ORIGINAL ARTICLE
Iran J Allergy Asthma Immunol
In press.

Exploring the Efficacy of Immunotherapy in Combination with Surgical Resection in High-risk Muscle-invasive Bladder Cancer

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Received: 1 June 2025; Received in revised form: 22 July 2025; Accepted: 8 August 2025

ABSTRACT

Traditional radical cystectomy has a high recurrence rate, a high probability of metastasis, and a reduced quality of life for patients. This study aimed to explore the efficacy of combining immunotherapy and surgical resection for high-risk muscle-invasive bladder cancer.

In a retrospective study, 231 patients with high-risk muscle-invasive bladder cancer admitted to Yueyang People's Hospital between January 2019 and May 2024 were selected. After exclusions, 200 cases were analyzed and divided into two groups according to the treatment modality: the control group (surgical resection alone, n=100) and the intervention group (combination of immunotherapy and surgical resection, n=100). The cellular immune function indexes ($CD3^+$, $CD4^+/CD8^+$, and natural killer cell levels), tumor markers (carcinoembryonic antigen, carbohydrate antigen 125, cytokeratin 21-1, and neuron-specific enolase), serum cytokines (basic fibroblast growth factor, vascular endothelial growth factor, and tumor necrosis factor-α), pathological complete remission (pCR), 1-year survival, and Functional Assessment of Cancer Therapy-Bladder (FACT-BL) quality-of-life scores were assessed in the two groups.

After 1 year of treatment, the indicators of the two groups of patients were statistically significant in comparison with each other. Patients in the intervention group had substantial improvements in immune function indexes, pCR, 1-year survival rate, and FACT-BL scores in comparison with the control group. Tumor markers and serum cytokines were lower than those in the control group.

The combination of immunotherapy and surgical resection can enhance clinical efficacy, providing a scientific basis for optimizing the clinical treatment plan.

Keywords: High-risk; Immunotherapy; Muscle invasive bladder neoplasms; Surgical margins

INTRODUCTION

Bladder cancer is a malignant tumor that occurs in the bladder mucosa, which is the most common malignant tumor of the urinary system and one of the ten most common tumors in the whole body.[1] Bladder cancer can occur at any age, even in children, and its incidence increases with age, with a high incidence at 50–70 years old. The incidence of bladder cancer in men is 3–4 times higher than that in women.[2] Pathologic

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types of bladder cancer include uroepithelial carcinoma of the bladder, squamous cell carcinoma of the bladder, adenocarcinoma of the bladder, and other rare ones, such as clear cell carcinoma of the bladder, small cell carcinoma of the bladder, and carcinoid tumor of the bladder. Among them, the most common is bladder uroepithelial cancer, which accounts for about 90% or more of the total number of bladder cancer patients. Commonly, "bladder cancer" is used in place of bladder urothelial cancer,[3] which can be categorized into non-muscle-invasive bladder cancer (NMIBC) (stage Ta, CIS, T1), muscle-invasive bladder cancer (MIBC) (stage T2–T4), and metastatic bladder cancer based on the progression of the disease.

The development of bladder cancer is a complex, multifactorial, and multistep pathological process, and its specific pathogenesis has not yet been elucidated. Both intrinsic genetic factors and extrinsic environmental factors play important roles. Extrinsic factors include smoking and long-term exposure to industrial chemicals, which are the two major extrinsic risk factors for bladder cancer and the most identified risk factors. About 50% of bladder cancer patients have a history of smoking, and the risk of bladder cancer in smokers increases by 2–3 times. The risk of the disease is directly proportional to the intensity and duration of smoking, which is mainly related to the aromatic amines contained in cigarettes, such as 4-aminobiphenyl. The effect of smoking on the recurrence and progression of bladder cancer is still unclear. Long-term occupational exposure to industrial chemicals is another important risk factor, and about 20% of bladder cancer patients are related to their occupations, such as textiles, dye manufacturing, rubber chemistry, pharmaceutical and pesticide production, paints, leather, and aluminum and steel production. Examples include naphthylamine and 4-aminobiphenyl.[4] Other causative factors include long-term chronic inflammatory stimuli in the bladder (bacteria, schistosomiasis,[5] human papillomavirus infection, etc.), long-term foreign body stimuli (indwelling catheters, stones), squamous cell carcinoma, and adenocarcinomas of the bladder. Past chemotherapy with cyclophosphamide, abuse of finasteride, a history of pelvic radiotherapy, and the treatment of diabetes mellitus with the drug pioglitazone can increase the risk of uroepithelial cancer of the bladder progression. High intake of fat, cholesterol, fried food, and red meat; long-term consumption of water with high arsenic content and chlorinated water; and coffee, artificial sweeteners, and hair coloring may increase the risk of bladder cancer. Intrinsic factors (genetic abnormalities) include the development of bladder cancer, which is related to heredity and genetic abnormalities.[6] The risk of bladder cancer in people with a family history is significantly increased by 2 times. The specific mechanism requires further study. The malignant transformation of normal bladder cells begins with the DNA alteration of the cells. Chemical carcinogens are the main extrinsic causative factors of bladder cancer, including aromatic compounds, such as 2-naphthylamine and 4-aminobiphenyl, which are present in tobacco and various chemical products. After being metabolized, the above-mentioned carcinogens enter the urine, leading to serious malignant changes in bladder epithelial cells. The oncogenes related to bladder cancer include *HER-2*, *HRAS*, *BCL-2*, *FGFR3*, *C-myc*, *MDM2*, and *MSH2*, among others. Another molecular mechanism is the inactivation of inhibitor genes encoding proteins that regulate cell growth, DNA repair, or apoptosis, which prevent apoptosis from occurring in DNA-damaged cells. Damaged cells do not undergo apoptosis, resulting in uncontrolled cell growth,[7] such as the deletion or heterozygous loss of chromosomes 17, 13, and 9 of oncogenes such as *P53*, *RB*, and *P21*, which is related to the onset of bladder cancer. Uroepithelial tumors are multicentric in time and space. If patients had upper urinary tract cancer in the past, they have an important risk factor for developing uroepithelial carcinoma, and the probability of bladder cancer in such patients is about 15%–50%.

The standard treatment modality for MIBC is radical cystectomy.[8] Radical cystectomy is a valid therapy proven to increase survival and eliminate local recurrence and distant metastasis. Surgery includes resection of the bladder and its adjacent organs, pelvic lymph node dissection, and urinary diversion. MIBC is not satisfactorily treated by surgery alone, and up to 50% of MIBC patients undergo radical cystectomy and develop distant recurrence, which can be improved by combining it with neoadjuvant therapy (NAT).[9] NAT includes neoadjuvant chemotherapy (NAC), radiotherapy (neoadjuvant radiotherapy, NAR), and immunotherapy.[10] NAC has the advantages of eliminating micrometastases, lowering tumor stage, decreasing the difficulty of surgery, reducing complications, and improving long-term survival. However, it may delay the time to surgery for those who are ineffective for NAC, and NAC based on the clinical

stage may have the problem of over-treatment. NAC[11] is mainly used in the cisplatin-based combination chemotherapy regimen, which is often used, including the gemcitabine plus cisplatin (GC) regimen,[12] methotrexate plus vincristine plus doxorubicin plus cisplatin (MVAC) regimen, dose-dense MVAC (ddMVAC) regimen, and cisplatin plus methotrexate plus vincristine (CMV) regimen. Cisplatin-based NAC combined with radical cystectomy has been recognized by the National Comprehensive Cancer Network (NCCN) as a class 1 recommended treatment option for stage II and IIIA MIBC.[13] Although cisplatin-containing NAC significantly prolonged overall survival (OS) compared with local surgery alone, the absolute improvement in the 5-year OS rate was only 8%. The number of patients who achieved pathological complete remission (pCR) or reached a pathologically descending stage of the tumor was not more than 30% and 50%, respectively. In addition, 20%–50% of MIBC patients are unable to withstand the cisplatin-containing chemotherapy due to hearing loss, neuropathy, poor physical status, renal insufficiency, or comorbidities, and at one time, these patients could only be treated with surgery. NAR is virtually a localized therapy. Its advantages indicate it can not only downstage the tumor but also has no influence on distant micrometastases, and its impact on survival is unclear, so it is used less frequently in the clinic and often not recommended. Therefore, there is an imminent need to establish more effective systemic adjuvant therapeutic strategies, especially for high-risk populations, to address the above unmet clinical needs.

Neoadjuvant immunotherapy is mainly the adjuvant treatment by applying immune checkpoint inhibitors, which are immunomodulatory drugs directly targeting programmed cell death protein 1 (PD-1), programmed death-ligand 1 (PD-L1), and cytotoxic T-lymphocyte-associated protein 4 (CTLA-4).[14] The efficacy of the treatment of uroepithelial carcinoma has been gradually confirmed by the clinic, and the US Food and Drug Administration (FDA) has approved natalizumab as an adjuvant therapy[15] for the treatment of resected patients with a high risk of recurrence after receiving excision of uroepithelial carcinoma. The high-risk group of MIBC relapsing and metastasis can be summarized into two categories: (1) patients who have not received NAC with postoperative pathological staging of pT3–pT4a or pN+; (2) patients who have received NAC with ypT2–ypT4a or ypN+.[16] Currently, nivolumab has been approved and marketed in China, and in recent years, a new drug has received China Food and Drug Administration (CFDA) approval, bringing a blessing for uroepithelial cancer patients. The tirilazumab and treprostinil injection was domestically developed and became a representative drug. Immunotherapy for bladder cancer has been a hotspot of continuous updates, and the congresses of the European Society for Medical Oncology (ESMO),[17] the American Society of Clinical Oncology Genitourinary Cancers Symposium (ASCO-GU), and the European Association of Urology (EAU) have successively announced the results of clinical studies of MIBC neoadjuvant immunotherapy (PURE-01[18] and ABACUS), with pCR rates of 37% (42/114) and 31% (21/68), respectively. In addition, NAT with immunotherapy combined with chemotherapy has shown encouraging preliminary results with pCR rates of 33%–49%, which are yet to be confirmed by survival data with long-term research.

In this study, we sought the efficacy of surgical resection combined with immunotherapy for patients with high-risk MIBC, mainly in terms of three indicators of cellular immune function ($CD3^+$, $CD4^+/CD8^+$, and natural killer [NK] cells); four indicators of tumor markers (carcinoembryonic antigen [CEA], carbohydrate antigen 125 [CA-125], cytokeratin 21-1 [CY21-1], and neuron-specific enolase [NSE]); and three indicators of cellular serum factors (basic fibroblast growth factor [bFGF], vascular endothelial growth factor [VEGF], and tumor necrosis factor-α [TNF-α]). We also assessed pCR, 1-year survival rate, and the Functional Assessment of Cancer Therapy-Bladder (FACT-BL) score for quality of life to comprehensively analyze the efficacy of the treatment. It is expected that the application of immunotherapy in the therapeutic strategy of high-risk MIBC will bring a more effective and personalized treatment plan for patients, and will contribute to the promotion of the development of the discipline of oncology and enhancement of patients' quality of life with both theoretical and practical value. It not only contributes to the development of oncology but also increases the patients' quality of survival in both theory and practice.

MATERIALS AND METHODS

General Information

This study retrospectively selected patients with high-risk MIBC admitted to Yueyang People's Hospital

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from January 2019 to May 2024, aiming to investigate the efficacy of immunotherapy combined with surgical resection on high-risk MIBC. As shown in the flowchart of experimental design in Figure 1, information was collected from 231 cases; 216 cases were included after exclusion, 3 cases were lost, 5 cases withdrew due to personal reasons, and 8 cases died. Finally, a total of 200 cases were analyzed, which were divided into a control group and an intervention group according to the treatment modality, with 100 cases in each group. Inclusion criteria were: (1) MIBC was diagnosed following the NCCN Bladder Cancer Guidelines (2024 edition) published by the *Journal of the National Comprehensive Cancer Network* written by Flaig et al[13]; (2) none of them received disease-related adjuvant treatment, such as radiotherapy, before enrollment; (3) aged 18–70 years old; and (4) complete clinical data and related examinations. Exclusion criteria were: (1) those with language, mental, or psychological disorders, which affect the smooth development of the study; (2) those with other malignant tumors; (3) those with severe damage to the heart, liver, brain, and other important organ functions; (4) those with urinary system infections; (5) patients with abnormal coagulation function; and (6) those with serious infectious diseases.

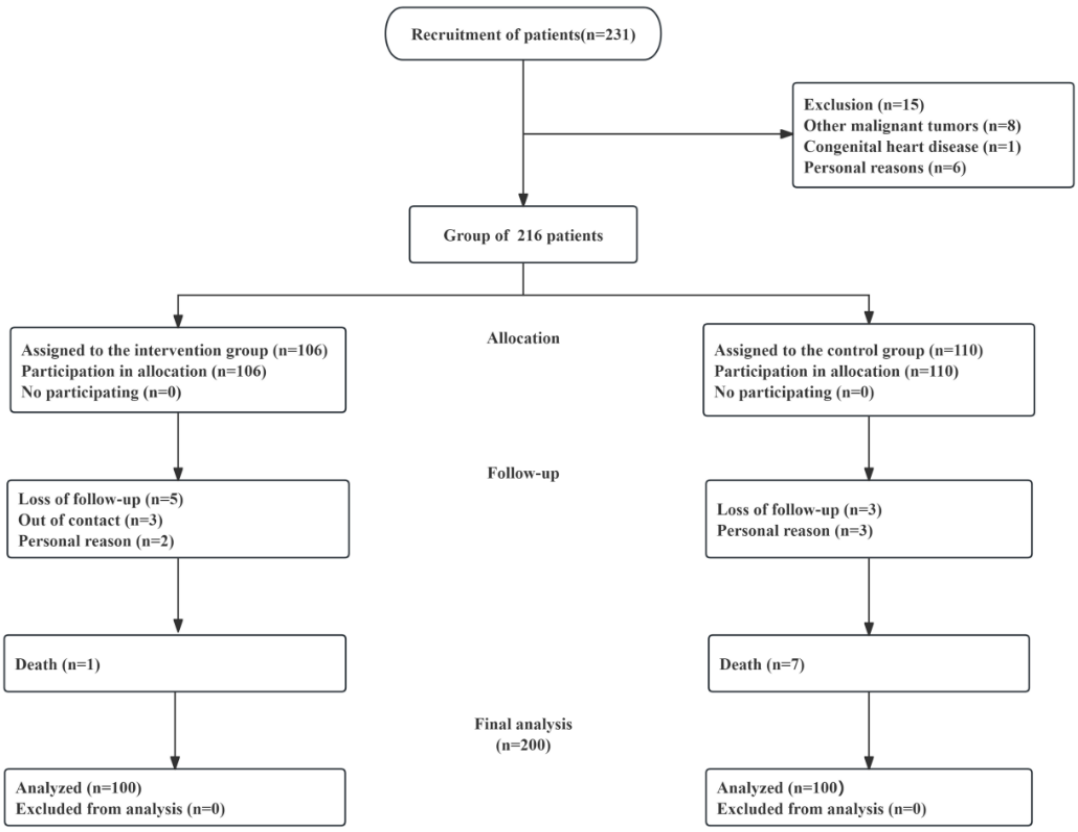

Figure 1. Flowchart of experimental design.

Ethics Statement

The clinical study followed the Declaration of Helsinki and other relevant ethical regulations and was reviewed and approved by Yueyang People's Hospital's Ethics Committee, which had explained in detail the purpose of the study, the process, and the potential risks to the subjects or their proxies, and obtained written informed consent.

Intervention Modalities

Control group: conventional treatment. (1) Radical cystectomy[13] is performed to remove the entire bladder as well as the surrounding adipose tissue, distal ureter, and pelvic lymph nodes with open surgery or laparoscopic assistance; it usually includes the prostate gland and seminal vesicles in men, and the uterus, a portion of the anterior vagina, and adnexa in women. If

the tumor invades the urethra, the neck of the bladder in females, or the prostate in males, or if intraoperative freezing shows positive margins, total urethral resection is required. (2) Pelvic lymph node dissection. The standard lymph node dissection extent is as follows: 92% of bladder lymphatic drainage is located below the plane where the ureter crosses the iliac vessels. The dissection includes the common iliac vessels (proximal), the genitofemoral nerve (lateral), the spinous iliac vein and Cloquet's lymph nodes (distal), and the internal iliac vessels (posterior), usually including the obturator, the internal and external iliac lymph nodes, and the presacral lymph nodes. (3) Urethral diversion. Urethral diversion is carefully selected according to the patient's specific situation, such as age, concomitant diseases, life expectancy, history of previous pelvic and abdominal surgery or radiotherapy, and the patient's wishes. The specific diversion program is chosen by the patient. (4) Postoperative general medications include pain medications, antibiotics, bowel-promoting medications, and nutritional supplements, and the dosage of medications is adjusted according to the patient's disease progression for 1 year.

Intervention group: conventional treatment, as in the above control group. In terms of postoperative medication, in addition to the general medication of the above control group, immune drug treatment was also carried out at the same time. Nivolumab injection was diluted into 0.9% sodium chloride injection or 5% dextrose injection for intravenous infusion. A dose of 3 mg/kg was injected intravenously every 2 weeks. The first dose was infused in 60 minutes, and then changed to 30 minutes after being well tolerated by the patients. The injected dose could be adjusted according to the disease progression of the patients, and the medication time lasted for 1 year.[19]

All study subjects were rechecked every 2 months with ultrasound and thoracic, abdominal, and pelvic computed tomography (CT). The patients were observed for disease progression or intolerable toxicity during the period in which the drug was taken.

Observational Indicators

Indicators of Cellular Immune Function

$CD3^+$, $CD4^+$, and $CD8^+$ are indicators used to mark different types of immune cells, and the normal range of values of $CD3^+$, $CD4^+$, and $CD8^+$ can reflect the normal functional status of the human immune system.[20] NK cells are the cells that play a killing role without pre-immunization or sensitization, and their killing action precedes that of other effector cells. They build up a first line of defense against tumors[21] and are usually measured by flow cytometry.[22] A procoagulant tube was used to collect 5 mL of fasting venous blood from each patient as test samples, and $CD3^+$, $CD4^+$, $CD8^+$, and NK cells were detected and quantified by flow cytometry using the BD FACSAria™ Fusion flow cytometer (U.S.A.).

Serum Tumor Markers

Serum CEA, glycan antigen CA-125, CY21-1, and NSE are usually detected by drawing a venous blood sample. A procoagulant tube was used to collect 5 mL of fasting venous blood from every patient. After centrifugation at 3500 r/min in a medical centrifuge with a semi-axis of 10 cm for 10 minutes, the serum fraction was separated, and CEA, CA-125, CY21-1, and NSE were measured by radioimmunoassay using a Threshold® Molecular Devices radioimmunoassay (U.S.A.).

Serum Cytokines

Serum was collected as above. bFGF, VEGF, and TNF-α were usually measured by enzyme-linked immunosorbent assay[23] using enzyme-linked immunoassay kits from Beyotime (PF323/PV963/PT518, China).

Pathological Complete Response

Pathological complete response is commonly used as a surrogate marker for clinical trials of NAT and is denoted as non-existent residual tumor cells after assessment of resected tumor tissue and regional lymph nodes.[24] In this study, a combination of imaging examinations and molecular biology tests was used to obtain a more comprehensive assessment. Imaging examination refers to the patients' ultrasound and thoracic, abdominal, and pelvic CT repeated every 2 months during the 1-year treatment period. Molecular biology testing refers to the patients' 5 mL fasting venous blood drawn every 2 months during the 1-year treatment period, and the circulating tumor DNA (ctDNA) in the blood was detected by polymerase chain reaction (PCR) technology to indirectly assess the residual tumor status. Pathological complete remission was achieved in patients with no residual tumor cells in both imaging and molecular biology tests. The pCR rate was calculated as (the number of cases with no residual

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tumor cells at the end of 1 year of treatment / the number of cases starting treatment) × 100%.

1-Year Survival Rate

We compared the death of patients in the two groups after a one-year treatment duration. The causes of death included complications, treatment side effects, psychological stress, organ failure, malnutrition, infection, bleeding, and embolism, among other causes. The 1-year survival rate was calculated as (number of cases surviving after 1 year of treatment / number of cases starting treatment) × 100%.

FACT-BL Score

The FACT-BL consists of two components: the Functional Assessment of Cancer Therapy-General (FACT-G) and bladder cancer-specific questions (BICS). These are categorized into physical well-being (PWB), social/family well-being (SWB), emotional well-being (EWB), functional well-being (FWB), and BICS. The BICS was developed from the Functional Assessment of Chronic Illness Therapy (FACIT) measure developed at Northwestern University in the United States and consists of 12 items, including urination, urinary control, sexual function, physical appearance, and other aspects related to urinary diversion. It is also scored on a 5-point Likert scale. As the earliest bladder cancer-specific scale, the FACT-BL scale has the advantage of reflecting the impact of disease and surgery on patients' quality of life better than general scales and has been widely used in assessing the quality of life of bladder cancer patients.[25]

Sample Size Calculation Methods

The number of samples chosen was based on a rigorous analysis using G*Power 3.1.9.7 software to determine the sample size required to detect statistically significant differences. The sample size was calculated based on the primary outcome of the Cellular Immune Function Indicator. Considering an alpha level of 0.05 and a statistical efficacy of 90%, we calculated that a sample size of 86 patients was required for each group. Considering the potential uncertainty, the sample sizes chosen for analysis in this study were the control group (n=100) and the intervention group (n=100). We believe that the sample sizes in this study allow us to draw reliable conclusions.

Statistical Methods

SPSS 28.0 statistical software was used to analyze the data. The data in this study were tested for normal distribution. Baseline characteristics were described as the number of persons and variables (expressed as mean±SD). The results of cellular immune function indexes, tumor markers, serum cytokines, and FACT-BL score results were expressed as mean±SD. Comparisons between groups were examined using the independent samples t-test. Pathologic complete remission and 1-year survival rate in the results were expressed as n (%). Comparison between the two groups was analyzed using the χ^2 test. All statistical tests were two-sided, and a p value <0.05 indicated a statistically significant difference.

RESULTS

Comparison of Baseline Data between the Two Groups

Comparing the baseline data of the patients in the control and intervention groups, it can be seen from Table 1 that there were no noteworthy variances between the two groups for the following indicators: age, body mass index (BMI), gender, tumor pathological stage, number of foci, and the presence of a smoking history (p=0.117, 0.968, 0.428, 0.771, 0.631, and 0.638, respectively), which indicated that the two groups were comparable in terms of their preoperative baseline data.

Comparison of Cellular Immune Function

The data from Table 2 suggested there were no noteworthy variances between the three cellular immune function indexes ($CD3^+$, $CD4^+/CD8^+$, and NK cell) of the 100 patients in the control group and the 100 patients in the intervention group on the day before the surgery (p=0.712, 0.326, and 0.104, respectively). One year after surgery, in the control group treated with surgery and in the intervention group of patients treated with surgery as well as postoperative combined immunotherapy, the three indicators of cellular immune function were noticeably elevated in the intervention patients group in comparison to those in the control group (p=0.002, 0.003, and 0.04, respectively), suggesting that after surgical resection, nivolumab enhances the immune response capacity of high-risk MIBC patients.

Table 1. Baseline information of patients.

Indicator	Control group (n=100)	Intervention group (n=100)	95% CI		*p*	Effect size
			Lower	Upper		
Age, y	49.41±10.23	51.70±10.32	-5.16	0.58	0.117	-0.22
BMI, kg/m^2	22.70±1.73	22.69±1.80	-0.48	0.50	0.968	0.006
Gender					0.428	0.056
Male	75	70	0.69	2.397		
Female	25	30				
Pathological stage					0.771	0.21
pT3	63	61	0.615	1.927		
pT4a	37	39				
Number of foci					0.631	0.034
Single	28	25	0.622	2.188		
Multiple	72	75				
Smoking history					0.638	0.033
Yes	73	70	0.627	2.143		
No	27	30				

BMI: body mass index; CI: confidence interval; pT3: tumor passes through the muscle into the fat layer and infiltrates the tissues surrounding the bladder; pT4a: tumor infiltrates the prostate, uterus, and vagina.

Table 2. Comparison of cellular immune function indicators between the two groups of patients (mean±SD).

Indicator	n	Time	Control group	Intervention group	95% CI		*p*	Effect size
					Lower	Upper		
CD3$^+$, %	100	1 day before surgery	32.53±8.09	32.97±8.59	-2.76	1.89	0.712	-0.05
	100	1 year after surgery	48.47±5.89	51.14±6.36	-4.37	-0.95	0.002	-0.44
CD4$^+$/CD8$^+$	100	1 day before surgery	1.35±0.12	1.37±0.12	-0.05	0.02	0.326	-0.17
	100	1 year after surgery	1.74±0.23	1.85±0.29	-0.18	-0.04	0.003	-0.42
NK, %	100	1 day before surgery	15.09±2.15	15.63±2.49	-1.19	-0.11	0.104	-0.23
	100	1 year after surgery	18.29±3.32	19.28±3.44	-1.93	-0.05	0.04	-0.29

CI: confidence interval; NK: natural killer; SD: standard deviation.

Comparison of Tumor Markers between the Two Groups

As can be seen in Table 3, there were no noteworthy variances between the four tumor markers (CEA, CA-125, CY21-1, and NSE) in the 100 patients in the control group and the 100 patients in the intervention group on the day before the surgery (p=0.13, 0.851, 0.125, and 0.159, respectively). One year after surgery, in the control group of patients treated with surgery and in the intervention group of patients treated with surgery as well as postoperative combined immunotherapy, the four tumor markers were significantly reduced in the patients in the intervention group compared to the control group (p=0.022, 0.007, 0.03, and 0.026, respectively). This suggests that immunotherapy with nivolumab after surgical resection can effectively inhibit the expression of tumor markers, and patients with high-risk MIBC have a better prognosis.

Comparison of Serum Cytokines between the Two Groups

As can be seen in Table 4, there were no noteworthy variances between the three serum cytokines (bFGF, VEGF, and TNF-α) in the 100 patients in the control group and the 100 patients in the intervention group on the day before the surgery (p=0.627, 0.505, and 0.137, respectively). One year after surgery, in the control group of patients treated with surgery and in the intervention group of patients treated with surgery as well as postoperative combined immunotherapy, the three serum cytokines were remarkably reduced in the patients in the intervention group compared to the control group (p=0.035, 0.037, and 0.013, respectively). This suggests that immunotherapy with nivolumab after surgical resection in patients with high-risk MIBC promotes normalization of tumor vasculature and enhancement of immune response function.

Comparison of Pathological Complete Remission Between the Two Groups

As can be seen from Table 5, after 1 year of treatment, by counting 200 patients, the pCR in the control group was 63%, and in the intervention group was 82%. Compared with the two groups, the pCR in the intervention group was more significant (p=0.03), suggesting that postoperative immunotherapy with nivolumab in patients with high-risk MIBC improves pCR and reduces the risk of recurrence in patients with better results.

Table 3. Comparison of tumor markers between the two groups of patients (mean±SD).

Indicator	n	Time	Control group	Intervention group	95% CI		*p*	Effect size
					Lower	Upper		
CEA, μg/L	100	1 day before surgery	123.87±6.26	122.39±7.49	-0.44	3.41	0.13	0.21
	100	1 year after surgery	37.05±8.01	33.97±10.71	0.44	5.72	0.022	0.33
CA-125, mg/L	100	1 day before surgery	263.53±19.10	263.04±17.88	-4.67	5.65	0.851	0.03
	100	1 year after surgery	30.78±6.81	28.25±6.19	0.72	4.35	0.007	0.39
CY21-1, μg/L	100	1 day before surgery	30.88±5.38	29.84±4.14	-0.29	2.39	0.125	0.22
	100	1 year after surgery	23.31±2.59	22.64±1.69	0.07	1.29	0.03	0.31
NSE, μg/L	100	1 day before surgery	17.34±3.11	18.06±4.01	-1.72	0.28	0.159	-0.20
	100	1 year after surgery	8.96±2.15	8.37±1.47	0.07	1.10	0.026	0.32

CA-125: carbohydrate antigen 125; CEA: carcinoembryonic antigen; CY21-1: cytokeratin 21-1; NSE: neuron-specific enolase.

Table 4. Comparison of serum cytokines between the two groups of patients (mean±SD).

Indicator	n	Time	Control group	Intervention group	95% CI		*p*	Effect size
					Lower	Upper		
bFGF, ng/L	100	1 day before surgery	26.39±5.09	26.87±8.36	-2.41	1.46	0.627	-0.07
	100	1 year after surgery	16.51±3.42	15.59±2.66	0.06	1.77	0.035	0.30
VEGF, ng/L	100	1 day before surgery	469.92±38.15	466.62±31.33	-6.44	13.03	0.505	0.09
	100	1 year after surgery	266.21±18.83	260.61±18.84	0.34	10.85	0.037	0.30
TNF-α, ng/L	100	1 day before surgery	463.76±36.34	470.76±29.49	-16.23	2.24	0.137	-0.21
	100	1 year after surgery	265.63±18.56	258.67±20.80	1.47	12.46	0.013	0.35

bFGF: basic fibroblast growth factor; TNF-α: tumor necrosis factor-α; VEGF: vascular endothelial growth factor.

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Table 5. Comparison of pCR between the two groups [n (%)].

Groups	n	pCR	95% CI		*p*	Effect size
			Lower	Upper		
Control group	100	63 (63%)	0.195	0.717	0.003	-0.213
Intervention group	100	82 (82%)				

pCR: pathological complete remission.

Comparison of 1-Year Survival Rate between the Two Groups

As can be seen from Table 6, after 1 year of treatment, by analyzing 208 patients, the 1-year survival rate of the control patient group was 93.5%, and that of the intervention group was 99%. The intervention group had a higher 1-year survival rate compared with the control group (p=0.037). This suggests that postoperative immunotherapy with nivolumab in patients with high-risk MIBC improved the 1-year survival rate of patients.

Comparison of FACT-BL Scores between the Two Groups

As prompted in Table 7, there was no marked difference between the FACT-BL scores of the 100 patients in the control group and the 100 patients in the intervention group on the day before surgery (p=0.097). After surgical treatment of the control patients and surgical treatment of the intervention group, as well as postoperative combined immunotherapy, one year after surgery, the FACT-BL scores of the patients in the intervention group were significantly elevated compared to those of the control group (p=0.03). This suggests that postoperative immunotherapy with nivolumab in patients with high-risk MIBC improved the quality of survival.

Table 6. Comparison of 1-year survival rate between the two groups of patients [n (%)].

Groups	n	1-year survival rate	95% CI		*p*	Effect size
			Lower	Upper		
Control group	107	100 (93.5%)	0.017	1.183	0.037	-0.144
Intervention group	101	100 (99%)				

Table 7. Comparison of FACT-BL scores between the two groups of patients (mean±SD, scores).

Indicator	n	Time	Control group	Intervention group	95% CI		*p*	Effect size
					Lower	Upper		
FACT-BL scores	100	1 day before surgery	68.65±5.31	67.41±5.20	-0.23	2.71	0.097	0.24
	100	1 year after surgery	86.37±5.42	88.00±5.11	-3.10	-0.16	0.03	-0.31

FACT-BL: Functional Assessment of Cancer Therapy-Bladder.

DISCUSSION

One of the fatal malignant tumors is MIBC. Based on the data, the survival rate of patients within 5 years is about 50%. The immune system has a dual character in the progression of epithelial cancer of the bladder, including killing and injuring cancer cells and increasing the survival and spread of tumor cells with the help of immune escape mechanisms. In a healthy state, cancer cells are eliminated when they are attacked by the host immune system, whereas when the body's immune protection mechanism is impaired, a delicate balance is

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formed between the immune system and the tumor. When the body's immune protection mechanism is impaired, this helps subpopulations of cancer cells to escape from the surveillance of the immune system by taking advantage of genetic instability, ultimately leading to the development of malignant diseases. Immunotherapy obviously enhances the body's immune response through the above pathogenesis or uses other targets to irritate the immune system to enhance, and then accomplish the purpose of resisting tumor cells.

Tumor markers are chemical substances composed of proteins or sugars. Monitoring the expression of tumor markers in serum can indirectly help understand the patient's disease. CEA, CA-125, CY21-1, and NSE are common tumor necrosis factors. CA-125[26] is highly expressed in cancer cells. When infiltrating tumor cells destroy tissues, CA-125 can rapidly enter the bloodstream, and its expression in the peripheral blood increases. CEA, as one of the relatively widespread tumor markers,[27] its high expression suggests more epidermal growth factor mutations, which directly affects the prognosis of patients. Hyeong Dong Yuk et al[28] also mentioned that CEA and CA-125 can usually be used to assess the prognosis of patients with bladder cancer after resection. NSE is present in neuroendocrine cells and neuronal cells.[29] CY21-1 belongs to differentiation-specific proteins and is mainly found in the cytoplasm of cancer cells.[30] In addition, tumorigenesis and disease progression are closely related to the total immune system. T-lymphocytes, as an important component of the body's immunity,[31] can counteract tumor immunity through cellular immunity. $CD3^+$, $CD4^+/CD8^+$, and NK cells, as indicators of immune function, directly mediate the body's positive and negative immune responses. Angiogenesis is a key factor in determining the growth of cancer foci. Cancer cells grow too fast, leading to local tissue hypoxia and ischemia changes, stimulating angiogenesis while promoting the secretion of a large number of angiogenic factors by tumor cells. Rohit Siddhartha et al[32] mentioned that bFGF and VEGF are cell growth factors, and the above factors are highly expressed in patients with malignant tumors, and the effect is not influenced by the histological and biological characteristics of the tumor. TNF-α can directly regulate the NF-κB signaling pathway and enhance the effect of cancer cell infiltration and proliferation. The finding of this research indicated that after a 1-year course of treatment, compared favorably with the control group, the expression of $CD3^+$, $CD4^+/CD8^+$, and NK cells was more effective in the intervention group, and the expression of CEA, CA-125, CY21-1, NSE, bFGF, VEGF, and TNF-α was lower in the intervention group. This suggests that the combined use of nivolumab immunotherapy after surgery can inhibit tumor markers, correct the expression of aberrant factors, and improve the body's immunity. The reason may be that nivolumab can activate the tumor-killing function mediated by T cells by blocking the binding of PD-1 and PD-L1, promote tumor cells to enter apoptosis, and expedite the death of tumor cells. It is also mentioned in the study of Patrick A. Ott et al[33] that nivolumab can accelerate the death of tumor cells by blocking PD-1 and PD-L1 to enhance the activation of $CD3^+$, $CD4^+$, and $CD8^+$ T cells. Activation of T cells also enhances immune surveillance, increases NK cell viability, stimulates bone marrow cell regeneration and differentiation, alleviates inflammatory response and immunosuppression, and inhibits the expression of tumor markers and the secretion of serum cytokines by tumor cells.

The results of this study showed that compared to the control group, the intervention group had significantly higher pCR, 1-year survival rate, and FACT-BL scores, suggesting that the combination of nivolumab immunotherapy after resection was effective in alleviating the risk of relapse and improving the quality of the patient's survival life. Elise F. Nassif et al[34] mentioned that improving T-lymphocyte immune function had a significant impact on the patient's survival, which is also consistent with the results of this study. The analysis of the reasons may be: (1) Nivolumab inhibits the signaling pathway of PD-L1 after entering the organism, and by blocking the T-lymphocyte immunosuppressive effect mediated by this signaling pathway, it can validly activate the body's anti-tumor immune response and enhance the immune system's competencies for identifying and removing cancer cells, which is important in helping to improve the therapeutic effect and reduce the tumor necrosis factor. (2) Nivolumab can help anti-tumor immune recovery by inactivating immune checkpoints through inhibiting the binding of receptor and ligand after entering the organism. MIBC belongs to the tumors with high cellular mutation frequency and high antigenic expression, so the immune checkpoints are the optimal targets of inhibitors, which was also mentioned in the study of Aimal Waqas et al.[35] By combining these targets, immune checkpoint inhibitors can restore the

immune response of cancer cells to T cells, and the immune escape of cancer cells is ineffective, which ultimately achieves the therapeutic effect. In conclusion, patients with high-risk MIBC are better treated by immunotherapy combined with surgical resection than by surgical resection alone, which is conducive to enhancing the body's immune function, correcting abnormal serum cytokines, lowering the level of tumor markers, decreasing the risk of relapse in patients, and improving the quality of the patient's survival.

In summary, high-risk MIBC patients treated with immunotherapy combined with surgical resection have better outcomes than those treated with surgical resection alone. This approach is conducive to enhancing the body's immune function, correcting abnormal serum cytokines, lowering the level of tumor markers, reducing the risk of relapse in patients, improving the quality of patient survival, and providing a scientific basis for the clinic to optimize the nursing program. Although studies have shown that high-risk MIBC patients have more positive treatment effects with immunotherapy combined with surgical resection, the following limitations still exist, which may guide the universalization of the results and the value of long-term application:

(1) Shortage of long-term follow-up data: The current results are based on a 12-month follow-up, and the peak of recurrence for MIBC patients is at 2–3 years postoperatively. The 5-year survival rate and long-term toxicity (e.g., immune-related endocrine disorders) have not been clarified.

(2) Surrogate endpoints for pathological remission: Although pCR boost is associated with improved survival, the long-term prognosis of non-pCR patients is highly heterogeneous, and some patients with residual lesions may still benefit, requiring more precise prognostic stratification models.

(3) Mechanisms of resistance and follow-up gaps: For patients with primary/secondary resistance to immunotherapy (e.g., tumor microenvironment immunosuppressive phenotypes), follow-up strategies (e.g., double-immunity combinations, antibody-drug conjugate [ADC] drugs)[36] have not been explored.

Although current evidence confirms the significant benefit of immunotherapy combined with surgical resection, the limited scope of application, lack of long-term data, and unknown mechanisms of resistance remain the core challenges. In the future, the individualized medical treatment of high-risk MIBC needs to be further improved by optimizing treatment intensity and exploring novel combination strategies.

STATEMENT OF ETHICS

The clinical study followed the Declaration of Helsinki and other relevant ethical regulations and was reviewed and approved by Yueyang People's Hospital's Ethics Committee. The study obtained written informed consent from all participants.

FUNDING

Not applicable.

CONFLICT OF INTEREST

The authors declare no conflicts of interest.

ACKNOWLEDGMENTS

Not applicable.

DATA AVAILABILITY

The data supporting the findings of this study can be obtained from the corresponding author upon request.

AI ASSISTANCE DISCLOSURE

Not applicable.

REFERENCES

1. Rozanec JJ, Secin FP. Epidemiology, etiology, and prevention of bladder cancer. Arch Esp Urol. 2020;73(10):872-8.
2. Viswambaram P, Hayne D. Gender discrepancies in bladder cancer: potential explanations. Expert Rev Anticancer Ther. 2020;20(10):841-9.
3. Xu N, Yao Z, Shang G, Chen J, Li Y, Wang Q, et al. Integrated proteogenomic characterization of urothelial carcinoma of the bladder. J Hematol Oncol. 2022;15(1):76.
4. Alouini S. Risk factors associated with urothelial bladder cancer. Int J Environ Res Public Health. 2024;21(7):954.
5. Santos LL, Santos J, Gouveia MJ, Vale N, Lopes C, Rinaldi G, et al. Urogenital schistosomiasis-history,

pathogenesis, and bladder cancer. J Clin Med. 2021;10(2):205.
6. Wu J, Jin S, Gu C, Wang H, Zhou L, Xu W, et al. Inherited mutations in Chinese patients with upper tract urothelial carcinoma. Cell Rep Med. 2023;4(1):100883.
7. Gupta G, Gupta R, Pasricha S, Sharma A, Kumar V, Yadav R, et al. Molecular stratification of high-grade urothelial carcinoma by immunohistochemistry with its histomorphological and clinical correlation. Indian J Pathol Microbiol. 2022;65(4):832-8.
8. Dobruch J, Oszczudłowski M. Bladder cancer: current challenges and future directions. Medicina (Kaunas). 2021;57(8):749.
9. Hu J, Chen J, Ou Z, Wang Y, Zhang H, Liu P, et al. Neoadjuvant immunotherapy, chemotherapy, and combination therapy in muscle-invasive bladder cancer: a multi-center real-world retrospective study. Cell Rep Med. 2022;3(11):100785.
10. Dyrskjøt L, Hansel DE, Efstathiou JA, Knowles MA, Malats N, Hartmann A, et al. Bladder cancer. Nat Rev Dis Primers. 2023;9(1):58.
11. Ahmadieh N, Zeidan T, Chebel JA, Haddad FG, Nemr E. Neoadjuvant chemotherapy for muscle-invasive bladder cancer in a Lebanese experience: in all aspects. Gulf J Oncol. 2023;1(43):33-9.
12. Facchini G, Cavaliere C, Romis L, Veneziani M, Porricelli R, Iorio B, et al. Advanced/metastatic bladder cancer: current status and future directions. Eur Rev Med Pharmacol Sci. 2020;24(22):11536-52.
13. Flaig TW, Spiess PE, Abern M, Agarwal N, Bangs R, Boorjian SA, et al. NCCN Guidelines® Insights: Bladder Cancer, Version 3.2024. J Natl Compr Canc Netw. 2024;22(4):216-25.
14. Ward Grados DF, Ahmadi H, Griffith TS, Warlick CA. Immunotherapy for bladder cancer: latest advances and ongoing clinical trials. Immunol Invest. 2022;51(8):2226-41.
15. Singh A, Osbourne AS, Koshkin VS. Perioperative immunotherapy in muscle-invasive bladder cancer. Curr Treat Options Oncol. 2023;24(9):1213-30.
16. Lopez-Beltran A, Cookson MS, Guercio BJ, Cheng L. Advances in diagnosis and treatment of bladder cancer. BMJ. 2024;384:e076743.
17. Powles T, Bellmunt J, Comperat E, Witjes JA, De Santis M, Necchi A, et al. ESMO clinical practice guideline interim update on first-line therapy in advanced urothelial carcinoma. Ann Oncol. 2024;35(6):485-90.
18. Basile G, Bandini M, Gibb EA, Gallina A, Fossati N, Gandaglia G, et al. Neoadjuvant pembrolizumab and radical cystectomy in patients with muscle-invasive urothelial bladder cancer: 3-year median follow-up update of PURE-01 trial. Clin Cancer Res. 2022;28(23):5107-14.
19. Galsky MD, Witjes JA, Gschwend JE, Bellmunt J, Merseburger AS, Huang Y, et al. Adjuvant nivolumab in high-risk muscle-invasive urothelial carcinoma: expanded efficacy from CheckMate 274. J Clin Oncol. 2025;43(1):15-21.
20. Wu Z, Zheng Y, Sheng J, Zhang W, Chen X, Li Q, et al. CD3(+)CD4(-)CD8(-) (double-negative) T cells in inflammation, immune disorders, and cancer. Front Immunol. 2022;13:816005.
21. Wu SY, Fu T, Jiang YZ, Shao ZM. Natural killer cells in cancer biology and therapy. Mol Cancer. 2020;19(1):120.
22. Schmit T, Klomp M, Khan MN. An overview of flow cytometry: its principles and applications in allergic disease research. Methods Mol Biol. 2021;2223:169-82.
23. Tabatabaei MS, Ahmed M. Enzyme-linked immunosorbent assay (ELISA). Methods Mol Biol. 2022;2508:115-34.
24. Saltalamacchia G, Bernardo A, Quaquarini E. Prognostic role of pathological complete response in early stage epithelial solid tumors. Cancer Control. 2023;30:10732748231161466.
25. Pazeto CL, Baccaglini W, Tourinho-Barbosa RR, Glina S, Cathelineau X, Sanchez-Salas R, et al. HRQOL related to urinary diversion in radical cystectomy: a systematic review of recent literature. Int Braz J Urol. 2019;45(6):1094-104.
26. Zhang M, Cheng S, Jin Y, Zhao Y, Wang Y. Roles of CA125 in diagnosis, prediction, and oncogenesis of ovarian cancer. Biochim Biophys Acta Rev Cancer. 2021;1875(2):188503.
27. Xiang W, Lv Q, Shi H, Xie B, Gao L. Aptamer-based biosensor for detecting carcinoembryonic antigen. Talanta. 2020;214(6):120716.
28. Yuk HD, Han JH, Jeong SH, Jeong CW, Kwak C, Ku JH, et al. Beta-human chorionic gonadotropin, carbohydrate antigen 19-9, cancer antigen 125, and carcinoembryonic antigen as prognostic and predictive biological markers in bladder cancer. Front Oncol. 2024;14:1479988.
29. Duggan B, O'Rourke D, Anderson N, Browne S, Fitzgerald R, Thomas V, et al. Biomarkers to assess the risk of bladder cancer in patients presenting with haematuria are gender-specific. Front Oncol. 2022;12:1009014.
30. Setianingsih YA, Djatisoesanto W, Laksita TB, Aryati A. Diagnostic accuracy of urinary cytokeratin fragment-19

(CYFRA21-1) for bladder cancer. Narra J. 2024;4(3):e1142.
31. Baessler A, Vignali D. T cell exhaustion. Annu Rev Immunol. 2024;42(1):179-206.
32. Siddhartha R, Goel A, Singhai A, Garg M. Matrix metalloproteinases -2 and -9, vascular endothelial growth factor, basic fibroblast growth factor and CD105-micro-vessel density are predictive markers of non-muscle invasive bladder cancer and muscle invasive bladder cancer subtypes. Biochem Genet. 2024.
33. Ott PA, Hu-Lieskovan S, Chmielowski B, Ribas A, Johnson DB, Daud A, et al. A phase Ib trial of personalized neoantigen therapy plus anti-PD-1 in patients with advanced melanoma, non-small cell lung cancer, or bladder cancer. Cell. 2020;183(2):347-62.e24.
34. Nassif EF, Thibault C, Oudard S, Galon J. Precision immunity: immunoscore and neoadjuvant treatment in bladder cancer. Oncoimmunology. 2021;10(1):1888488.
35. Waqas A, Zaffar J, Jalil A, Butt S. Nivolumab-induced isolated neutropenia. Cureus. 2023;15(9):e45675.
36. Ren J. Advances in combination therapy for gastric cancer: integrating targeted agents and immunotherapy. Adv Clin Pharmacol Ther. 2024;1(1):1-15.