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Comparative Analysis of the Efficacy of Neoadjuvant Therapy on Primary Lesions and Metastatic Lesions in Bladder Cancer: A Multicenter Retrospective Study

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Research Article

Keywords: bladder cancer, neoadjuvant therapy, primary tumor efficacy, metastatic lesion efficacy

Posted Date: November 6th, 2026

DOI: https://doi.org/10.21203/rs.3.rs-11349687/v1

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Additional Declarations: No competing interests reported.

Abstract

Background

Neoadjuvant therapy (NAT) plays a significant role in bladder cancer (BC) treatment, but comparative data on its efficacy between primary and metastatic lesions are limited. This study aims to compare the treatment response between primary and metastatic lesions and to analyze the impact of clinical characteristics, treatment regimens, and histological types on such differences.

Methods

This multicenter retrospective study included BC patients with evaluable metastatic lesions who received NAT followed by radical cystectomy with bilateral pelvic lymph node dissection. Treatment response was assessed using TNM staging combined with imaging for both primary and metastatic lesions. Statistical analyses were performed using R software.

Results

Among 72 eligible patients, the overall response rate of primary lesions (73.61%) was higher than that of metastatic lesions (63.89%) and pelvic lymph node metastases (64.41%). Significant differences in treatment efficacy were observed between primary lesions and metastatic lesions ($P = 0.046$), and the difference was even greater between primary lesions and pelvic lymph node metastases ($P = 0.034$). Variant histology (OR = 12.40, $P < 0.001$) and smoking history (OR = 5.31, $P = 0.02$) were significant factors influencing efficacy differences. The integrated T-stage and size assessment reclassified primary tumor response in 6 patients (8.33%) and showed better consistency than conventional RECIST criteria (Kappa: 0.24 vs. 0.19).

Conclusion

NAT efficacy significantly differed between primary and metastatic lesions, as well as between primary lesions and pelvic lymph node metastases in BC. Variant histology and smoking history were independent predictors of such differences. In terms of efficacy assessment methodology, the novel "T-stage and size integrated evaluation" method provided more accurate primary tumor response assessment. This study offers important evidence for personalized treatment strategies in BC.

Trial registration

Clinical trial number: not applicable.

Background

Bladder cancer (BC) ranks as the tenth most common cancer globally, with the 13th highest mortality rate. In 2018, it accounted for approximately 3% of all new cancer diagnoses, and its incidence has been steadily increasing worldwide [1, 2]. Approximately 90% of BC cases are histologically classified as Urothelial Carcinoma (UC), while the remaining cases include squamous cell carcinoma, sarcomatoid carcinoma, lymphoma, and adenocarcinoma [3]. These non-UC histological subtypes are collectively referred to as Variant Histology (VH), which studies have shown to be associated with poorer prognosis and a higher likelihood of Occult Lymph Node Metastasis (OLNM) [4, 5]. Smoking and exposure to specific occupational/environmental toxins are the most well-established risk factors for BC, with smoking implicated in approximately 50%–65% of cases [3]. BC can be classified into Non-Muscle Invasive Bladder Cancer (NMIBC) and Muscle Invasive Bladder Cancer (MIBC) based on the depth of tumor invasion. Approximately 75% of newly diagnosed cases each year are NMIBC, but 15%–20% of these will progress to MIBC over time [6]. According to current guidelines, the primary treatment for NMIBC involves Transurethral Resection of Bladder Tumor (TURBT) combined with intravesical instillation of cytotoxic agents (such as epirubicin). In contrast, the standard treatment for MIBC is Radical Cystectomy (RC) [7–9]. Regarding prognosis, NMIBC demonstrates a relatively high 5-year survival rate following standard therapy. However, the 5-year survival rate for MIBC after RC is only approximately 50%–60%, with a high recurrence rate, indicating limited survival benefit from RC alone [10, 11]. Consequently, to improve outcomes, perioperative systemic therapy has been incorporated into the standard of care for MIBC, with a series of significant benefits reported in current studies [12–14].

Neoadjuvant Therapy (NAT) involves the administration of chemotherapy, immunotherapy, or targeted therapy prior to RC, aiming to reduce tumor size, eliminate micrometastases, achieve pathological downstaging, and improve prognosis. Current evidence suggests that NAT does not increase the risk of surgery-related complications [15]. For MIBC, Neoadjuvant Chemotherapy (NAC) represents the most established NAT modality. Cisplatin-based regimens, primarily Methotrexate/Vinblastine/Doxorubicin/Cisplatin (M-VAC) and Gemcitabine/Cisplatin (GC), have been proven to improve overall survival and are considered the first-line standard of care [9, 16–18]. However, due to factors such as renal insufficiency, some patients are ineligible for cisplatin-based chemotherapy, leading to growing interest in neoadjuvant immunotherapy (NIT). Immune checkpoint inhibitors (ICIs) like atezolizumab, nivolumab, and tislelizumab have demonstrated promising efficacy in locally advanced and metastatic bladder cancer. Clinical trials in MIBC have reported high pathological response rates with acceptable toxicity profiles for neoadjuvant ICIs, although mature survival data are still lacking [17, 19, 20]. Consequently, their current use in the neoadjuvant setting is primarily reserved for cisplatin-ineligible patients. Furthermore, antibody-drug conjugates (ADCs) such as enfortumab vedotin and sacituzumab govitecan have been approved for patients with locally advanced or metastatic UC. They are also showing potential application value in MIBC patients who are unsuitable for or decline cisplatin-based chemotherapy [21, 22]. In summary, the role of NAT in MIBC management is becoming increasingly established. While NAC remains the standard, the advent of immunotherapy and ADC

agents offers more personalized options for patients and holds promise for further optimizing comprehensive treatment strategies for MIBC.

Traditionally, the assessment of lesion response to NAT in oncology is categorized into preoperative clinical evaluation and postoperative pathological evaluation [23, 24]. Both methods are routinely employed in research investigating NAT for BC [25–27]. Since NAT in BC is predominantly applied to patients with locally advanced disease, the vast majority of studies focus on the impact of primary tumor response on survival benefit, often overlooking changes in metastatic lesions. However, clinical observation has indicated that responses in primary and metastatic sites are frequently discordant. Studies have shown that patients achieving a complete response (CR) in the primary tumor may still present with OLNM postoperatively [28]. Furthermore, multiple studies have indicated that lymph node status following NAC is a more critical prognostic factor than the status of the primary tumor [29–31]. Therefore, in patients with well-defined "visible metastatic lesions" (e.g., those radiologically or pathologically confirmed as having distant metastases or locally advanced disease with regional lymph node involvement), the assessment of treatment response in these metastatic sites after NAT is also of paramount importance. Nevertheless, there is a paucity of studies dedicated to a direct comparison of NAT efficacy at primary versus metastatic sites. This topic has only been sporadically addressed, typically within small retrospective cohorts or as a secondary note in larger investigations [15, 25, 32–34]. Given this gap, no study has specifically compared the efficacy of NAT between primary and metastatic lesions in BC. Notably, a preclinical study conducted by Ebos et al. in breast, kidney, and melanoma models demonstrated that the response of primary tumors to neoadjuvant antiangiogenic therapy does not reliably predict postoperative metastatic recurrence or survival benefit. Furthermore, the same regimen yielded discordant outcomes between primary and metastatic sites: in certain models, despite significant primary tumor shrinkage following NAT, postoperative survival was unchanged or even worsened, suggesting a fundamental divergence in therapeutic efficacy. [35] Therefore, this study aims to conduct a multicenter, retrospective statistical analysis comparing the response of BC primary tumors and metastatic sites to NAT.

Methods and materials

Clinical data

This study retrospectively analyzed patients with bladder cancer who underwent neoadjuvant therapy followed by radical cystectomy and pelvic lymph node dissection at Sun Yat-sen Memorial Hospital, Sun Yat-sen University Cancer Center, and The First Affiliated Hospital of Sun Yat-sen University between January 2015 and January 2025.

Inclusion Criteria: (1) Age ≥ 18 years, regardless of gender; (2) Histologically or cytologically confirmed diagnosis of bladder cancer; (3) Received NAT (regardless of the specific regimen) before radical surgery and completed one or more planned cycles; (4) Underwent radical cystectomy combined with bilateral pelvic lymph node dissection (PLND) following NAT; (5) The extent of bilateral pelvic lymph node

dissection met at least the standard template, including bilateral obturator, internal iliac, and external iliac lymph nodes; (6) Availability of complete and traceable imaging data and pathological records before and after NAT, as well as postoperatively; (7) Provided written informed consent for the use of clinical data in this retrospective study. Exclusion Criteria: (1) Pathological type of the primary or metastatic lesions included other tumors; (2) Inability to evaluate the neoadjuvant regimen and cycle number, or receipt of less than one full cycle of therapy; (3) History of pelvic surgery during the period between NAT initiation and radical surgery, which could preclude accurate assessment of treatment response; (4) Insufficient research data to objectively evaluate efficacy or key outcome measures (e.g., interrupted treatment records, or missing imaging/pathological data at critical time points); (5) Absence of metastatic lesions indicated by both clinical and pathological assessments prior to neoadjuvant therapy. Based on these criteria, a total of 72 eligible cases were identified, with patient contributions from Sun Yat-sen Memorial Hospital, The First Affiliated Hospital of Sun Yat-sen University, and Sun Yat-sen University Cancer Center being 39, 13, and 20 cases, respectively. Of these, 44 cases received NAC alone, 26 received NAC combined with ICIs, 1 received NAC combined with targeted therapy, and 1 received neoadjuvant ICIs combined with targeted therapy. Ethical approval was obtained from the Ethics Committee of Sun Yat-sen Memorial Hospital.

Treatment regimen

The NAC regimens included GC, gemcitabine plus carboplatin (GP), or nab-paclitaxel plus nedaplatin. The NIT agents comprised tislelizumab, toripalimab, camrelizumab, and sintilimab. The targeted therapies utilized were nimotuzumab and disitamab vedotin.

Data collection

Clinical data were collected from surgical records, electronic medical records, imaging databases (PACS), and pathological databases. Variables included gender, age at initial diagnosis of bladder cancer, smoking history, and comorbidities before neoadjuvant therapy (hypertension, coronary heart disease, diabetes, renal insufficiency). Treatment-related information encompassed intravesical instillation regimens (after pre-neoadjuvant TURBT), neoadjuvant therapeutic regimens and cycle numbers, surgical approach for radical cystectomy, extent and number of lymph nodes dissected. Imaging outcomes consisted of pre-neoadjuvant imaging reports and pre-radical surgery imaging reports following neoadjuvant therapy (tumor size, depth of invasion, size and number of enlarged lymph nodes, size and number of distant metastases). Pathological outcomes included post-radical surgery pathological reports (pathological type and grade of primary lesion, depth of invasion, number and location of positive lymph nodes). Tumor characteristics, including pre-neoadjuvant clinical TNM (cTNM) stage and post-neoadjuvant pathological TNM (ypTNM) stage based on postoperative pathology. The cTNM and ypTNM stages were assessed according to the 8th edition of the TNM classification system of the AJCC and UICC[36, 37].

Evaluation of primary tumor response

Recognizing tumor invasion depth as a pivotal determinant of both therapeutic strategy and prognosis in BC [38, 39], we propose a unique approach to response assessment: the "T-stage and Size Integrated Evaluation Method" (Fig. 1). The primary reference standard for efficacy evaluation was the comparison between the post-neoadjuvant therapy pathologic tumor stage (ypT) and the pre-neoadjuvant clinical tumor stage (cT). The ypT stage reflects the pathologic assessment of tumor size and invasion depth after surgery, while the cT stage was determined based on pre-NAT imaging and pathological findings from transurethral resection of bladder tumor (TURBT). Based on this comparison, treatment efficacy was defined as follows: response for downstaging; progression for upstaging. In cases of unchanged stage, the Response Evaluation Criteria in Solid Tumors (RECIST 1.1, a standard imaging-based size evaluation method) were applied [24]. For the purpose of statistical analysis, RECIST outcomes were consolidated into three discrete categories: pathological complete response / clinical complete response / partial response (pCR / cCR / PR) was classified as “response”; stable disease (SD) as “stable”; and progressive disease (PD) as “progression”.

Evaluation of Metastatic Lesion Response

The efficacy of NAT on metastatic lesions was evaluated using an integrated method that combined assessment of lymph node (N) stage, distant metastasis (M) stage, and radiographic findings (Fig. 2). The primary efficacy evaluation standard constituted the comparison of post-RC pathological NM stages (ypNM) versus pre-NAT clinical NM stages (cNM). Progression was defined as upstaging in either N or M stage. Downstaging was defined when both N and M stages were downstaged, or when one was downstaged while the other remained stable. If both N and M stages remained unchanged, NAT efficacy was assessed radiographically with reference to RECIST 1.1 criteria: (1) “Response” was defined as either a decrease in the number of metastatic lesions or a ≥ 30% reduction in the sum of the longest diameters of target lesions (in the absence of new lesions); (2) “Progression” was defined as an increase in the number of lesions or a ≥ 20% increase in the sum of the longest diameters of target lesions; (3) “Stable” was assigned when the changes met neither the criteria for Response nor for Progression.

Given the limited sample size of patients with distant metastases prior to NAT, a separate evaluation of response was performed specifically for lymph node metastases, in accordance with the methodology described above.

Outcome measures

Primary outcomes: Treatment response for primary tumors, metastatic lesions, and lymph node-only metastases was classified as “response”, “stable”, or “progression” based on a novel assessment framework. Unlike conventional size-based criterion (RECIST 1.1), which is suboptimal for BC due to its hollow-organ nature, our assessment integrated changes in both TNM stage and tumor size to capture the depth of invasion—a prognostically critical parameter. The response categories were compared between: (1) primary tumors and all metastatic lesions; and (2) primary tumors and lymph node metastases.

Secondary outcomes: Heterogeneous response, defined as discordant efficacy between primary and metastatic lesions, was analyzed to identify independent risk factors using univariate and multivariate logistic regression. Additionally, the concordance between primary tumor response (assessed by two different methods) and metastatic lesion response was evaluated.

Statistical analysis

Statistical analyses were performed using R software (version 4.4.2) and IBM SPSS Statistics (version 31.0.2.0). A two-sided significance level of α = 0.05 was set for all tests, with $P < 0.05$ considered statistically significant. Treatment response for primary tumors and metastatic lesions was independently classified into three ordinal categories: progression, stable, or response. Response heterogeneity was defined as discordance in the assigned categories between the two sites, and its statistical significance was assessed using the Wilcoxon signed-rank test. Univariate and multivariate binary logistic regression analyses, along with subgroup analyses, were conducted to identify factors associated with response heterogeneity. Additionally, weighted kappa statistics were used to evaluate the concordance between primary tumor response (assessed by two different methods) and metastatic lesion response.

Ethics statement

This study received ethics approval from the main center's ethics committee (Ethical approval number: SYSKY-2026-193-01). The authors assume full responsibility for all aspects of this work and will ensure that any queries regarding its accuracy or integrity are duly investigated and resolved.

Results

Comparison of baseline characteristics

A total of 72 eligible patients with preoperative confirmation of metastases who received NAT followed by RC and PLND were enrolled, of whom 44 (61.11%) exhibited heterogeneous responses between primary tumors and metastatic lesions, while 28 (38.89%) showed concordant responses. The study cohort comprised predominantly males, with a mean age of 59.5 ± 10.1 years. Among the NAT regimens, chemotherapy was administered to 98.61% of patients and immunotherapy to 38.89%, reflecting the use of combination regimens in a subset of patients. Regarding treatment duration, most patients (51.39%) received ≥ 4 cycles of NAT. Baseline demographic and clinicopathological characteristics stratified by response heterogeneity are summarized in Table 1. Characteristics including center, age, gender, comorbidities, intravesical instillation, clinical TNM stage, and NAT cycles were well balanced between the two groups (all $P > 0.05$). However, significant differences were observed in smoking history ($P = 0.04$), histologic subtype ($P < 0.001$), and NAT regimen ($P = 0.04$), with a higher proportion of variant histology and smoking history in the heterogeneity group, and more combination therapy in the concordance group. Pathologic responses also differed markedly: patients in the concordance group were more likely to achieve ypT0 (18.06% vs. 1.39%) and ypN0 (40.28% vs. 5.56%), while those in the

heterogeneity group had higher rates of ypT4 (18.06% vs. 5.56%) and ypN3 (34.72% vs. 2.78%) (both $P < 0.01$). According to RECIST criteria, the concordance group had a higher proportion of pCR and PR, whereas the heterogeneity group showed more SD and PD ($P = 0.03$). These findings suggest that histologic subtype, smoking status, NAT regimen, and pathologic response patterns are associated with response discordance, underpinning the subsequent regression analyses.

Table 1
Baseline patient characteristics

Variable	Overall, n (%)	Response Heterogeneity Group, n (%)	Response Concordance Group, n (%)	method	Statistic	*P* value
Center				Chisq test	3.70	0.16
SYSMH	39 (54.17%)	14 (19.44%)	25 (34.72%)			
SYSUCC	20 (27.78%)	11 (15.28%)	9 (12.50%)			
FAH-SYSU	13 (18.06%)	3 (4.17%)	10 (13.89%)			
Gender				Yates' correction	< 0.001	0.95
Male	68 (94.44%)	27 (37.50%)	41 (56.94%)			
Female	4 (5.56%)	1 (1.39%)	3 (4.17%)			
Age, mean ± sd	59.5 ± 10.1	58.1 ± 9.0	60.5 ± 10.7	T test	-0.98	0.33
Hypertension	14 (19.44%)	7 (9.72%)	7 (9.72%)	Chisq test	0.9	0.34
Coronary heart disease	3 (4.167%)	1 (1.39%)	2 (2.78%)	Yates' correction	< 0.001	1.00
Diabetes	6 (8.33%)	3 (4.17%)	3 (4.17%)	Yates' correction	0.02	0.88
Renal insufficiency	16 (22.22%)	8 (11.11%)	8 (11.11%)	Chisq test	1.07	0.30
Smoking history	23 (31.94%)	13 (18.06%)	10 (13.89%)	Chisq test	4.42	0.04
Intravesical instillation	23 (31.94%)	7 (9.72%)	16 (22.22%)	Chisq test	1.02	0.31
Histologic type				Chisq test	15.46	< 0.001
UC	48 (66.67%)	11 (15.3%)	37 (51.4%)			
VH	24 (29.17%)	17 (23.6%)	7 (9.7%)			
NAT regimen				Chisq test	4.45	0.04

Variable	Overall, n (%)	Response Heterogeneity Group, n (%)	Response Concordance Group, n (%)	method	Statistic	*P* value
NAC	43 (59.72%)	21 (29.17%)	22 (30.56%)			
Combo	29 (40.28%)	7 (9.72%)	22 (30.56%)			
NAT cycles				Chisq test	0.04	0.85
< 4	35 (48.61%)	14 (19.4%)	21 (29.2%)			
≥ 4	37 (51.39%)	14 (19.4%)	23 (31.9%)			
cT stage				Yates' correction	2.56	0.28
2	11 (15.28%)	4 (5.56%)	7 (9.72%)			
3	28 (38.89%)	8 (11.11%)	20 (27.78%)			
4	33 (45.83%)	16 (22.22%)	17 (23.61%)			
cN stage				Yates' correction	0.08	0.96
1	22 (30.56%)	9 (12.50%)	13 (18.06%)			
2	47 (65.28%)	18 (25.00%)	29 (40.28%)			
3	3 (4.17%)	1 (1.39%)	2 (2.78%)			
cM stage				Yates' correction	0.09	0.76
0	60 (83.33%)	21 (29.17%)	39 (54.17%)			
1	12 (16.67%)	7 (9.72%)	5 (6.94%)			
ypT stage				Yates' correction	19.57	0.002
0	14 (19.44%)	1 (1.39%)	13 (18.06%)			
tis	2 (2.78%)	5 (6.94%)	2 (2.78%)			

Variable	Overall, n (%)	Response Heterogeneity Group, n (%)	Response Concordance Group, n (%)	method	Statistic	*P* value
1	7 (9.72%)	1 (1.39%)	6 (8.33%)			
2	17 (23.61%)	6 (8.33%)	11 (15.28%)			
3	15 (20.83%)	7 (9.72%)	8 (11.11%)			
4	17 (23.61%)	13 (18.06%)	4 (5.56%)			
ypN stage				Yates' correction	21.45	< 0.001
0	34 (47.22%)	4 (5.56%)	29 (40.28%)			
1	12 (16.67%)	15 (20.83%)	8 (11.11%)			
2	20 (27.78%)	4 (5.56%)	5 (6.94%)			
3	6 (8.33%)	25 (34.72%)	2 (2.78%)			
ypM stage				Yates' correction	0.02	0.88
0	66 (91.67%)	3 (4.17%)	41 (56.94%)			
1	6 (8.33%)	1 (1.39%)	3 (4.17%)			
Efficacy (RECIST)				Yates' correction	10.51	0.03
pCR	14 (19.44%)	2 (2.78%)	12 (16.67%)			
cCR	8 (11.11%)	3 (4.17%)	5 (6.94%)			
PR	30 (41.67%)	10 (13.89%)	3 (4.17%)			
SD	14 (19.44%)	10 (13.89%)	20 (27.78%)			
PD	6 (8.33%)	3 (4.17%)	4 (5.56%)			

Variable	Overall, n (%)	Response Heterogeneity Group, n (%)	Response Concordance Group, n (%)	method	Statistic	*P* value
Note: Categorical variables are presented as n (%), and compared using the chi-square test (Chisq test) or Yates' continuity correction (Yates' correction) when appropriate; Continuous variables are presented as mean ± standard deviation (SD) and compared using the independent samples t-test (T test). A two-sided P value < 0.05 was considered statistically significant. FAH-SYSU, The First Affiliated Hospital, Sun Yat-sen University; SYSMH, Sun Yat-sen Memorial Hospital; SYSUCC, Sun Yat-sen University Cancer Center; The row for intravesical instillation records the number of instillations performed prior to NAT; Age, Age at initial bladder cancer diagnosis; UC, Urothelial carcinoma; VH, Variant Histology; NAT, Neoadjuvant Therapy; NAC, Neoadjuvant Chemotherapy; Combo, Combination neoadjuvant therapy (including C + I, C + T, and I + T; n = 27, 1, 1, respectively; C: chemotherapy, I: immunotherapy, T: targeted therapy); cT, pre-neoadjuvant clinical tumor stage; cN, pre-neoadjuvant clinical lymph node stage; cM, pre-neoadjuvant clinical distant metastasis stage; ypT, post-neoadjuvant therapy pathologic tumor stage; tis, carcinoma in situ; ypN, post-neoadjuvant therapy pathologic lymph node stage; ypM, post-neoadjuvant therapy pathologic distant metastasis stage; RECIST, Response Evaluation Criteria in Solid Tumors; pCR, Pathological Complete Response; cCR, Clinical Complete Response; PR, Partial Response; SD, Stable Disease; PD, Progressive Disease.						

Comparative analysis of response heterogeneity between primary tumors and metastatic lesions

The response to NAT was evaluated for both primary tumors and metastatic lesions, and the consistency of treatment efficacy between the two sites was examined. Overall, 38.89% (28/72) of patients exhibited response heterogeneity between primary and metastatic lesions. Among these discordant cases, the majority (53.57%, 15/28) showed a better response in the primary tumor than in metastatic lesions, consistent with the overall higher response rate for primary tumors (73.61%) compared with metastases (63.89%). This pattern is illustrated in the agreement plot (Fig. 3), where the most common discordant scenario was a better response in the primary site. Notably, even among patients achieving pathological complete response (pCR) in the primary tumor, one case (1/14, 7.14%) had persistent positive lymph nodes (ypN1), underscoring the potential for metastatic-site resistance despite primary-site clearance. Moreover, response rates were significantly lower in the heterogeneity group than in the concordance group at both primary (50% vs. 88.6%) and metastatic sites (25% vs. 88.6%), further highlighting the systemic nature of treatment resistance in patients with discordant responses. The Wilcoxon signed-rank test (Table 2) indicated a statistically significant difference in treatment response between primary tumors and metastatic lesions ($P = 0.046$). The test statistic ($V = 288$), representing the sum of positive ranks, further supports the significance of this response heterogeneity. Furthermore, all 13 patients presenting with distant metastases had evaluable lymph node disease prior to NAT. To isolate the differential response between primary tumors and pelvic lymph nodes, we analyzed the 59 patients with lymph node-only involvement (no distant metastases). In this subgroup, the Wilcoxon signed-rank test also demonstrated a significant difference in response ($P = 0.034$, Table 2).

Table 2
Results of the wilcoxon signed-rank test comparing treatment response to NAT between primary tumors and metastatic/pelvic lymph node lesions.

Wilcoxon Signed-Rank Test	Sample Size (n)	Statistic	*P* Value
Primary Tumors vs. Metastatic Lesions	72	288	0.046
Primary Tumors vs. LNM	59	175	0.034
Note: LNM, Lymph Node Metastases.			
Response heterogeneity analysis across different NAT regimens			

In this study, among 43 patients receiving NAC alone and 27 receiving NAC+ICIs, a stratified analysis revealed distinct patterns of response heterogeneity (Table 3). In the NAC-alone group, response differed significantly between primary tumors and lymph node metastases (n = 36, V = 106, $P = 0.045$) but not between primary tumors and overall metastatic lesions ($P = 0.133$). In contrast, the combination therapy group (NAC+ICIs) showed no significant response differences for either comparison (primary vs. overall metastases, $P = 0.330$; primary vs. lymph node metastases, $P = 0.49$).

Table 3
Impact of NAT regimen on response heterogeneity (Primary vs. Metastatic Sites)

Wilcoxon Signed-Rank Test	Sample Size (n)	Statistic	*P* Value
Primary Tumors vs. Metastatic Lesions (NAC)	43	157.5	0.133
Primary Tumors vs. LNM (NAC)	36	106	0.045
Primary Tumors vs. Metastatic Lesions (NAC+ICIs)	27	15.5	0.330
Primary Tumors vs. LNM (NAC+ICIs)	23	10.5	0.490
Note: NAC, Neoadjuvant Chemotherapy; ICIs, Immune Checkpoint Inhibitors; LNM, Lymph Node Metastases.			

Logistic regression analysis of factors associated with response heterogeneity

Univariate and multivariate logistic regression analyses were performed to identify predictors of response heterogeneity between primary and metastatic lesions in the 72 patients who received NAT. The results are presented in detail in Table 4 and summarized visually in forest plots (Fig. 4 for univariate analysis, Fig. 5 for multivariate analysis). In univariate analysis, VH (OR = 8.17, 95% CI: 2.70–24.74, $P < 0.001$), smoking history (OR = 2.95, 95% CI: 1.06–8.21, $P = 0.04$), and combination neoadjuvant therapy (OR = 0.33, 95% CI: 0.12–0.94, $P = 0.04$) were significantly associated with response heterogeneity. Other conventional prognostic factors (age, gender, comorbidities, clinical T/N/M stage) and treatment parameters (intravesical therapy, number of NAT cycles) showed no significant association in univariate analysis (all $P > 0.05$). In the multivariate model adjusting for potential confounders, VH (OR = 12.40, 95% CI: 3.14–49.02, $P < 0.001$) and smoking history (OR = 5.31, 95% CI: 1.27–22.16, $P = 0.02$) remained

independent predictors of response heterogeneity. However, the protective effect of combination neoadjuvant therapy observed in univariate analysis was attenuated and lost statistical significance after multivariate adjustment (OR = 0.74, 95% CI: 0.20–2.81, *P* = 0.66), suggesting that its apparent association was confounded by other factors. These findings identify VH and smoking history as independent risk factors for response heterogeneity.

Table 4
Logistic regression analysis of independent risk factors associated with response heterogeneity

Variable	Univariate Analysis				Multivariate Analysis			
	P Value	OR	Lower	Upper	*P* Value	OR	Lower	Upper
Gender								
Male	Ref.							
Female	0.56	0.51	0.05	5.12				
Age	0.32	0.98	0.93	1.02				
Hypertension	0.35	1.76	0.54	5.71				
Diabetes	0.56	1.64	0.31	8.76				
Renal insufficiency	0.31	1.80	0.59	5.53				
Intravesical instillation	0.32	0.58	0.20	1.67				
Smoking history	0.04	2.95	1.06	8.21	0.02	5.31	1.27	22.16
Histological type								
UC	Ref.							
VH	< 0.001	8.17	2.70	24.74	< 0.001	12.40	3.14	49.02
NAT regimen								
NAC	Ref.							
Combo	0.04	0.33	0.12	0.94	0.66	0.74	0.20	2.81
NAT cycles								
< 4	Ref.							
≥ 4	0.85	0.91	0.35	2.36				
cT stage								

Note: OR, Odds Ratio; An OR > 1 indicates an increased odds of the event, whereas an OR < 1 indicates a decreased odds; Ref., Reference; Age, Age at initial bladder cancer diagnosis; UC, Urothelial Carcinoma; VH, Variant Histology; NAT, Neoadjuvant Therapy; NAC, Neoadjuvant Chemotherapy; Combo, Combination Neoadjuvant Therapy; cT, pre-neoadjuvant clinical tumor stage; cN, pre-neoadjuvant clinical lymph node stage; cM, pre-neoadjuvant clinical distant metastasis stage.

Variable	Univariate Analysis				Multivariate Analysis			
	P Value	OR	Lower	Upper	*P* Value	OR	Lower	Upper
Gender								
cT2	Ref.							
cT3	0.64	0.7	0.16	3.07				
cT4	0.49	1.65	0.4	6.72				
cN stage								
cN1	Ref.							
cN2	0.84	0.9	0.32	2.52				
cN3	0.80	0.72	0.06	9.22				
cM stage								
cM0	Ref.							
cM1	0.14	2.60	0.73	9.21				

Note: OR, Odds Ratio; An OR > 1 indicates an increased odds of the event, whereas an OR < 1 indicates a decreased odds; Ref., Reference; Age, Age at initial bladder cancer diagnosis; UC, Urothelial Carcinoma; VH, Variant Histology; NAT, Neoadjuvant Therapy; NAC, Neoadjuvant Chemotherapy; Combo, Combination Neoadjuvant Therapy; cT, pre-neoadjuvant clinical tumor stage; cN, pre-neoadjuvant clinical lymph node stage; cM, pre-neoadjuvant clinical distant metastasis stage.

Logistic regression analysis in the UC subgroup

Exploratory univariate logistic regression analysis was performed to identify potential predictors of response heterogeneity specifically in the UC subgroup (n = 48). Due to the limited sample size of the VH subgroup (n = 24), regression analysis was not feasible in that cohort to avoid model overfitting and unreliable estimates. The results of the univariate analysis for the UC subgroup are presented in detail in Table 5. In univariate analysis, smoking history (OR = 4.73, 95% CI: 1.13–19.68, $P = 0.03$) and combination neoadjuvant therapy (OR = 0.17, 95% CI: 0.03–0.89, $P = 0.04$) were significantly associated with response heterogeneity in UC patients. Smoking history emerged as a risk factor for discordant responses, while combination therapy showed a protective effect. Other clinicopathological variables, including age, hypertension, diabetes, renal insufficiency, intravesical instillation, number of NAT cycles, and clinical T/N/M stage, showed no significant association with response heterogeneity in this subgroup (all $P > 0.05$). For the VH subgroup, descriptive analysis revealed a high proportion of response heterogeneity (17/24, 70.83%), but the small sample size precluded meaningful statistical inference. These findings in the UC subgroup, although preliminary and limited by sample size, suggest that

smoking history and combination neoadjuvant therapy may be associated with response discordance, warranting further validation in larger, histology-stratified cohorts.

Table 5
Logistic regression analysis in the urothelial carcinoma subgroup

Variable	Univariate Analysis			
	***P* Value**	**OR**	**Lower**	**Upper**
Gender				
Male	Ref.			
Female	-	-	-	-
Age	0.18	0.95	0.88	1.02
Hypertension	0.70	1.42	0.24	8.59
Diabetes	0.35	2.52	0.36	17.42
Renal insufficiency	0.81	0.81	0.14	4.50
Intravesical instillation	0.94	1.06	0.26	4.29
Smoking history	0.03	4.73	1.13	19.68
NAT regimen				
NAC	Ref.			
Combo	0.04	0.17	0.03	0.89
NAT cycles	0.73	0.79	0.21	3.05
cT stage				
cT2	Ref.			
cT3	0.40	0.42	0.05	3.22
cT4	0.94	1.07	0.16	7.15
cN stage				
cN1	Ref.			
cN2	0.93	1.07	0.23	4.86
cN3	-	-	-	-
cM stage				

Note: OR, Odds Ratio; An OR > 1 indicates an increased odds of the event, whereas an OR < 1 indicates a decreased odds; Ref., Reference; Age, Age at initial bladder cancer diagnosis; NAT, Neoadjuvant Therapy; NAC, Neoadjuvant Chemotherapy; Combo, Combination Neoadjuvant Therapy; cT, pre-neoadjuvant clinical tumor stage; cN, pre-neoadjuvant clinical lymph node stage; cM, pre-neoadjuvant clinical distant metastasis stage.

Variable	Univariate Analysis			
	***P* Value**	**OR**	**Lower**	**Upper**
Gender				
cM0	Ref.			
cM1	0.70	1.42	0.24	8.59

Note: OR, Odds Ratio; An OR > 1 indicates an increased odds of the event, whereas an OR < 1 indicates a decreased odds; Ref., Reference; Age, Age at initial bladder cancer diagnosis; NAT, Neoadjuvant Therapy; NAC, Neoadjuvant Chemotherapy; Combo, Combination Neoadjuvant Therapy; cT, pre-neoadjuvant clinical tumor stage; cN, pre-neoadjuvant clinical lymph node stage; cM, pre-neoadjuvant clinical distant metastasis stage.

Comparative analysis of integrated assessment and RECIST criteria for primary tumor response

The integrated assessment method (ypT stage + tumor size) reclassified the primary tumor response in 6 patients (8.33%) compared to conventional size-based (RECIST) criteria. The improvement is driven by incorporating pathological invasion depth—a key prognostic factor in BC not captured by size alone—thereby better aligning the assessed primary tumor response with systemic disease behavior after NAT.

When comparing the concordance between primary tumor response and metastatic lesion response, the weighted kappa statistic was 0.24 for integrated assessment Method and 0.19 for conventional assessment Method, indicating a slightly higher level of agreement for the prior (Table 6). However, both values remained within the range of fair to slight agreement, reflecting the substantial discordance between primary and metastatic treatment responses observed in our cohort.

Table 6
Comparison of Overall Response Agreement Between the Two Primary Tumor Assessment Methods

Assessment Method	Weighted Kappa	*P* Value	Strength of Agreement
T-stage & Size (RECIST)	0.24	0.04	Fair
Size-based (RECIST)	0.19	0.10	Slight

Note: RECIST, Response Evaluation Criteria in Solid Tumors.

Discussion

While NAT is an integral component of multimodal management for locally advanced and metastatic BC, the consistency of its efficacy across primary versus metastatic sites remains underexplored. Notably, the existing literature reveals a paradox: some studies report concurrent regression at both sites [25], whereas others describe clear discordance in response, even without direct comparative analysis [4, 34, 40]. This observed inter-site heterogeneity likely stems from fundamental biological disparities, including

intratumoral heterogeneity, distinct tumor microenvironments, and differential mechanisms of therapeutic agents.

This study presents the first systematic, multicenter analysis to directly compare NAT efficacy between primary and metastatic lesions in BC, characterizing the extent of response heterogeneity and defining its clinical determinants. In this analysis, which employed an innovative method integrating TNM staging with quantitative imaging assessment, we found that the overall response rate was significantly higher in primary tumors than in metastatic lesions (73.61% vs. 63.89%), with a response concordance rate of only 61.11% (44/72) between the two sites. Further statistical validation supported this finding. As shown in Table 2, the signed-rank test revealed a statistically significant difference ($P = 0.046$), implying a potential disparity in therapeutic efficacy where NAT may achieve local control at the primary site but demonstrate weaker control over metastatic lesions. Notably, in a subgroup analysis (Table 2), an even more pronounced difference in efficacy was observed between primary tumors and pelvic lymph node metastases ($P = 0.034$). This risk of insufficient systemic control is starkly highlighted in a key prognostic site: lymph node metastasis. As a critical determinant of BC outcomes, it is alarming that despite a primary tumor pCR rate of 19.44% (14 patients), 7.14% of these individuals (1/14) had persistent nodal disease. This finding carries significant clinical implications that local response does not equate to systemic control. The traditional paradigm of guiding therapeutic decisions primarily by primary tumor response may require reevaluation, underscoring the imperative to reinforce the independent value of metastatic site monitoring in treatment response assessment. Subsequently, regimen-specific analysis yielded critical clinical insights (Table 3). The significant efficacy heterogeneity between primary tumors and lymph node metastases observed in the NAC-alone group ($P = 0.045$) suggests potentially suboptimal penetration of conventional chemotherapeutic agents into nodal sites. In contrast, the absence of a significant difference in the combination immunotherapy group implies that the systemic action of immunotherapy may compensate for this limitation. The underlying mechanism for these differential efficacy may involve divergent clonal evolution. The primary tumor, as the clonal reservoir of origin, typically exhibits a more homogeneous genomic landscape and treatment sensitivity. In contrast, metastatic lesions undergo iterative clonal selection throughout the metastatic cascade, a process that can foster the acquisition of enhanced drug-resistant phenotypes [41, 42]. Furthermore, anatomical and microenvironmental heterogeneities may provide a complementary mechanism. The primary tumor typically harbors a more favorable milieu with efficient drug delivery, while metastatic niches—particularly in lymph nodes—are often characterized by an immunosuppressive environment. This immunosuppression, mediated through mechanisms such as PD-L1 upregulation and regulatory T cell recruitment, may ultimately compromise treatment efficacy at metastatic sites [43–46].

Building on the finding of overall response heterogeneity, this study further explored clinicopathological factors influencing this discordance. As summarized in Table 4, the results identified VH and smoking history as independent predictors of differential efficacy between primary and metastatic lesions. These findings are biologically coherent. The association with VH is mechanistically grounded, as this subtype is robustly linked to aggressive tumor biology and treatment resistance [4, 5, 47]. This linkage may be explained by distinct molecular pathways inherent to specific differentiations. For example, in squamous

differentiation, upregulation of basement membrane genes such as COL17A1 potentially enhances cellular adhesion and invasiveness, while concurrently diminishing chemosensitivity [48]. In sarcomatoid differentiation, studies have reported upregulation of EMT markers (e.g., ZEB1, SNAIL), implicating this pathway in its aggressive and metastatic behavior [49, 50]. Similarly, a plausible biological link exists for smoking history. As an established risk factor, smoking may exacerbate therapeutic resistance by inducing DNA damage to promote tumor heterogeneity and by altering the immune microenvironment, thereby impairing treatment response [2, 3, 51]. These findings suggest that lifestyle interventions may represent a novel strategy to enhance the overall response to neoadjuvant therapy. It is noteworthy that patients receiving neoadjuvant combination therapy showed an improved trend toward consistency of treatment efficacy between primary and metastatic lesions (OR = 0.33, 95% CI 0.12–0.94, P = 0.04, Table 4); however, this effect did not reach statistical significance in the multivariate model (P = 0.66). The inconsistency between univariate and multivariate analyses can be interpreted from several perspectives. Firstly, the limited sample size, especially within the heterogeneous response subgroup (n = 28), compromises statistical power and increases the risk that variable interactions in the multivariable model obscured the independent effect of NIT. Similar limitations have been reported in other studies. For example, research by Samuel Rosner (n = 21) noted that the small sample size impeded the establishment of a significant association between histopathological subgroups and long-term clinical outcomes [52]. Secondly, variability in the specific immunotherapy agents represents a key source of heterogeneity. In this study, the NIT protocols comprised various agents (e.g., tislelizumab, toripalimab). Differences in the immune activation profiles among these regimens likely contributed to an underestimation of the true treatment effect in the multivariable model. Thirdly, the complexity underlying immunotherapy response is a pertinent factor. The response to immunotherapy is dictated by a confluence of factors, including tumor mutational burden, PD-L1 expression levels, and the tumor immune microenvironment, with high PD-L1 expression being strongly associated with greater clinical benefit [53]. This complexity, coupled with our study's lack of analysis stratified by PD-L1 status, could have concealed meaningful efficacy variations across patient subgroups.

This study provides clinically relevant insights by identifying VH as a biomarker for pretreatment stratification and highlighting smoking as a potential modulator of response consistency, thereby underscoring the clinical significance of lifestyle interventions. From a methodological standpoint, the assessment framework integrating T-stage with tumor size measurement yielded a more accurate evaluation of primary tumor response. This was achieved by reclassifying 8.73% (6/72) of primary lesions that would have been misjudged using size criteria alone, thereby eliminating bias from the lack of pathological correlation. Owing to the unique anatomy of bladder cancer as a hollow-organ tumor, where T-stage provides prognostically critical information on the depth of invasion, this integrated approach is particularly valuable for research requiring high-precision assessment of primary tumor response. Furthermore, weighted kappa analysis showed only fair to slight agreement between primary and metastatic tumor responses (Table 6), regardless of the assessment method used. This highlights that, owing to inherent biological heterogeneity, primary tumor response alone cannot fully capture

overall treatment efficacy. Future studies integrating primary and metastatic responses with survival outcomes are needed to establish composite endpoints for neoadjuvant therapy evaluation.

The limitations of this work primarily relate to its retrospective nature, which may introduce selection bias despite the use of multi-center data and statistical corrections; further validation in a prospective setting is needed. A further constraint is the limited sample size in the VH subgroup (n = 21), potentially affecting the stability of the multivariable model. In addition, the absence of histopathological verification for baseline metastatic lesions may lead to potential imprecision in efficacy assessment. To address these limitations, future studies should be designed with larger sample sizes, adopt prospective designs to better control for confounders, and explore novel biomarkers or advanced imaging techniques to enhance the accuracy of therapeutic response assessment.

Conclusion

This study confirms significant spatial heterogeneity in treatment response between primary and metastatic lesions following NAT for BC. VH and smoking history were identified as independent predictors of such response discordance. Although NIT may improve response consistency, further validation is warranted. The innovative assessment system integrating T-stage with tumor size measurement enhanced the precision of efficacy evaluation, leading to the reclassification of 8.33% of cases. Of particular clinical relevance, the persistence of lymph node metastasis in patients achieving pCR of the primary tumor underscores the necessity for independent monitoring of metastatic sites.

Declarations

Ethics approval and consent to participate

This retrospective study was approved by the Ethics Committee of Sun Yat-sen Memorial Hospital (Approval No. SYSKY-2026-193-01) and was conducted in accordance with the Declaration of Helsinki. Informed consent was waived by the Ethics Committee due to the retrospective nature of this study.

Consent for publication

Not applicable.

Competing interests

The authors have no relevant financial or non-financial interests to disclose.

Funding

The authors did not receive support from any organization for the submitted work.

Author Contribution

L.C. and B.B. wrote the main manuscript text and performed data analysis; L.C. also collected and managed the data; J.Z. collected data and edited the manuscript; R.W. and Y.Z. edited the manuscript; Z.L. and J.H. developed the project; J.K. developed the protocol. All authors reviewed and approved the final manuscript.

Acknowledgements

Not applicable.

Availability of data and materials

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Figures

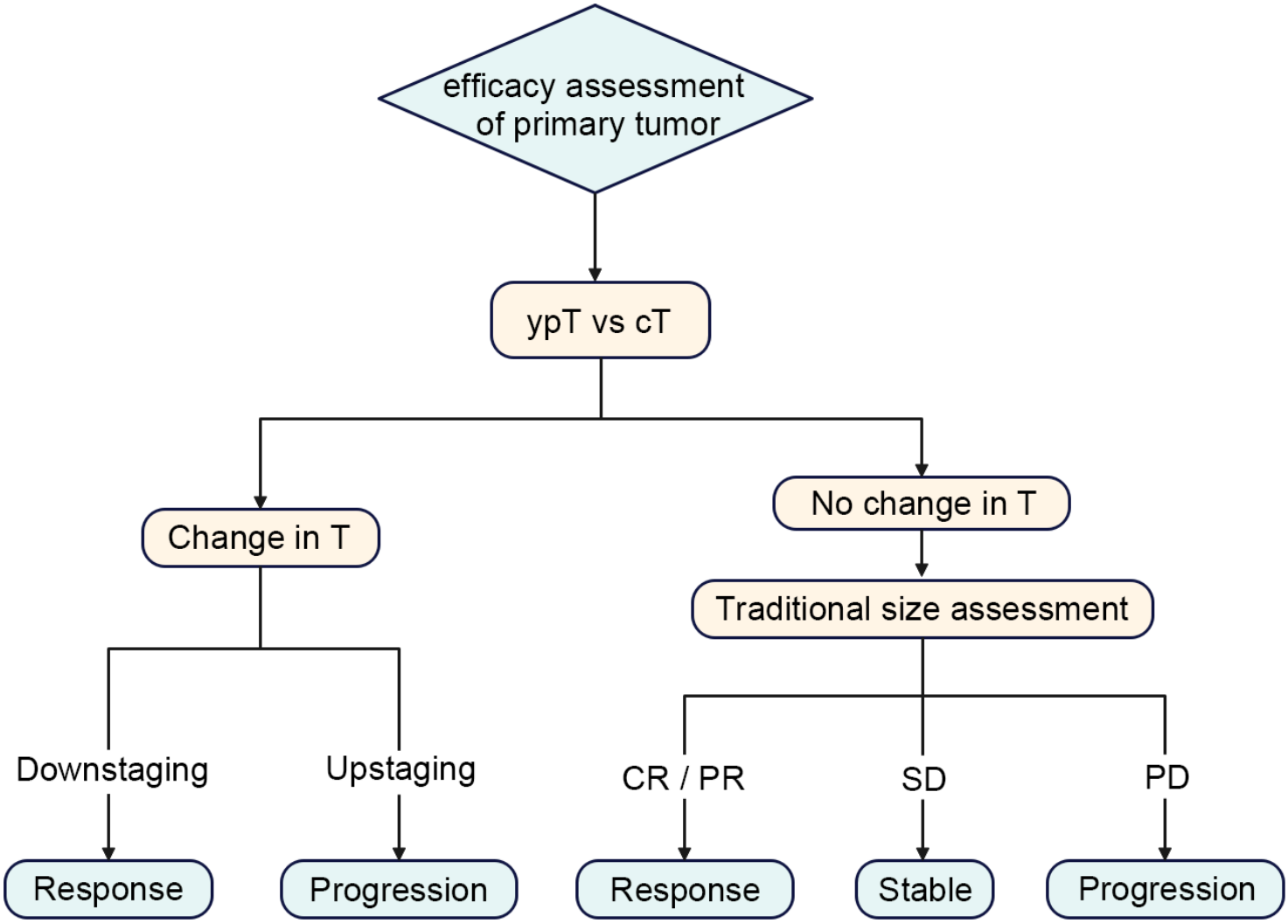


Figure 1

Method for assessing primary tumor response (T-stage combined with size assessment). Note: ypT, post-neoadjuvant therapy pathologic tumor stage; cT, pre-neoadjuvant clinical tumor stage; CR, Complete Response; PR, Partial Response; SD, Stable Disease; PD, Progressive Disease.

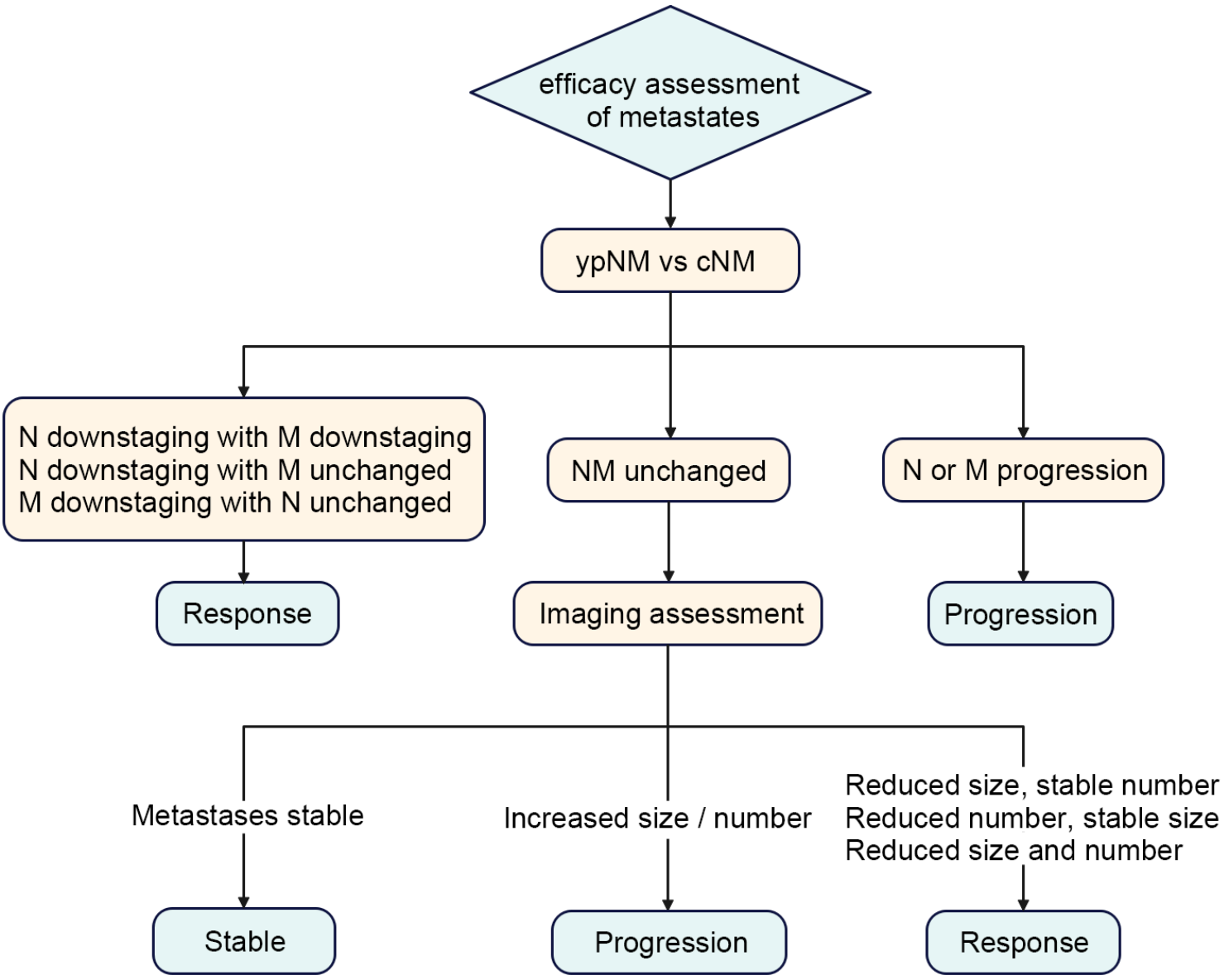


Figure 2

Method for assessing metastases response (NM-stages combined with size assessment). Note: ypNM, postneoadjuvant therapy pathological lymph node and distant metastasis stage; cNM, pre-neoadjuvant clinical lymph node and distant metastasis stage.

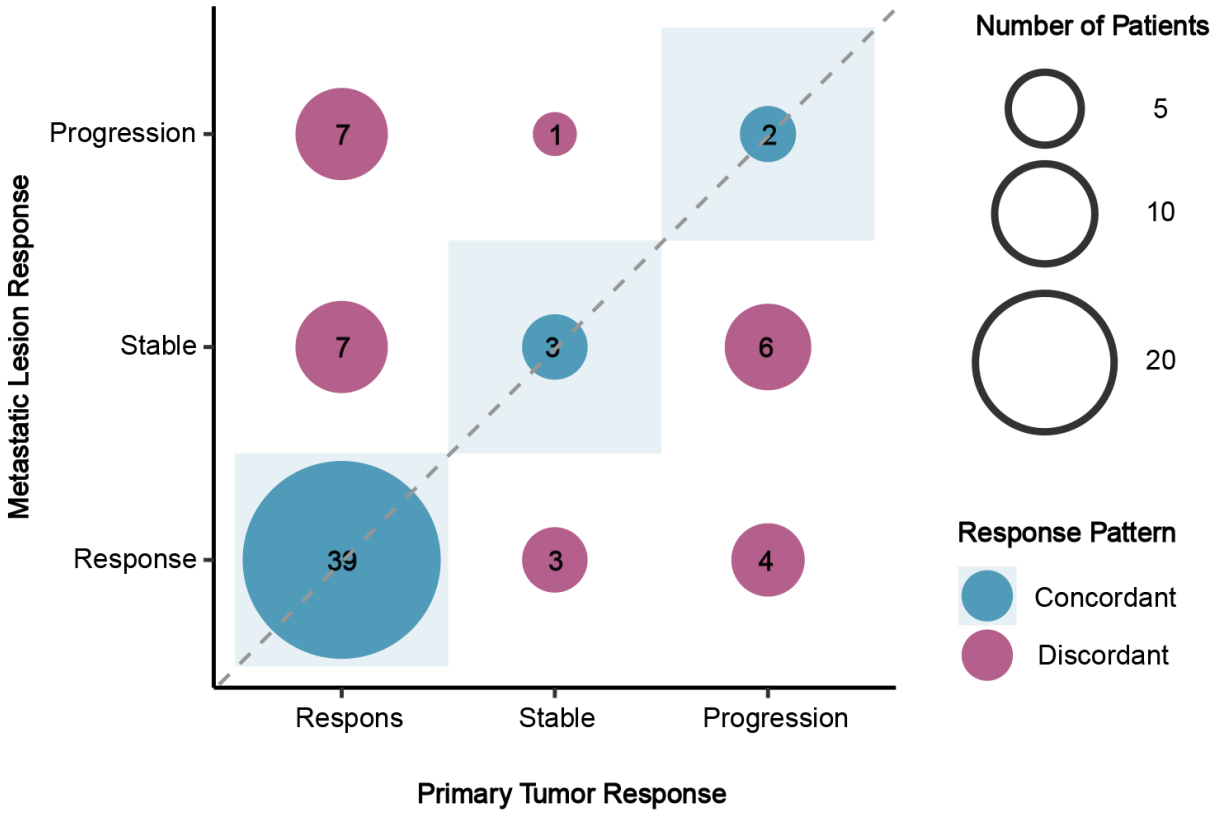


Figure 3

Agreement plot depicting paired treatment responses for primary tumors versus metastatic lesions. Note: Bubble size corresponds to patient number (n values shown). Diagonal bubbles (blue) represent concordant responses; off-diagonal bubbles (red) represent response heterogeneity. Discordance most frequently involved a better response in the primary tumor.

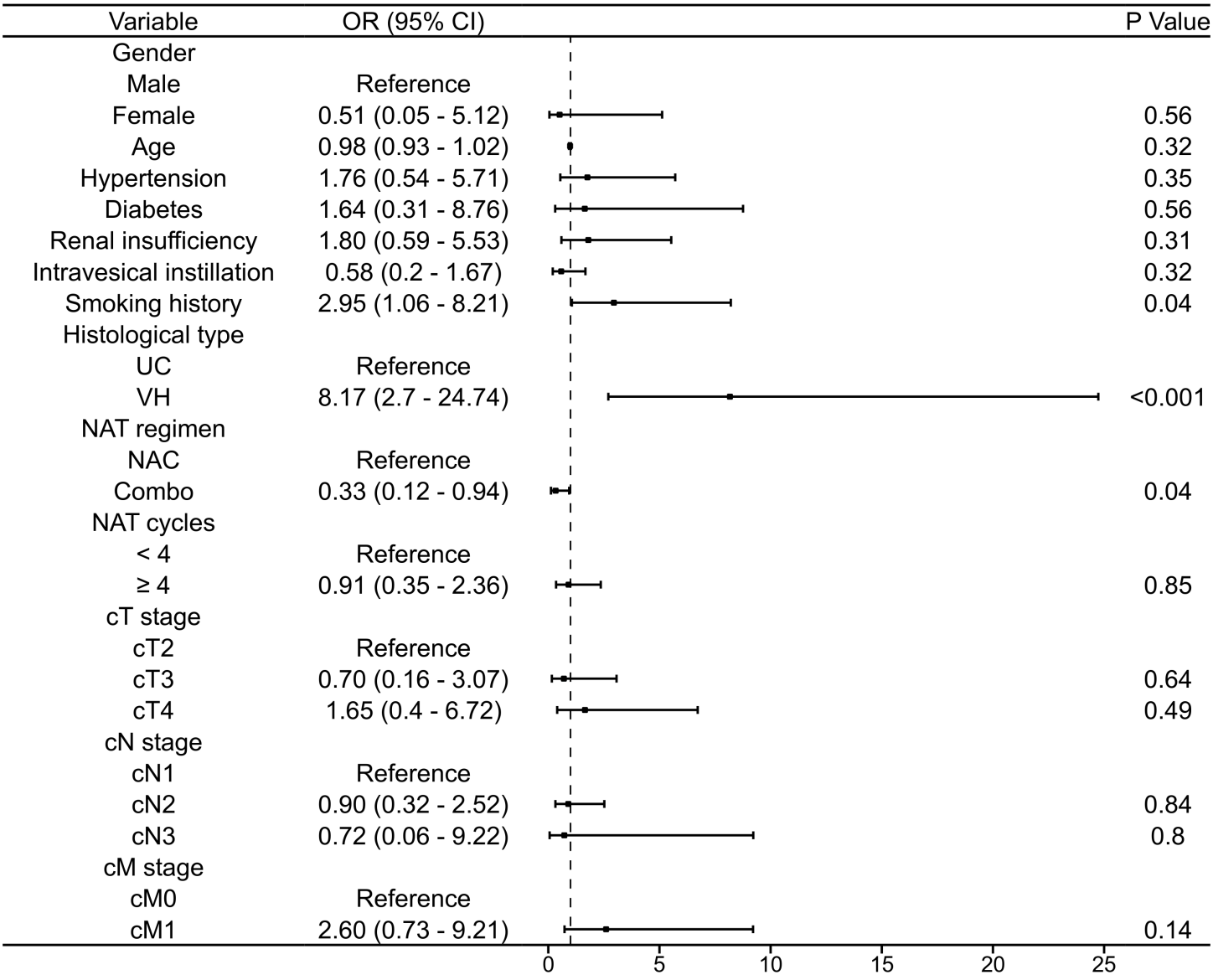


Figure 4

Forest plot of significant predictors from the univariate logistic regression analysis. Note: Age, Age at initial bladder cancer diagnosis; UC, Urothelial Carcinom; VH, Variant Histology; NAT, Neoadjuvant Therapy; NAC, Neoadjuvant Chemotherapy; Combo, Combination Neoadjuvant Therapy; cT, pre-neoadjuvant clinical tumor stage; cN, pre-neoadjuvant clinical lymph node stage; cM, pre-neoadjuvant clinical distant metastasis stage.

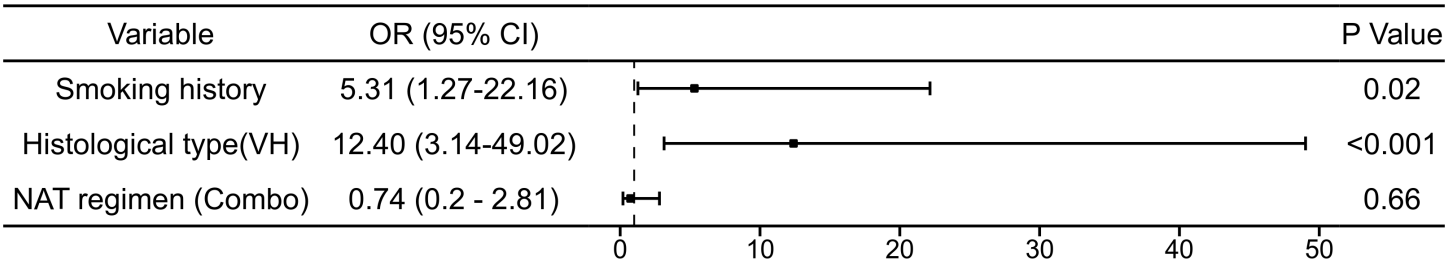


Figure 5

Forest plot of significant predictors from the multivariate logistic regression analysis. Note: Odds ratios (OR) and 95% confidence intervals (CI) are shown. See Table 4 for complete regression results. Note: VH, Variant Histology; Combo, Combination Neoadjuvant Therapy.