Tucatinib and Trastuzumab for Previously Treated Human Epidermal Growth Factor Receptor 2–Positive Metastatic Biliary Tract Cancer (SGNTUC-019): A Phase II Basket Study

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ABSTRACT

PURPOSE To evaluate the efficacy and safety of tucatinib and trastuzumab in patients with previously treated human epidermal growth factor receptor 2–positive (HER2+) metastatic biliary tract cancer (mBTC).

METHODS SGNTUC-019 (ClinicalTrials.gov identifier: NCT04579380) is an open-label phase II basket study evaluating the efficacy and safety of tucatinib and trastuzumab in patients with HER2-altered solid tumors. In the biliary tract cancer cohort, patients had previously treated HER2 overexpressing or amplified (HER2+) tumors (identified with local testing) with no prior HER2-directed therapy. The primary end point was confirmed objective response rate (cORR) per investigator assessment. Patients were treated on a 21-day cycle with tucatinib (300 mg orally twice daily) and trastuzumab (8 mg/kg intravenously followed by 6 mg/kg every 3 weeks).

RESULTS Thirty patients were enrolled. As of data cutoff (January 30, 2023), the median duration of follow-up was 10.8 months. The cORR was 46.7% (90% CI, 30.8 to 63.0), with a disease control rate of 76.7% (90% CI, 60.6 to 88.5). The median duration of response and progression-free survival were 6.0 months (90% CI, 5.5 to 6.9) and 5.5 months (90% CI, 3.9 to 8.1), respectively. At data cutoff, 15 patients (50.0%) had died, and the estimated 12-month overall survival rate was 53.6% (90% CI, 36.8 to 67.8). The two most common treatment-emergent adverse events (TEAEs) were pyrexia (43.3%) and diarrhea (40.0%). Grade ≥3 TEAEs were reported in 18 patients (60.0%), with the most common being cholangitis, decreased appetite, and nausea (all 10.0%), which were generally not treatment related. TEAEs led to treatment regimen discontinuation in one patient, and there were no deaths due to TEAEs.

CONCLUSION Tucatinib combined with trastuzumab had clinically significant antitumor activity and was well tolerated in patients with previously treated HER2+ mBTC.

ACCOMPANYING CONTENT

Appendix
Data Sharing Statement
Data Supplement
Protocol

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INTRODUCTION

Biliary tract cancer (BTC) is an aggressive malignancy, with the majority of patients having metastatic or locally advanced disease at diagnosis.[1,2] First-line systemic therapy for advanced BTC, including metastatic BTC (mBTC), includes gemcitabine plus cisplatin with or without durvalumab, with a median overall survival (OS) of 12.8 months and 11.5 months, respectively.[3] A recent report has also shown that pembrolizumab added to gemcitabine and cisplatin results in a significantly longer OS compared with gemcitabine and cisplatin alone in the first-line setting (12.7 months *v* 10.9 months).[4] However, for patients whose BTC progresses beyond first-line therapy, treatment options are limited and provide modest clinical benefit.[5,6] Currently used second-line treatments, such as infusional fluorouracil, leucovorin, and oxaliplatin and S-1, yield objective response rates of 5% and 7.5%, with a median OS of 6.2 months and 6.8 months, respectively.[7,8] Therefore, patients with mBTC that progresses on first-line therapy need well-tolerated treatment options with higher efficacy.

CONTEXT

Key Objective

Is tucatinib combined with trastuzumab effective and safe in patients with previously treated human epidermal growth factor receptor 2–positive (HER2+) metastatic biliary tract cancer (mBTC)?

Knowledge Generated

Tucatinib and trastuzumab showed clinical activity in patients with HER2+ mBTC, with a confirmed objective response rate of 46.7%; the treatment regimen also showed a tolerable and manageable safety profile with low rates of treatment-related serious and high-grade adverse events and treatment discontinuations. The exploratory biomarker analyses demonstrated that multiple HER2 testing methods can be used to help identify patients with HER2+ mBTC who may respond to the treatment regimen.

Relevance *(A.H. Ko)*

HER2 represents a viable target for biliary tract cancers. The promising results from this study, while requiring confirmation in larger cohorts, suggest that the combination of tucatinib and trastuzumab may become a useful therapeutic strategy for the subset of patients with HER2+ disease.*

*Relevance section written by *JCO* Associate Editor Andrew H. Ko, MD, FASCO.

Human epidermal growth factor receptor 2 (HER2) overexpression/amplification (HER2-positive [HER2+]) has been identified as an oncogenic driver in multiple malignancies and may be associated with a poorer prognosis.[9] HER2-directed treatments have been shown to be efficacious in several HER2+ solid tumors,[10-20] leading to their approval for treatment of HER2+ metastatic breast, gastric, and colorectal cancers. HER2 overexpression/amplification is observed in up to 20% of mBTC, with varying rates on the basis of tumor location.[21-23] A recent report has shown that 7.9% of mBTC are HER2+.[24] HER2 is emerging as an important actionable target in this patient population,[23,25-28] as investigational anti-HER2 therapies have demonstrated clinical activity in mBTC with reported objective response rates (ORRs) ranging from 12% to 41.3%.[29-34]

Tucatinib is an oral tyrosine kinase inhibitor highly selective for HER2.[35] Preclinical data have shown that tucatinib and trastuzumab in various HER2+ tumor types results in superior antitumor activity compared with either agent alone.[35,36] Consistent with the preclinical data, clinical trials of tucatinib have demonstrated that vertical receptor inhibition of HER2 is highly effective in patients with HER2+ metastatic cancer. The HER2CLIMB trial (ClinicalTrials.gov

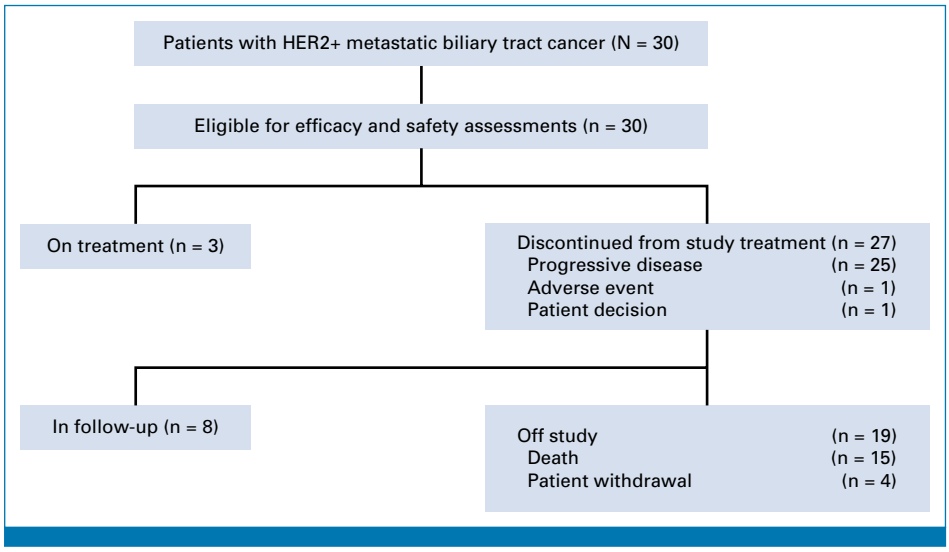


FIG 1. Flow diagram of the BTC cohort. BTC, biliary tract cancer; HER2+, human epidermal growth factor receptor 2–positive.

TABLE 1. Patient Demographics and Baseline Characteristics

Characteristic	Total (N = 30)
Age, years, median (range)	68.5 (33-79)
Sex, No. (%)	
Male	15 (50.0)
Female	15 (50.0)
Ethnicity, No. (%)	
Hispanic, Latino/a, or of Spanish origin	1 (3.3)
Not Hispanic, Latino/a, or of Spanish origin	26 (86.7)
Not reportable	3 (10.0)
Race, No. (%)	
Asian	23 (76.7)
Black or African American	1 (3.3)
White	3 (10.0)
Not reportable	3 (10.0)
ECOG performance status score, No. (%)	
0	17 (56.7)
1	13 (43.3)
Tumor location, No. (%)	
Cholangiocarcinoma extrahepatic	8 (26.7)
Cholangiocarcinoma intrahepatic	7 (23.3)
Gallbladder	15 (50.0)
HER2 status, No. (%)	
Amplification, overexpression status unknown	19 (63.3)
Amplification, no overexpression	4 (13.3)
Overexpression, amplification unknown	4 (13.3)
Amplification and overexpression	3 (10.0)
Stage at initial diagnosis, No. (%)	
I	1 (3.3)
II	5 (16.7)
III	6 (20.0)
IV	18 (60.0)
Previous lines of systemic therapy in any setting, No., median (range)	2.0 (1-4)
Previous lines of systemic therapy in metastatic or recurrent settings, No., median (range)	1.0 (1-4)

Abbreviations: ECOG, Eastern Cooperative Oncology Group; HER2, human epidermal growth factor receptor 2.

TABLE 2. Summary of Responses

Response	Total (N = 30)
Best overall response,[a] No. (%)	
CR	1 (3.3)
PR	13 (43.3)
Stable disease	9 (30.0)
Progressive disease	6 (20.0)
Not available[b]	1 (3.3)
Confirmed objective response rate,[c] No. (%)	14 (46.7)
Median duration of response, months (90% CI)	6.0 (5.5 to 6.9)
Disease control rate,[d] No. (%)	23 (76.7)
Progression-free survival, months, median (90% CI)	5.5 (3.9 to 8.1)
Overall survival, months, median (90% CI)	15.5 (6.5 to 16.7)

NOTE. Data cutoff, January 30, 2023.
Abbreviations: CR, complete response; PR, partial response.
[a]Per RECIST v1.1.
[b]Postbaseline assessment unavailable.
[c]Objective response is confirmed CR or PR, according to RECIST v1.1.
[d]Defined as confirmed CR, PR, or stable disease.

identifier: NCT02614794) has shown that adding tucatinib to trastuzumab and capecitabine is well tolerated and improves the OS of patients with previously treated HER2+ metastatic breast cancer, with or without brain metastases.[14,37-39] In the phase II MOUNTAINEER study (ClinicalTrials.gov identifier: NCT03043313), tucatinib and trastuzumab were well tolerated and highly effective in patients with previously treated HER2+ metastatic colorectal cancer.[20] These data suggest that tucatinib in combination with trastuzumab may have clinical activity in other HER2+ solid tumors. Herein, we present the efficacy and safety results of tucatinib and trastuzumab in a cohort of patients with previously treated HER2+ mBTC from the SGNTUC-019 study.

METHODS

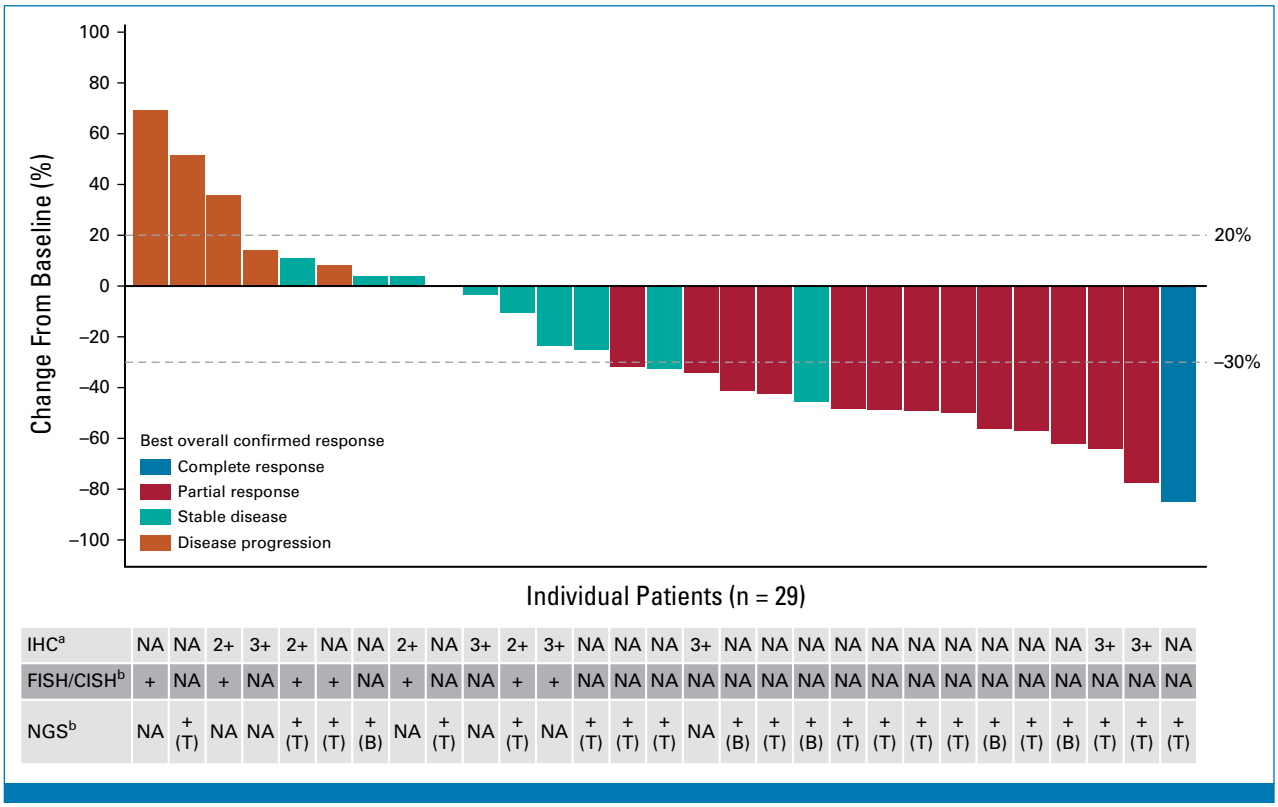
Study Overview

SGNTUC-019 (ClinicalTrials.gov identifier: NCT04579380) is an open-label phase II basket study of patients with previously treated, locally advanced, unresectable or metastatic HER2-altered solid tumors. The study was conducted in accordance with regulatory requirements and International Council for Harmonisation Good Clinical Practice guidelines. All patients provided written informed consent. The Protocol (online only; available with the full text of this article) was approved by institutional review boards and ethics committees according to the practice at each participating trial site.

Study Population

The BTC cohort comprises patients with HER2+ mBTC with measurable disease as per RECIST v1.1. Patients must have progressed during or after at least one prior line of systemic therapy or be intolerant of the most recent line of systemic therapy. Patients with an Eastern Cooperative Oncology Group performance status 0 or 1 and adequate baseline cardiac, hepatic, renal, and hematologic function were eligible. Patients previously treated with any systemic anticancer therapy, radiation therapy, major surgery, or experimental agent within 3 weeks of the first dose of study treatment were excluded. In addition, patients must have not received prior HER2-directed therapy. Full inclusion and exclusion criteria are available in the Protocol.

HER2 overexpression or amplification was determined locally using archival or fresh tumor tissue or blood via any of the following methods: (1) immunohistochemistry



IHC[a]	NA	NA	2+	3+	2+	NA	NA	2+	NA	3+	2+	3+	NA	NA	NA	3+	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	3+	3+	NA
FISH/CISH[b]	+	NA	+	NA	+	+	NA	+	NA	NA	+	+	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
NGS[b]	NA	+ (T)	NA	NA	+ (T)	+ (T)	+ (B)	NA	+ (T)	NA	+ (T)	NA	+ (T)	+ (T)	+ (T)	NA	+ (B)	+ (T)	+ (B)	+ (T)	+ (T)	+ (T)	+ (T)	+ (B)	+ (T)	+ (B)	+ (T)	+ (T)	+ (T)

FIG 2. Maximum percentage reduction in the sum of tumor diameters from baseline per investigator assessment and local HER2 testing results for each patient. One patient was excluded because of missing postbaseline measurement. [a]Numbers refer to the IHC scores. NA indicates a missing evaluable sample, including quality control fails. [b]+ is defined as amplification. NA indicates a missing evaluable sample, including quality control fails. B, blood-based; CISH, chromogenic in situ hybridization; IHC, immunohistochemistry; FISH, fluorescence in situ hybridization; HER2, human epidermal growth factor receptor 2; NA, not available; NGS, next-generation sequencing; T, tissue-based.

(IHC; overexpression defined as IHC 3+), (2) in situ hybridization (fluorescence in situ hybridization [FISH] or chromogenic in situ hybridization [CISH], amplification defined as *HER2/CEP17* signal ratio ≥2.0 or gene copy number >6), or (3) next-generation sequencing (NGS) amplification.

Procedures

Patients in the BTC cohort were treated with tucatinib 300 mg orally twice daily and trastuzumab 8 mg/kg intravenously then 6 mg/kg every 3 weeks in a 21-day cycle. Disease response to study treatment and the occurrence of disease progression were determined according to RECIST v1.1, as assessed by the investigator. Disease assessments were performed at baseline, every 6 weeks for the first 24 weeks, then every 12 weeks until the occurrence of documented disease progression per RECIST v1.1, death, withdrawal of consent, loss to follow-up, or study closure.

Safety was assessed by the incidence of treatment-emergent adverse events (TEAEs), graded according to the National Cancer Institute Common Terminology Criteria for Adverse Events v5.0, recording of concomitant medication, physical examination findings, vital signs, laboratory tests, pregnancy testing, and cardiac function. Cardiac ejection fraction was assessed via echocardiogram or a multigated acquisition scan at screening and every 12 weeks thereafter.

For the exploratory biomarker assessments, central HER2 testing was performed in Clinical Laboratory Improvement Amendments–accredited laboratories. Patients' blood samples and archival or fresh tumor tissue biopsies (if available) were collected during prescreening, screening, or on day 1 of cycle 1. Central HER2 testing was performed using IHC (PATHWAY anti-HER-2 assay [Roche, Tucson, AZ]), FISH (HER2 IQFISH pharmDx assay [Agilent, Singapore, Singapore]), and blood-based NGS assay (Guardant360, Redwood City, CA). IHC and FISH results were evaluated using the ASCO-College of American Pathologists Gastric Scoring criteria.[40]

Assessments

The primary end point was confirmed objective response rate (cORR), defined as the proportion of patients with confirmed complete response (CR) or partial response

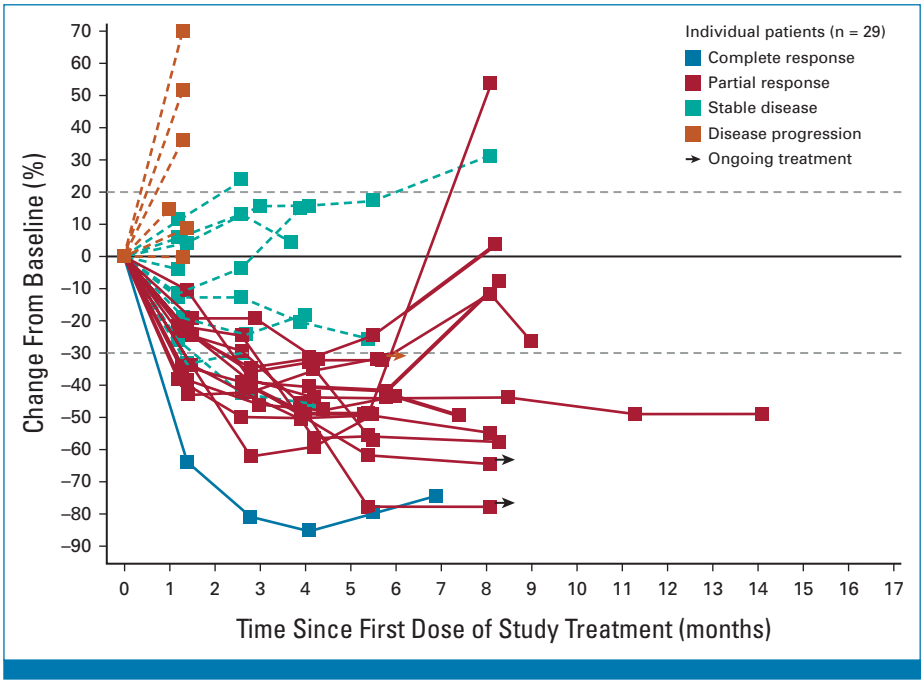


FIG 3. Percentage change from baseline in sum of diameter of target lesions per investigator assessment. One patient was excluded because of no postbaseline measurement.

according to RECIST v1.1, per investigator assessment. Secondary end points included disease control rate (DCR), duration of response (DOR), progression-free survival (PFS), OS, and safety. The exploratory end points included time to first response, the percent agreement among results from different local and central testing methods of HER2 overexpression/amplification, and cORR of patients who had HER2+ tumors on the basis of different central testing methods.

Statistical Analysis

The BTC cohort aimed to enroll up to 30 patients, a number calculated per the 90% exact CI given a range of expected cORR of 10%-30%. An interim analysis was to be performed when 12 patients were enrolled or two confirmed responses were observed, whichever was earlier. If there were at least two responders observed among the 12 patients, the predictive probability of success would be >20%, indicating that it is possible that the cORR will be higher than the current standard of care once all 30 patients were enrolled and assessed.

All enrolled patients received at least one dose of tucatinib and trastuzumab and were included in the evaluation for efficacy and safety. Two-sided 90% exact CIs for response rates were calculated by using the Clopper-Pearson method. Median PFS and OS were estimated by using the Kaplan-Meier method; the associated 90% CI was calculated on the basis of the complementary log-log transformation. Safety and concordance of local versus central HER2 testing results were assessed by descriptive statistics. The term percent agreement was used instead of concordance in comparing local and central testing results since all patients had HER2+ tumors per local testing assays. All analyses except the biomarker analyses were performed with SAS, version 9.4 (SAS Institute, Cary, NC). Biomarker analyses were performed with R, version 4.0.2 (R Core Team and the R Foundation for Statistical Computing, Vienna, Austria).

RESULTS

Patient Characteristics

Thirty patients were enrolled in the BTC cohort from June 7, 2021, to May 30, 2022. As of the data cutoff (January 30, 2023), three remain on study treatment, eight are in long-term follow-up, and 19 are off study. One patient discontinued the treatment regimen (both tucatinib and trastuzumab) because of a TEAE, with 25 patients discontinuing study treatment because of progressive disease and one patient because of patient decision. The patient disposition is summarized in Figure 1. The median duration of follow-up was 10.8 months (range, 1.5-17.1).

Demographics and baseline characteristics of patients enrolled in the BTC cohort are shown in Table 1. The median age was 68.5 years (range, 33-79). The majority of patients were Asian (76.7%). Most patients (80.0%) had locally advanced or metastatic disease at initial diagnosis, and all patients had a history of metastatic disease. The median number of prior lines of therapy in any setting and in the locally advanced or metastatic setting was 2.0 (range, 1-4) and 1.0 (range, 1-4), respectively. All 30 patients previously received a gemcitabine

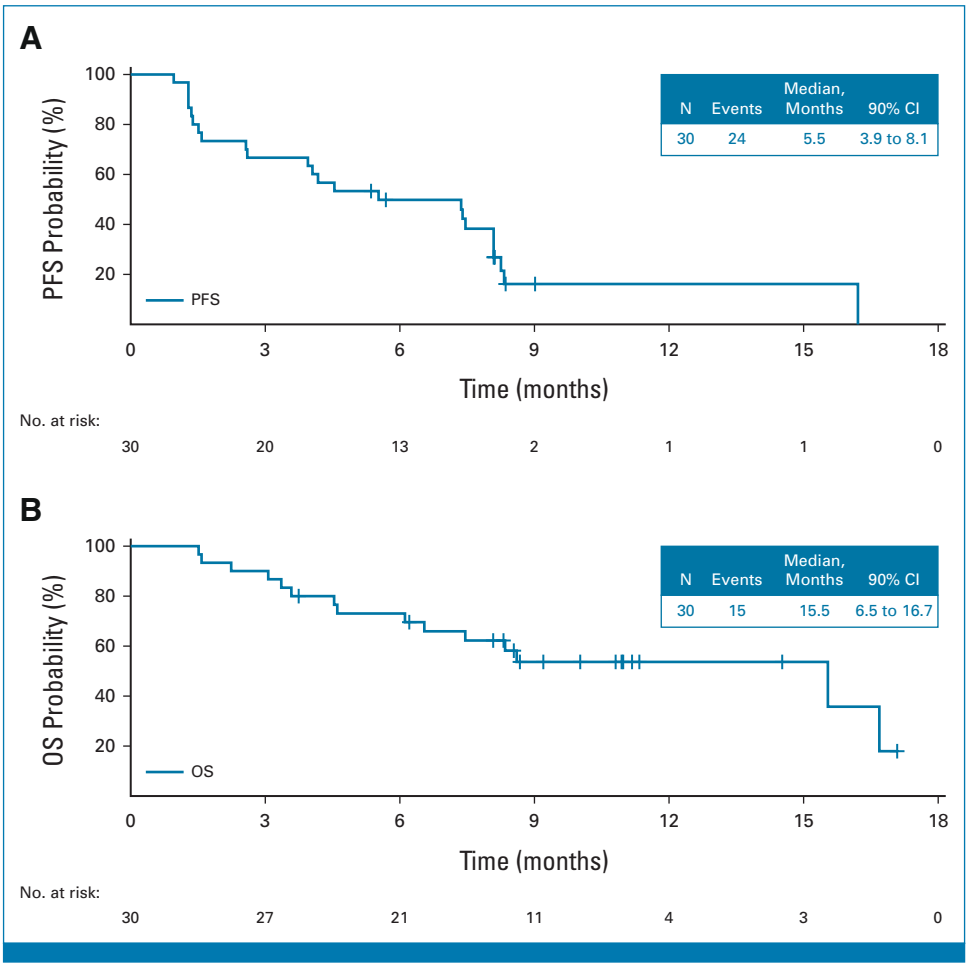


FIG 4. Kaplan-Meier curves for (A) PFS and (B) OS. Associated 90% CIs were calculated on the basis of the complementary log-log transformation. OS, overall survival; PFS, progression-free survival.

and cisplatin-containing regimen, and two patients (6.7%) were previously treated with a PD-1 or PD-L1 inhibitor. All enrolled patients had positive HER2 status on the basis of local testing.

Efficacy

The cORR per investigator assessment was 46.7% (14 of 30; 90% CI, 30.8 to 63.0; Table 2). The median DOR was 6.0 months (range, 1.4-13.4). The DCR was 76.7% (90% CI, 60.6 to 88.5). The change in tumor size from baseline among the patients with a baseline and at least one postbaseline measurement is shown in Figures 2 and 3. Twenty-one patients (70.0%) had a reduction in the tumor size (Fig 2). The median time to first response was 2.1 months (range, 1.2-4.3; Fig 3).

At time of data cutoff, the median PFS was 5.5 months (90% CI, 3.9 to 8.1; Fig 4A), with an estimated 6-month PFS of 49.8% (90% CI, 34.1 to 63.6) and estimated 12-month PFS of 16.1% (90% CI, 5.9 to 30.6). The median OS was 15.5 months (90% CI, 6.5 to 16.7; Fig 4B), with an estimated 6-month OS of 73.0% (90% CI, 56.9% to 83.9%) and 12-month OS of 53.6% (90% CI, 36.8 to 67.8). Fifteen (50.0%) patients had died at data cutoff (Fig 1).

At data cutoff, 27 patients (90.0%) were off treatment, and among these patients, 7 (25.9%) received at least one subsequent anticancer therapy, including three (11.1%) who received a subsequent HER2-directed therapy.

Safety

The median treatment duration with tucatinib was 5.1 months (range, 0.3-16.2) and with trastuzumab was 5.6 months (range, 0.7-16.8). TEAEs were reported in all patients (Table 3; Appendix Table A1, online only). The five most common TEAEs were pyrexia (13 patients [43.3%]), diarrhea (12 [40.0%]), blood creatinine increased, infusion related reaction, and ALT increased (each in eight [26.7%]; Table 3). Eighteen (60.0%) patients had grade ≥3 TEAEs. The most common grade ≥3 events were nausea, decreased appetite, and cholangitis (each in three [10.0%]). The majority of grade ≥3 TEAEs were not related to study treatment, with grade ≥3 tucatinib-related events reported in seven patients (23.3%) and trastuzumab-related events in three (10.0%). Thirteen patients (43.3%) had serious TEAEs, with three (10.0%) related to tucatinib and two (6.7%) related to trastuzumab. Tucatinib was discontinued because of TEAEs in three patients (10.0%), due to cholangitis (grade 3),

TABLE 3. Most Common TEAEs

	Total (N = 30), No. (%)	
TEAE	Any Grade	Grade ≥3[a]
Any TEAE	30 (100)	18 (60.0)
Pyrexia	13 (43.3)	0
Diarrhea	12 (40.0)	2 (6.7)
Infusion-related reaction	8 (26.7)	0
Blood creatinine increased	8 (26.7)	1 (3.3)
ALT increased	8 (26.7)	2 (6.7)
Nausea	7 (23.3)	3 (10.0)
Chills	7 (23.3)	0
Decreased appetite	7 (23.3)	3 (10.0)
Aspartate aminotransferase increased	6 (20.0)	2 (6.7)
Malaise	5 (16.7)	0
Vomiting	4 (13.3)	0
Fatigue	4 (13.3)	1 (3.3)
Anemia	4 (13.3)	2 (6.7)
Stomatitis	4 (13.3)	0
Hyponatremia	4 (13.3)	1 (3.3)
Abdominal pain upper	4 (13.3)	0
Abdominal pain	4 (13.3)	0
Hepatic function abnormal	4 (13.3)	1 (3.3)
Cholangitis	4 (13.3)	3 (10.0)
COVID-19	4 (13.3)	1 (3.3)
Dry skin	3 (10.0)	0
Dizziness	3 (10.0)	0
Back pain	3 (10.0)	0

NOTE. Adverse events reported in ≥10.0% of the patients who received at least a single dose of the study drug are listed here. The events are reported as per the preferred terms in the *Medical Dictionary for Regulatory Activities*.

Abbreviation: TEAE, treatment-emergent adverse event.

[a]Grade ≥3 TEAEs not listed here included acute kidney injury, amylase increased, biliary tract infection, blood bilirubin increased, gastritis hemorrhagic, hemobilia, hepatic encephalopathy, hepatic infection, hepatotoxicity, hypertension, interstitial lung disease, liver disorder, and respiratory failure. Each of the above was reported in one patient (3.3%).

interstitial lung disease (grade 3), and liver disorder (grade 4). At the time of data cutoff, the events of liver disorder and cholangitis were resolved, and the event of interstitial lung disease was grade 1. One patient (3.3%) discontinued trastuzumab because of interstitial lung disease (grade 3). Tucatinib dose reductions because of TEAEs were reported in six patients (20.0%). No TEAEs resulted in death. All 15 deaths (50.0%) that occurred at the time of the data cutoff date were related to disease progression.

Of the 12 patients with diarrhea, 10 patients had grade ≤2 events, two had grade 3 events, and there were no grade 4 events. No events of diarrhea led to treatment discontinuation. Events of ALT and aspartate aminotransferase increased were reported in eight and six patients, respectively, and two patients had grade 3 events for both.

Agreement Among HER2 Testing Methods and Treatment Response by HER2 Testing Methods

Among the 30 patients in the BTC cohort, 24 patients had tissue samples evaluable for the exploratory analysis comparing results from local versus central HER2 testing using central IHC and FISH (Appendix Fig A1, online only). Twenty-nine patients had evaluable samples for central blood-based NGS (Appendix Fig A1). The percent agreement between local testing and central IHC/FISH, central FISH, and central blood-based NGS results were, respectively, 87.5% (21 of 24; 90% CI, 70.8 to 96.5; Appendix Table A2, online only), 87.5% (21 of 24; 90% CI, 70.8 to 96.5; Appendix Table A3, online only), and 75.9% (22 of 29; 90% CI, 59.4 to 88.1; Appendix Table A4, online only). The percent agreement between different central HER2 testing assays ranged from 82.4% to 100.0% (Appendix Tables A5-A8, online only).

The cORR for patients confirmed as HER2+ by central IHC/FISH was 57.1% (12 of 21; 90% CI, 37.2 to 75.5; Appendix Table A9, online only) and confirmed as HER2+ by FISH was 57.1% (12 of 21; 90% CI, 37.2 to 75.5; Appendix Table A10, online only). The cORR for patients confirmed by blood-based NGS as HER2+ was 63.6% (14 of 22; 90% CI, 43.9% to 80.4%; Appendix Table A11, online only). All patients who responded were HER2+ by central testing. All patients who tested HER2-negative by any central testing method did not have an objective response to the treatment (Appendix Fig A1).

DISCUSSION

Most patients with BTC are diagnosed with locally advanced or metastatic disease and have a poor prognosis.[1,2,9] The median OS for patients with mBTC on first-line therapy is approximately 1 year and for second-line therapy approximately 6 months.[3,5-8] Current second-line options for patients with mBTC are limited and yield modest benefit; hence, effective treatment options are needed.

BTC is a heterogeneous group of rare diseases with varied actionable molecular alterations (eg, *FGFR2* and *IDH1*),[41] and recent reports have shown that several molecular agents targeting specific genomic alterations result in antitumor activity in patients with mBTC.[42] HER2 is a validated target for HER2+ metastatic breast, gastric, and colorectal cancers,[10-20] and results from studies with HER2-directed agents also suggest activity in patients with HER2+ mBTC.[29-34] Previous studies have demonstrated meaningful antitumor activity treating HER2+ mBTC with HER2-directed agents (ORR of 12%-41.3%).[29-34] The results presented in this study indicate that tucatinib and trastuzumab appear to be effective in patients with previously treated HER2+ mBTC, and clinical activity was observed in patients with IHC 3+ or 2+ tumors. Patients treated with the combination had a cORR of 46.7%, which, to our knowledge, is one of the highest reported response rates among investigational HER2-directed therapies for mBTC to date. Responses to treatment with tucatinib and trastuzumab were rapid and durable, with a median time to

first response of 2.1 months and a median DOR of 6.0 months. The majority of all enrolled patients experienced a reduction in tumor size, with one patient achieving a CR. Of note, the treatment regimen resulted in a median PFS of 5.5 months (90% CI, 3.9 to 8.1) and a median OS of 15.5 months (90% CI, 6.5 to 16.7). Because of the short follow-up, the median OS should be interpreted with caution. These data support the meaningful clinical activity of tucatinib and trastuzumab in patients with HER2+ mBTC.

The combination of tucatinib and trastuzumab was well tolerated and consistent with the previously reported safety profile of the regimen.[20] Chemotherapy-related toxicities are of considerable concern for patients with mBTC on first- and second-line therapies. Grade ≥3 TEAEs are reported in approximately 69%-78% of patients, highlighting a need for better tolerated, chemotherapy-free treatment options.[3,7] Tucatinib and trastuzumab were well tolerated with a low incidence of treatment-related events; only 23.3% and 10.0% of the patients had grade ≥3 TEAEs related to tucatinib and trastuzumab, respectively. In addition, only one patient discontinued the study regimen because of TEAEs. Diarrhea was reported in 40.0% of patients, but most events were grade 1. Given the primary tumor location in the biliary tract and the propensity for the tumor to spread locally, the increase in aminotransferase levels may be explained by complications associated with the primary site of the tumor, such as cholangitis.[43]

There is currently no consistent HER2 testing guideline for BTC.[44] A more extensive application of HER2 testing may help with informing treatment decisions, and patients with mBTC with molecular alterations may have improved outcomes when treated with a tailored targeted therapy.[41,45,46] A comparative analysis of results from local and central testing showed a high level of agreement between central and local and between central HER2 status determination. In addition, cORR for patients whose samples tested positive for HER2 centrally (IHC/FISH, 57.1%; FISH only, 57.1%; NGS, 63.6%) was similar to the cORR reported in the overall cohort (46.7%). The percent agreement and cORR data suggest that in clinical setting, various HER2 testing modalities can reliably be used to identify HER2+ patients with mBTC who may respond to tucatinib and trastuzumab. No responses were seen in patients who tested negative by the central blood-based NGS assay, suggesting clinical utility of this platform in HER2+ mBTC.

This analysis is limited by a small cohort, short follow-up, lack of control group, and absence of independent central radiology review. Despite the absence of a control arm, the encouraging results from this analysis support that HER2 is an actionable biomarker for HER2+ mBTC, justifying further investigation of HER2-directed agents in this tumor type. Most patients enrolled in the BTC cohort were Asian, which is consistent with the global statistics for BTC; Japan and South Korea are known to have some of the highest incidences of the disease.[47] Additionally, to contextualize our data, we have referenced previous studies with other HER2-directed agents in similar patient populations while acknowledging the limitations of cross-trial comparisons. Finally, the patients were enrolled on the basis of various local HER2 testing modalities that could be performed on archival tissues, and the assessments were heterogeneous because of lack of standardized testing guidelines for BTC. Local and central HER2 test results had a high level of agreement. These results highlight the importance of HER2 testing for patients with mBTC to optimize clinical treatment, and the testing methods used in this analysis could serve as a framework for future studies involving patients with HER2+ mBTC or other types of tumors.

The results from the BTC cohort of SGNTUC-019 study further validate HER2 as an actionable biomarker in mBTC, and additional investigations of HER2-directed agents are warranted in patients with HER2+ mBTC. The combination of tucatinib and trastuzumab in this analysis demonstrated clinically meaningful activity and favorable tolerability for patients with HER2+ mBTC, a population with historically poor outcomes.

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No other potential conflicts of interest were reported.

APPENDIX

TABLE A1. Summary of Safety Events

TEAE	Total (N = 30), No. (%)
Any TEAE	30 (100)
Tucatinib-related	23 (76.7)
Trastuzumab-related	20 (66.7)
Grade ≥3 TEAE	18 (60.0)
Tucatinib-related	7 (23.3)
Trastuzumab-related	3 (10.0)
Any serious TEAE	13 (43.3)
Tucatinib-related	3 (10.0)
Trastuzumab-related	2 (6.7)
TEAEs leading to discontinuation	3 (10.0)
Tucatinib	3 (10.0)
Trastuzumab	1 (3.3)
TEAEs leading to death	0

Abbreviation: TEAE, treatment-emergent adverse event.

TABLE A2. Percent Agreement of HER2 Status for Local Versus Central IHC/FISH Testing

	Central IHC/FISH			
Local HER2 Status	Evaluable Samples	Positive[a]	Negative[b]	Agreement, % (90% CI)
Positive	24	21	3	87.5 (70.8 to 96.5)

Abbreviations: FISH, fluorescence in situ hybridization; HER2, human epidermal growth factor receptor 2; IHC, immunohistochemistry.
[a]Defined as IHC 3+ or 2+ with FISH amplification.
[b]Defined as IHC 0, 1+, or 2+ with no FISH amplification.

TABLE A3. Percent Agreement of HER2 Status for Local Versus Central FISH Testing

	Central FISH			
Local HER2 Status	Evaluable Samples	Positive[a]	Negative[b]	Agreement, % (90% CI)
Positive	24	21	3	87.5 (70.8 to 96.5)

Abbreviations: FISH, fluorescence in situ hybridization; HER2, human epidermal growth factor receptor 2.
[a]Defined as FISH amplification.
[b]Defined as no FISH amplification.

TABLE A4. Percent Agreement of HER2 Status for Local Versus Central Blood-Based NGS Testing

	Central Blood-Based NGS			
Local HER2 Status	Evaluable Samples	Positive[a]	Negative[b]	Agreement, % (90% CI)
Positive	29	22	7	75.9 (59.4 to 88.1)

Abbreviations: HER2, human epidermal growth factor receptor 2; NGS, next-generation sequencing.
[a]Defined as NGS amplification.
[b]Defined as no NGS amplification.

TABLE A5. Percent Agreement of HER2 Status Between Central IHC/FISH and Blood-Based NGS Testing

	Central Blood-Based NGS		
Central IHC/FISH	Positive[a]	Negative[b]	Agreement, % (90% CI)
Positive[c]	18	3	83.3 (65.8 to 94.1)
Negative[d]	1	2	

Abbreviations: FISH, fluorescence in situ hybridization; HER2, human epidermal growth factor receptor 2; IHC, immunohistochemistry; NGS, next-generation sequencing.
[a]Defined as NGS amplification.
[b]Defined as no NGS amplification.
[c]Defined as IHC 3+ or 2+ with FISH amplification.
[d]Defined as IHC 0, 1+, or 2+ with no FISH amplification.

TABLE A6. Percent Agreement of HER2 Status Between Central FISH and Blood-Based NGS Testing

	Central Blood-Based NGS		
Central FISH	Positive[a]	Negative[b]	Agreement, % (90% CI)
Positive[c]	18	3	83.3 (65.8 to 94.1)
Negative[d]	1	2	

Abbreviations: FISH, fluorescence in situ hybridization; HER2, human epidermal growth factor receptor 2; NGS, next-generation sequencing.
[a]Defined as NGS amplification.
[b]Defined as no NGS amplification.
[c]Defined as FISH amplification.
[d]Defined as no FISH amplification.

TABLE A7. Percent Agreement of HER2 Status Between Central IHC and FISH Testing

	Central FISH		
Central IHC	Positive[a]	Negative[b]	Agreement, % (90% CI)[c]
Positive[d]	16	0	100.0 (83.8 to 100.0)
Negative[e]	0	1	
Equivocal[f]	5	2	

Abbreviations: FISH, fluorescence in situ hybridization; HER2, human epidermal growth factor receptor 2; IHC, immunohistochemistry.
[a]Defined as FISH amplification.
[b]Defined as no FISH amplification.
[c]IHC equivocal results were not included in the calculation since there were no FISH equivocal results. The FISH assay does not return "equivocal" as a result.
[d]Defined as IHC 3+.
[e]Defined as IHC 0 or 1+.
[f]Defined as IHC 2+.

TABLE A8. Percent Agreement of HER2 Status Between Central IHC and Blood-Based NGS Testing

	Central Blood-Based NGS		
Central IHC	Positive[a]	Negative[b]	Agreement, % (90% CI)[c]
Positive[d]	13	3	82.4 (60.4 to 95.0)
Negative[e]	0	1	
Equivocal[f]	6	1	

Abbreviations: HER2, human epidermal growth factor receptor 2; IHC, immunohistochemistry; NGS, next-generation sequencing.
[a]Defined as NGS amplification.
[b]Defined as no NGS amplification.
[c]IHC equivocal results were not included in the calculation since there were no NGS equivocal results. The NGS assay does not return equivocal as a result.
[d]Defined as IHC 3+.
[e]Defined as IHC 0 or 1+.
[f]Defined as IHC 2+.

TABLE A9. HER2 Status by Central IHC/FISH Testing Versus Treatment Response

IHC/FISH Result	Responder	Nonresponder	ORR, % (90% CI)
Positive[a]	12	9	57.1 (37.2 to 75.5)
Negative[b]	0	3	0 (0 to 63.2)

Abbreviations: FISH, fluorescence in situ hybridization; HER2, human epidermal growth factor receptor 2; IHC, immunohistochemistry; ORR, objective response rate.
[a]Defined as IHC 3+ or 2+ with FISH amplification.
[b]Defined as IHC 0, 1+, or 2+ with no FISH amplification.

TABLE A10. HER2 Status by Central FISH Testing Versus Treatment Response

FISH Result	Responder	Nonresponder	ORR, % (90% CI)
Positive[a]	12	9	57.1 (37.2 to 75.5)
Negative[b]	0	3	0 (0 to 63.2)

Abbreviations: FISH, fluorescence in situ hybridization; HER2, human epidermal growth factor receptor 2; ORR, objective response rate.
[a]Defined as FISH amplification.
[b]Defined as no FISH amplification.

TABLE A11. HER2 Status by Central Blood-Based NGS Testing Versus Treatment Response

NGS Result	Responder	Nonresponder	ORR, % (90% CI)
Positive[a]	14	8	63.6 (43.9 to 80.4)
Negative[b]	0	7	0 (0 to 34.8)

Abbreviations: HER2, human epidermal growth factor receptor 2; NGS, next-generation sequencing; ORR, objective response rate.
[a]Defined as NGS amplification.
[b]Defined as no NGS amplification.

FIG A1. Summary of HER2 testing results and best overall response. Each column represents one patient. One patient was not included because of missing postbaseline measurement. The one excluded patient had IHC 3+ tumor per local testing (NA for FISH/CISH and NGS). Per central testing, this patient had IHC 1+ and no amplification per FISH and blood-based NGS. [a]Numbers refer to the IHC scores. NA indicates a missing evaluable sample, including quality control fails. [b]No scoring criteria were specified for local IHC. [c]+ is defined as amplification. NA indicates a missing evaluable sample, including quality control fails. [d]Evaluated by using the ASCO-College of American Pathologists Gastric Scoring criteria. [e]+ is defined as amplification. – is defined as no amplification. NA indicates a missing evaluable sample, including quality control fails. B, blood-based; CISH, chromogenic in situ hybridization; CR, complete response; FISH, fluorescence in situ hybridization; HER2, human epidermal growth factor receptor 2; IHC, immunohistochemistry; NA, not available; NGS, next-generation sequencing; PD, progressive disease; PR, partial response; SD, stable disease; T, tissue-based.