Doi.org/10.36721/PJPS.2025.38.1.REG.195-206.1

Impact of tislelizumab combined with albumin-bound paclitaxel and carboplatin on intestinal flora and gastrointestinal toxicities in advanced lung cancer

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Abstract: To evaluate the impact of tislelizumab combined with albumin-bound paclitaxel and carboplatin on intestinal flora and gastrointestinal toxicities in advanced lung cancer patients. A retrospective study of 431 patients (control group: albumin-bound paclitaxel + carboplatin; study group: Tislelizumab + albumin-bound paclitaxel + carboplatin). Intestinal micro biota, tumor markers, treatment efficacy and gastrointestinal adverse reactions were compared. The study group showed lower levels of pathogenic bacteria and higher levels of beneficial bacteria. The study group also had higher objective response rate (71.81%) and disease control rate (92.02%). No significant difference in gastrointestinal toxicities was observed. The combination therapy enhances treatment efficacy and reduces gastrointestinal toxicities.

Keywords: Tislelizumab, albumin-bound paclitaxel, carboplatin, advanced lung cancer, intestinal flora.

Submitted on 23-08-2024 – Revised on 19-12-2024 – Accepted on 03-02-2025

INTRODUCTION

Lung cancer ranks as the primary cause of cancer-related mortalities worldwide. Its incidence and fatality rates have been on a persistent upward trend. Annually, there are around 2 million newly diagnosed cases and 1.76 million deaths. More than 85% of these cases are non-small cell lung cancer, imposing a substantial social burden (Chen *et al*., 2022; Abu Rous *et al*., 2022). The global incidence of lung cancer (ASIR) in 2019 was 27.66 cases per 100,000 population and the global ASIR showed a downward trend between 1990 and 2019, with an average annual rate of change (EAPC) of -0.09 (Chen and Yi, 2022). By 2050, the global lung cancer burden is expected to reach 4.62 million new cases and 3.55 million deaths (Tu *et al*., 2021).

According to the non-small cell lung cancer guidelines issued by the National Comprehensive Cancer Network in the United States, for certain patients with advanced lung squamous cell carcinoma who have no detected driver gene mutations, a chemotherapy protocol centered around albumin-bound paclitaxel and carboplatin is suggested as the preferred treatment approach (Meyer *et al*., 2024). Paclitaxel is a new type of anti-microtubule drug that can specifically bind to the β-site of microtubules, causing microtubules to aggregate into lumps and bundles and stabilize microtubules by preventing polymerization, effectively limiting the proliferation of tumor cells. Paclitaxel is also crucial for immunomodulation *in vivo*. At low doses, it can regulate immune cells such as dendritic cells, natural killer cells and T cells in the tumor microenvironment by promoting the maturation and antigen-presenting function of dendritic cells, enhancing the cytotoxic activity of natural killer cells and modulating the differentiation and function of T cells (Wang *et al.,* 2023). Since albumin is mainly responsible for transporting nutrients in the human body and tumor cells proliferate and differentiate faster and take in more nutrients, albumin-bound paclitaxel will accumulate in the tumor tissue, enhancing the targeting of paclitaxel and its efficacy is better than that of paclitaxel injection (Yardley *et al*., 2013). Besides chemotherapy and immunotherapy, radiotherapy is a common lung cancer treatment. It uses high-energy radiation like X-rays or protons to kill cancer cells or shrink tumors. For early-stage non-small cell lung cancer patients unfit for surgery, radiotherapy can be curative. Stereotactic ablative radiotherapy (SABR), for example, shows good local tumor control with acceptable toxicity. In advanced-stage cases, it's used palliatively to relieve tumor-caused pain, dyspnea and bleeding (Diez *et al*., 2022).

Targeted therapy is another key treatment. It targets molecules or pathways vital for cancer cell growth, survival, and metastasis. Epidermal growth factor receptor (EGFR)-tyrosine kinase inhibitors (TKIs) are effective in treating non-small cell lung cancer patients with EGFR mutations. Drugs like gefitinib, erlotinib, and osimertinib can notably improve progression-free and overall survival (Karlsen *et al*., 2021). Anaplastic lymphoma kinase (ALK) inhibitors such as crizotinib, ceritinib, and alectinib benefit ALK-positive non-small cell lung cancer patients (Luo *et al*., 2023). Although first-line chemotherapy has a certain effect on advanced lung cancer, its gastrointestinal toxic and side effects are obvious. In recent times, the advent of immune checkpoint inhibitors (ICIs) has revolutionized the landscape of cancer treatment, particularly in the context of lung cancer. These agents have emerged as a cornerstone in therapeutic strategies, playing a pivotal and

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transformative role in enhancing the prognosis of lung cancer patients. ICIs are a class of immunotherapeutic drugs that target specific proteins on immune cells, thereby modulating the body's immune response against cancer cells. Among the most well-known and widely studied ICIs are those represented by programmed death-1 (PD-1)/programmed death ligand 1 (PD-L1) and cytotoxic T-lymphocyte-associated antigen 4 (CTLA-4) inhibitors (Guo *et al*., 2022). The PD-1/PD-L1 axis is a key regulatory pathway in the immune system. Cancer cells often exploit this pathway to evade the immune system by expressing PD-L1, which binds to PD-1 on T cells, suppressing their anti-tumor activity. PD-1 and PD-L1 inhibitors block this interaction, thus unleashing the T cells' ability to recognize and attack cancer cells. Similarly, CTLA-4 inhibitors work by preventing CTLA-4 from dampening the immune response, allowing T cells to be more effective in eliminating cancer cells. The success of these ICIs has led to a paradigm shift in the treatment of lung cancer, offering new hope for patients who previously had limited treatment options. However, when advanced lung cancer patients receive first-line chemotherapy combined with immunotherapy, they often experience gastrointestinal toxic and side effects such as nausea, vomiting and diarrhea. Nearly a third or more of patients are likely to encounter gastrointestinal adverse reactions triggered by immune checkpoint inhibitors (ICIs). These adverse reactions can significantly impinge on the patients' quality of life and treatment effectiveness, potentially undermining the overall success of the treatment plan (Gonroy *et al.,* 2023). A multitude of earlier studies have demonstrated that the gut micro biota plays a pivotal role in the immunotherapy response among patients with diverse types of cancers, such as colorectal, lung, gastric and liver cancers. The composition and balance of the gut microbiota are closely intertwined with the responsiveness of immunotherapy. In fact, fluctuations in the gut microbiota can impact the way the immune system reacts to cancer treatment, either promoting or impeding the efficacy of immunotherapeutic interventions (Zhao *et al*., 2021; Shah and Ng, 2023). Consequently, investigating the effect of the combinatorial regimen of Tislelizumab Injection, albumin-bound paclitaxel and carboplatin on the gut microbiota of advanced lung cancer patients is of paramount importance. Simultaneously, deciphering the relationship between gut microbiota alterations and gastrointestinal toxicities and side-effects is crucial. This exploration can provide valuable guidance for optimizing treatment protocols. By doing so, we aim to enhance patients' quality of life, mitigate the impact of treatment-related adverse events and potentially achieve superior treatment outcomes. Such an approach acknowledges the intricate interplay between the therapeutic agents, the gut micro biota and the overall health of the patients, thereby adhering to a comprehensive and evidence-based strategy in oncological care.

MATERIALS AND METHODS

Sample size estimation

This study adopts the sample size calculation method based on the hypothesis of superiority (Farrington and Manning, 1990; Bacchetti and Leung, 2022). The methodology for calculating the sample size incorporated in this research is presented in equation ①. Drawing upon the findings of preliminary experiments and relevant prior studies, the following assumptions were made. In the control group, the efficacy rate of the drug treatment regimen was postulated to be 50%, denoted as π_c = 0.5. For the study group, the treatment efficacy rate was assumed to be 70%, represented as π_t =0.7. In this investigation, it was determined that for the treatment effect of the drug treatment regimen in the study group to be clinically meaningful, it should exceed that of the control group by at least 5%, thus Δ=0.05. With a significance level of α = 0.025, the corresponding critical value $Z_{\alpha/2}$ was calculated as 1.96. Additionally, considering a power of 1-β =0.80 (where β = 0.20), the value of Z_β was found to be 0.84. Through computational analysis, it was determined that a minimum of 161 patients were needed in each group. However, factoring in an anticipated 20% sample attrition rate, the actual sample size required for each group was adjusted to 201 subjects. Consequently, a total of 402 cases were deemed necessary for inclusion across the two groups.

$$n = \frac{\pi_c \times (1-\pi_c) + \pi_t \times (1-\pi_t)}{(\pi_t - \pi_c - \Delta)^2} \times (\mu_{\alpha/2} + \mu_\beta)^2 \quad ①$$

Patients' selection

A retrospective assessment was carried out on 431 advanced-stage lung cancer patients who received treatment at our hospital between July 2022 and June 2024. These patients were categorized based on their treatment protocols. The control group consisted of 243 patients, while the study group was composed of 188 patients. Inclusion criteria: diagnosed as primary lung cancer by diagnosis and pathological examination, with at least one measurable tumor lesion; a Karnofsky Performance Status (KPS) score ≥60; diagnosed as Tumor Node Metastasis (TNM) stage IIIb or IV by pathology; and complete clinical data. Exclusion Criteria: Patients presenting with nausea, vomiting and loss of appetite resulting from gastrointestinal neoplasms. Individuals diagnosed with coagulation abnormalities. Those having hepatic and renal functional impairments. Patients suffering from anorexia nervosa. Subjects who had been administered other treatment regimens prior to study enrollment. Individuals with allergic reactions or contraindications to the medications utilized in this research. Patients demonstrating severe organ dysfunction. Those afflicted with other grave disorders, including those of the immune, digestive and hematopoietic systems. The schematic diagram of the study's workflow is depicted in fig. 1.

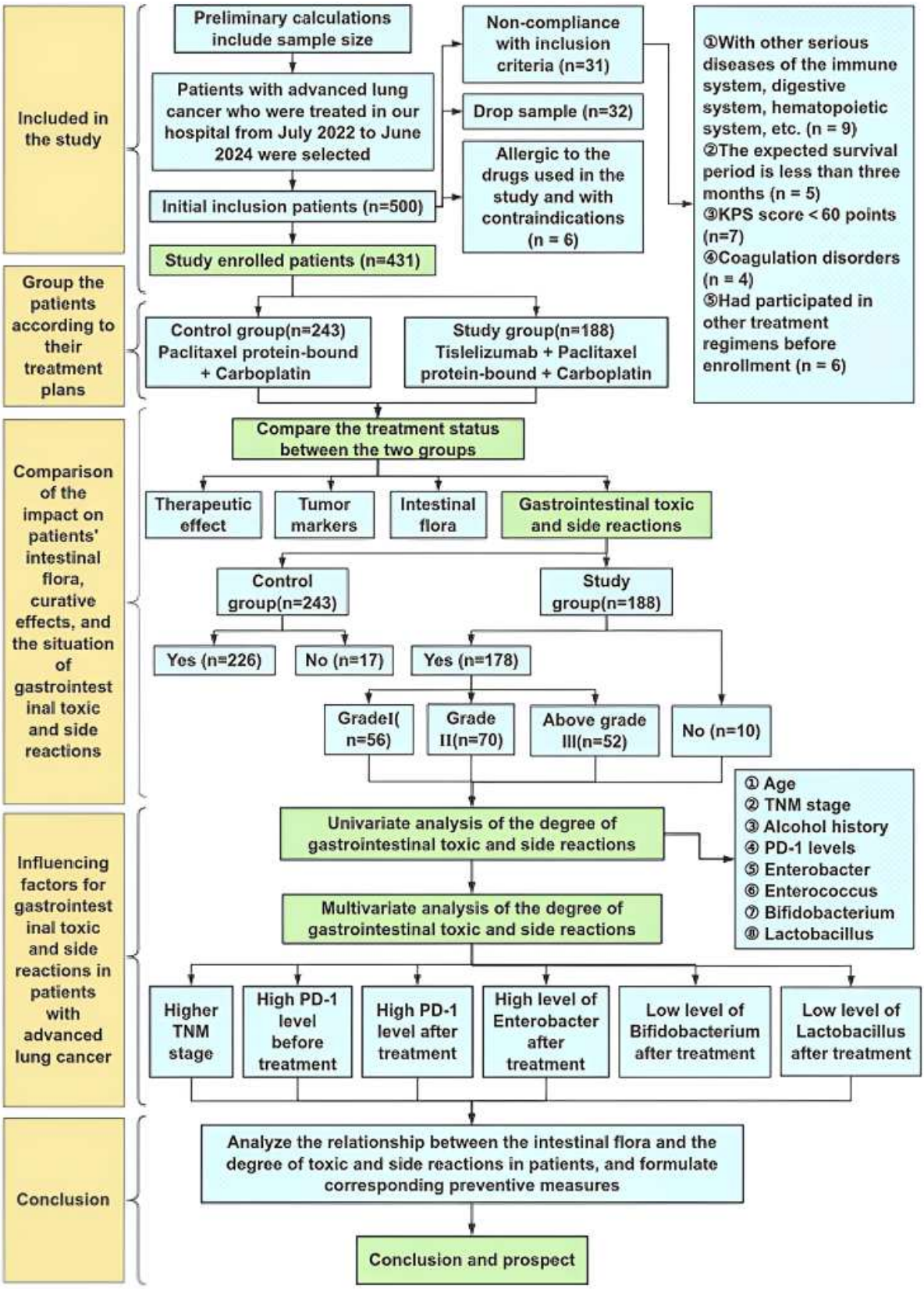
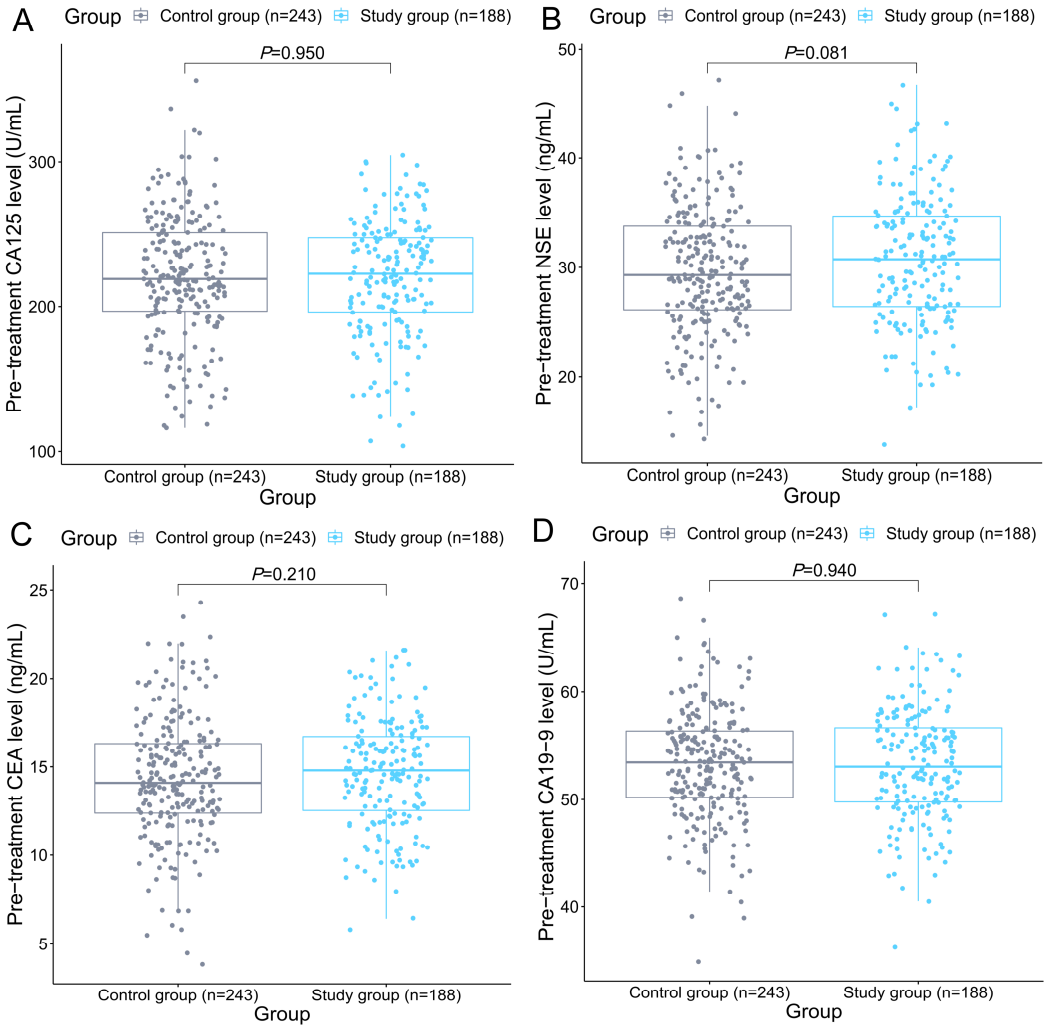


Fig. 1: Research flow chart

Table 1: Comparison of General Information of Patients [($\bar{x} \pm s$), n(%)]

Item		Control group (n = 243)	Study group (n = 188)	χ^2	*P*
Age (years)		55.62±10.91	56.13±11.09	0.250	0.803
Gender	Male (n=230) Female (n=201)	129(53.09) 114(46.91)	101(53.72) 87(46.28)	0.017	0.985
BMI (kg/m^2)		22.95±2.03	23.12±2.15	1.420	0.156
TNM stage	III b (n=319) IV (n=112)	181(74.49) 62(25.51)	138(73.40) 50(26.60)	0.064	0.800
Smoking history (n=206)		116(47.74)	90(47.87)	0.001	0.978
Drinking history (n=156)		86(35.39)	70(47.87)	0.156	0.693
Transfer situation	Yes(n=157) No(n=274)	87(35.39) 156(35.80)	70(37.23) 118(37.23)	0.094	0.759
History of radiotherapy	Yes(n=143) No(n=188)	141(64.20) 102(58.02)	102(62.77) 86(54.26)	0.612	0.434
KPS score		76.51±5.42	76.63±5.20	1.768	0.078
Pathological type	SCLC (n=43) NSCLC (n=388)	26(10.70) 217(89.30)	17(9.04) 171(90.96)	0.324	0.569



A: CA125, B: NSE, C: CEA, D: CA19-9

Fig. 2: Comparison of Tumor Markers between the Two Groups of Patients before Treatment A-D

Treatment methods

Control Group: Patients in the control group received treatment with albumin-bound paclitaxel and carboplatin. Albumin-bound paclitaxel injection was intravenously infused at a dosage of 260mg/m^2 and the infusion had to be accomplished within 180 minutes. Carboplatin injection was administered via intravenous injection at a daily dose of 25mg/m^2 from day 1 to day 3. The entire treatment regimen was structured in a 21-day cycle, with a total of 4 cycles being carried out. Study Group: In the study group, Tislelizumab injection was added to the treatment protocol of the control group. Patients received a treatment regimen consisting of 200 mg of Tislelizumab injection intravenously infused (90 minutes in the first cycle and 60 minutes in subsequent cycles), along with the control group's regimen of albumin-bound paclitaxel (260mg/m² infused within 180 minutes) and carboplatin (25mg/m² daily from day 1 to day 3) in 21-day cycles for a total of 4 cycles.

Data Collection

Collect general information of patients (age, gender, body mass index (BMI), TNM stage, pathological type (Small Cell Lung Cancer (SCLC), Non-Small Cell Lung Cancer (NSCLC)), drinking history, smoking history, tumor metastasis status, radiotherapy history, Karnofsky Performance Status (KPS) score); collect data before and after four courses of treatment: tumor markers (Carbohydrate Antigen 19-9 (CA19-9), Carcinoembryonic Antigen (CEA), Neuron-Specific Enolase (NSE), Carbohydrate Antigen 125 (CA125)), intestinal flora levels (*Enterobacter*, *Enterococcus*, *Bifidobacterium*, *Lactobacillus*, *Escherichia coli*, *Streptococcus*); collect the treatment efficacy and the occurrence of gastrointestinal toxic and side reactions within six months after the start of treatment.

(1) Efficacy Assessment

The evaluation of treatment effect was carried out in accordance with the Response Evaluation Criteria in Solid Tumors (RECIST) 1.1 (Schwartz *et al*., 2016). The efficacy outcomes were classified into the following categories: Complete Remission (CR): All visible lesions vanished completely and this state was sustained for over one month. Partial Remission (PR): The product of the maximum diameter and the maximum perpendicular diameter of the tumor decreased by at least 50%. Stable Disease (SD): The product of the maximum diameter and the maximum perpendicular diameter of the tumor decreased by less than 50% and increased by 25% or less. Progressive Disease (PD): The product of the maximum diameter and the maximum perpendicular diameter of one or more lesions in the patient increased by more than 25%.

Two key metrics, the Objective Response Rate (ORR) and the Disease Control Rate (DCR), were calculated using the following formulas. The formula for calculating the Objective Response Rate (ORR) is shown as Formula ②:

$$ORR = \frac{Number\ of\ CR\ cases + Number\ of\ PR\ cases}{Total\ number\ of\ cases} \times 100\% \quad ②$$

The formula for calculating the Disease Control Rate (DCR) is shown as Formula ③:

$$DCR = \frac{Number\ of\ CR\ cases + Number\ of\ PR\ cases + Number\ of\ SD\ cases}{Total\ number\ of\ cases} \times 100\% \quad ②$$

(2) Intestinal flora: 4-6 g of fresh feces of patients were collected before and after treatment. After sample collection, they were stored in a-80 °C refrigerator. After being dissolved in normal saline, they were inoculated on agar medium. For the cultivation of bacteria, anaerobic bacteria were incubated at a temperature of 37 degrees Celsius for a duration of 48 hours. In contrast, aerobic bacteria were subjected to incubation at the same temperature of 37 degrees Celsius, yet for a relatively shorter period of 24 hours. The colony counts of *Enterobacter*, *Enterococcus*, *Bifidobacterium*, *Lactobacillus*, *Escherichia coli* and *Streptococcus* in each gram of feces of patients were detected and expressed as lg CFU/g.

(3) Gastrointestinal toxic and side reactions: The Common Terminology Criteria for Adverse Events (CTCAE) 5.0 (Freites-Martinez *et al*., 2021) of the American Cancer Institute was utilized to grade the gastrointestinal toxic and side reactions such as nausea, vomiting, loss of appetite, diarrhea, constipation, and bloody stools in patients. The grades were defined as follows: Grade I represented mild symptoms, Grade II indicated moderate symptoms that required treatment, Grade III signified severe symptoms necessitating interventional treatment but not being life-threatening, Grade IV denoted life-threatening symptoms that required emergency treatment, and Grade V meant death. The toxic and side-reaction scores of patients were categorized into mild (Grade I), moderate toxic and side reactions (Grade II) and severe toxic and side reactions (Grade III and above).

(4) PD-1 Level Measurement: A volume of 3 ml of venous blood was drawn from the patient's elbow and transferred into an anticoagulant tube. Subsequently, the tube was placed in a centrifuge, which was set to rotate at a speed of 3500 revolutions per minute (r/min) for a duration of 10 minutes. This centrifugation process enabled the separation of the upper serum. The prepared test sample was then stored in a freezer maintained at-80°C. The determination of the PD-1 level was carried out using an enzyme-linked immunosorbent assay, with the assistance of an enzyme-linked immunoassay analyzer.

Ethical approval

This study was approved by the Medical Ethics Committee of Hebei Petrochina Central Hospital, China vide reference No.KYLL-2022-17.

STATISTICAL ANALYSIS

Statistical analyses were conducted with the aid of SPSS

27.0 statistical software. For measurement data that adhered to a normal distribution, they were presented as the mean ± standard deviation ($\bar{x}\pm s$). When comparing between two groups, independent-sample t-tests were employed. In the case of comparisons among multiple groups, one-way ANOVA (Analysis of Variance) tests were utilized. Count data were represented as the number of cases (n) along with the corresponding percentage (%). To compare between groups, chi-square tests were applied. Ordered regression was utilized to analyze the factors influencing the severity of gastrointestinal toxic and side reactions in patients with advanced lung cancer. *P*-value less than 0.05 was regarded as indicating statistical significance.

RESULTS

General Information

As depicted in table 1, the general information of the two groups of patients demonstrated no statistically significant differences.

Comparison of Intestinal Flora

Prior to treatment, no statistically significant difference was detected in the intestinal flora between the two patient groups ($P>0.05$). Post-treatment, a remarkable decrease was noted in the intestinal flora of both patient groups when compared to their pre-treatment states. Moreover, all the differences between the groups were statistically significant. Specifically, in the study group, the levels of *Enterobacter*, *Enterococcus*, *Escherichia coli*, and *Streptococcus* were found to be lower than those in the control group. Conversely, the levels of *Bifidobacterium* and *Lactobacillus* were higher in the study group ($P<0.05$). These findings are presented in table 2.

Comparison of tumor markers

As depicted in fig. 2, prior to treatment, no statistically significant difference was observed in the tumor markers between the two groups of patients. Following treatment, a substantial reduction in tumor markers was evident in both groups. Moreover, as illustrated in fig. 3, the tumor marker levels in the study group were significantly lower than those in the control group ($P<0.05$).

Comparison of efficacy

The objective response rate in the study group, which stood at 71.81%, was markedly higher than that of the control group, which was 51.44%. Additionally, the disease control rate in the study group, reaching 92.02%, was significantly greater than the 83.54% observed in the control group. All these differences were statistically significant ($P<0.05$), as presented in table 3.

Comparison of gastrointestinal toxic and side reactions

The incidence of gastrointestinal toxic and side-effects was 94.68% in the Study Group and 93.00% in the Control Group. Statistically, there was no significant difference between the two groups. Moreover, as shown in table 4, when inspecting the distribution of the degrees of these gastrointestinal toxic and side-effects, no significant statistical difference was found between the two groups ($P > 0.05$).

Analysis of influencing factors for gastrointestinal toxic and side reactions in chemotherapy combined with immunotherapy

Univariate Analysis of Gastrointestinal Toxic and Side Reactions

For patients receiving treatment with Tislelizumab Injection in conjunction with albumin-bound paclitaxel and carboplatin, a univariate analysis of their gastrointestinal toxic and side reactions was conducted. The patients were classified into groups according to the grading of the severity of their gastrointestinal toxic and side reactions, namely: none, Grade I, Grade II and Grade III or above. The outcomes of this univariate analysis revealed that multiple factors were related to the occurrence of gastrointestinal toxic and side reactions in these patients. These factors included the patients' age, TNM stage, drinking history, PD-1 levels prior to and following treatment, as well as the post-treatment levels of *Enterobacter*, *Bifidobacterium* and *Lactobacillus*. The relevant data can be found presented in table 5.

Multivariate analysis of gastrointestinal toxic and side reactions

Taking the grading of the degree of gastrointestinal toxic and side reactions as the dependent variable, and the patient's age, TNM stage, drinking history, PD-1 levels before and after treatment, the levels of *Enterobacter*, *Bifidobacterium*, and *Lactobacillus* after treatment as the independent variables, these were substituted into the ordered logistic regression analysis model. The results showed that a higher TNM stage, high PD-1 levels before and after treatment and a high level of *Enterobacter* after treatment were risk factors for the occurrence of gastrointestinal toxic and side reactions in patients, while high levels of *Bifidobacterium* and *Lactobacillus* after treatment were protective factors, as shown in table 6.

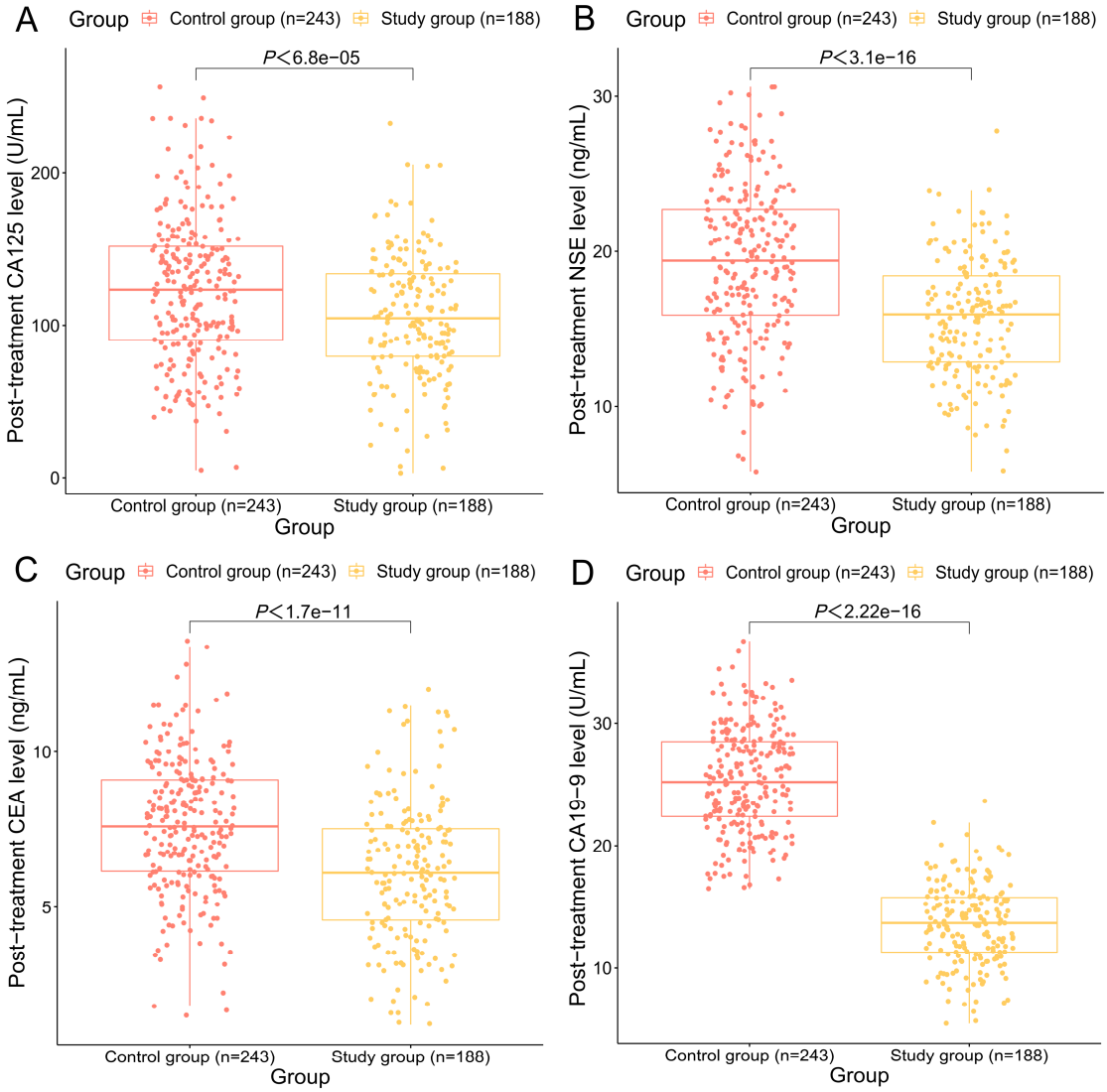
DISCUSSION

Albumin-bound paclitaxel combined with platinum agents is a first-line chemo cornerstone for advanced lung cancer patients. Paclitaxel, a microtubule-inhibitor, disrupts cell division and has immunomodulatory effects, like curbing TNF release and upregulating interferon and IL-1, acting against neoplastic cells (Tian and Yao, 2022). Carboplatin, a 2nd-gen platinum agent, induces DNA cross-linking, halting tumor cell proliferation (Tsuruoka *et al*., 2024). Yet, this dual-drug regimen's efficacy is limited. The 19%-28% response rate hampers patient outcomes; NSCLC patients' median survival is just 8 months. Toxic side effects like nausea and neuropathy reduce treatment compliance (Schiller *et al*., 2022; Kelly *et al*., 2001). Immunotherapy has become crucial.

Table 2: Comparison of intestinal flora of patients [($\bar{x} \pm s$)]

Item		Control group (n = 243)	Study group (n = 188)	χ^2	*P*
Enterobacter (cfu/g)	Before treatment	6.48±1.77	6.60±1.82	0.122	0.903
	After treatment	4.37±1.99[a]	6.02±1.93[a]	9.444	<0.001
Enterococcus (cfu/g)	Before treatment	6.23±1.82	6.36±1.94	1.121	0.263
	After treatment	5.82±1.57[a]	3.23±1.42[a]	18.870	<0.001
Bifidobacterium (cfu/g)	Before treatment	6.24±1.82	6.12±1.90	1.264	0.207
	After treatment	3.76±1.04[a]	5.57±1.43[a]	15.921	<0.001
Lactobacillus (cfu/g)	Before treatment	8.92±1.76	8.83±1.88	0.158	0.875
	After treatment	5.28±1.96[a]	8.25±1.74[a]	16.544	<0.001
E. coli (cfu/g)	Before treatment	10.73±2.16	10.51±2.22	1.524	0.128
	After treatment	10.11±1.90[a]	9.27±1.03[a]	5.308	<0.001
Streptococcus (cfu/g)	Before treatment	5.23±1.48	5.26±1.52	0.005	0.996
	After treatment	4.85±1.14[a]	4.22±0.91[a]	6.357	<0.001

Note: The difference compared with that before treatment within the group was statistically significant, $^a p < 0.05$.



A: CA125, B: NSE, C: CA, D: CA19-9

Fig. 3: Comparison of tumor markers between the two groups of patients after treatment A-D

Table 3: Comparison of treatment efficacy of patients [n(%)]

	CR	PR	SD	PD	ORR	DCR
Control group (n = 243)	1(0.41)	124(51.03)	78(32.10)	40(16.46)	125(51.44)	203(83.54)
Study group (n = 188)	3(1.60)	132(70.21)	38(20.21)	15(7.98)	135(71.81)	173(92.02)
χ^2					18.373	6.850
P					<0.001	0.009

Table 4: Comparison of gastrointestinal toxic and side reactions in patients [n(%)]

	Grade I (n=56)	Grade II (n=70)	Grade III and above (n=52)	Overall occurrence
Control group (n = 243)	55(22.63)	96(39.51)	75(30.86)	226(93.00)
Study group (n = 188)	56(29.79)	70(37.23)	52(27.66)	178(94.68)
χ^2	2.837	0.231	0.524	0.507
p	0.092	0.631	0.469	0.476

Table 5: Univariate analysis of gastrointestinal toxic and side reactions in chemotherapy combined with immunotherapy [(x± s), n(%)]

Item		None(n=10)	Grade I (n=56)	Grade II (n=70)	Grade III and above (n=52)	F/χ^2	*p*
Age (years)		50.30±11.80	56.66±10.78	54.06±9.69	58.92±12.42	2.951	0.034
Gender	Male (n=101)	4(40.00)	30(53.57)	38(54.29)	29(55.77)	0.854	0.836
	Female (n=87)	6(60.00)	26(46.43)	32(45.71)	23(44.23)		
TNM stage	IIIb (n=138)	10(100.00)	51(91.07)	55(78.57)	22(42.31)	20.666	<0.001
	IV (n=50)	0(0.00)	5(8.93)	15(21.43)	30(57.69)		
BMI (kg/m^2)		21.94±1.40	23.55±1.89	23.370±2.32	23.04±2.22	1.853	0.139
KPS score		76.20±4.47	77.64±5.34	78.04±5.36	77.31±5.06	0.460	0.710
Drinking history (n=70)		2(20.00)	15(26.79)	26(37.14)	27(51.92)	8.688	0.034
Smoking history (n=90)		4(40.00)	24(42.86)	36(51.43)	26(50.00)	1.261	0.738
Transfer situation	Yes (n=70)	6(60.00)	16(28.57)	31(44.29)	19(36.54)	5.365	0.147
	No (n=118)	4(40.00)	40(71.43)	39(55.71)	33(63.46)		
Radiotherapy history	Yes (n=102)	5(50.00)	26(46.43)	41(58.57)	30(57.69)	2.228	0.526
	No (n=86)	5(50.00)	30(53.57)	29(41.43)	22(42.31)		
Pathological type	SCLC (n=17)	2(20.00)	8(14.29)	5(7.14)	2(3.85)	5.346	0.148
	NSCLC (n=171)	8(80.00)	48(85.72)	65(92.86)	50(96.15)		
PD-1(pg/mL)	Before treatment	254.65±11.06	253.63±15.32	259.96±15.94	271.45±17.41	12.003	<0.001
	After treatment	178.06±9.16	180.95±14.24	189.68±15.21	203.83±18.46	21.605	<0.001
Enterobacter (cfu/g)	Before treatment	6.22±2.36	7.01±1.93	6.50±1.61	6.23±1.83	1.873	0.136
	After treatment	2.10±0.98	3.35±1.14	4.27±1.00	6.14±1.37	69.475	<0.001
Enterococcus (cfu/g)	Before treatment	7.30±2.32	6.36±1.90	6.38±1.98	6.22±1.90	0.857	0.465
	After treatment	4.75±1.16	4.55±1.58	4.47±1.71	4.06±1.79	1.071	0.363
Bifidobacterium (cfu/g)	Before treatment	4.91±2.27	6.02±1.75	6.26±1.87	6.09±2.03	1.484	0.220
	After treatment	6.96±1.09	6.45±1.22	5.62±0.84	4.43±1.44	32.324	<0.001
Lactobacillus (cfu/g)	Before treatment	9.40±2.02	8.87±1.63	8.68±2.05	9.07±1.92	0.681	0.565
	After treatment	10.16±1.68	9.47±1.29	8.28±1.07	6.67±1.52	48.813	<0.001
E. coli (cfu/g)	Before treatment	10.29±2.24	10.75±1.76	10.27±1.62	10.59±1.62	0.900	0.442
	After treatment	9.10±1.18	9.14±1.07	9.35±1.01	9.13±0.96	0.689	0.560
Streptococcus (cfu/g)	Before treatment	5.31±0.89	5.12±1.57	5.35±1.43	5.29±1.72	0.247	0.863
	After treatment	4.65±0.65	4.36±0.87	4.14±0.97	4.14±0.89	1.496	0.217

Table 6: Multivariate analysis of gastrointestinal toxic and side reactions in chemotherapy combined with immunotherapy

Factor	*Estimate*	*S.E.*	*Wald*X^2	*P*	*exp*	95% CI lower limit	95% CI upper limit
Age	-0.003	0.017	0.023	0.879	0.997	-0.036	0.031
TNM Stage IV	1.027	0.481	4.557	0.033	1.618	0.084	1.969
Drinking history	0.037	0.394	0.009	0.925	1.483	-0.736	0.81
Pre-treatment PD-1	0.031	0.012	7.215	0.007	1.031	0.008	0.054
PD-1 after treatment	0.034	0.012	8.697	0.003	1.035	0.011	0.057
Enterobacter after treatment	1.078	0.165	42.665	<0.001	1.180	0.755	1.402
Enterococcus after treatment	-0.105	0.108	0.938	0.333	1.114	-0.316	0.107
Bifidobacteria after treatment	-0.65	0.162	16.21	<0.001	1.176	-0.967	-0.334
Lactobacillus after treatment	-0.781	0.149	27.57	<0.001	1.161	-1.072	-0.489

Tumor cells evade immunity via immune checkpoints like the PD-1/PD-L1 axis. Tislelizumab, an ICI approved in Dec 2019 for Hodgkin lymphoma, shows promise in lung cancer. It targets PD-1 but can cause irAEs, especially gastrointestinal ones (Lu *et al*., 2021). The gut microbiota impacts treatment. It interacts with the host immune system, and specific compositions are linked to chemotherapy tolerance, response, and prognosis (Dong *et al*., 2021). Lung cancer patients have dysregulated gut microbiota. Qian *et al.* (2022) found certain bacteria abundance changes in their samples. Jin *et al*. (2019) showed high gut microbial diversity linked to enhanced immune cells, hinting at its role in anti-PD-1/PD-L treatment. The gut microbiome could be a biomarker and treatment target.

This study aimed to compare gut microbiota in advanced lung cancer patients before and after chemo-immunotherapy, explore its link to gut toxic side-effects, identify related microbial signatures and optimize treatment through micro biota-targeted means like probiotics or dietary changes.

Efficacy of combination therapy

In this study, patients receiving dual-drug chemotherapy combined with Tislelizumab showed greater efficacy. Their tumor markers like carcinoembryonic antigen (CEA) and cytokeratin 19 fragments (CYFRA21-1) were significantly lower compared to those on dual-drug chemo alone, quantifying the combined treatment's enhanced effect (Zhu *et al*., 2023). Albumin-bound paclitaxel, a new form of paclitaxel, can boost paclitaxel concentration in tumor tissues. Its albumin part can recognize gp60 receptors on vascular endothelial cell membranes, interact with membrane vesicles, and enable receptor-mediated drug-complex translocation. This increases drug uptake and tumor-cell killing, and may more thoroughly disrupt the tumor's microtubule network, curbing growth and metastasis. However, in traditional chemotherapy, high drug-induced toxicity often reduces patient tolerance. Side effects like severe nausea, vomiting and myelosuppression can affect quality of life and treatment continuation, harming treatment outcomes. islelizumab, a humanized anti-PD-1 monoclonal antibody, can reverse T-cell anergy or exhaustion. In the body, it activates PD-1, countering the immunosuppressive tumor microenvironment. PD-1 on T-cell surfaces binds to PD-L1 and PD-L2, suppressing T-cell proliferation and cytokine activity, hindering immune surveillance. Tislelizumab blocks this, enhancing the anti-tumor immune response and inhibiting tumor growth. It may also modulate other immune-related pathways involving dendritic cells to boost overall anti-tumor immunity (Lee and Keam, 2020). Designed to minimize FcγR-macrophage interaction, Tislelizumab can effectively stop antibody-dependent phagocytosis and has a high affinity for PD-1. This reduces unwanted immune-mediated side-effects. Adding Tislelizumab injection to dual-drug chemotherapy can halt disease progression. This combination, merging chemo's direct cytotoxicity with Tislelizumab's immune-modulating properties, offers a more comprehensive and potent anti-tumor response, bringing new hope to advanced-malignancy patients.

Impact on intestinal flora

In this study, both patient groups had a high incidence of gastrointestinal side-effects. Adding tislelizumab didn't raise this rate, and stats showed no significant difference between the two (Bum *et al*., 2024). The gut flora affects lung cancer onset via the gut-lung axis, influencing the lung's immune and inflammatory responses, and producing metabolites. During treatment, both groups' gut flora was disrupted, but the Study Group had more *Bifidobacterium* and *Lactobacillus*. Li *et al.* (2023) found 40 gut-microbiota groups linked to lung-cancer subtypes. *Bifidobacterium*, a probiotic, has antioxidant subspecies that may protect against lung cancer. Vernocchi *et al*. (2022) found NSCLC patients had imbalanced gut flora. Short-chain fatty acids (SCFAs) from *Lactobacillus* and *Bifidobacterium* are linked to a healthy gut and could be anti-PD-1 treatment biomarkers. The Study Group had lower levels of *Enterobacter*, *Enterococcus*, *Escherichia coli* and *Streptococcus*. This means less immune- function damage in those on combined immunotherapy. The treatment likely stabilized the gut-flora imbalance, cut pathogenic-strain levels, and stopped the immune system's abnormal activation from imbalanced gut flora.

Factors influencing gastrointestinal toxicities

Analysis of influencing factors indicated that an advanced TNM stage was a major risk factor for gastrointestinal toxic and side reactions. As the TNM stage progresses, tumors become more aggressive, requiring higher-dose ICI treatment. A higher ICI concentration in the gut microenvironment stimulates the mucosal layer, disrupting the intestinal flora balance, which compromises gut functions and raises the risk of adverse reactions. Elevated PD-1 levels before and after treatment were recognized as risk factors. PD-1, part of the immunoglobulin B7-CD38 family, is expressed on key immune cells. Its interaction with PD-L1 on tumor cells is crucial for tumor immune evasion, making PD-1 a vital biomarker in lung cancer research (Han *et al.,* 2020). A high post-treatment *Enterobacter* level was a risk factor, while high *Bifidobacterium* and *Lactobacillus* levels were protective. Chemotherapy drugs damage intestinal epithelial villi and tight junctions, causing flora imbalance and more opportunistic pathogens (El Tekle and Garrett, 2023). *Lactobacillus* modulates the immune response by enhancing dendritic cell differentiation and cytokine secretion, and regulating innate lymphoid cell proliferation (Zhu *et al*., 2023). *Bifidobacterium* exerts protective effects through its metabolites, regulating intestinal immunity and reducing inflammation-related side-effects (Zheng *et al*., 2020).

Lung cancer patients often endure high-incidence gastrointestinal side-effects like nausea, vomiting, constipation, and anorexia due to chemotherapy drugs. These not only harm their quality of life but also impede treatment. So, improving the intestinal flora's micro-environment is crucial for managing these issues. Duttagupta *et al*. (2023) show fecal microbiota transplantation (FMT) can directly alter the intestinal microbiota. In cancer immunotherapy, FMT has shown safety and effectiveness. It transfers fecal microbes from a healthy donor to the patient's gut (Yadegar *et al*., 2024). Since the gut microbiota's role in anti-cancer immunity is recognized, FMT may enhance immunotherapy outcomes. But its long-term effects and mechanisms need more study for use as ICI adjuvant treatment. During chemotherapy, tailored nutritional support is key. Supplementing probiotics, prebiotics, and synbiotics can strengthen the intestinal barrier and reduce reactions. A rational diet plan should encourage nutrient-rich foods. After vomiting, fresh produce helps, and for nausea, easy-to-digest foods are best. Also, a good work-rest and exercise routine benefits patients. This single-center retrospective study has limitations. It's prone to sample-size selection bias, and the single-center setup limits sample representativeness. The lack of long-term follow-up hinders understanding the treatment's long-term impact. Future multi-center, large-sample, and prospective studies are needed to better evaluate the link between intestinal flora, immunotherapy, and side-effects.

CONCLUSION

In summary, in the context of advanced lung cancer patients, incorporating tislelizumab into the treatment protocol that already includes albumin-bound paclitaxel and carboplatin can effectively augment treatment efficacy. This integrated approach not only significantly enhances the therapeutic outcome but also serves to restore the equilibrium of the intestinal flora. Additionally, it alleviates the severity of gastrointestinal toxic and side-effects. As such, this treatment combination may contribute to an improved quality of life and enhanced treatment compliance among patients suffering from advanced lung cancer.

ACKNOWLEDGMENT

We would like to express our sincere thanks to Hebei Petrochina Central Hospital. It provides a site for research, facilitates sample acquisition and facilitates data collection. The advanced technical equipment of the hospital guarantees the experimental detection and analysis. The medical staff was actively involved in patient recruitment, management and data collection, and their professional dedication facilitated the smooth conduct of the study.

FUNDING

Langfang Science and Technology Support Plan Project (No.2022013013)

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