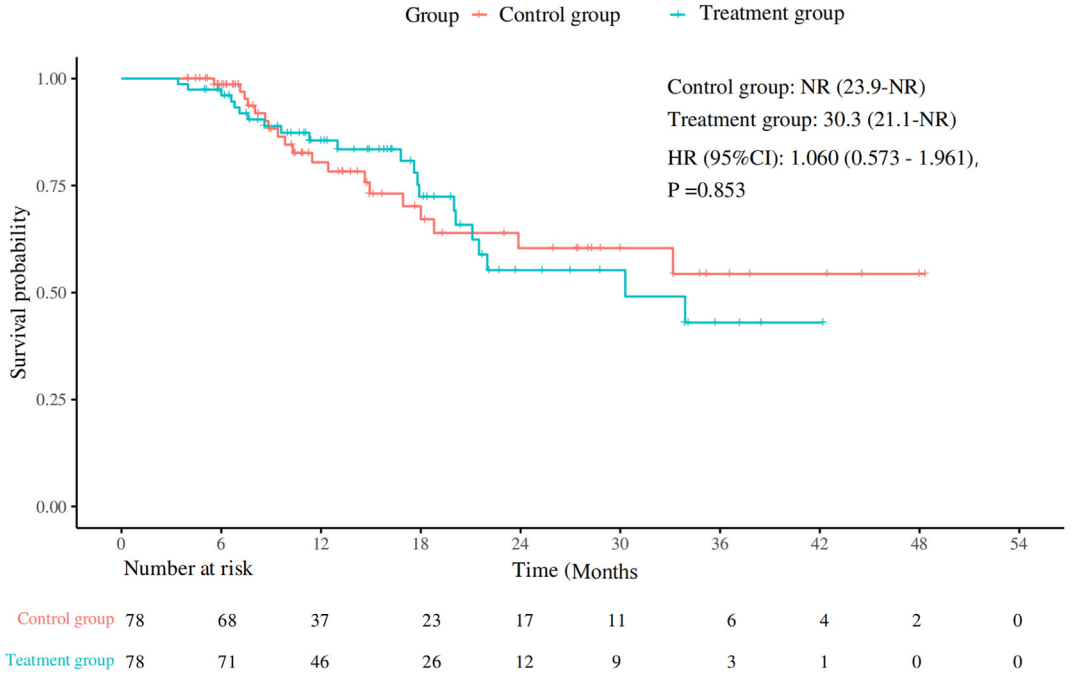


Figure 6. Kaplan-Meier analysis of OS after propensity score matching. Control group median OS: NR (23.9-NR); treatment group median OS: 30.3 (21.1-NR); HR = 1.06 (95% CI: 0.57-1.96), $p = 0.85$. CI, confidence interval; HR, hazard ratio; NR, not reached; OS, overall survival.

Table 3. Summary of All-Cause AEs

	Treatment Group (N = 87)		Control Group (N = 133)	
AEs	Any grade	Grades 3-5	Any grade	Grades 3-5
TRAE	84 (96.6)	24 (27.6)	122 (91.7)	41 (30.8)
AE leading to dose interruption of treatment[a]	2 (2.3)		14 (10.5)	
AE leading to dose reduction of treatment	4 (4.6)		10 (7.5)	
AE leading to withdrawal from treatment	2 (2.3)		8 (6.0)	
AE caused by immunotherapy	32 (36.8)	4 (4.6)	43 (32.3)	11 (8.3)
Leukopenia[a]	32 (36.8)	10 (11.5)	56 (42.1)	20 (15.1)
Neutropenia[b]	35 (31.3)	4 (4.6)	61 (45.9)	23 (17.3)
Anemia	72 (82.8)	8 (9.2)	120 (91.2)	11 (8.3)
Thrombopenia	48 (55.2)	8 (9.2)	66 (40.1)	8 (6.1)
Alanine aminotransferase increased	22 (25.3)	3 (3.3)	43 (32.3)	3 (3.0)
Aspartate aminotransferase increased	25 (28.7)	2 (2.2)	46 (34.6)	2 (1.5)
Febrile neutropenia	1 (1.1)		7 (5.3)	
Diarrhea	7 (8.0)	0	14 (10.5)	1 (0.8)
Vomit	10 (11.4)	0	14 (10.5)	0
Hypothyroidism	5 (5.7)	0	9 (6.9)	0
Immune-mediated pneumonitis	4 (4.6)	3 (3.4)	12 (9.0)	10 (7.5)
Rash	25 (28.7)	0	27 (20.3)	0
Immune-mediated hepatitis		1 (1.1)		1 (0.8)
Immune-mediated myocarditis	1 (1.1)		0	

Note: Data are no. (%) unless otherwise indicated.
[a]There was a statistically significant difference in the incidence of chemotherapy interruptions and any-grade leukopenia between treatment group and control group ($p = 0.022$, 0.035).
[b]There was a statistically significant difference in the incidence of any-grade and grades 3 to 5 neutropenia between treatment group and control group ($p = 0.034$ and $p = 0.005$).
AEs, adverse events; TRAE, treatment-related AE.

standard first-line immunochemotherapy, while minimizing side effects such as myelosuppression and optimizing the benefits of immunochemotherapy. Our retrospective investigation aimed to understand the impact of primary prophylaxis with PEG-rhG-CSF on the efficacy and safety profile of such therapy. The study findings revealed that prophylactic PEG-rhG-CSF did not undermine the therapeutic efficacy of immunochemotherapy. Instead, it facilitated a safer and more consistent treatment regimen by mitigating the incidence of severe neutropenia and reducing antibiotic requirements, without exacerbating irAEs.

Notably, this study addressed a significant gap in clinical knowledge, as there had previously been no studies evaluating the prophylactic effects of PEG-rhG-CSF on immunochemotherapy outcomes. Our findings indicated that post-immunochemotherapy prophylaxis with PEG-rhG-CSF did not affect the efficacy of the treatment. These findings were consistent with previous phase III randomized controlled trials (RCTs) revealing the superiority of immunochemotherapy over chemotherapy alone in advanced NSCLC. These trials reported ORRs ranging from 48.1% to 65.7% for PD-1 inhibitors plus chemotherapy, with median PFS and OS of 9.7 to 11.3 and 21.6 to 27.8 months, respectively.[15–18] After propensity score matching, the PFS (12.6 versus 10.5 mo), OS (30.3 versus NR months), and ORR (66.7% versus 67.9%) in the treatment and control groups, respectively, observed in our study echoed these precedents, confirming that prophylaxis with PEG-rhG-CSF did not compromise the antitumor efficacy of the treatment.

Our study results indicate that the prophylactic use of PEG-rhG-CSF after immunochemotherapy alleviates severe myelosuppressive side effects, specifically leukopenia and neutropenia, and significantly reduced incidence of chemotherapy deferral during treatment. In several phase III RCTs focused on first-line immunochemotherapy for advanced NSCLC, the incidence of any-grade leukopenia and neutropenia within immunochemotherapy cohorts were approximately 80% and 60%, respectively, whereas the incidence of grade 3 or higher leukopenia and neutropenia was 30% to 36.3% and 47.8% to 55.0%, respectively.[3–5] Our investigation revealed a lower incidence of all grades of leukopenia, neutropenia, and febrile neutropenia in the intervention group compared with the control group, indicating a significant myeloprotective effect exerted by PEG-rhG-CSF. Notably, these findings are consistent with those observed in the era of chemotherapy alone, suggesting that the benefits of PEG-rhG-CSF persist in the context of immunochemotherapy.[19–21]

Among these studies, a prospective RCT involving 130 participants compared the efficacy and tolerability

of PEG-rhG-CSF with rhG-CSF for preventing neutropenia during successive cycles of chemotherapy in patients with NSCLC. The study found a significantly reduced incidence of grade 3/4 neutropenia in the PEG-rhG-CSF group compared with the control group (1.2% versus 11.6%, $p < 0.05$), along with a decreased rate of chemotherapy delays (43.7% versus 54.8%),[19] further validating the enhanced myelosupportive properties of PEG-rhG-CSF. In another study of PEG-rhG-CSF for the prevention of leukopenia in NSCLC, 151 patients were included, and the incidence of grades 3 to 4 neutropenia was significantly lower in the treatment group than in the control group, with 14.6% versus 50.0% ($p < 0.05$).[20] Consequently, administering PEG-rhG-CSF prophylactically after immunochemotherapy decreased hematological toxicities, such as severe leukopenia and neutropenia, ensuring uninterrupted chemotherapy at optimal dosages and schedules. Notably, our safety assessment did not reveal significant differences in irAE frequencies between groups (36.8% versus 32.3, $p > 0.05$), indicating that prophylactic PEG-rhG-CSF after immunochemotherapy did not exacerbate immune-related side effects.

The prophylactic use of PEG-rhG-CSF after immunochemotherapy ensures uninterrupted progression of chemotherapy while minimizing antibiotic use throughout treatment. Neutropenia-associated infections and fevers are common chemotherapy complications, with studies revealing a 10% to 30% increased risk of infection accompanying grades 3 to 4 neutropenia, leading to high antibiotic use.[22] Emerging studies emphasize the correlation between gut microbiome diversity and the efficacy of PD-1 monoclonal antibody treatments, suggesting that antibiotic-induced disruptions to the microbiota may affect immunotherapy efficacy.[23,24] Such disruptions can impair systemic T cell function and counts, hinder dendritic cell migration, and reduce B-lymphocyte activity, collectively affecting immunotherapy effectiveness.[25,26] Ahmed et al. observed that patients with cancer receiving broad-spectrum antibiotics had reduced immunotherapy efficacy, leading to shorter OS and PFS compared with those not taking antibiotics.[12] A meta-analysis by Lurienne et al.[27] underscored the negative impact of antimicrobial exposure on the outcomes of ICI therapy in NSCLC, linking it with unfavorable OS and PFS. This finding was supported by another prospective RCT in advanced NSCLC, which noted reduced antibiotic dependence with prophylactic PEG-rhG-CSF, although not statistically significant.[19] Our study also revealed that prophylactic PEG-rhG-CSF reduced antibiotic use, albeit not significantly. Importantly, we observed significantly shorter survival in patients using antibiotics compared with those who did not (23.9 versus NR, $p = 0.03$), and multivariate regression analysis suggested that the absence of antibiotics was an independent predictor of good OS. Therefore, by mitigating the incidence of severe neutropenia, prophylactic PEG-rhG-CSF may mitigate the detrimental immune-efficacy consequences of antibiotic use, potentially enhancing immunotherapy success and improving patient survival. Nevertheless, there was a difference in antibiotic utilization between the two groups, but it did not reflect a difference in OS, possibly due to insufficient follow-up time.

The prophylactic use of PEG-rhG-CSF did not significantly increase the NLR. Recent studies have revealed that a lower NLR was associated with a better prognosis in patients with NSCLC with metastases.[28,29] Furthermore, in patients treated with ICIs, a high NLR was associated with lower PFS, OS, and response rates, suggesting that NLR could serve as a prognosticator for immunotherapy efficacy.[30] Rossi et al.,[31] in their retrospective study, analyzing neutrophil and platelet counts as predictors of navulizumab efficacy in advanced NSCLC, revealed that changes in NLR from initial imaging evaluation relative to baseline NLR could predict treatment response. Notably, patients with declining NLR exhibited significantly longer median OS compared with those with increasing NLR (15.6 versus 7.8 mo; HR: 0.32; 95% CI: 0.16–0.64; $p = 0.0001$).[31] Consistent with these findings, our study identified the reduction in NLR as an independent predictor of favorable OS, with significantly prolonged OS observed in the NLR decline group (NR versus 30.3 mo, $p = 0.043$). Our results indicated a congruent pattern of NLR dynamics post–PEG-rhG-CSF prophylaxis compared with the control group, confirming that the prophylactic use of PEG-rhG-CSF did not significantly elevate NLR, effectively maintained neutrophil counts, and did not adversely affect the therapeutic efficacy of immunochemotherapy in terms of NLR.

The PEG-rhG-CSF agent, functioning as a myeloprotective compound, also influences the immune status. Lymphocytes, particularly $CD3^+$, $CD4^+$, and $CD8^+$ T cells, play a pivotal role in immunotherapies. Sustained antitumor effects can be achieved by stimulating $CD4^+$ T cells, isolating tumor antigen-specific $CD4^+$ T subsets and enhancing the Th/Treg ratio, working in concert with DCs, NK cells, and $CD8^+$ T cells.[32] Studies revealed that G-CSF medications exhibited multifaceted potential impact on the immune microenvironment.[33] Specifically, tumor cells themselves have been found to secrete G-CSF,[34,35] which can promote tumor expansion and metastasis by facilitating angiogenesis and promoting immature granulocyte recruitment.[36,37] Furthermore, G-CSF promotes immune cell phenotypes that, in turn, promote tumor growth, such as M2 macrophages, myeloid-derived suppressor cells, and

regulatory T cells, leading to the suppression of antitumor immune response.[38] Laboratory investigations have revealed the expression of G-CSF in various subsets of T lymphocytes, including $CD4^+$, $CD8^+$, regulatory T cells, and helper T cells, which influence T cell proliferation and cytokine secretion.[39]

PEG-rhG-CSF, a protein engineered by covalently attaching PEG to the amino terminus of rhG-CSF, operates through a mechanism similar to that of rhG-CSF, but few studies have explored whether it similarly affects tumors and the immune microenvironment in a multifaceted manner. A clinical study revealed that prophylactic use of PEG-rhG-CSF during chemotherapy significantly increased the proportions of $CD3^+$ and $CD4^+$ T cells in the peripheral blood of patients with SCLC and enriched T cell receptor diversity.[10] Another small-scale study comparing PEG-rhG-CSF with rhG-CSF for post-chemotherapy myelosuppression prevention in breast cancer therapy suggested that PEG-rhG-CSF could increase $CD4^+/CD8^+$ T cell ratios and NK cell counts more effectively, hinting at its potential to modulate immune function in patients with cancer.[11] Our study observed no significant difference in Th/Ts ratios, Ts cells, Th cells, NK cells, or B cells in the peripheral blood pre- and posttreatment between the two groups, implying that PEG-rhG-CSF enhanced bone marrow protection without disturbing immune cell subsets. Consequently, based on the existing evidence, the immunomodulatory potential of PEG-rhG-CSF may hold promise for enhancing cancer therapies, but further validation through extensive research is imperative given the current scarcity of relevant studies.

Despite these encouraging findings, our study had inherent limitations due to its retrospective design, relatively small sample size, and short follow-up duration, necessitating careful interpretation. Nevertheless, the adoption of PSM aimed to mitigate variable discrepancies between cohorts, thus reducing the potential bias in our findings. The intricate actions of G-CSF analogues in the immune microenvironment warrant attention, as these agents can either stimulate immune cells to enhance antitumor responses or promote immunosuppression, potentially facilitating tumor progression through multifaceted mechanisms. Our study confirmed that PEG-rhG-CSF did not impair the therapeutic activity or efficacy of immunochemotherapy; nevertheless, a cautious evaluation of its use and its immunomodulatory potential in real-world settings is essential to ensure maximal therapeutic benefits while minimizing adverse effects. Consequently, large prospective studies are urgently needed comprehensively to assess the role of PEG-rhG-CSF in immunochemotherapy regimens and thoroughly assess its safety implications.

Conclusion

In summary, this study pioneered evaluating the antitumor efficacy and safety profile associated with the prophylactic administration of PEG-rhG-CSF in the contemporary era, where immunochemotherapy stands as the first-line therapeutic approach for NSCLC. Our findings revealed that prophylactic use of PEG-rhG-CSF neither exacerbated irAEs nor decreased the antitumor potency of immunochemotherapy in patients with advanced NSCLC receiving first-line immunochemotherapy. Instead, it seemed to facilitate a safer and more consistent therapeutic course by mitigating the likelihood of severe neutropenia and necessitating less antibiotic intervention. Consequently, this study provided a foundational argument for incorporating prophylactic PEG-rhG-CSF into clinical immunochemotherapy regimens for oncological management. Future large-scale prospective studies are imperative to validate the role of PEG-rhG-CSF in immunochemotherapy and its comprehensive safety impact.

CRediT Authorship Contribution Statement

Li Sun: Data curation, Formal analysis, Writing - Original draft.

Yuan Tian: Data curation, Resources.

Shuling Zhang: Methodology, Validation.

Letian Huang: Software, Validation.

Jietao Ma: Funding acquisition, Project administration, Visualization, Writing - review & editing.

Chengbo Han: Conceptualization, Funding acquisition, Project administration, Supervision, Writing - review & editing.

Disclosure

The authors declare no conflict of interest.

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Supplementary Data

Note: To access the supplementary material accompanying this article, visit the online version of the *JTO Clinical and Research Reports* at www.jtocrr.org and at https://doi.org/10.1016/j.jtocrr.2024.100780.

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