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ORIGINAL RESEARCH

Phase II study of a trastuzumab biosimilar in combination with paclitaxel for HER2-positive recurrent or metastatic urothelial carcinoma: KCSG GU18-18

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Available online 27 June 2023

Background: Human epidermal growth factor receptor 2 (HER2) is a widely explored therapeutic target in solid tumors. We evaluated the efficacy and safety of trastuzumab-pkrb, a biosimilar of trastuzumab, in combination with paclitaxel, in HER2-positive recurrent or metastatic urothelial carcinoma (UC).

Patients and methods: We enrolled 27 patients; they were administered a loading dose of 8 mg/kg trastuzumab-pkrb on day 1, followed by 6 mg/kg and 175 mg/m^2 paclitaxel on day 1 every 3 weeks, intravenously. All patients received six cycles of the combination treatment and continued to receive trastuzumab-pkrb maintenance until disease progression, unacceptable toxicity, or for up to 2 years. HER2 positivity (based on immunohistochemistry analysis) was determined according to the 2013 American Society of Clinical Oncology /College of American Pathologists HER2 testing guidelines. The primary endpoint was objective response rate (ORR); the secondary endpoints were overall survival (OS), progression-free survival (PFS), and safety.

Results: Twenty-six patients were evaluated via primary endpoint analysis. The ORR was 48.1% (1 complete and 12 partial responses) and the duration of response was 6.9 months [95% confidence interval (CI) 4.4-9.3 months]. With a median follow-up of 10.5 months, the median PFS and OS were 8.4 months (95% CI 6.2-8.8 months) and 13.5 months (95% CI 9.8 months-not reached), respectively. The most common treatment-related adverse event (TRAE) of any grade was peripheral neuropathy (88.9%). The most common grade 3/4 TRAEs were neutropenia (25.9%), thrombocytopenia (7.4%), and anemia (7.4%).

Conclusions: Trastuzumab-pkrb plus paclitaxel demonstrates promising efficacy with manageable toxicity profiles in patients with HER2-positive recurrent or metastatic UC.

Key words: HER2, paclitaxel, trastuzumab-pkrb, targeted therapy, urothelial carcinoma

INTRODUCTION

Platinum-based combination therapy is the standard first-line treatment for metastatic urothelial carcinoma (UC).[1] Although the treatment outcomes of patients with metastatic UC who progress after first-line treatment have improved, a significant number of patients still show poor prognosis.[2,3]

Recent advances in molecular profiling techniques have facilitated the application of targeted therapies for the management of various tumors, including UC. Human epidermal growth factor receptor 2 (HER2), encoded by the *ERBB2* gene, is one of the most extensively studied therapeutic targets in solid tumors. HER2-driven tumorigenesis can occur via multiple mechanisms, including *ERBB2* amplification, *ERBB2* mutation, and HER2 protein overexpression. A comprehensive analysis of molecular alterations in 131 muscle-invasive, high-grade urothelial bladder cancers was carried out in The Cancer Genome Atlas project, which led to the identification of 32 recurrent mutations.[4] Of the genomic alterations identified, *ERBB2* mutation or amplification, which could offer potential targets for HER2-directed therapy, was found in 9% of the UC cases. In another genomic profiling study of 295 cases of advanced UC of the bladder, *ERBB2* genomic alteration was one of the most common

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events (17%).[5] The incidence of HER2 overexpression ranges from 0% to 25% in patients with UC;[6] however, the prognostic value of HER2 in UC is unknown.

Clinical trials of HER2-targeting agents have been conducted in patients with advanced UC; however, the results are controversial. Trastuzumab, a monoclonal antibody against HER2, resulted in an objective response rate (ORR) of 70% with a median time to progression of 9.3 months in advanced HER2-positive UC when used as first-line therapy in combination with paclitaxel, carboplatin, and gemcitabine.[7] In contrast, addition of trastuzumab to gemcitabine plus platinum did not lead to significant clinical benefit in terms of both ORR and survival in patients with advanced UC overexpressing HER2.[8] Addition of maintenance lapatinib, an HER1 and HER2 tyrosine kinase inhibitor, to the standard of care did not yield a favorable outcome after first-line chemotherapy in patients with HER2-positive metastatic UC.[9] However, dual blockage with trastuzumab plus pertuzumab showed promising results in advanced bladder cancer with HER2 amplification/overexpression.[10]

As paclitaxel showed substantial activity in advanced UC,[11-13] we designed a phase II trial of trastuzumab-pkrb, a biosimilar of trastuzumab,[14] plus paclitaxel. Herein, we evaluated the efficacy and safety of trastuzumab-pkrb in combination with paclitaxel in HER2-positive recurrent or metastatic UC.

PATIENTS AND METHODS

Patients

Patients with pathologically confirmed recurrent/metastatic UC were eligible to participate in the study. The inclusion criteria were as follows: age $\geq$20 years; an Eastern Cooperative Oncology Group performance status of 0 or 1; at least one platinum-based chemotherapy for recurrent or metastatic UC; one or more measurable lesions; tumors with HER2 immunohistochemistry (IHC) 3+ according to the 2013 American Society of Clinical Oncology (ASCO)/College of American Pathologists (CAP) HER2 testing guidelines;[15] adequate organ function, including bone marrow, liver, and kidney; and left ventricular ejection fraction >50%. Patients were excluded if they had received prior treatment(s) with HER2-targeting agents or paclitaxel or had experienced myocardial infarction or clinically significant heart disease within 6 months before enrollment. HER2 IHC was carried out using either archival tumor tissue or baseline re-biopsy. All patients provided written informed consent before participation, and all procedures associated with this study were conducted in accordance with Good Clinical Practice and the Declaration of Helsinki. The study was reviewed and approved by the research board of each participating institution and is registered at ClinicalTrials.gov (NCT03698383).

Study design and treatments

The study was a phase II, multicenter, open-label, single-arm study of a trastuzumab biosimilar in combination with paclitaxel in patients with recurrent or metastatic UC who had not responded to or had progressed after first-line platinum-based chemotherapy. The patients received a loading dose of 8 mg/kg of trastuzumab-pkrb (HERZUMA®) on day 1, followed by 6 mg/kg intravenously on day 1 every 3 weeks. Paclitaxel was administered on day 1 every 3 weeks at a dose of 175 mg/m^2. All patients received six cycles of the combination treatment and continued to receive trastuzumab-pkrb monotherapy after the six cycles until disease progression, unacceptable toxicity, or up to 2 years. Tumor responses were evaluated according to RECIST version 1.1. Radiologic assessments using chest and abdominopelvic computed tomography were carried out at baseline and every three cycles thereafter. Adverse events were assessed based on the National Cancer Institute Common Terminology Criteria for Adverse Events (NCI-CTCAE) version 4.03. Echocardiography was carried out at baseline and every four cycles thereafter. The intention-to-treat (ITT) population included all patients who received at least one cycle of trastuzumab-pkrb and paclitaxel treatment.

Study endpoints and statistical analysis

The primary endpoint was the ORR to the trastuzumab-pkrb and paclitaxel combination treatment in the ITT population. The ORR was defined as the proportion of patients with either complete response (CR) or partial response (PR). The secondary endpoints included overall survival (OS), progression-free survival (PFS), and safety.

A null hypothesis of 30% ORR and an alternative hypothesis of 55% ORR were established. To prove this hypothesis, a sample size of 24 patients was determined using the one-arm binomial method with a one-sided type I error of 5% and power of 80%. Considering a dropout rate of 10%, 27 patients for inclusion were calculated. All statistical analyses were carried out using SAS® (version 9.4 or higher, SAS Institute Inc., Cary, NC), and all data were summarized using descriptive statistics.

Targeted next-generation sequencing

Formalin-fixed paraffin-embedded tumor samples or blood samples, if tumor tissue was not available, were collected before the initiation of treatment. Targeted next-generation sequencing was carried out using the K-MASTER cancer panel v1.1 and Axen Cancer Panel. Detailed methods for targeted sequencing and variant annotation were the same as those described previously.[16]

HER2 immunohistochemistry analysis using an artificial intelligence-powered HER2 analyzer

An artificial intelligence (AI)-powered HER2 analyzer, Lunit SCOPE HER2, was developed; it comprised an area of 1.04 $\times$ 1010 μm^2 and 7.31 $\times$ 10^5 tumor cells from 1133 HER2 IHC-stained whole-slide images of breast cancer that were annotated by 113 board-certified pathologists, similar to that for previous models developed by Lunit (Seoul, Republic of Korea).[17,18] Briefly, the AI model was developed based on a semantic segmentation algorithm, which consists of two

atrous spatial pyramid pooling blocks for tissue-level and tumor cell-level classifications. It classifies tumor cells according to HER2 staining intensity (H0, H1, H2, or H3) and quantifies the cells in each staining category. In addition, it calculates the AI HER2 IHC categories in accordance with the latest ASCO/CAP HER2 IHC evaluation algorithm.[15]

RESULTS

Patient characteristics

Between April 2019 and December 2020, 27 patients with HER2-positive recurrent/metastatic UC were enrolled (Supplementary Figure S1, available at https://doi.org/10.1016/j.esmoop.2023.101588). The baseline patient characteristics are listed in Table 1. The median patient age was 66 years (range 44-85 years). The primary tumor sites were the bladder (59.3%), ureter (22.2%), renal pelvis (14.8%), and urethra (7.4%). The most prevalent metastatic site was the lymph nodes (81%), followed by the lungs (44%), liver (22%), and bones (22%). All patients received prior lines of gemcitabine plus cisplatin or carboplatin, and 74% of patients received immune checkpoint inhibitors.

Treatment response and survival outcome

Of the 27 patients who had received trastuzumab-pkrb and paclitaxel treatment, 26 were evaluated for treatment response. The ORR was 48.1%, with CR in 1 patient (3.7%) and PR in 12 patients (44.4%). Stable disease and progressive disease (PD) were observed in 10 (37.0%) and 3 (11.1%) patients, respectively, resulting in a disease control rate of 85.1%. A reduction in the sum of the target lesion sizes is depicted in a waterfall plot (Figure 1A). All patients with CR and PR progressed, with one patient still undergoing treatment beyond progression (Figure 1B). Duration of response was 6.9 months [95% confidence interval (CI) 4.4-9.3 months].

Table 1. Baseline characteristics of the patients

Characteristics (n = 27)		No. of patients (%)
Median age, years (range)		66 (44-85)
Sex	Male	22 (81.5)
	Female	5 (18.5)
ECOG performance status	0	2 (7.4)
	1	25 (92.6)
Site of primary tumor	Bladder	16 (59.3)
	Ureter	6 (22.2)
	Renal pelvis	4 (14.8)
	Urethra	2 (7.4)
Metastasis sites	Lymph nodes	22 (81.5)
	Lung	12 (44.4)
	Liver	6 (22.2)
	Bone	6 (22.2)
Lines of prior palliative systemic therapies	1	6 (22.2)
	2	18 (66.7)
	3	3 (11.1)
Prior therapies	Gemcitabine plus cisplatin or carboplatin	27 (100)
	Immune checkpoint inhibitor	20 (74.1)

ECOG, Eastern Cooperative Oncology Group.

As of 31 October 2021, the median follow-up time in this study was 10.5 months (range 1.0-6.8 months). The Kaplan–Meier curves for PFS and OS are shown in Figure 2. The median PFS and OS were 8.4 (95% CI 6.2-8.8 months) and 13.5 (95% CI 9.8 months-not reached) months, respectively.

Safety

Safety was assessed for all 27 patients who had received at least one cycle of the study treatment. Table 2 shows the adverse events associated with trastuzumab-pkrb plus paclitaxel therapy in the safety population. Of the 27 patients, 26 (96.3%) experienced at least one treatment-related adverse event (TRAE) during therapy. The most common TRAE of any grade was peripheral neuropathy (88.9%). Grade $\geq$3 neutropenia occurred in seven patients (25.9%), whereas grade $\geq$3 febrile neutropenia was observed in two patients (7.4%). Grade $\geq$3 thrombocytopenia and anemia developed in two patients (7.4%). All other toxicities were of grades 1 or 2. One patient died of sepsis. The incidences of permanent discontinuation and dose interruption were 3.7% and 25.9%, respectively.

Genomic profiling by targeted sequencing and AI-powered HER2 analysis

Target sequencing was carried out in 23 patients using tumor tissues, and blood-based target sequencing was carried out in 3 patients whose tumor tissues were not available (Figure 3). The most common genetic mutation was in *TP53* (63%). No *FGFR2/3* fusions or *FGFR3* mutations sensitive to erdafitinib were observed. *ERBB2* amplification was observed in five patients (19.2%), and *ERBB2* and *CDK2* co-amplification was observed in two patients (7.7%). Among patients with *ERBB2* amplification, as the best response, CR and PR (one in each) were observed, but PD was not observed. No significant difference in PFS or OS was observed between patients with and without *ERBB2* amplification.

AI-powered HER2 analysis was carried out in 24 patients. Among all samples classified as HER2 IHC 3+ by a local pathologist, 33.3% (8/24) and 54.2% (13/24) were classified as HER2 3+ and HER2 3+ or 2+, respectively, by AI analysis. A representative patient sample determined to be HER2 3+ by AI analysis is shown in Supplementary Figure S2, available at https://doi.org/10.1016/j.esmoop.2023.101588. When the cut-off of the proportion of HER2 3+ tumor cells was set to 10%, 25%, and 50%, the ORRs were 62.5%, 75.0%, and 100%, respectively (Supplementary Table S1, available at https://doi.org/10.1016/j.esmoop.2023.101588).

DISCUSSION

Herein, we found that trastuzumab-pkrb plus paclitaxel has promising efficacy, with an ORR of 48.1% and PFS of 8.4 months in patients with HER2 overexpressing UC who failed platinum-based chemotherapy. Toxicities were

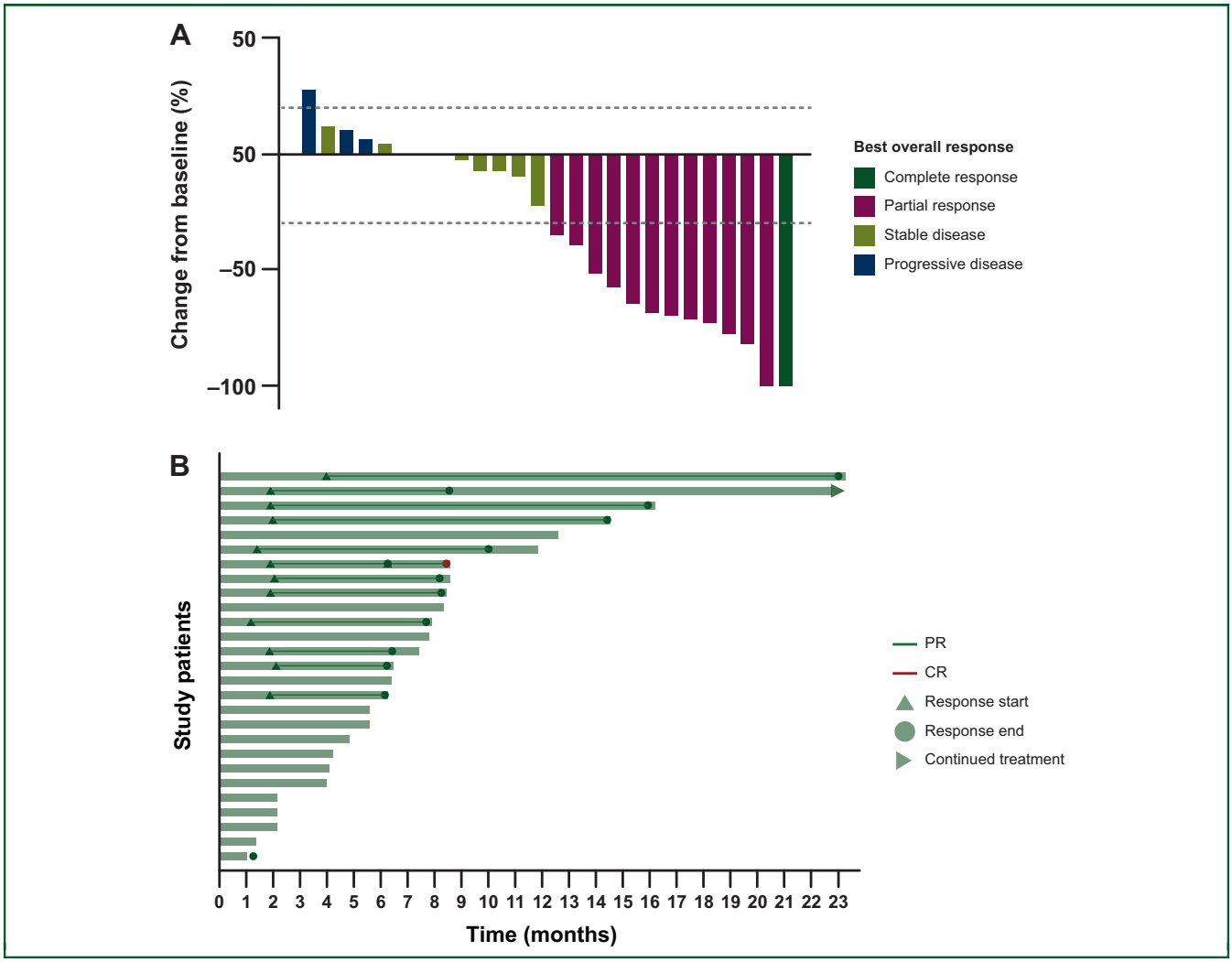

Figure 1. Response and treatment duration. Waterfall plot of maximum percent changes in tumor size of target lesions from baseline (A). Response to trastuzumab-pkrb in combination with paclitaxel and treatment duration (B).
CR, complete response; PR, partial response.

manageable and generally well tolerated, as reported for breast cancer.[19]

Given the high prevalence of HER2 overexpression and/or amplification in advanced UC, HER2-targeted therapy appears to be a reasonable option in this patient population. However, to date, there is little evidence supporting this rationale. Unlike the appealing data from initial studies,[7,20] the addition of trastuzumab to cytotoxic chemotherapy failed to improve survival over that achieved with chemotherapy alone in a randomized phase II trial.[8] Despite the disappointing results from trastuzumab with cytotoxic chemotherapy, promising results were obtained from dual HER2 blockade and HER2-targeted antibody-drug conjugate (ADC) agents. In the MyPathway study, 33% (3/9) of patients with HER2-positive metastatic UC responded to trastuzumab and pertuzumab.[10] A phase II study of disitamab-vedotin, a humanized anti-HER2 ADC, showed an ORR of 51.2% and favorable survival outcomes (median PFS and OS of 6.9 and 13.9 months, respectively) in locally advanced or metastatic UC.[21] In another recent phase Ib study, the combination of anti-HER2 ADC trastuzumab deruxtecan and nivolumab showed an ORR of 36.7% and a median PFS of 6.9 months in patients with advanced UC expressing HER2.[22]

Although previous phase II and III studies have shown conflicting data, considering the results of previous studies reporting tumor responses to trastuzumab monotherapy or dual HER2 blockade treatments in metastatic UC, it is clear that some patients with UC benefit from HER2-directed therapy.[10,20] The inconsistent results among studies might be explained by non-standardized criteria during HER2 evaluation, such as the use of different techniques, interpretations, or cut-off values.

According to a recent study by Moktefi et al., HER2 overexpression and amplification were found in approximately 10% and 8% of metastatic UC cases, respectively, as classified using the 2013 ASCO/CAP guidelines for breast cancer.[6] The frequency of *ERBB2* amplification was in general agreement with that reported in other recent studies.[4,5,23-26] However, the reported frequency of HER2 overexpression covers a wide range.[6] In this study, we defined HER2 positivity as IHC 3+ using the 2013 ASCO/CAP guidelines for breast cancer, and this was not confirmed by FISH. Considering the false-positive rate of the IHC 3+ score

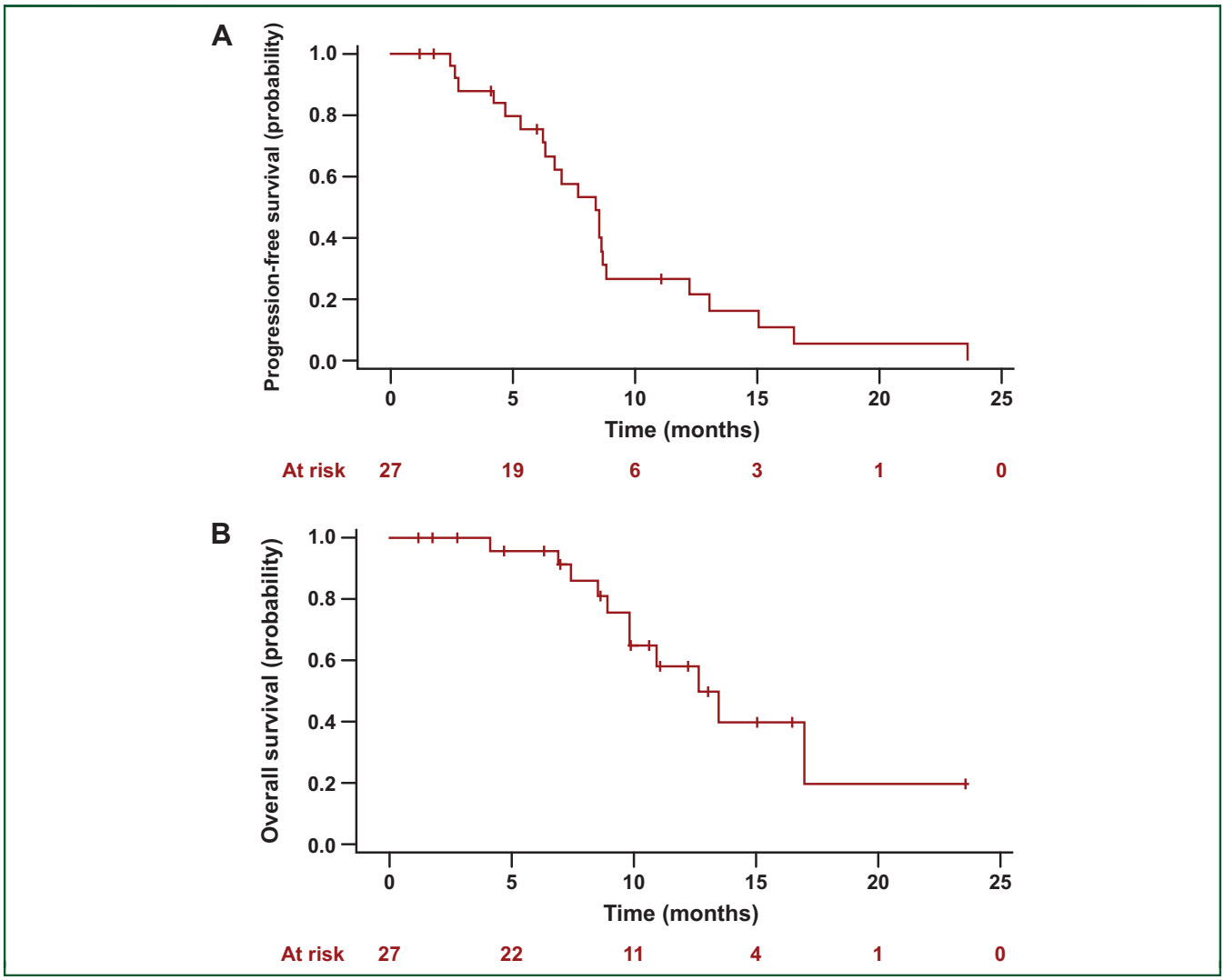

Figure 2. Kaplan–Meier survival curves. Progression-free survival (A) and overall survival (B) in the intention-to-treat population.

for UC as reported in previous studies,[6,27-29] all our IHC 3+ specimens might not have been indicative of HER2 positivity in the study cohort. In addition, only five patients had *ERBB2* amplification, and their treatment outcomes did not differ from those of the patients without *ERBB2* amplification. Although *ERBB2* amplification is generally correlated with protein overexpression, it is not always associated with HER2 overexpression, and the mechanisms by which HER2 overexpression occurs without gene amplification are also known.[6,30] This suggests that *ERBB2*

Table 2. Treatment-related adverse events (n = 27)

No. of patients (%)	Grade 1	Grade 2	Grade 3	Grade 4	Total
Peripheral neuropathy	16 (59.3)	8 (29.6)	0	0	24 (88.9)
Neutropenia	0	4 (14.8)	4 (14.8)	3 (11.1)	11 (40.7)
Myalgia	3 (11.1)	3 (11.1)	0	0	6 (22.2)
Anemia	0	2 (7.4)	2 (7.4)	0	4 (14.8)
Thrombocytopenia	0	2 (7.4)	2 (7.4)	0	4 (14.8)
Chills	3 (11.1)	1 (3.7)	0	0	4 (14.8)
Fever	3 (11.1)	0	0	0	3 (11.1)
Fatigue	1 (3.7)	1 (3.7)	1 (3.7)	0	3 (11.1)
Diarrhea	1 (3.7)	2 (7.4)	0	0	3 (11.1)
Rash	2 (7.4)	0	0	0	2 (7.4)
Febrile neutropenia	0	0	1 (3.7)	1 (3.7)	2 (7.4)
Nausea	2 (7.4)	0	0	0	2 (7.4)
Pneumonitis	1 (3.7)	1 (3.7)	0	0	2 (7.4)
Sepsis	0	0	1 (3.7)	0	2 (7.4)[a]

[a]One patient died of sepsis (grade 5).

Figure 3. Integrated clinical and genomic profiling data. Mutational profiles of 26 patients in this trial were subject to targeted next-generation sequencing. Each column represents one patient.

amplification alone is not a satisfactory predictive marker of HER2-directed therapy, which provides a rationale for IHC for HER2 overexpression as a screening tool for HER2-directed therapy in UC.

Nonetheless, there are also limitations to using IHC for HER2 overexpression as an eligibility criterion. Firstly, the intratumoral heterogeneity of UC might contribute to the inaccurate assessment of HER2 positivity.[6,25,31] A recent study assessing HER2 expression in primary UC and paired metastatic tumors showed that 44% of HER2-positive primary tumors had HER2 intratumoral heterogeneity, and 55% of paired metastatic tumors lost HER2 overexpression.[31] Secondly, interobserver variability in the interpretation of HER2 IHC expression exists among pathologists. In an attempt to reduce such variability, AI analysis has been used as an assistive tool. A recent study demonstrated that an AI-powered HER2 analyzer can significantly improve the concordance of HER2 evaluations by pathologists in breast cancer.[32] In our study, the tumor slides from 24 patients were evaluated using an AI-powered HER2 analyzer. However, all samples were classified as HER2 3+ by a local pathologist but only one-third were labeled as HER2 3+ using the AI model. The ORR of HER2 3+ based on the AI model was 62.5%, with a higher response rate being observed with a higher cut-off of HER2 3+ tumor cells. Therefore, reducing the interobserver variability in the interpretation of HER2 IHC expression would be one way to enrich patients with UC eligible for HER2-targeted therapy.

This study has several limitations. Firstly, this was not a randomized trial. Secondly, the ORR was 48.1% (<55%); thus, the primary endpoint was not attained. Although a direct comparison of studies is difficult to carry out, the ORR of trastuzumab-pkrb plus paclitaxel in our study was higher than that reported in previous studies of paclitaxel monotherapy in patients with advanced UC.[11-13] Our findings suggest that HER2-directed therapy still has potential benefits when HER2 positivity is confined to IHC 3+. AI-based HER2 image analysis could be an enrichment strategy and assistant tool for HER2 heterogeneity. Lastly, the OS observed in this study was longer than the data from

studies of paclitaxel monotherapy; however, careful interpretation is required due to the potential impact of subsequent treatment on OS.

In conclusion, trastuzumab-pkrb plus paclitaxel showed promising outcomes with manageable toxicity profiles in patients with recurrent or metastatic HER2-positive UC who had previously received platinum-based chemotherapy. Further investigation through better patient selection and randomized trials is required to confirm the role of HER2-directed therapy in HER2-positive UC.

ACKNOWLEDGEMENTS

We thank all the participating patients and their families as well as the research nurses and study coordinators. This work was supported by Celltrion (who provided the trastuzumab-pkrb supply) and Samyang Holdings (who provided the paclitaxel supply). This work was also supported by the Korean Cancer Study Group. The funder or drug supplier had no role in the study design, data collection, analyses, and interpretation of data, in the writing of the report, or in the decision to submit the article for publication. We also thank Sung Soo Kim, who carried out the statistical analyses.

FUNDING

This work was supported by the Korea Health Technology R&D Project through the Korea Health Industry Development Institute (KHIDI), funded by the Ministry of Health & Welfare, Republic of Korea [grant number HI17C2206] and by the National R&D Program for Cancer Control through the National Cancer Center (NCC) funded by the Ministry of Health & Welfare, Republic of Korea [grant number HA22C0012].

DISCLOSURE

BK received research funding from MSD, AstraZeneca, and Ono Pharmaceutical Co., Ltd., and has served as an advisor for Handok, NeoImmuneTec, Trialinformatics, and ImmuneOncia outside of the current work. CYO, SK, and HS are employees of Lunit, and report support from Lunit during the conduct of this study, as well as other support from Lunit outside of the submitted work. All other authors have declared no conflicts of interest.

DATA SHARING

The data generated in this study are available from the corresponding author upon reasonable request.

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