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ORIGINAL RESEARCH

Safety and efficacy of trastuzumab biosimilar plus irinotecan or gemcitabine in patients with previously treated HER2 (*ERBB2*)-positive non-breast/non-gastric solid tumors: a phase II basket trial with circulating tumor DNA analysis

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Background: Human epidermal growth factor receptor 2 (HER2) (*ERBB2*)-directed agents are standard treatments for patients with HER2-positive breast and gastric cancer. Herein, we report the results of an open-label, single-center, phase II basket trial to investigate the efficacy and safety of trastuzumab biosimilar (Samfenet®) plus treatment of physician's choice for patients with previously treated HER2-positive advanced solid tumors, along with biomarker analysis employing circulating tumor DNA (ctDNA) sequencing.

Methods: Patients with HER2-positive unresectable or metastatic non-breast, non-gastric solid tumors who failed at least one prior treatment were included in this study conducted at Asan Medical Center, Seoul, Korea. Patients received trastuzumab combined with irinotecan or gemcitabine at the treating physicians' discretion. The primary endpoint was the objective response rate as per RECIST version 1.1. Plasma samples were collected at baseline and at the time of disease progression for ctDNA analysis.

Results: Twenty-three patients were screened from 31 December 2019 to 17 September 2021, and 20 were enrolled in this study. Their median age was 64 years (30-84 years), and 13 patients (65.0%) were male. The most common primary tumor was hepatobiliary cancer (seven patients, 35.0%), followed by colorectal cancer (six patients, 30.0%). Among 18 patients with an available response evaluation, the objective response rate was 11.1% (95% confidence interval 3.1% to 32.8%). *ERBB2* amplification was detected from ctDNA analysis of baseline plasma samples in 85% of patients ($n = 17$), and the *ERBB2* copy number from ctDNA analysis showed a significant correlation with the results from tissue sequencing. Among 16 patients with post-progression ctDNA analysis, 7 (43.8%) developed new alterations. None of the patients discontinued the study due to adverse events.

Conclusions: Trastuzumab plus irinotecan or gemcitabine was safe and feasible for patients with previously treated HER2-positive advanced solid tumors with modest efficacy outcomes, and ctDNA analysis was useful for detecting HER2 amplification.

Key words: HER2-directed agents, basket trial, circulating tumor DNA, biosimilar

INTRODUCTION

Amplification of the human epidermal growth factor receptor 2 (HER2) (*ERBB2*) gene is a common oncogenic driver in solid tumors, and HER2 positivity, defined as overexpression of ERBB2 protein or amplification of the *ERBB2* gene, is found in various types of cancer.[1] In HER2-positive breast cancer or gastric adenocarcinoma, HER2-directed agents have proven efficacy in large randomized, controlled trials and are currently part of standard treatments.[2-5] Several studies have shown the substantial efficacy of HER2-directed agents among patients with

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HER2-positive solid tumors in addition to breast and gastric cancer.[1] The phase IIa MyPathway basket trial of treatment-refractory solid tumors found that a dual HER2-directed regimen consisting of pertuzumab and trastuzumab had a considerable response against patients with HER2-positive treatment-refractory solid tumors, with an objective response rate (ORR) of 26% [95% confidence interval (CI) 19% to 35%] as per Response Evaluation Criteria in Solid Tumor (RECIST) version 1.1.[6]

With the advancement of precision oncology and the development of many targeted agents, genomic profiling of the tumor is essential to provide optimal treatment options for patients. Sequencing of plasma circulating tumor DNA (ctDNA) can be useful to evaluate the potential actionable genomic alterations in cancer patients, especially when a biopsy is not feasible or sequencing from a tissue sample does not provide adequate data.[7] Also, ctDNA sequencing may reflect the tumor heterogeneity within a patient and be used to evaluate the response to treatment and the evolution of resistance clones.[8,9] Several targeted next-generation sequencing (NGS) panels for ctDNA analysis have been developed to evaluate actionable alterations in patients with advanced solid tumors, including *ERBB2* amplification.[10,11]

Samfenet®, a trastuzumab biosimilar, has shown bioequivalence with the original trastuzumab and is approved by the Ministry of Food and Drug Safety of Korea for the treatment of HER2-positive early and metastatic breast cancer and advanced gastric cancer in South Korea.[12,13] Herein, we report the results from a phase II, open-labeled prospective clinical trial to investigate the efficacy and safety of trastuzumab plus treatment of the physician's choice in patients with previously treated HER2-positive unresectable locally advanced or metastatic solid tumor, excluding breast and gastric cancer. We also carried out an exploratory analysis with ctDNA sequencing to evaluate potential biomarkers associated with patient outcomes of HER2-directed agents used to treat HER2-positive solid tumors.

MATERIALS AND METHODS

Study design and patients

This study was a single-center, open-label prospective phase II trial (ClinicalTrials.gov identifier: NCT04215159). Patients with histologically or cytologically confirmed HER2-positive solid tumors who progressed on or discontinued at least one prior treatment, except those with breast cancer and gastric cancer, were included as HER2-directed therapies are currently the standard treatment of these patients. HER2 positivity was defined as *ERBB2* overexpression confirmed by immunohistochemistry 3+ and/or *ERBB2* gene amplification confirmed by *in situ* hybridization (ISH). Patients with *ERBB2* amplification detected with tissue targeted NGS were also included in the study. Other key eligibility criteria were Eastern Cooperative Oncology Group (ECOG) performance status of 0 to 1, at least one measurable or evaluable lesion by RECIST version 1.1, at least 3 months of life expectancy, left ventricle ejection fraction (LVEF) $\geq$50%, and adequate organ function. Patients with symptomatic or uncontrolled central nervous system metastasis, a cardiovascular or cerebrovascular event within 6 months, a history of symptomatic interstitial pneumonitis, hypersensitivity to the study treatments, and other uncontrolled medical conditions were ineligible.

Procedure

Patients received trastuzumab (Samfenet®) 8 mg/kg intravenously (i.v.) as a loading dose for the first cycle, then received 6 mg/kg for the second cycle on day 1 combined with a treatment of the physician's choice of either gemcitabine 1000 mg/m^2 i.v. on day 1 and day 8 or irinotecan 100 mg/m^2 i.v. on day 1 and day 8 every 3 weeks. Patients were monitored for possible infusion reactions during and after the administration of trastuzumab every cycle, and LVEF was monitored every 12 weeks during the study treatment to evaluate the potential cardiac toxicity of trastuzumab. Response evaluation was carried out every 8 weeks with computed tomography or magnetic resonance imaging per RECIST version 1.1. Patients were continued on study treatment until radiologic disease progression by RECIST version 1.1, unacceptable adverse event, patient's refusal, pregnancy, or death. For patients with radiologic disease progression, survival follow-up was carried out every 12 weeks. For those with study discontinuation without disease progression, disease evaluation using imaging studies was carried out every 12 weeks.

Outcomes

The primary endpoint of the study was the ORR, defined as the proportion of patients who achieved a complete response (CR) or partial response (PR) as the best response to the study treatment by RECIST version 1.1. Secondary endpoints included progression-free survival (PFS), defined as the time from enrollment to radiologic progressive disease or any cause of death, whichever came first, and overall survival (OS), defined as the time from study enrollment to any cause of death. Patients were evaluated for safety outcomes every 3 weeks according to National Cancer Institute Common Terminology Criteria for Adverse Events (NCI CTCAE) version 5.0.

Biomarker analysis

Exploratory analysis using plasma ctDNA sequencing was carried out to investigate potential biomarkers associated with the efficacy outcomes. Blood samples were collected at the time of screening for study inclusion and at the time of disease progression, and targeted NGS was carried out using the CT-ULTRA panel, which includes 118 genes. Details of the process of plasma ctDNA sequencing are described in the Supplementary Methods, available at https://doi.org/10.1016/j.esmoop.2023.101583. Plasma *ERBB2* copy number was estimated from the sequencing results as follows. First, considering that somatic alterations of the tumor cells were heterozygous, tumor cellularity was assumed based on

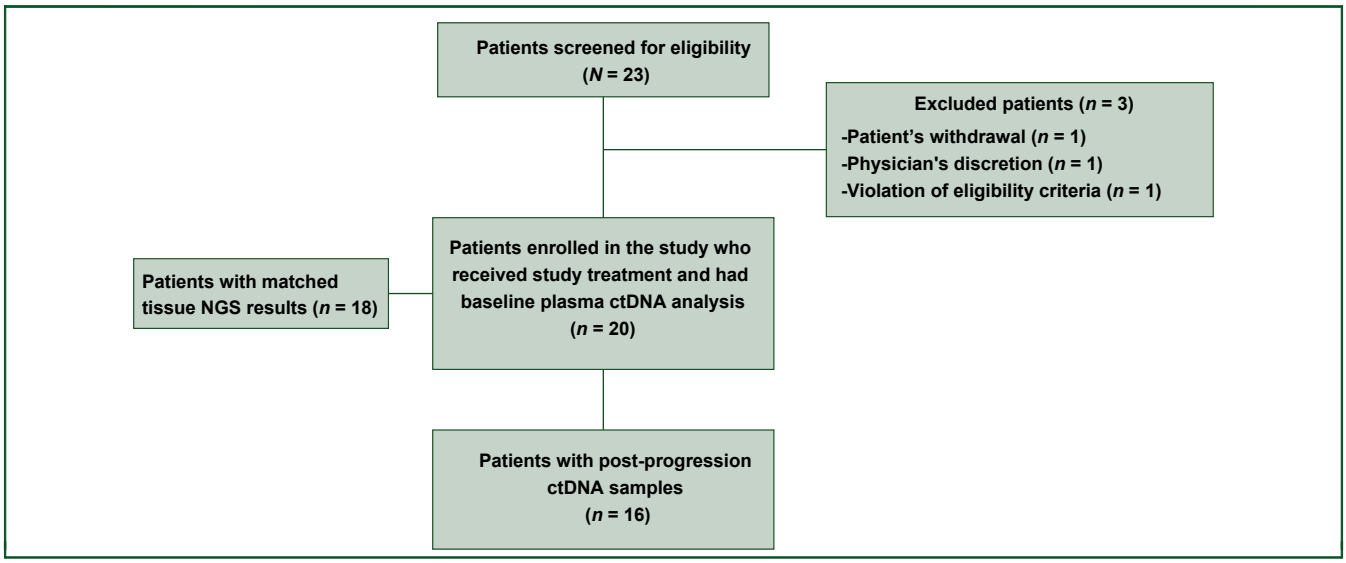

Figure 1. Study outline.
ctDNA, circulating tumor DNA; NGS, next-generation sequencing.

the highest variant allele frequency (hVAF) among the somatic alterations detected, excluding copy number variations: *Tumor cellularity* $=$ *hVAF* $\times 2$. In cases where loss-of-heterozygosity (LOH) of the gene with hVAF was assumed, except for those with copy-neutral LOH, tumor cellularity was estimated as: *Tumor cellularity* $= \frac{2\times hVAF}{1+hVAF}$.[14] Using the assumed tumor cellularity, the *ERBB2* copy number was inferred from the bin-level log2 ratios analyzed using the CNVkit (0.9.6). Genomic profiling of the archived tissue was also carried out using an in-house targeted NGS panel (OP_v4.3 RNA bait) using the NextSeq 550 platform (Illumina, San Diego, CA). The sequencing data from the plasma ctDNA and tissue were analyzed together with the clinical outcomes. Patients with an *ERBB2* copy number of $\geq$5 were defined as having *ERBB2* amplification.

Statistical analysis

This study was designed to have 80% power to detect an ORR of 25% in the study population with a one-sided type 1 error rate of $\leq$5%, with a null hypothesis of the ORR being 10%. Considering a drop-out rate of 10%, 42 patients were planned to be screened for inclusion. Since the start of the study, however, the development of new-anti HER2 agents accelerated, and many early-phase clinical trials targeting HER2-positive solid tumors opened. This resulted in a low enrollment rate for the current study and early termination of this trial. Baseline characteristics, efficacy outcomes, and safety profiles were analyzed using descriptive methods. The CI for proportional variables was calculated using Wilson's method. Survival curves were calculated using Kaplan—Meier methods and compared using a log-rank test. The association between two continuous variables was analyzed using Pearson's correlation coefficient. All statistical analysis was carried out in R version 4.1.2 (R Foundation for Statistical Computing, Vienna, Austria). A two-sided *P* value $<$0.05 was considered statistically significant.

RESULTS

Study population

A total of 23 patients from 31 December 2019 to 17 September 2021, were screened for inclusion in the study, and 20 were enrolled, including 18 patients with matched tissue NGS results and 16 patients with post-progression plasma ctDNA sequencing results (Figure 1). Their median age was 64 years (range, 30-84 years), 13 patients (65.0%) were male, and 11 patients (57.9%) received $>$2 lines of previous treatment with a median of 3 lines of previous treatment (range, 2-5) (Table 1). Hepatobiliary cancer was the most common type of solid tumor included, with seven patients (35.0%), followed by colorectal cancer (six patients, 30.0%) and non-small-cell lung cancer (three patients, 15.0%). Seventeen patients (85.0%) received irinotecan as the treatment of the physician's choice, while three patients (15.0%) received gemcitabine, and a median of 5.5 cycles (range, 1-17) of treatment was given to the patients.

Clinical outcomes and safety profiles

Among the 20 patients treated in the trial, 18 patients were available for response evaluation. Two patients (11.1%) had PR as the best response, and none of the patients had a CR (Table 1). The ORR was 11.1% (95% CI 3.1% to 32.8%), and the disease control rate, defined as the proportion of patients who had a CR, PR, or stable disease as the best response as per RECIST version 1.1, was 77.8% (95% CI 54.8% to 91.0%). With a median follow-up duration of 11.5 months [95% CI 4.64 months-(not applicable)], the median PFS and OS were 3.8 months (95% CI 3.6-4.1 months) and 6.9 months (95% CI 0.4 months-NA), respectively (Figure 2A).

Among patients with primary colorectal cancer (six patients), the median PFS and OS were 5.6 months (95% CI 3.68 months-NA) and 9.3 months (95% CI 6.94 months-NA), respectively. The median PFS and OS of patients with

Table 1. Baseline clinical characteristics and best response to treatment as per RECIST v1.1

Variables	(*N* = 20)
Age, years, *n* (%)	
≥60	12 (60.0)
<60	8 (40.0)
Sex, *n* (%)	
Male	13 (65.0)
Female	7 (35.0)
ECOG PS, *n* (%)	
0	6 (30.0)
1	14 (70.0)
Prior Tx, *n* (%)	
≤2	8 (40.0)
>2	12 (60.0)
Location of disease, *n* (%)	
Colorectal cancer	6 (30.0)
Non-small-cell lung cancer	3 (15.0)
Hepatobiliary cancer[a]	7 (35.0)
Ampulla of Vater cancer	1 (5.0)
Bladder cancer	1 (5.0)
CUP	1 (5.0)
Extramammary Paget's disease	1 (5.0)
Confirmation of *ERBB2* amplification/overexpression, *n* (%)	
IHC 2+/ISH (+)	2 (10.0)
IHC 3+	16 (80.0)
ERBB2 amplification from tissue NGS	2 (10.0)
Combination drug, *n* (%)	
Gemcitabine	3 (15.0)
Irinotecan	17 (85.0)
Median cycles	5.5 (range, 1-17)
Best response, *n* (%)	(*n* = 18)
PR	2 (11.1)
SD	14 (66.7)
PD	4 (22.2)
ORR (95% CI)	11.1 (3.1-32.8)
DCR (95% CI)	77.8 (54.8-91.0)

CI, confidence interval; CUP, carcinoma of unknown primary; DCR, disease control rate; ECOG PS, Eastern Cooperative Oncology Group performance status; IHC, immunohistochemistry; ISH, *in situ* hybridization; NGS, next-generation sequencing; ORR, objective response rate; PD, progressive disease; PR, partial response; RECIST, Response Evaluation Criteria in Solid Tumors; SD, stable disease.

[a]Includes four patients with gallbladder cancer, one patient with perihilar cholangiocarcinoma, and one patient with combined hepatocellular carcinoma and cholangiocarcinoma.

hepatobiliary cancer (seven patients) were 3.6 months (95% CI 1.81 months-NA) and 5.1 months (95% CI 3.0 months-NA), respectively. Changes in the sum of the diameters of target lesions throughout the treatment course are shown in Figure 2B. All seven patients with hepatobiliary cancer and all six patients with colorectal cancer received irinotecan as the physician's choice of combination agent.

Overall, 14 patients (70.0%) showed adverse events of grade 3 or 4 according to NCI CTCAE version 5.0. The most common grade 3-4 adverse events were anemia (six patients, 30.0%) and gastrointestinal (six patients, 30.0%) (Supplementary Table S1, available at https://doi.org/10.1016/j.esmoop.2023.101583). Five patients (25.0%) had an infusion reaction to trastuzumab, and none had an infusion reaction of grade 3 or higher. None of the patients showed a decrease in LVEF of more than 20% compared with baseline or below 50% through the treatment course (Supplementary Figure S1, available at https://doi.org/10.1016/j.esmoop.2023.101583). There was no treatment-related mortality, and none of the patients discontinued the study treatment due to toxicity.

Genomic profiles and clinical outcomes

The genomic alterations detected by ctDNA sequencing are shown in Figure 3A. A median of 3 mutations (range, 1-10) was found in each patient, and *ERBB2* amplification (copy number ≥5) in baseline plasma ctDNA was found in 17 patients (85%). The gene most altered other than *HER2* was *TP53* (16 patients, 80%). There were no exclusively found alterations according to the best response or primary tumor type. PFS and the best response to treatment according to the primary tumor location and plasma *ERBB2* copy number in each patient are shown in Figure 3B.

Two patients (AMC-001, AMC-002) showed a substantially longer PFS duration than the others, and both patients had colorectal cancer as a primary tumor with a plasma *ERBB2* copy number of 10 or higher (Figure 3A). There was no difference in survival outcomes (PFS and OS) when the patients were dichotomized according to the plasma *ERBB2* copy number (median and copy number of 10) (Supplementary Figure S2, available at https://doi.org/10.1016/j.esmoop.2023.101583). Among 16 patients with matched ctDNA sequencing data at baseline and disease progression, 7 patients (43.8%) developed new genomic alterations at the time of progression compared with baseline (Figure 3C).

Concordance of plasma and tissue genomic alteration profiles. Genomic profiling of the archived biopsy specimen was carried out for 18 patients and was compared with the baseline ctDNA analysis results (Figure 4A). Among the 18 patients with matched baseline tissue and plasma NGS results, 16 patients (88.9%) had *ERBB2* amplification (copy number ≥5) from both tissue and plasma, whereas 2 patients (11.1%) had *ERBB2* amplification detected in tissue only. Overall, 91.8% of the alterations (45 out of 49) other than *ERBB2* amplification found in the tissue sequencing data were also detected by ctDNA, whereas 75.0% of the alterations (45 out of 60) detected by ctDNA sequencing were found in the tissue analysis (Figure 4B). There were 15 alterations exclusively found in the ctDNA analysis, and the VAF of alterations found in the plasma only were lower than those found in both plasma and tissue (Figure 4B). When comparing the *ERBB2* copy number between plasma and tissue, there was a significant correlation between the two results with a Pearson's correlation coefficient of 0.52 ($P = 0.027$) (Figure 4C). Details of the *ERBB2* copy numbers from the plasma ctDNA and tissue sequencing data and the sample collection dates of the patients are described in Supplementary Table S2, available at https://doi.org/10.1016/j.esmoop.2023.101583.

DISCUSSION

In this single-arm phase II basket trial, trastuzumab (Samfenet®) plus irinotecan or gemcitabine was a safe and tolerable treatment of patients with treatment-refractory

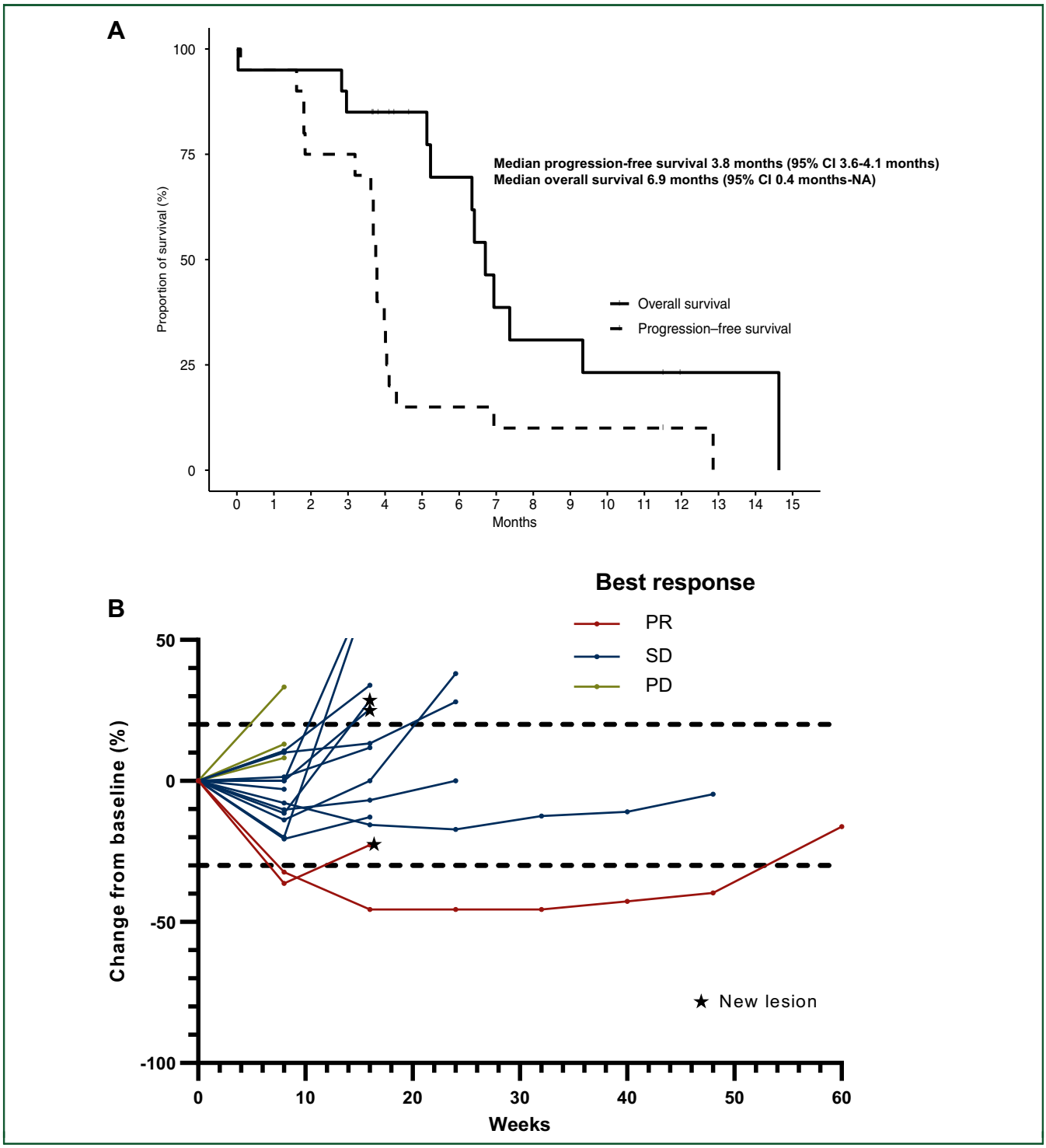

Figure 2. Efficacy outcomes of patients receiving study treatment.
(A) Kaplan–Meier estimates of progression-free survival and overall survival. (B) Spider plot of the changes in the sum of the target lesions among patients with measurable lesions during the treatment.
NA, not applicable; PD, progressive disease; PR, partial response; SD, stable disease.

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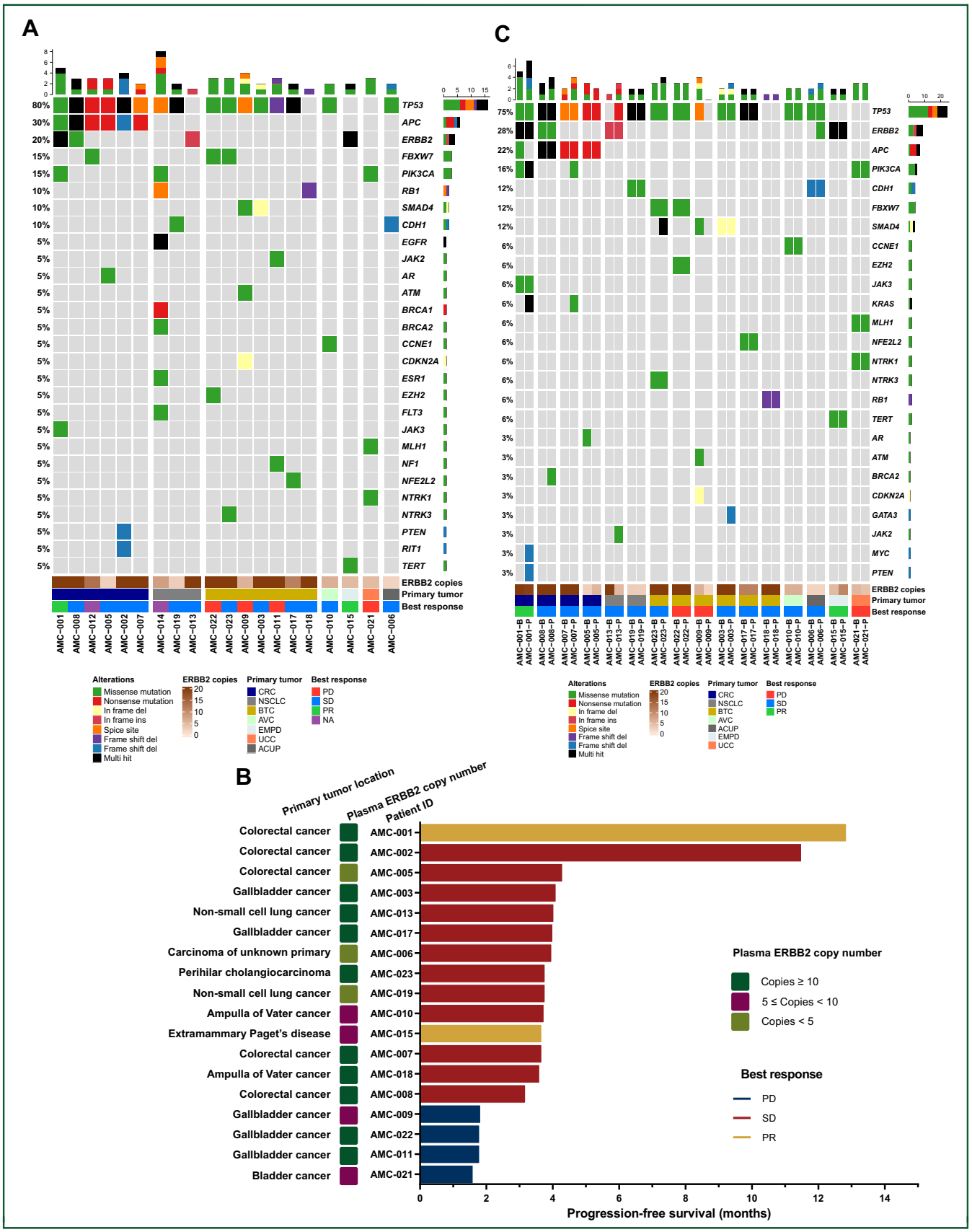

Figure 3. Genomic profiles and clinical outcomes of the patients.
(A) Genomic landscape of the baseline circulating tumor DNA (ctDNA) analysis ($n = 20$). (B) Swimmer's plot of progression-free survival and the best response of each patient, along with the primary tumor location and baseline plasma *ERBB2* copy number. (C) Comparison of genomic alterations between baseline and post-progression ctDNA ($n = 13$).
ACUP, adenocarcinoma of unknown primary; AVC, ampulla of Vater cancer; BTC, biliary tract cancer; CRC, colorectal cancer; EMPD, extramammary Paget's disease; NA, not applicable; NSCLC, non-small-cell lung cancer; PD, progressive disease; PR, partial response; SD, stable disease; UCC, urothelial carcinoma.

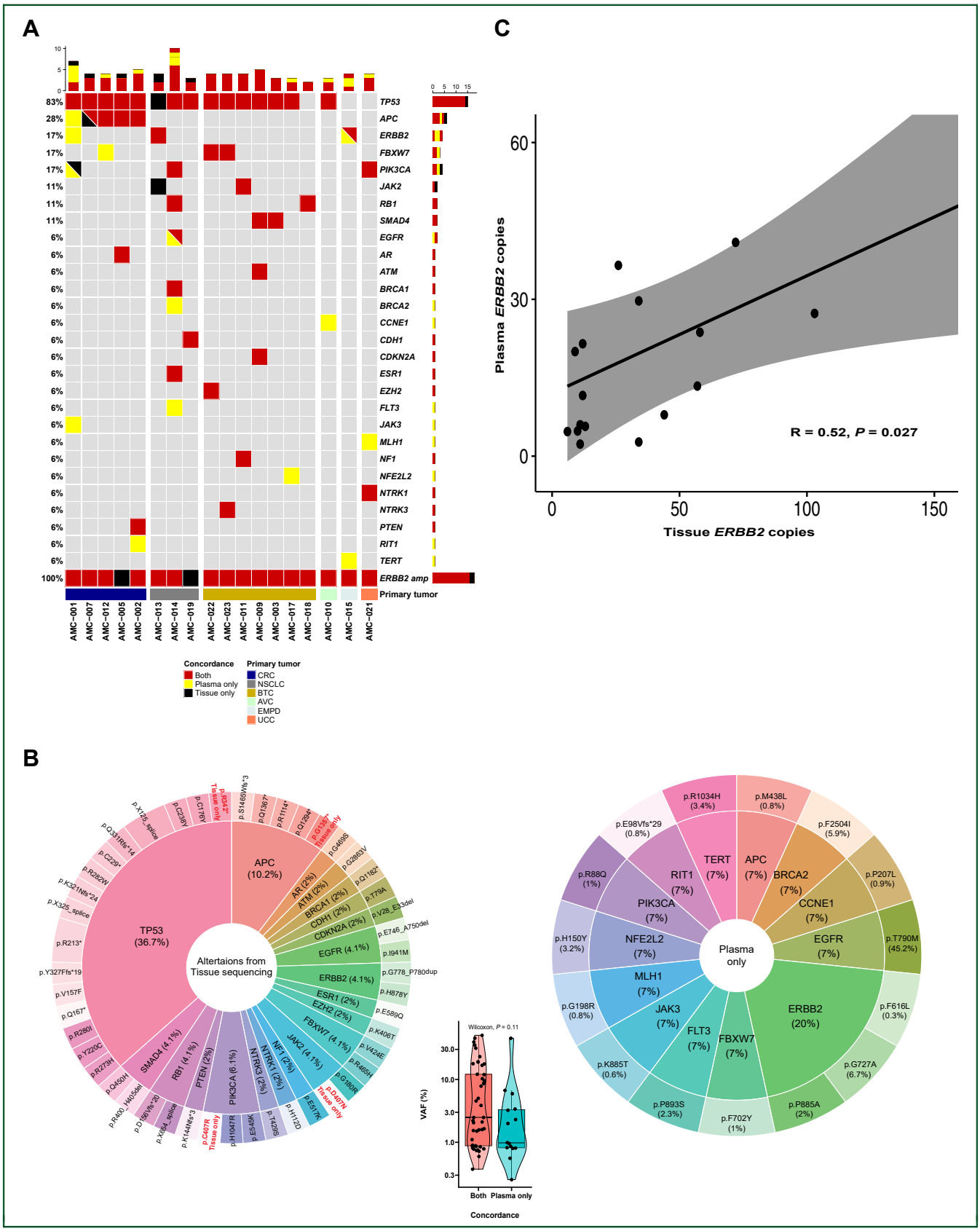

Figure 4. Genomic alterations detected with baseline ctDNA analysis and archival tissue sequencing analysis.
(A) Comparison between circulating tumor DNA (ctDNA) and tissue genomic profiles. (B) Concordance of genomic alterations between ctDNA and tissue. The pie chart on the left shows the alterations detected by tissue sequencing, and four alterations exclusively found in tissue are described in red letters. The pie chart on the right shows the alterations exclusively found by ctDNA sequencing. The violin plot in the middle shows the variant allele frequency (%) of alterations in the ctDNA. The alterations found in both the tissue and ctDNA showed a numerically higher frequency compared with those found in the ctDNA only (Wilcoxon $P = 0.11$). (C) Correlation between tissue and plasma ctDNA *ERBB2* copy number.
AVC, ampulla of Vater cancer; BTC, biliary tract cancer; CRC, colorectal cancer; EMPD, extramammary Paget's disease; NSCLC, non-small-cell lung cancer; UCC, urothelial carcinoma.

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HER2-positive non-breast, non-gastric advanced solid tumors. It showed modest efficacy outcomes with an ORR of 11.1% and a median PFS and OS of 3.8 months and 6.9 months, respectively. In the previous phase IIa MyPathway trial, including 114 patients with previously treated HER2-positive non-breast, non-gastric advanced solid tumors, the ORR was 26% (2 patients with a CR and 28 patients with a PR) for patients treated with pertuzumab plus trastuzumab.[6] Although the response rate in the current study was numerically lower compared with the phase IIa MyPathway, a direct comparison is difficult as any difference may be due to the treatment regimen (HER2-directed agent + cytotoxic agent versus dual HER2-directed agents), ethnicity, and the number of patients included (20 patients versus 114 patients).

Based on the ctDNA genomic biomarker analysis, there was no genomic alteration associated with response or resistance, and a higher plasma *ERBB2* copy number was not associated with the survival outcomes. Genomic alterations were heterogeneous among the patients, which may imply molecular heterogeneity among heavily treated HER2-positive advanced solid tumors, contributing to the low response rate. At progression, 43.8% of patients with matched post-progression ctDNA sequencing results showed new alterations, which may implicate the evolving tumor clonality. There were, however, no commonly occurring new alterations at the time of progression.

The median PFS and OS among patients with colorectal cancer as a primary tumor were 5.6 months and 9.3 months, respectively, numerically longer than the total population. In the phase IIa MyPathway HER2-amplified colorectal cancer patient cohort analysis, 57 patients were treated with pertuzumab plus trastuzumab, and the median PFS and OS were 2.9 months and 11.5 months, respectively.[15] The phase II HERACLES trial of dual HER2 blockade with trastuzumab plus lapatinib for 27 patients with previously treated HER2-positive metastatic colorectal cancer showed a median PFS and OS of 4.8 months and 10.6 months, respectively.[16] Although a direct comparison is difficult, colorectal cancer patients treated with trastuzumab plus irinotecan or gemcitabine in this study showed comparable outcomes to those treated with a dual HER2 blockade, and the efficacy of trastuzumab and a cytotoxic chemotherapy combination regimen may need further investigation for HER2-positive colorectal cancer.

Among patients with hepatobiliary cancer, the median PFS and OS were 3.6 months and 5.1 months, respectively. In the phase IIa MyPathway trial HER2-amplified biliary tract cancer cohort, 39 patients received pertuzumab plus trastuzumab and had a median PFS and OS of 4.0 months and 10.9 months, respectively.[17] From another phase II trial including 34 patients with HER2-positive advanced biliary tract cancer patients who progressed on first-line treatment, trastuzumab combined with fluorouracil and leucovorin plus oxaliplatin had a median PFS and OS of 5.1 months and 10.7 months, respectively.[18] The difference in treatment regimen may be attributable to the shorter survival outcomes in our trial compared with the other two trials, since the patients received irinotecan as the combination agent, whereas a fluoropyrimidine-based regimen is known to have efficacy for pretreated biliary tract cancer.[19,20] Direct comparisons, however, should be made cautiously considering the small sample size and given the fact that biliary tract cancer is a highly heterogeneous disease.[21]

The concordance of genomic alterations detected from tissue and ctDNA sequencing was high, with 91.8% of alterations found by tissue NGS analysis also detected on plasma ctDNA analysis, and 75.0% of alterations detected with ctDNA were also detected by the tissue analysis. Also, the VAF of alterations found exclusively from ctDNA analysis was numerically lower compared with that of alterations found in both tissue and plasma. These results may reflect the tumor heterogeneity within a patient, which may be better assessed with ctDNA analysis compared with genomic profiling of biopsy samples. In another study comparing mutational profiles of ctDNA and primary tumors in gastric cancer, ctDNA showed a low concordance rate with a single tumor sample. In contrast, sequencing with multiple tumor samples showed a higher concordance rate.[22] The estimated plasma *ERBB2* copy number was significantly correlated with the *ERBB2* copy numbers from the tissue sequencing data. Several patients had discrepancies in *ERBB2* copy numbers between their plasma and tissue sequencing results, however, which may be caused by *ERBB2* heterogeneity within a patient. Temporal heterogeneity may also have affected the results, as the sampling dates of plasma and tissue were different for most patients.

In this study, higher plasma *ERBB2* copy numbers did not have an association with better survival outcomes. As a higher plasma *ERBB2* copy number may imply that *ERBB2*-amplified tumor cells are the dominant clone within a patient, it is reasonable to assume that a higher plasma *ERBB2* copy number may be associated with a better response to an ERBB2-targeted agent. Biomarker analysis of 28 patients with HER2-positive colorectal cancer treated in the HERACLES A trial showed that patients with an estimated plasma *HER2* copy number higher than the median had a better response and a significantly longer PFS.[23] From the phase II TRIUMPH trial, including 30 patients with HER2-positive colorectal cancer from tissue and/or ctDNA analysis, patients with higher plasma *ERBB2* copy number and without concurrent alterations in receptor tyrosine kinase genes (*RAS*, *PI3K*) had a significantly better PFS compared with those with a lower copy number and/or such alterations. In addition, those with a decreased ctDNA at 3 weeks after treatment initiation had a significantly better PFS compared with those without.[24] From a previous analysis including 24 patients with HER2-positive advanced gastric cancer who had serial plasma ctDNA analyses during the treatment course, patients with primary resistance showed an increase in plasma *HER2* copy numbers, whereas patients with acquired resistance showed a decrease in *HER2* copy number.[25] Additionally, a case report of a patient with advanced, previously treated cholangiocarcinoma treated with off-label trastuzumab plus pertuzumab based on *ERBB2*

amplification detected from ctDNA analysis showed a durable response of >12 months.[26] Whereas these studies included cohorts of patients with the same type of primary tumor, our study included patients with heterogeneous solid tumors, which may explain the lack of an association between the plasma *ERBB2* copy number and the outcomes. Some solid tumors with *ERBB2* amplification may have other concurrent mechanisms resulting in resistance to HER2-directed therapy, even in those with high plasma *ERBB2* copy numbers.

The importance of an accurate assessment of *ERBB2* amplification in patients with advanced solid tumors is increasing as novel HER2-directed agents are developed. Recently, trastuzumab deruxtecan has shown promising outcomes in various HER2-positive solid tumors, including gastric cancer, breast cancer, and colon cancer.[27-29] Moreover, trastuzumab deruxtecan has been proven to exhibit significantly better efficacy compared with standard chemotherapy in breast cancer patients with low *ERBB2* amplification or ERBB2 overexpression.[30] In this study, 85% of patients had *ERBB2* amplification detected from plasma ctDNA analysis, which implies that ctDNA analysis may have substantial variability in sensitivity or positive predictive value. Additional studies are required to evaluate the robustness of ctDNA testing for detecting patients with HER2-positive solid tumors.[8] Despite its limitations, *ERBB2* amplification evaluation with plasma ctDNA may be useful when it is not feasible to perform a biopsy. Also, ctDNA analysis may have advantages in assessing the tumor heterogeneity within a patient and the dynamics of genomic profiles throughout treatment compared with tissue-based analysis. The phase II TRIUMPH study has shown a high correlation in detecting *ERBB2* amplification from tissue and plasma among patients with colorectal cancer, and patients with *ERBB2* amplification detected from plasma showed outcomes comparable to those with detection from tissue analysis.[24]

Our study has several limitations. At the time of the study initiation, HER2-directed agents were not part of the standard of care for colorectal cancer patients, and new anti-HER2 antibody—drug conjugates, including trastuzumab deruxtecan, had not been introduced in the clinic. Consequently, we designed our trial to evaluate the efficacy of trastuzumab plus cytotoxic chemotherapy, and patients with colorectal and hepatobiliary cancer were mainly enrolled. During the study period, there were changes to the standard practice and clinical trials involving new HER2-directed agents were initiated, which led to an early termination of this trial. This led to limitations in evaluating the efficacy of the treatment based on the pre-planned analysis. Also, only a small proportion of patients with non-small-cell lung cancer, one of the major target populations during planning, were included in this trial due to competing trials recruiting simultaneously, contributing to the low proportion of patients who received gemcitabine compared with irinotecan.

Despite its limitations, our study includes biomarker analysis using plasma ctDNA, which showed a high concordance in genomic profiles with tissue NGS data from diverse solid tumors, including *ERBB2* amplification. Our results suggest that plasma ctDNA could play a role in selecting patients eligible for HER2-directed therapy in future trials and clinical practice.

Conclusion

In this phase II basket trial of patients with previously treated HER2-positive non-gastric and non-breast solid tumors, trastuzumab plus irinotecan or gemcitabine was a safe and feasible treatment with modest efficacy outcomes. Although our study was underpowered to show clinical outcomes due to early termination, biomarker analysis revealed the genomic profiles of *ERBB2*-amplified advanced solid tumors and demonstrated the usefulness of plasma ctDNA analysis for evaluating HER2 status.

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ROLE OF THE FUNDER

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DISCLOSURE

The authors have declared no conflicts of interest.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

All procedures in studies involving human participants were carried out in accordance with the ethical standards of the Institutional Review Board of Asan Medical Center and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards (IRB approval no. 2019-0989).

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