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*Discovery Medicine* 2025; 37(199): 1586–1594
https://doi.org/10.24976/Discov.Med.202537199.137

# Efficacy and Safety of Neoadjuvant Chemoimmunotherapy in Patients With Muscle-Invasive Bladder Cancer

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Published: 20 August 2025

**Background: Muscle-invasive bladder cancer (MIBC) is an aggressive malignancy associated with high mortality. While neoadjuvant chemotherapy (NAC) remains the standard of care, its overall benefit is limited. The addition of immune checkpoint blockade (ICB) to NAC holds potential for improving patient outcomes. This study aimed to compare the pathological response and safety profile of NAC alone versus NAC combined with the programmed cell death protein 1 (PD-1) inhibitor tislelizumab (NAC+ICB) in a real-world cohort of MIBC patients.**

**Methods: A total of 153 MIBC patients treated between October 2021 and December 2024 were retrospectively reviewed. Among them, 88 received gemcitabine/cisplatin-based NAC, while 65 received the same regimen combined with the PD-1 inhibitor tislelizumab (NAC+ICB). The primary endpoints were pathological complete response (pCR, ypT0N0) and the incidence of grade ≥3 treatment-related adverse events (TRAEs). Multivariate logistic regression was applied to identify factors associated with pCR.**

**Results: The NAC+ICB group showed a significantly higher pCR rate compared to the NAC group (33.85% vs. 18.18%, $p$ = 0.027). Pathological downstaging rate (≤ypT1N0) was also significantly higher in the combination group (58.46% vs. 38.64%, $p$ = 0.015). The incidence of grade ≥3 TRAEs did not significantly differ between groups (41.54% vs. 38.64%, $p$ = 0.717). Multivariate analysis revealed that lower platelet counts (OR = 0.960, $p$ = 0.003), higher hemoglobin levels (OR = 1.229, $p$ = 0.002), and lower total cholesterol levels (OR = 0.010, $p$ = 0.004) were independently associated with a higher pCR rate in the NAC+ICB group.**

**Conclusion: Compared with chemotherapy alone, NAC combined with ICB significantly improves pathological response in MIBC patients without increasing severe adverse events, suggesting favorable efficacy and safety. This combination represents a novel strategy for perioperative optimization, though prospective studies are needed to validate its long-term survival benefits.**

**Keywords:** bladder cancer; muscle-invasive bladder cancer; neoadjuvant chemotherapy; immune checkpoint inhibitors

## Introduction

Bladder cancer is among the most prevalent urologic malignancies globally, with nearly 600,000 new cases diagnosed annually [1]. Muscle-invasive bladder cancer (MIBC) represents a more aggressive subtype, accounting for approximately 30% of all cases [2], and has a 5-year overall survival (OS) rate of only about 50% [3]. Clinical guidelines recommend cisplatin-based neoadjuvant chemotherapy (NAC) prior to radical cystectomy (RC) for MIBC, as it improves downstaging and survival outcomes [4].

However, the benefit of conventional NAC remains limited. Even with standard NAC followed by surgery, approximately 40% of patients experience disease recurrence or death within three years [5]. Research indicates that NAC improves 5-year survival by only 5–8% [6]. Therefore, novel perioperative treatment strategies aimed at improving pathological downstaging and long-term survival are urgently needed in MIBC management [7].

Recently, immune checkpoint blockade (ICB), particularly inhibitors targeting programmed cell death protein 1/programmed cell death ligand 1 (PD-1/PD-L1), has demonstrated substantial efficacy in advanced urothelial carcinoma and is now a standard second-line therapy after platinum-based chemotherapy failure [8]. Encouraged by its success in metastatic disease, ICB is being actively investigated in the neoadjuvant setting for MIBC. Several Phase II trials have explored single-agent ICB prior to radical cystectomy (RC). For example, the PURE-01 trial evaluated three cycles of the PD-1 inhibitor pembrolizumab and reported a pathological complete response (pCR; pT0) rate of 42% [9]. Similarly, the ABACUS trial assessed two cycles of the PD-L1 inhibitor atezolizumab in cisplatin-

ineligible MIBC patients, achieving a 31% pCR rate [10]. These single-arm studies suggest that checkpoint inhibitors can induce complete remission in a substantial subset of MIBC patients, although long-term outcomes and optimal patient selection remain undefined. However, these trials were predominantly single-arm or enrolled highly selected patient populations, and their survival benefits require longer follow-up. Moreover, evidence on the efficacy and safety of ICB in broader, real-world populations remains limited.

Building on the modest activity of immunotherapy alone, a logical next step has been to combine ICB with standard chemotherapy in the neoadjuvant setting. One study assessed pembrolizumab with a split-dose gemcitabine/cisplatin regimen, reporting a pCR rate of 36% and meeting its prespecified endpoint for improved tumor downstaging compared to prior NAC [11]. Despite these encouraging findings, significant knowledge gaps remain. Most earlier trials were conducted in Western populations or under the strict inclusion criteria for controlled clinical trial conditions. Real-world data on the efficacy and safety of NAC combined with ICB (NAC+ICB) in unselected MIBC patients remain limited, especially in Asian cohorts. Chinese MIBC patients may have unique genetic, environmental, or healthcare-related factors that influence the outcomes of chemoimmunotherapy. Moreover, optimal patient selection and predictive biomarkers for chemoimmunotherapy remain under investigation.

In this context, we conducted a real-world study evaluating neoadjuvant chemoimmunotherapy (NAC+ICB) in MIBC patients. We aimed to compare its efficacy with standard NAC and to explore factors associated with pathological response. By examining patient populations and clinical settings underrepresented in prior trials, this study addresses a critical gap and offers insights into the use of neoadjuvant immunotherapy for MIBC in the Chinese population. Accordingly, we performed a retrospective real-world analysis to assess the efficacy and safety of gemcitabine/cisplatin-based NAC combined with toripalimab (NAC + ICB) during the neoadjuvant phase in MIBC patients, and to identify potential predictors of response, thereby contributing evidence for optimizing perioperative treatment strategies.

## Methods

### *Study Design*

This retrospective, single-center cohort study was conducted at Jinhua Municipal Central Hospital. The study was approved by the Ethics Committee of Jinhua Municipal Central Hospital (Approval No. 2021-315-001), and written informed consent was obtained from all participants. The study was conducted following the ethical principles outlined in the Declaration of Helsinki.

### *Patient Population*

Patients diagnosed with MIBC and treated at Jinhua Municipal Central Hospital between October 2021 and December 2024 were enrolled.

**Inclusion criteria were as follows**:

(1) Pathologically confirmed MIBC according to the latest guidelines of the European Association of Urology (EAU) or the American Society of Clinical Oncology (ASCO) [12,13];

(2) Receipt of at least two cycles of standardized neoadjuvant therapy;

(3) Underwent radical cystectomy (RC), maximal transurethral resection of bladder tumor (TURBT), or partial cystectomy following neoadjuvant therapy for accurate pathological staging.

**Exclusion criteria included**:

(1) Histopathological diagnosis of metastatic cancer other than MIBC;

(2) Complete TURBT was performed before neoadjuvant therapy, as the absence of a residual baseline tumor would preclude accurate assessment of pathological response to treatment, the primary endpoint of this study;

(3) Incomplete perioperative pathological data.

### *Treatment Groups and Neoadjuvant Regimens*

During the neoadjuvant phase, patients were categorized into two treatment groups based on treatment regimens:

(1) NAC group: Received gemcitabine plus cisplatin, consisting of gemcitabine (CHIATAI TIANQING, Lianyungang, China) 1000 mg/m$^2$ intravenously on days 1 and 8, and cisplatin (Qilu Pharmaceutical, Jinan, China) 70 mg/m$^2$ intravenously on day 2, every 21 days per cycle.

(2) NAC+ICB group: Received the same chemotherapy regimen combined with the PD-1 inhibitor tislelizumab (Jiangsu Hengrui Pharmaceuticals, Lianyungang, China) 200 mg intravenously on day 1 of each 21-day cycle, concurrently with chemotherapy.

All patients underwent 2–4 cycles of neoadjuvant therapy, followed by RC and pelvic lymph node dissection. Tumor response was evaluated based on postoperative pathological findings.

### *Data Collection*

Clinical data were extracted from the hospital's electronic health records system. All data were anonymized to maintain patient confidentiality. Baseline characteristics collected included age, sex, body mass index (BMI), comorbidities (hypertension, diabetes, cardiovascular disease), smoking history, clinical TNM stage (cTNM), tumor histology and grade, tumor multiplicity, and Eastern Cooperative Oncology Group (ECOG) performance status.

Pre-treatment laboratory parameters were also recorded, including white blood cell count (WBC), C-reactive protein (CRP), neutrophil count, lymphocyte

**Table 1. Baseline characteristics of patients in the NAC and NAC+ICB groups.**

| Variable | Total (n = 153) | NAC (n = 88) | NAC+ICB (n = 65) | Statistic | *p*-value |
|---|---|---|---|---|---|
| Age, years | 64.72 ± 4.92 | 65.01 ± 4.66 | 64.33 ± 5.28 | $t = 0.841$ | 0.402 |
| Gender, n (%) | | | | $\chi^2 = 0.008$ | 0.930 |
| Male | 129 (84.31) | 74 (84.09) | 55 (84.62) | | |
| Female | 24 (15.69) | 14 (15.91) | 10 (15.38) | | |
| BMI, kg/m$^2$ | 21.70 ± 3.21 | 21.84 ± 3.32 | 21.52 ± 3.07 | $t = 0.598$ | 0.551 |
| Hypertension, n (%) | | | | $\chi^2 = 1.011$ | 0.315 |
| No | 119 (77.78) | 71 (80.68) | 48 (73.85) | | |
| Yes | 34 (22.22) | 17 (19.32) | 17 (26.15) | | |
| Diabetes, n (%) | | | | $\chi^2 = 0.517$ | 0.472 |
| No | 123 (80.39) | 69 (78.41) | 54 (83.08) | | |
| Yes | 30 (19.61) | 19 (21.59) | 11 (16.92) | | |
| Heart disease, n (%) | | | | $\chi^2 = 0.001$ | 0.976 |
| No | 139 (90.85) | 80 (90.91) | 59 (90.77) | | |
| Yes | 14 (9.15) | 8 (9.09) | 6 (9.23) | | |
| Smoking, n (%) | | | | $\chi^2 = 1.304$ | 0.253 |
| No | 86 (56.21) | 46 (52.27) | 40 (61.54) | | |
| Yes | 67 (43.79) | 42 (47.73) | 25 (38.46) | | |
| Clinical stage, n (%) | | | | $\chi^2 = 0.307$ | 0.579 |
| >T2N0M0 | 69 (45.10) | 38 (43.18) | 31 (47.69) | | |
| T2N0M0 | 84 (54.90) | 50 (56.82) | 34 (52.31) | | |
| Histology, n (%) | | | | $\chi^2 = 1.043$ | 0.594 |
| Squamous | 39 (25.49) | 20 (22.73) | 19 (29.23) | | |
| Adenocarcinoma | 82 (53.59) | 50 (56.82) | 32 (49.23) | | |
| Others | 32 (20.92) | 18 (20.45) | 14 (21.54) | | |
| Grade, n (%) | | | | $\chi^2 = 2.411$ | 0.120 |
| Low | 43 (28.10) | 29 (32.95) | 14 (21.54) | | |
| High | 110 (71.90) | 59 (67.05) | 51 (78.46) | | |
| Tumor number, n (%) | | | | $\chi^2 = 1.500$ | 0.221 |
| Single | 125 (81.70) | 69 (78.41) | 56 (86.15) | | |
| Multiple | 28 (18.30) | 19 (21.59) | 9 (13.85) | | |
| ECOG, n (%) | | | | $\chi^2 = 2.674$ | 0.102 |
| 0 | 116 (75.82) | 71 (80.68) | 45 (69.23) | | |
| 1 | 37 (24.18) | 17 (19.32) | 20 (30.77) | | |

Abbreviations: NAC+ICB, neoadjuvant chemotherapy combined with immune checkpoint blockade; NAC, neoadjuvant chemotherapy; BMI, body mass index; ECOG, Eastern Cooperative Oncology Group.

count, monocyte count, platelet count (PLT), red blood cell count (RBC), hemoglobin (HB), albumin (ALB), alanine aminotransferase (ALT), triglycerides (TG), total cholesterol (TC), high-density lipoprotein cholesterol (HDL), low-density lipoprotein cholesterol (LDL), neutrophil-to-lymphocyte ratio (NLR), and platelet-to-lymphocyte ratio (PLR).

Histopathological diagnosis was confirmed through diagnostic TURBT. All pathological assessments were independently reviewed by two experienced pathologists.

### *Definitions*

Pathological complete response (pCR): Absence of residual invasive cancer in the surgical specimen after RC or adequate pathological evaluation (ypT0N0) [5].

Pathological partial response (pPR): Tumor downstaging to <ypT2N0.

Overall pathologic downstaging rate: Proportion of patients with pT < T2 and pN = N0 (pTis-T1N0M0), including both pCR and pPR cases [5].

Treatment-related adverse events (TRAEs): Adverse events were graded according to the National Cancer Institute's Common Terminology Criteria for Adverse Events, version 5.0 (NCI-CTCAE v5.0) [14]. The primary safety outcome was the incidence of grade ≥3 TRAEs.

Tumor grade: Classified according to the 2022 World Health Organization (WHO) classification system as either low-grade or high-grade [15]. High-grade indicates poorly differentiated carcinomas, whereas low-grade refers to well-differentiated tumors.

**Table 2. Pathological response to NAC versus NAC+ICB regimens.**

| Variable | Total (n = 153) | NAC (n = 88) | NAC+ICB (n = 65) | Statistic | *p*-value |
|---|---|---|---|---|---|
| Pathological response, n (%) | | | | $\chi^2 = 9.048$ | 0.029 |
| CR | 38 (24.84) | 16 (18.18) | 22 (33.85) | | |
| PD | 35 (22.88) | 20 (22.73) | 15 (23.08) | | |
| PR | 34 (22.22) | 18 (20.45) | 16 (24.62) | | |
| SD | 46 (30.07) | 34 (38.64) | 12 (18.46) | | |
| pCR (ypT0N0), n (%) | | | | $\chi^2 = 4.914$ | 0.027 |
| No | 115 (75.16) | 72 (81.82) | 43 (66.15) | | |
| Yes | 38 (24.84) | 16 (18.18) | 22 (33.85) | | |
| Overall downstaging (≤ypT1N0), n (%) | | | | $\chi^2 = 5.898$ | 0.015 |
| Nonresponder (SD + PD) | 81 (52.94) | 54 (61.36) | 27 (41.54) | | |
| Responder (CR + PR) | 72 (47.06) | 34 (38.64) | 38 (58.46) | | |

Abbreviations: CR, complete response; PR, partial response; SD, stable disease; PD, progression disease; pCR, pathological complete response.

### *Statistical Analysis*

Data were analyzed using R software (version 4.4.2; R Foundation for Statistical Computing, Vienna, Austria). Continuous variables were expressed as mean ± standard deviation for normally distributed data or median with interquartile range (IQR) for non-normally distributed data. The Shapiro-Wilk test was used to assess normality. Between-group comparisons of normally distributed variables were performed using independent *t*-tests, while non-normally distributed variables were compared using the Mann-Whitney U test. Categorical variables were presented as frequencies and percentages and compared using the chi-square test.

To identify factors independently associated with pCR in the NAC+ICB group, variables with a $p$-value $< 0.05$ in univariate analysis were included in a multivariate logistic regression model. Correlation analysis was performed to evaluate collinearity among these variables. A two-sided $p < 0.05$ was considered statistically significant.

## Results

### *Baseline Characteristics of the Study Population*

A total of 153 patients with MIBC were included in this study. Among them, 88 patients (57.5%) received neoadjuvant chemotherapy alone (NAC group), while 65 patients (42.5%) received neoadjuvant chemotherapy combined with tislelizumab (NAC+ICB group).

There were no statistically significant differences between the two groups in terms of age, sex, comorbidities (hypertension, diabetes, cardiovascular disease), smoking history, clinical stage, histological subtype, tumor grade, tumor multiplicity, or ECOG performance status (all $p > 0.05$). These findings indicate that the baseline characteristics of the two groups were comparable (Table 1).

### *Comparison of Pathological Responses Between the Two Treatment Regimens*

Postoperative pathological responses of the two groups are summarized in Table 2. Compared with the NAC group, the NAC+ICB group demonstrated a significantly higher pCR rate. The pCR rate in the NAC+ICB group was 33.85%, significantly greater than the 18.18% observed in the NAC group ($p = 0.027$). The overall pathological downstaging rate (≤ypT1N0) was also significantly higher in the NAC+ICB group (58.46% vs. 38.64%, $p = 0.015$).

### *Comparison of Clinical Characteristics Between pCR and Non-pCR Patients in the NAC+ICB Group*

Given the favorable pathological response observed in the NAC+ICB group, we focused on this cohort to identify clinical factors associated with achieving pCR. Baseline clinical characteristics of patients who achieved pCR (n = 22) were compared with those who did not (n = 43), as presented in Table 3.

The results revealed that patients who achieved pCR had significantly lower PLT levels and higher HB and RBC levels (all $p < 0.05$). In addition, TC levels were significantly lower in the pCR group compared to the non-pCR group ($p < 0.001$). No significant association was observed between traditional inflammatory markers, such as the NLR, and pCR status ($p > 0.05$), suggesting the need for more specific predictive biomarkers.

### *Factors Associated With Pathological Complete Response in the NAC+ICB Group*

To identify factors independently associated with pCR, variables with $p < 0.05$ in the univariate analysis were entered into a multivariate logistic regression model. The results indicated that lower PLT counts, higher HB levels, and lower TC levels were independently associated with achieving pCR (Table 4). Correlation analysis was also performed to assess the relationships between these variables and pCR (Table 5). PLT and TC were negatively correlated

**Table 3. Comparison of clinical characteristics between pCR and non-pCR patients in the NAC+ICB group.**

| Variable | Total (n = 65) | Non-pCR (n = 43) | pCR (n = 22) | Statistic | *p*-value |
|---|---|---|---|---|---|
| Age, years | 64.33 ± 5.28 | 65.01 ± 5.75 | 63.01 ± 4.00 | $t$ = 1.458 | 0.150 |
| BMI, kg/m$^2$ | 21.52 ± 3.07 | 21.70 ± 3.07 | 21.17 ± 3.10 | $t$ = 0.661 | 0.511 |
| WBC, 10$^9$/L | 5.93 ± 1.49 | 6.13 ± 1.35 | 5.56 ± 1.69 | $t$ = 1.476 | 0.145 |
| CRP, mg/L | 6.44 (3.34, 11.15) | 6.39 (3.24, 10.84) | 7.17 (3.75, 11.48) | $Z$ = –0.340 | 0.734 |
| Neutrophil, 10$^9$/L | 3.98 ± 1.53 | 4.08 ± 1.54 | 3.80 ± 1.53 | $t$ = 0.698 | 0.488 |
| Lymphocyte, 10$^9$/L | 1.39 ± 0.29 | 1.40 ± 0.28 | 1.36 ± 0.31 | $t$ = 0.472 | 0.639 |
| Monocyte, 10$^9$/L | 0.37 ± 0.14 | 0.37 ± 0.14 | 0.35 ± 0.12 | $t$ = 0.699 | 0.487 |
| PLT, 10$^9$/L | 214.44 ± 52.78 | 227.16 ± 55.53 | 189.58 ± 36.65 | $t$ = 2.865 | 0.006 |
| RBC, 10$^{12}$/L | 3.95 ± 0.61 | 3.83 ± 0.56 | 4.18 ± 0.63 | $t$ = –2.302 | 0.025 |
| HB, g/L | 129.49 ± 10.50 | 125.96 ± 9.76 | 136.38 ± 8.36 | $t$ = –4.265 | <0.001 |
| ALB, g/L | 35.47 ± 3.95 | 35.03 ± 3.83 | 36.33 ± 4.14 | $t$ = –1.261 | 0.212 |
| TG, mmol/L | 1.11 ± 0.26 | 1.10 ± 0.28 | 1.12 ± 0.23 | $t$ = –0.300 | 0.765 |
| TC, mmol/L | 4.18 ± 0.43 | 4.32 ± 0.38 | 3.91 ± 0.39 | $t$ = 4.089 | <0.001 |
| HDL, mmol/L | 1.23 ± 0.17 | 1.24 ± 0.17 | 1.20 ± 0.15 | $t$ = 1.014 | 0.315 |
| LDL, mmol/L | 2.65 ± 0.40 | 2.68 ± 0.44 | 2.59 ± 0.32 | $t$ = 0.840 | 0.404 |
| NLR | 3.02 ± 1.37 | 3.08 ± 1.37 | 2.90 ± 1.41 | $t$ = 0.500 | 0.619 |
| PLR | 162.42 ± 58.37 | 171.55 ± 64.78 | 144.57 ± 38.56 | $t$ = 1.794 | 0.078 |

Abbreviations: BMI, body mass index; WBC, white blood cell count; CRP, C-reactive protein; PLT, platelet count; RBC, red blood cell count; HB, hemoglobin; ALB, albumin; TG, triglycerides; TC, total cholesterol; HDL, high-density lipoprotein cholesterol; LDL, low-density lipoprotein cholesterol; NLR, neutrophil-to-lymphocyte ratio; PLR, platelet-to-lymphocyte ratio.

**Table 4. Multivariate logistic regression of factors associated with pCR in the NAC+ICB group.**

| Variable | $\beta$ | S.E. | Wald | *p*-value | OR (95% CI) |
|---|---|---|---|---|---|
| PLT | –0.040 | 0.013 | 9.042 | 0.003 | 0.960 (0.936–0.986) |
| RBC | 1.737 | 1.038 | 2.799 | 0.094 | 5.681 (0.743–43.450) |
| HB | 0.206 | 0.066 | 9.784 | 0.002 | 1.229 (1.080–1.399) |
| TC | –4.635 | 1.621 | 8.174 | 0.004 | 0.010 (0.000–0.233) |

Abbreviations: PLT, platelet count; RBC, red blood cell count; HB, hemoglobin; TC, total cholesterol; OR, odds ratio; CI, confidence interval.

**Table 5. Correlation between laboratory parameters and pCR.**

| Parameters | *r*-value | *p*-value |
|---|---|---|
| PLT | –0.340 | 0.006 |
| RBC | 0.279 | 0.025 |
| HB | 0.473 | <0.001 |
| TC | –0.470 | <0.001 |

Abbreviations: PLT, platelet count; RBC, red blood cell count; HB, hemoglobin; TC, total cholesterol.

with pCR (r = –0.340, $p$ = 0.006; r = –0.470, $p < 0.001$, respectively), while RBC and HB showed positive correlations (r = 0.279, $p$ = 0.025; r = 0.473, $p < 0.001$, respectively).

### *Comparison of Treatment-Related Adverse Events Between the Two Groups*

The incidence of TRAEs was evaluated in both groups. No statistically significant difference was observed in the occurrence of grade ≥3 TRAEs between the two groups ($p$ = 0.717). The incidence of grade ≥3 TRAEs was 38.64% in the NAC group and 41.54% in the NAC+ICB group (Table 6). These findings suggest that the addition of tislelizumab to the gemcitabine/cisplatin regimen did not significantly increase the risk of severe adverse events in patients with MIBC.

## Discussion

This retrospective study compared the efficacy and safety of NAC alone versus NAC+ICB in patients with MIBC. Our findings demonstrate a significant advantage of the combination regimen in improving pathological responses, without an unacceptable increase in toxicity. The NAC+ICB group exhibited a notably higher pCR rate and an improved pathological downstaging rate (≤ypT1) compared with NAC alone. These findings are consistent with the growing body of evidence suggesting that the addition of PD-1/PD-L1 inhibitors to neoadjuvant chemotherapy significantly increases the likelihood of achieving a pathological response in MIBC patients [16]. Moreover,

**Table 6. Comparison of treatment-related adverse events between the NAC and NAC+ICB groups.**

| Variable | Total (n = 153) | NAC (n = 88) | NAC+ICB (n = 65) | Statistic | *p*-value |
|---|---|---|---|---|---|
| TRAEs, n (%) | | | | $\chi^2 = 0.131$ | 0.717 |
| <Grade 3 | 92 (60.13) | 54 (61.36) | 38 (58.46) | | |
| ≥Grade 3 | 61 (39.87) | 34 (38.64) | 27 (41.54) | | |

Abbreviations: TRAEs, treatment-related adverse events; NAC+ICB, neoadjuvant chemotherapy combined with immune checkpoint blockade; NAC, neoadjuvant chemotherapy.

the comparable incidence of severe adverse events between the two groups indicates that the combination regimen does not markedly increase perioperative risks, supporting its clinical feasibility.

Our findings align with previous studies reporting the superiority of the NAC+ICB over chemotherapy alone. For example, single-center studies by Yu *et al.* [16] and real-world analyses by Ding *et al.* [7] reported pCR rates of approximately 50% in NAC+ICB cohorts, significantly higher than the 20% observed in NAC alone. Similarly, a multicenter retrospective study by Hu *et al.* [5] compared three neoadjuvant strategies, immunotherapy alone, chemotherapy alone, and combination therapy, and demonstrated that the combined approach achieved the highest rates of pCR and pathological downstaging. Notably, our study also identified several pre-treatment clinical features, which are associated with pCR, including lower platelet counts, higher hemoglobin levels, and reduced total cholesterol. These findings may inform the development of predictive models for identifying patients most likely to benefit from chemoimmunotherapy.

In monotherapy trials using ICB agents as neoadjuvant treatment, pCR rates have typically been around 30% [10], while classical cisplatin-based NAC regimens report pCR rates of 20–38% in clinical trials [17]. By contrast, multiple phase II trials have shown that NAC combined with ICB yields superior outcomes, with pCR rates of 40–50% and overall downstaging rates of 70–80% [18]. For instance, a phase II study combining gemcitabine/cisplatin with pembrolizumab achieved a pCR rate of 35.9% [11], whereas a multicenter, single-arm phase II trial of gemcitabine/cisplatin plus tislelizumab reported a pCR rate of 50.9% and a downstaging rate of 75.4% [19]. These results are consistent with our findings and underscore the potential synergistic effect between immunotherapy and chemotherapy in the neoadjuvant phase. Nevertheless, variations in pCR rates across studies may reflect differences in ICB agents used, patient selection criteria, and treatment protocols. Taken together, current evidence, including our real-world findings, supports the use of neoadjuvant chemoimmunotherapy as a promising strategy for appropriately selected MIBC patients.

Gemcitabine and cisplatin-based NAC agents demonstrate marked synergistic antitumor effects when combined with ICB, supported by multiple biological mechanisms. First, chemotherapeutic drugs act via a dual pathway: they exert direct cytotoxic effects to kill tumor cells and induce immunogenic cell death (ICD). This process leads to the release of tumor-associated antigens (TAAs) and damage-associated molecular patterns (DAMPs), such as calreticulin and high mobility group box 1 (HMGB1). These DAMPs promote the maturation and activation of dendritic cells (DCs), initiating T-cell-mediated immune responses [20–22]. This process remodels the tumor microenvironment, converting an immunosuppressive "cold" tumor into an immunologically active "hot" tumor. However, tumor cells can evade immune surveillance via checkpoint pathways such as programmed death-1/programmed death-ligand 1 (PD-1/PD-L1) [23]. Accordingly, PD-1 inhibitors, such as tislelizumab or toripalimab, block this pathway, restoring T-cell function and amplifying the immune response primed by chemotherapy [24,25]. Notably, the synergy between NAC and ICB rests on their mechanistic interplay: chemotherapy-induced immunogenic remodeling provides the foundation for ICB, while ICB unleashes the full antitumor potential of activated T cells. Together, this interaction enhances pCR rates and tumor downstaging. It is also notable that the immunomodulatory effects of chemotherapy are dynamic. For example, prostaglandin $E_2$ ($PGE_2$) blockade can boost $CD8^+$T-cell infiltration and synergize with PD-1 inhibition [26], and different chemotherapeutic agents vary in their ICD-inducing capacity. Although cisplatin and gemcitabine are not classical ICD inducers, their combination with ICB still triggers potent antitumor immunity.

Current evidence indicates that the efficacy of PD-1 or PD-L1 blockade is broadly comparable across agents, despite differences in patient populations and trial designs. Pembrolizumab and nivolumab, whether used alone or in combination with chemotherapy, achieve pCR rates of approximately 40–50% [9], while tislelizumab, a PD-1 inhibitor developed in China to minimize Fc$\gamma$ receptor binding and potentially reduce T-cell clearance, achieved a 50.9% pCR rate when combined with NAC in a recent Chinese Phase II trial, closely mirroring pembrolizumab and nivolumab outcomes [19]. Although no head-to-head trials exist in MIBC, all three agents (tislelizumab, pembrolizumab, nivolumab) appear effective when combined with chemotherapy, with accessibility varying geographically, tislelizumab being more readily available in China, while pembrolizumab and nivolumab have predominated in Western trials. Regarding safety, checkpoint inhibitors

carry a risk of immune-related adverse events (irAEs), but adding ICB to NAC has not resulted in prohibitive toxicity, with relatively low rates of grade 3–4 irAEs and most patients completing therapy and proceeding to surgery without significant delay [11]. In our real-world cohort, we likewise observed no unexpected toxicities, and the safety profile of tislelizumab was comparable to other PD-1 inhibitors, with no unique organ-specific toxicities reported. Thus, from a pragmatic perspective, clinicians may select any approved PD-1/PD-L1 agent for combination with NAC, expecting similar efficacy while remaining vigilant for class-effect immunotoxicities.

Moreover, our study identified several baseline clinical parameters in the NAC+ICB group that were associated with achieving pCR. Patients with lower platelet counts, higher hemoglobin and RBC levels, and lower TC levels were more likely to achieve pCR. These hematological parameters may reflect systemic inflammatory status, nutritional-metabolic homeostasis, and tumor microenvironment characteristics. Reduced platelet counts correlate with favorable outcomes, as thrombocytosis, a recognized poor prognostic factor in malignancies, promotes tumor immune evasion by shielding circulating tumor cells from immune recognition, secreting pro-metastatic factors (e.g., transforming growth factor beta (TGF-$\beta$), vascular endothelial growth factor (VEGF)), and suppressing effector lymphocyte function [27,28]. Thus, lower platelet levels may indicate attenuated pro-inflammatory states and diminished immunosuppressive microenvironments. Elevated hemoglobin and red blood cell counts suggest superior oxygen-carrying capacity and physiological reserve, with non-anemic patients demonstrating enhanced antitumor immune signatures, consistent with observations of improved efficacy across cancer immunotherapies [29]. These observations support the hypothesis that well-oxygenated tumor milieus optimize ICB responses. Decreased total cholesterol also appears relevant, reflecting the immunomodulatory role of cholesterol metabolism; while aberrant lipid metabolism in tumor cells, including cholesterol accumulation, inhibits T-cell function and facilitates immune escape [30,31]. Epidemiological evidence links high dietary cholesterol to an increased bladder cancer risk [32], while experimental data indicate that modulating cholesterol metabolism (e.g., via statins) may enhance immune-mediated tumor control [31]. Although the direct correlation between serum cholesterol and pCR in our case requires larger-scale validation in large cohorts, the underlying biology supports a role for metabolic status in influencing therapeutic sensitivity. Collectively, these baseline hematologic parameters (platelet count, hemoglobin level, cholesterol concentration) may serve as biomarkers of systemic inflammation, oxygen dynamics, and metabolic balance, modulating pCR rates through tumor microenvironment interactions. Future multimodal omics studies are warranted to further elucidate these mechanisms.

Despite these encouraging findings, several limitations must be acknowledged. First, as a single-center retrospective study, it is inherently susceptible to selection bias, as patient allocation was not randomized. Second, the sample size, while representing all eligible patients over a three-year period, remains relatively small, limiting the statistical power for detailed subgroup analyses and the identification of additional predictive factors. Third, and most importantly, this study lacks long-term survival outcomes such as event-free survival (EFS) and OS. Given that patient enrollment concluded in late 2024, the follow-up duration is insufficient to conduct a mature and meaningful survival analysis, and presenting premature survival data could be misleading. Therefore, while pCR is a strong surrogate endpoint, confirmation of long-term benefit requires prospective, multicenter randomized trials. Lastly, our findings are based on a specific PD-1 inhibitor in a predominantly Chinese cohort, and their generalizability to other populations and treatment contexts requires further validation.

## Conclusion

This study demonstrates that NAC combined with ICB significantly improves the pathological response in patients with MIBC compared to NAC alone, particularly in terms of pathological complete response and overall response downstaging rates. Moreover, the incidence of severe treatment-related adverse events was comparable between the two groups, supporting the safety and feasibility of the combination regimen. Prospective, multicenter studies are warranted to validate these findings, assess long-term survival benefits, and identify predictive biomarkers, with the ultimate goal of optimizing and expanding the clinical application of this therapeutic approach.

## Availability of Data and Materials

The data and materials in the current study are available from the corresponding author on reasonable request.

## Author Contributions

QL, PZ and DT contributed to the study design. QL conducted the literature search. PZ and DT acquired the data. QL wrote the article and performed data analysis. DT revised the article. All authors contributed to important editorial changes in the manuscript. All authors read and approved the final manuscript. All authors have participated sufficiently in the work and agreed to be accountable for all aspects of the work.

## Ethics Approval and Consent to Participate

The study was approved by the Ethics Committee of Jinhua Municipal Central Hospital (Approval No. 2021-315-001), and written informed consent was obtained from

all participants. The study was conducted following the ethical principles outlined in the Declaration of Helsinki.

## Acknowledgment

Not applicable.

## Funding

This work was partially supported by fund of Jinhua City Science and Technology Program Project Social Development Key Projects (No.2021-3-094).

## Conflict of Interest

The authors declare no conflict of interest.

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