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Original Article

HER2 Expression Associated with Clinical Characteristics and Prognosis of Urothelial Carcinoma in a Chinese Population

Li Zhou[1,†], Zhiting Shao[2,†], Yiqiang Liu[3], Xieqiao Yan[1], Juan Li[1], Xiaowen Wu[1], Bixia Tang[1], Siming Li[1], Chuanliang Cui[1,4], Zhihong Chi[4], Lu Si[4], Yan Kong[4], Lili Mao[4], Bin Lian[4], Xuan Wang[4], Xue Bai[4], Jie Dai[4], Jun Guo[1], Xinan Sheng[*,1]

[1]Key Laboratory of Carcinogenesis and Translational Research (Ministry of Education/Beijing), Department of Genitourinary Oncology, Peking University Cancer Hospital & Institute, Beijing, People's Republic of China
[2]Department of Hematology, Oncology, and Tumor Immunology, Charité-Universitätsmedizin Berlin, Berlin, Germany
[3]Key Laboratory of Carcinogenesis and Translational Research (Ministry of Education/Beijing), Department of Pathology, Peking University Cancer Hospital & Institute, Beijing, People's Republic of China
[4]Key Laboratory of Carcinogenesis and Translational Research (Ministry of Education/Beijing), Department of Melanoma and Sarcoma, Peking University Cancer Hospital & Institute, Beijing, People's Republic of China

[*]Corresponding author: Xinan Sheng, MD, Key Laboratory of Carcinogenesis and Translational Research (Ministry of Education/Beijing), Department of Genitourinary Oncology, Peking University Cancer Hospital & Institute, 52 Fucheng Road, Haidian District, Beijing 100142, People's Republic of China. Tel: +86 1088196951; Fax: +86 1088196951. Email: doctor_sheng@126.com
[†]Contributed equally.

Abstract

Background: The frequency of HER2 overexpression in bladder cancer is reported as 9%-61%. HER2 alteration correlates with aggressive disease in bladder cancer. Traditional anti-HER2 targeted therapy has failed to show clinical benefits in patients with advanced urothelial carcinoma .

Methods: The information on pathologically proven patients with urothelial carcinoma with detected HER2 status was collected from the database of Peking University Cancer Hospital. The HER2 expression, as well as its association with clinical characteristics and prognosis, was analyzed.

Results: A total of 284 consecutive patients with urothelial carcinoma were enrolled. HER2 was positive (IHC 2+/3+) in 44% of urothelial carcinoma. HER2 positivity was found more frequent in UCB than in UTUC (51% vs. 38%). Stage, radical surgery, and histological variant were associated with survival ($P < .05$). For metastatic patients, multivariate analysis shows that 3 indicators, including liver metastasis, the number of involved organs, and anemia, are independent risk factors of prognosis. Receiving immunotherapy or disitamab vedotin (DV) treatment is an independent protecting factor. The survival of patients with low HER2 expression was also significantly improved by the treatment of DV ($P < .001$). HER2 expression (IHC 1+, 2+, 3+) was associated with a better prognosis in this population.

Conclusion: DV has improved the survival of patients with urothelial carcinoma in the real world. With the new-generation anti-HER2 ADC treatment, HER2 expression is no longer a poor prognostic factor.

Key words: HER2; urothelial carcinoma; targeted therapy; prognosis; antibody-drug conjugate

Implications for Practice

This study describes the HER2 expression in urothelial carcinoma from a Chinese population and analyzes its association with clinical characteristics and prognosis. The survival of patients with HER2 positive and low expression was significantly improved by disitamab vedotin. HER2 expression was associated with a better prognosis in this population. Therefore, we propose for the first time to classify UC patients as HER2-expressive (IHC 1+, 2+, and 3+) or negative (IHC 0) in clinical practice. The role of FISH in treatment selection is limited.

Introduction

HER2, or ERBB-2, is a member of the human epidermal growth factor receptor family. The activation of HER2 signaling results from ERBB2 amplification or somatic mutations.[1] Heterodimerization of HER family receptors results in the autophosphorylation of the intracellular tyrosine kinase domain. Then, various downstream signaling pathways, including PI3K/AKT/mTOR, Ras/Raf/MAPK, and STAT, are initiated, promoting cell proliferation and leading to tumorigenesis.[2]

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In breast cancer, HER2 overexpression is associated with poor prognosis. The incidence of HER2 overexpression is reported as 9.2%-61.1% in urothelial carcinoma (UC),[3-6] which was mostly evaluated by immunochemistry (IHC) or fluorescent in situ hybridization (FISH). Studies have shown that the HER2-positive status was more frequent in micropapillary carcinomas[7,8] and significantly more frequent in lymph node metastases than in primary tumors.[9] In addition, HER2 amplification is enriched in muscle invasive bladder cancer (MIBC) and metastatic disease compared with non-muscle invasive bladder cancer (NMIBC).[10] Therefore, HER2 alteration might be correlated with aggressive disease in bladder cancer.

Anti-HER2 targeted therapy has achieved great success in breast and gastric cancer[11] and is also continuously explored in bladder cancer. However, there was no obvious benefit in clinical trials of traditional targeted therapy, including trastuzumab, lapatinib, or trastuzumab emtansine, until disitamab vedotin (DV, or RC48-ADC), and the new-generation antibody-drug conjugate.[12-15] Disitamab vedotin demonstrated a promising efficacy[16,17] and has been approved in China for HER2-positive (IHC 2+ or 3+) patients with advanced urothelial carcinoma after the failure of chemotherapy.

Asian populations are characterized by a high prevalence of upper tract urothelial carcinoma (UTUC).[18] There are currently no detailed reports of HER2 expression in Asian populations with urothelial carcinoma. This study aimed to investigate the HER2 expression of UC patients, analyze its association with clinical characteristics and prognosis, and explore the efficacy of DV in the real world.

Materials and Patients

Databases of consecutive patients with urothelial carcinoma were prospectively designed at Peking University Cancer Hospital. The information on pathologically proven urothelial carcinoma patients with detected HER2 status was collected. The HER2 expression, as well as its association with clinical characteristics and prognosis, were analyzed. This study was approved by the Ethics Committee of the Beijing University Cancer Hospital.

Clinicopathological characteristics and survival data were collected including age, gender, smoking history, primary location, surgery, metastatic sites, histological variant, HER2 status, East Operation Cooperative Group performance score (ECOG PS), time from diagnosis to metastasis, treatment regimens, overall survival (OS), hemoglobin (Hb), and lactate dehydrogenase (LDH). Patients without complete information were excluded. Patients were followed up every 3 months for survival outcomes until death.

HER2 expression status of patients treated with disitamab vedotin in this study was evaluated by IHC staining. HER2 IHC 3+ and 2+ were defined as HER2 positive or else as HER2 negative (including 1+ as low expression and 0). The IHC scores were reviewed by 2 experienced pathologists according to the 2018 American Society of Clinical Oncology (ASCO)/College of American Pathologists (CAPs) guidelines for breast cancer.[19] The Ventana anti-HER2/Neu (4B5) rabbit monoclonal antibody and ultra-View Universal DAB Detection Kit (Roche) were used for staining and testing. Some patients were also assessed for HER2 amplification by FISH.

Continuous variables were described as median (range) and compared with the Mann-Whitney *U* test. Categorical variables were presented as frequencies and compared with Chi-square or Fisher's test. OS was analyzed using the Kaplan-Meier method, and prognostic factors were compared with log-rank tests. Variables with a *P*-value < .1 in univariate analyses were incorporated in the multivariate COX proportional hazard model. Statistical analyses were performed using SPSS software (SPSS Inc., Chicago, IL), version 25.0, and GraphPad Prism version 9.

Results

A total of 284 patients with consecutive urothelial carcinoma were enrolled in this study from October 2016 to January 2021 of Peking University Cancer Hospital. Basic demographic characteristics are demonstrated in Table 1. Male patients were predominant, with a proportion of 63%. The median age was 64 (range: 23-86). UTUC comprises approximately half of the population. Almost all patients (97.5%) were classified as high-grade urothelial carcinoma. Seventy patients (27%) were diagnosed as UC with variant histology (VH), namely 10.6% squamous cell carcinoma, 4.6% adenocarcinoma, 4.6% sarcomatoid, and 7.4% others, including micropapillary, plasmacytoid, signet ring cell, clear cell, poorly differentiated, neuroendocrine, and mixed variants. Patients with NMIBC, MIBC, positive lymph nodes, or metastatic disease were 9.5%, 59.5%, 16.2%, or 14.8%, respectively. Seventy percent of the patients underwent radical surgery (including cystectomy or nephroureterectomy, excluding local resection). Twenty-nine patients (10%) received palliative surgery (with a residual lesion or positive surgical margin), and 8 were recorded to have received radiation therapy. Ninety-three percent of the patients developed recurrence or metastasis by the cutoff date of October 15, 2022.

Of the 284 cases, 125 (44%) were assessed as HER2 IHC 2+ or 3+. In 18 patients of IHC 2+ tested by FISH, 3 cases (16.7%) were observed with gene amplification. In addition, 3 of the 5 HER2 IHC 3+ cases (60%) were proven to have gene amplification. Seven of the 8 patients with multiple samples for HER2 testing turned out to have different IHC scores. Intratumoral heterogeneity existed among different areas within the same sample. HER2 positivity was more common in male patients than females (50% vs. 33%). There were 51% of patients with HER2 positivity in bladder cancer and 38% in UTUC (Fig. 1A). No significant differences were found between age, histological variant, or stage at diagnosis (Table 1). Baseline features of HER2 low-expression (IHC 1+) and negative patients were shown in Supplementary Table S1.

With a median follow-up time of 63.5 months (95%CI: 53.0-74.0), the median OS is 46.9 months (95%CI: 39.2-54.6) for this population. Survival of patients with positive lymph nodes and distant metastases was significantly worse than the local disease. Patients who underwent radical surgery had the best survival. Palliative surgery failed to improve clinical benefit compared to those without surgery. The histological variants were associated with a worse prognosis. There is no significant difference of survival in the primary locations. Patients with HER2 expression (IHC 1+, 2+, and 3+) had a better outcome than HER2-negative patients, considering 38% of the metastatic patients receiving disitamab vedotin (Supplementary Fig. 1F).

For 198 patients who underwent radical surgery, 29 cases (14.6%) developed urinary recurrence, and the median

Table 1. Baseline characteristics of patients with urothelial carcinoma.

HER2 IHC expression[a]	Total	Negative (0/1+)	Positive (2+/3+)	*P*
N (%)	284	159 (56.0)	125 (44.0)	
Gender				.005
Male	179 (63.0)	89 (49.7)	90 (50.3)	
Female	105 (37.0)	70 (66.7)	35 (33.3)	
Age, years (median)	64	64	63	.415
Range	23-86	23-86	29-81	
Smoking history				.051
Yes	118 (41.5)	58 (49.2)	60 (50.8)	
No	166 (58.5)	101 (60.8)	65 (39.2)	
Primary site				.001
UTUC	145 (51.1)	90 (62.1)	55 (37.9)	
UCB[b]	135 (47.5)	66 (48.9)	69 (51.1)	
Urethral	4 (1.4)	3 (75.0)	1 (25.0)	
High grade	277 (97.5)	155 (97.5)	122 (97.6)	—
Histology				.267
Pure UC	207 (72.9)	111 (53.6)	96 (46.4)	
With squamous	30 (10.6)	19 (63.3)	11 (36.7)	
With glandular	13 (4.6)	6 (46.2)	7 (53.8)	
With sarcomatoid	13 (4.6)	11 (84.6)	2 (15.4)	
Other variants[c]	21 (7.4)	12 (57.1)	9 (42.9)	
Initial stage				.276
NMIUC[d]	27 (9.5)	11 (40.7)	16 (59.3)	
MIUC[d]	169 (59.5)	94 (55.6)	75 (44.4)	
Lymph nodes positive	46 (16.2)	27 (58.7)	19 (41.3)	
Metastatic	42 (14.8)	27 (64.3)	15 (35.7)	
Surgery				.706
None	57 (20.1)	30 (52.6)	27 (47.4)	
Radical	198 (69.7)	111 (56.1)	87 (43.9)	
Palliative	29 (10.2)	18 (62.1)	11 (37.9)	

[a]18 cases tested with metastatic samples;
[b]Including multifocal lesions;
[c]Including micropapillary, plasmacytoid, signet ring cell, clear cell, poorly differentiated, neuroendocrine, and mixed variants;
[d]Lymph nodes negative.
Abbreviations: IHC, immunohistochemistry; UTUC, upper tract urothelial carcinoma; UCB, urothelial carcinoma of bladder; NMIUC, non-muscle invasive urothelial cancer; MIUC, muscle invasive urothelial cancer.

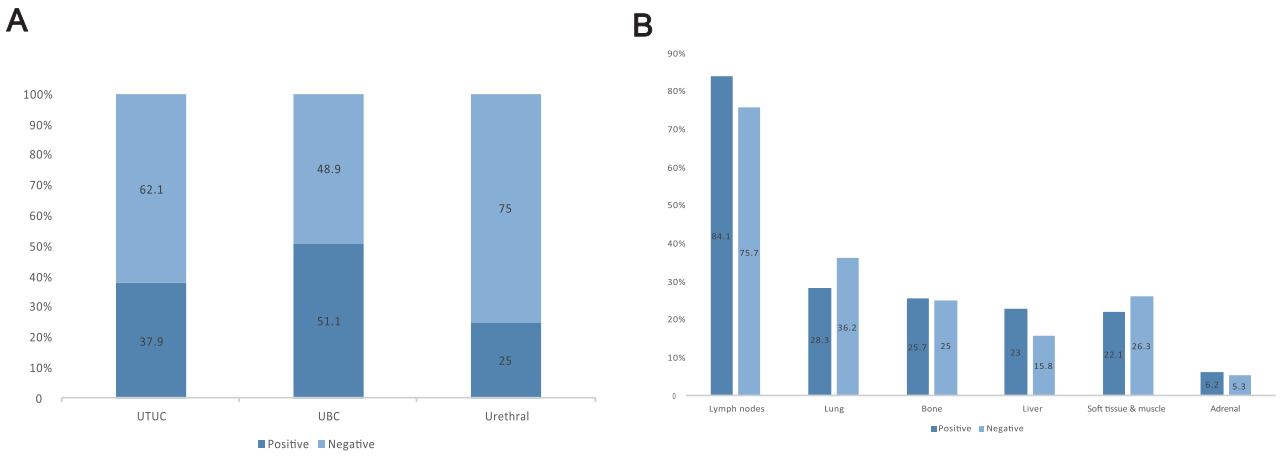

Figure 1. The proportion of HER2 expression in primary and metastatic sites.

recurrent time was 12.8 months (range 1.1-77.9). The median disease-free survival (DFS) was 13.1 months (95%CI: 11.0 -15.2).

As of October 2022, a total of 265 patients developed recurrence or metastases, of which 42.6% were HER2 positive (Table 2). The most common metastatic sites were lymph nodes (79.2%), lung (32.8%), bone (25.3%), soft tissue and muscle (24.5%), liver (18.9%), and other sites, including the adrenal gland, pleura or peritoneum, pancreas, kidney, prostate, penile, stomach, and brain (Fig. 1B). Among these patients, 55.5% had an ECOG PS score of over 1. Anemia and elevated LDH were observed in 10.9% and 30.9% of patients, respectively. There were no significant differences regarding the metastatic sites, number of involved organs, anemia, or LDH level. Baseline features of HER2 low-expression and negative patients after metastases were shown in Supplementary Table S2.

Almost all patients (98%) received chemotherapy in adjuvant, neoadjuvant, or metastatic settings, including cisplatin, carboplatin, gemcitabine, paclitaxel, etc. There were 154 patients (58%) treated with immunotherapy, 25% in the first line, and 75% in the second or subsequent line after chemotherapy. One hundred patients (38%) received disitamab vedotin, of which 31% progressed on immunotherapy, with a median treatment duration of 6.5 months (range 1-15). No patients received enfortumab vedotin or sacituzumab govitecan due to the unavailability of the drug.

With a median follow-up time of 44.1 months (95%CI: 41.0-47.2), the median OS was 27.4 months (95%CI: 23.9-30.9) in the metastatic setting. In univariate analysis, liver metastasis, bone metastasis, the number of involved organs, ECOG PS ≥1, and anemia were significantly related to the prognosis (Fig. 2A-C). Besides, no apparent correlations were observed with lymph node metastasis, lung metastasis, elevated LDH, and metastasis at diagnosis. Survival of patients was significantly improved by immunotherapy compared to the others (Fig. 2D). In addition to HER2-positive patients, patients with low HER2 expression also benefited from the treatment of DV ($P < .001$, Supplementary Fig. S2). The combined ORR for patients with HER2 IHC 1+/2+/3+ treated by DV was 54%. Even without consideration of HER2 status, the survival of the whole population was improved by the treatment of DV (Fig. 2E). The ROC curves for different HER2 positivity definitions demonstrated that IHC 1+, 2+, 3+ have the best predictive power (Supplementary Fig. S3). HER2 expression (IHC 1+, 2+, 3+) was associated with a better prognosis in this population (Fig. 2F). In multivariate analysis, liver metastases, the number of involved metastatic organs, and anemia were determined to be the independent risk factors of prognosis, and receiving immunotherapy or DV treatment was an independent protecting factor (Fig. 3).

Discussion

In this retrospective study, we studied the association of HER2 expression with clinical characteristics and prognosis in urothelial carcinoma patients from Asian populations. The majority of patients incorporated in this study had recurrences or metastases. The incidence of UTUC is much higher in East Asia, such as China.[20,21] The consumption of herb drugs containing aristolochic acid (AA) may contribute to the prevalence of UTUC, with mutation signatures of AA's DNA

Table 2. Baseline characteristics of 265 metastatic patients with urothelial carcinoma.

HER2 IHC expression	Total	Negative (0/1+)	Positive (2+/3+)	*P*
N	265	152 (57.4)	113 (42.6)	
Metastatic at initial				.322
Yes	42	27(64.3)	15 (35.7)	
No	223	125 (56.1)	98 (43.9)	
Metastatic organs				
Lymph nodes	210 (79.2)	115 (75.7)	95 (84.1)	.094
Lung	87 (32.8)	55 (36.2)	32 (28.3)	.177
Bone	67 (25.3)	38 (25.0)	29 (25.7)	.902
Liver	50 (18.9)	24 (15.8)	26 (23.0)	.137
Adrenal gland	15 (5.7)	8 (5.3)	7 (6.2)	.745
SFT and muscle	65 (24.5)	40 (26.3)	25 (22.1)	.432
Others[a]	29 (10.9)	21 (13.8)	8 (7.1)	-
No. of involved organs				.735
1	105 (39.6)	63 (41.4)	42 (37.2)	
2	90 (34.0)	49 (32.2)	41 (36.3)	
≥3	70 (26.4)	40 (26.3)	30 (26.5)	
ECOG PS				.037
0	118 (44.5)	76 (50.0)	42 (37.2)	
1-2	147 (55.5)	76 (50.0)	71 (62.8)	
HGB < 10 g/dL	29 (10.9)	21 (13.8)	8 (7.1)	.082
LDH elevated	82 (30.9)	48 (31.6)	34 (30.1)	.795

SFT, soft tissue; ECOG PS, East Operation Cooperative Group performance score; HGB, hemoglobin; LDH, lactate dehydrogenase.
[a]Including pleura, peritoneum, pancreas, kidney, prostate, penile, stomach, and brain.

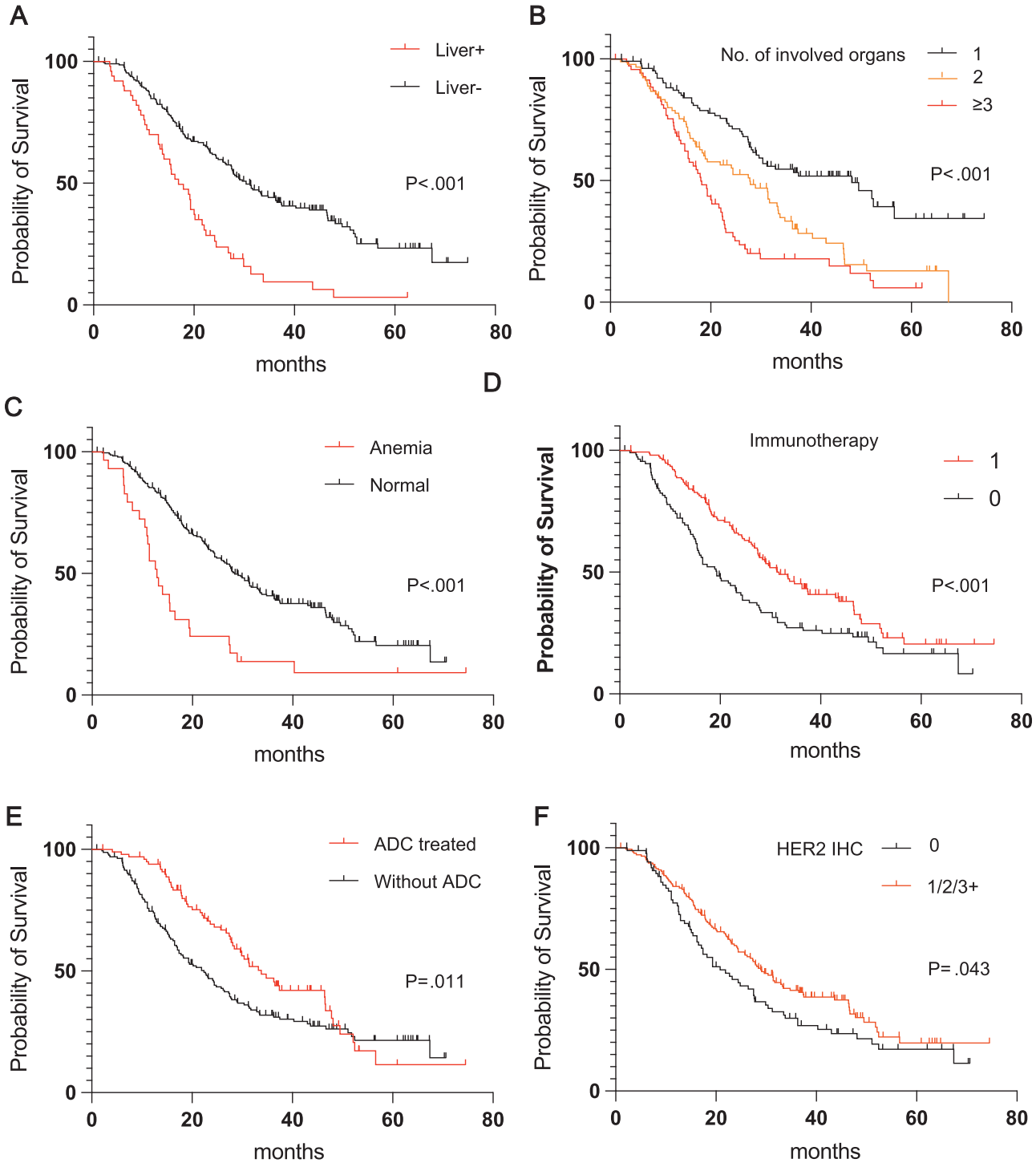

Figure 2. Kaplan-Meier analyses of OS for metastatic patients according to different strata by prognostic factors: (**A**) liver metastasis, (**B**) number of metastatic organs, (**C**) anemia, (**D**) treated by immunotherapy, (E) treated by disitamab vedotin, (F) HER2 expression.

damage footprint.[20-22] We found that HER2 was overexpressed in 44% of urothelial carcinoma. Moreover, disitamab vedotin has improved the survival of the whole population, and HER2 expression was associated with improved overall survival.

The evaluation of HER2 IHC in this study was according to the criteria of breast cancer. However, there was significant inter- and intratumoral heterogeneity in urothelial carcinoma. The HER2 IHC scores of different samples from the same patient were mostly inconsistent. Moreover, HER2 expression was not consistent between the FISH and IHC method, with 60% FISH positive in HER2 IHC 3+ and 16.7% FISH positive in IHC 2+ patients. Patients with HER2 FISH negative but protein expression, such as IHC 1+ or some IHC 2+ cases, may respond to DV. Besides, the efficacy of DV between IHC 2+ FISH+ and FISH- subgroups is not significantly different in the report of RC48-C005 clinical trial.[16] Therefore, the role of FISH in treatment selection was limited in clinical practice.

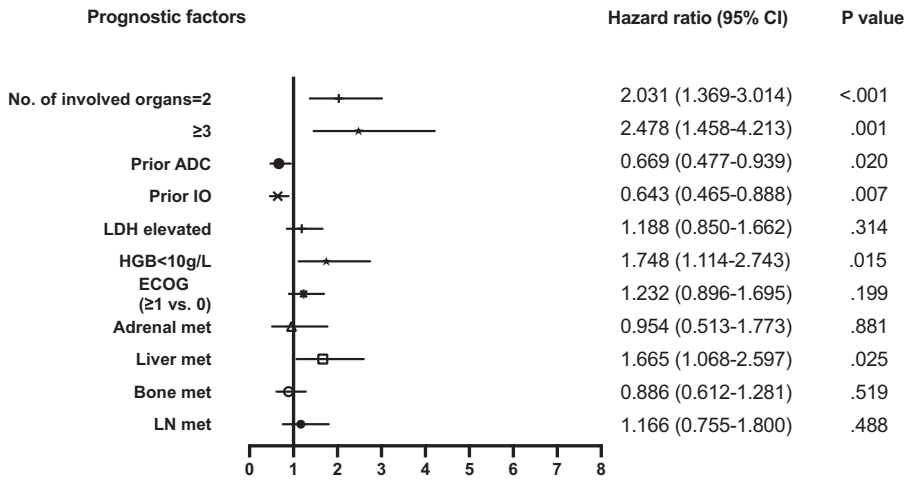

Figure 3. Multivariate analyses of prognostic factors for OS in patients with metastatic urothelial carcinoma.

There were 51% of HER2-positive patients in bladder cancer and 38% in UTUC, which is consistent with previous UTUC and UCB's genomic characteristics.[23,24] Recent molecular studies suggest that UTUC has a different genomic landscape from UCB. For patients with metastatic disease, ERBB2 was found to be more frequently altered in UCB than in UTUC, while FGFR3 and HRAS mutation were less frequently altered.[25,26] In addition, we also found that HER2 positivity was more frequent in male patients than in females (50% vs. 33%). HER2 status was not correlated to histological variants, involved organs, anemia, or elevated LDH in metastatic patients.

As indicated in this study, localized stage, radical surgery, and pure UC histology were associated with better survival. For metastatic patients, liver metastasis, the number of involved metastatic organs, and anemia were the independent risk factors of prognosis. Poor ECOG PS is significantly related to the prognosis in univariate analysis. The prognostic factors identified in this study are consistent with the Bellmunt risk factors.[27]

In terms of anti-HER2 treatment, the previous monoclonal antibody and tyrosine kinase inhibitors have not demonstrated improvement for HER2-positive urothelial carcinoma patients, including trastuzumab, lapatinib, or trastuzumab emtansine. Disitamab vedotin is approved in China for HER2-positive patients with advanced urothelial carcinoma after the failure of chemotherapy, which demonstrated superior efficacy and improved survival. In the analysis of RC48-C005 and C009 studies,[17] the objective response rate (ORR) was 50.5%. The median progression-free survival (PFS) and median overall survival (OS) were 5.9 months and 14.2 months, respectively. A randomized phase III trial of disitamab vedotin in advanced urothelial carcinoma is now recruiting in China (NCT 05302284).

Patients with low HER2 expression can also benefit from the treatment of HER2-directed ADC, such as DS8201 for patients with HER2 low-expressive breast cancer.[28] The experimental results of DS8201 monotherapy in urothelial carcinoma have not been disclosed. The RC48-C011 study tested disitamab vedotin in HER2 low-expressive patients with urothelial carcinoma. The ORR reached 26.3%, and the median PFS and median OS were 5.5 months and 16.4 months.[29] This anti-tumor activity may be related to the heterogeneity of HER2 expression and the bystander antitumor effect. Therefore, we propose to classify UC patients as negative (IHC 0) or expressive (1+, 2+, and 3+) in the future.

Retrospective studies suggested that HER2 expression is associated with the poor prognosis of urothelial carcinoma.[30] In this study, the outcome of patients expressing HER2 is better than those who do not, with 38% of metastatic patients treated by DV. In the metastatic setting, patients who received DV treatment survived longer than the control group. The trailing curve in the control group may be due to the early application and durable effect of immunotherapy. In multivariate analysis, receiving immunotherapy or DV treatment is an independent protecting factor. In this study population, HER2 expression is no longer an adverse risk factor with the treatment of new-generation anti-HER2 ADC.

There are some limits to this study. First, bias exists for the retrospective nature of the study. Secondly, HER2 evaluation in urothelial carcinoma does not rely on a standardized scoring system. The criteria of breast cancer are applied in this study, and further verification will be required in the future. Finally, HER2 expression is currently assessed from the protein level, and the consistency with FISH is not high. The concordance of HER2 overexpression and gene amplification in urothelial carcinoma by FISH or next-generation sequencing (NGS) has yet to be investigated. In the future, HER2 expression may be detected from multiple dimensions, including DNA, RNA, and protein, to enhance our understanding of HER2 expressive urothelial carcinoma and help to choose treatment strategies.

Conclusions

Disitamab vedotin has improved the survival of urothelial carcinoma in the real world, including in HER2 low-expression patients. With the new-generation anti-HER2 ADC treatment, HER2 expression is no longer a poor prognostic factor. It may be more appropriate to classify UC patients as HER2– expressive (IHC 1+, 2+, and 3+) or negative (IHC 0) in the future.

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Conflict of Interest

The authors indicated no financial relationships.

Author Contributions

Conception/design: X.S. Provision of study material or patients: X.S., C.C., Z.C., L.S., J.G., Y.L. Collection and/or assembly of data: X.Y., J.L., X.W., B.T., S.L., Y.K., L.M., B.L., X.W., X.B., J.D. Data analysis and interpretation: L.Z., Z.S. Manuscript writing: L.Z. Final approval of manuscript: All authors.

Data Availability

The datasets analyzed during the current study will be available on reasonable request by contacting Dr. Li Zhou (zhoulilucky@126.com).

Supplementary Material

Supplementary material is available at *The Oncologist* online.

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