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Tucatinib plus trastuzumab for chemotherapy-refractory, HER2 + , *RAS* wild-type metastatic colorectal cancer (MOUNTAINEER): final analysis

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MOUNTAINEER was a multicenter, open-label, phase 2 trial (NCT03043313) that evaluated the efficacy and safety of tucatinib plus trastuzumab, a dual HER2-targeted chemotherapy-free regimen. Patients were included if they had chemotherapy-refractory, HER2+, *RAS* wild-type unresectable or metastatic colorectal cancer. This final analysis reports updated efficacy and safety after a median follow-up of 32.4 months. Of the 84 patients who received tucatinib plus trastuzumab, the confirmed objective response rate was 39.3%; median duration of response was 15.2 months. Median progression-free survival was 8.1 months and overall survival was 23.9 months. Efficacy was relatively similar across central HER2+ testing methods. No clear association of treatment response with co-occurring biomarker alterations was seen. Few patients discontinued treatment due to adverse events; no treatment-emergent deaths occurred. Tucatinib plus trastuzumab showed clinically meaningful efficacy and favorable safety. Efficacy was observed irrespective of central HER2+ testing methods and in patients with heterogeneous tumor biomarker profiles.

Human epidermal growth factor receptor 2 (HER2) overexpression/amplification (HER2+) is present in 3% to 5% of patients with metastatic colorectal cancer (mCRC) and ranges from 4% to 7% among those with *RAS* wild-type mCRC[1]. Phase 2 trials have demonstrated improved treatment responses to dual HER2-targeted combination therapies compared with standard later-line treatments in patients with treatment-refractory HER2+ mCRC[2–6]. One such dual combination regimen is tucatinib, an oral, highly selective HER2-targeted tyrosine kinase inhibitor, plus trastuzumab, an anti-HER2 monoclonal antibody, which was granted accelerated approval by the United States Food and Drug Administration (FDA) on January 19, 2023[6,7]. This chemotherapy-free regimen was approved based on the findings of the global, multicenter, phase 2, open-label MOUNTAINEER trial, which evaluated the safety and efficacy of tucatinib plus trastuzumab in patients with HER2+, *RAS* wild-type, unresectable or metastatic CRC that had progressed after fluoropyrimidine-, oxaliplatin-, and irinotecan-based

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chemotherapy[6,7]. The primary analysis showed a 38.1% confirmed objective response rate (cORR) by blinded independent central review (BICR) with a 12.4-month median duration of response (DOR); 8.2-month median progression-free survival (PFS), also by BICR, and 24.1-month overall survival (OS)[6].

In this report of the final analysis of the MOUNTAINEER trial, now with a 32.4-month extended median follow-up duration (16.1 months of additional follow-up duration since the primary analysis), we present the updated efficacy and safety findings of the tucatinib plus trastuzumab regimen in patients with HER2+ mCRC. Furthermore, in expanded exploratory analyses, we evaluated the concordance between HER2 central testing methods, as well as treatment response, and additionally characterized response according to co-occurring tumor biomarker alterations.

Results

Patient disposition and characteristics

Between August 8, 2017 and September 22, 2021, 140 patients were screened, of whom 117 were enrolled; 116 patients received ≥1 dose of study treatment (45 in Cohort A, 41 in Cohort B, and 30 in Cohort C; safety population; Fig. 1a). Baseline characteristics of the study population have been described previously[6]. Briefly, among the 114 patients who had locally assessed HER2+ disease and received treatment (45 in Cohort A, 39 in Cohort B, and 30 in Cohort C; efficacy population), median age was 56.0 years, 58% ($n = 66$) were male, 77% ($n = 88$) were White and 5% ($n = 6$) were Black/African American (Supplementary Table 1). The majority of patients had an Eastern Cooperative Oncology Group performance status of 0 (Cohorts A + B: 60%; Cohort C: 57%). A left-sided primary tumor was recorded in 85% of patients in

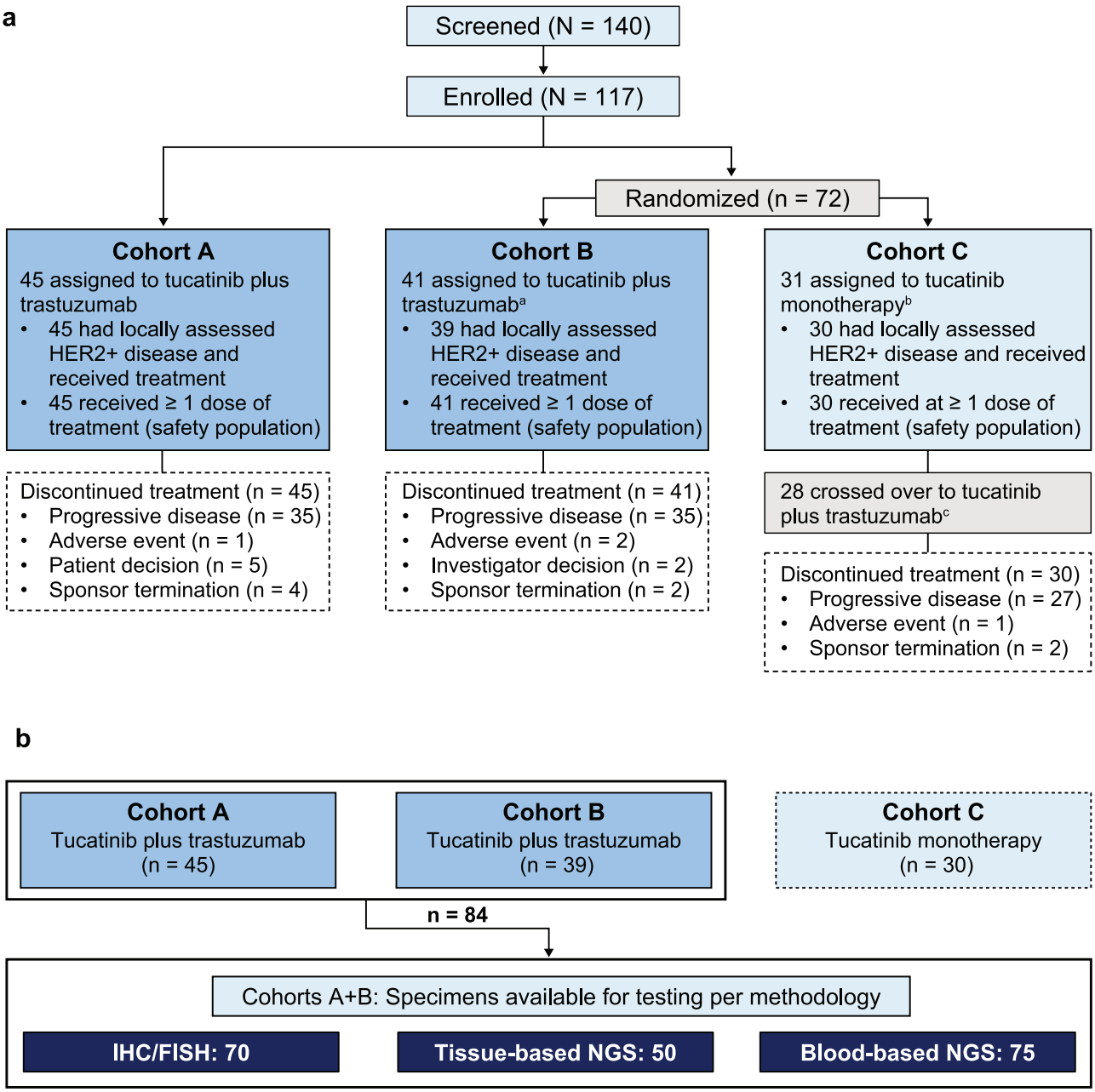


Fig. 1 | Patient disposition and specimens for central testing. a Patient disposition in final analysis and **b** patient specimens available for central testing by methodology. FISH fluorescence in situ hybridization, IHC immunohistochemistry, NGS next-generation sequencing. [a]Two patients were excluded from the antitumor activity analysis because of negative local HER2 test results. [b]One patient discontinued before receiving treatment. [c]Two patients did not cross over to receive trastuzumab in addition to tucatinib due to clinical progression, which resulted in death.

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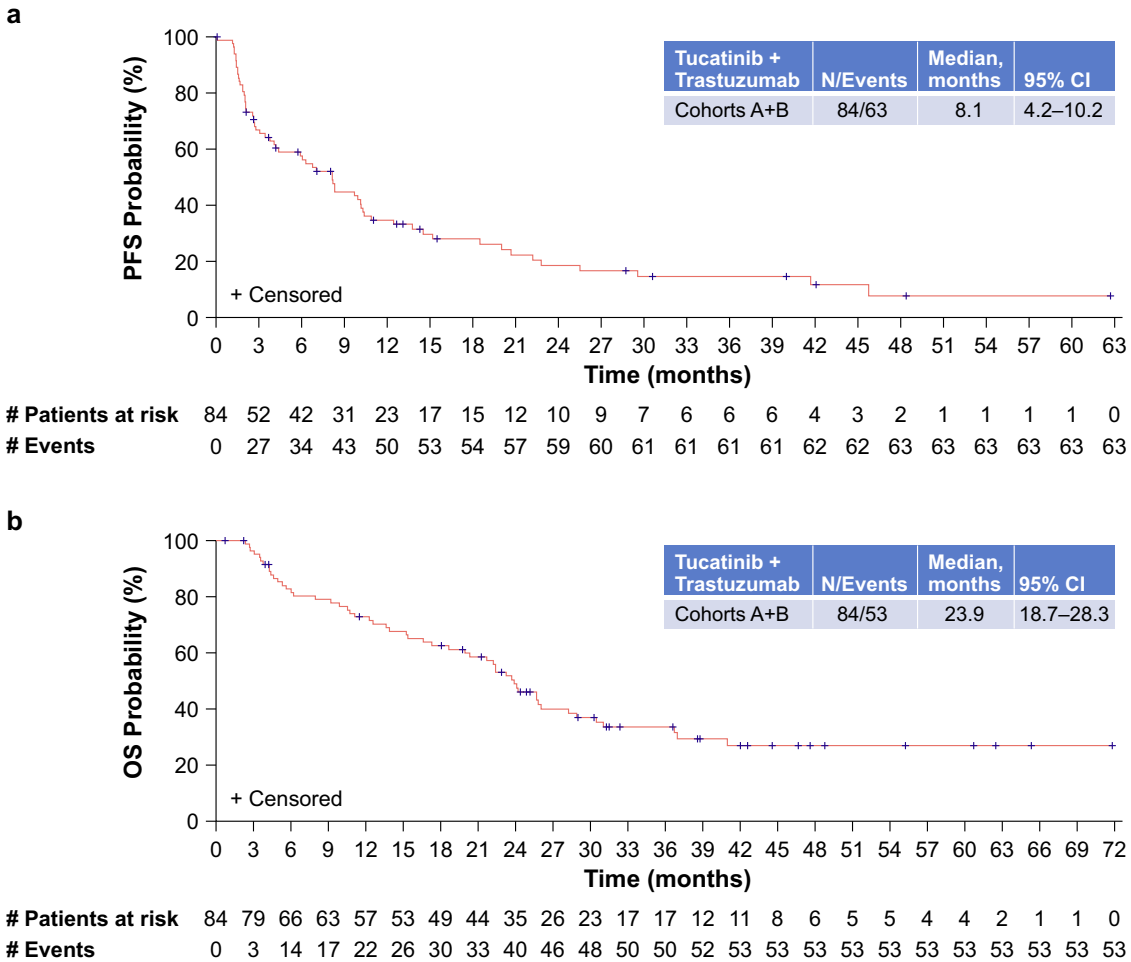


Fig. 2 | Progression-free survival and overall survival. a PFS per BICR and **b** OS in patients treated with tucatinib and trastuzumab (Cohorts A + B). BICR blinded independent central review, CI confidence interval, PFS progression-free survival, OS overall survival. **a** Kaplan–Meier plot of PFS per BICR in patients in Cohorts A + B. **b** Kaplan–Meier plot of OS per BICR in patients in Cohorts A + B ($n = 84$). Log-log transformation was used to estimate the associated 95% CIs.

Cohorts A + B and 90% of those in Cohort C. For Cohorts A + B, median prior lines of therapy was 3.0 in any setting and 2.0 in metastatic or recurrent settings.

Efficacy

At the November 2, 2023 data cut-off, median follow-up duration was 32.4 months (interquartile range, 25.1–46.7), an additional 16.1 months of follow-up since the primary analysis. Among the patients who were HER2+ in Cohorts A + B ($n = 84$) who received tucatinib plus trastuzumab, the cORR by BICR was 39.3% (95% confidence interval [CI]: 28.8–50.5%) with a median DOR of 15.2 months (95% CI: 8.9–20.5). Median PFS by BICR was 8.1 months (95% CI: 4.2–10.2), and median OS was 23.9 months (95% CI: 18.7–28.3) (Fig. 2). Among patients with baseline and postbaseline lesion measurements available in Cohorts A + B ($n = 80$), a target lesion size reduction per BICR was observed in 52 (65.0%) patients (Fig. 3a); Fig. 3b presents the percent change in tumor burden from baseline per BICR.

In patients treated with tucatinib monotherapy (Cohort C), 28 patients crossed over to receive tucatinib plus trastuzumab. Among these patients after crossover, cORR was 28.6%; median DOR was not reached, and median OS was 21.1 months (95% CI: 17.0–not estimable [NE]).

Safety

Median treatment duration in Cohorts A + B was 7.9 months with tucatinib and 7.5 months with trastuzumab. In Cohorts A + B ($n = 86$), the most commonly reported treatment-emergent adverse events (TEAEs) were diarrhea (66.3%), fatigue (44.2%) and nausea (34.9%) (Table 1). Most TEAEs were low-grade (54.7%) and not related to tucatinib treatment; 33.7% of patients had grade 3 TEAEs and 7.0% had grade 4 TEAEs. The most common grade 3 TEAEs were hypertension (7.0%), diarrhea (3.5%) and abdominal pain (3.5%). Grade 4 TEAEs that occurred in more than one patient included elevated alanine aminotransferase in two patients (2.3%), elevated aspartate aminotransferase in two patients (2.3%), and COVID-19 pneumonia in two patients (2.3%). Five patients (5.8%) discontinued tucatinib due to an AE, which was unchanged from the primary analysis. No TEAE resulted in death.

The most common tucatinib-related TEAEs in Cohorts A + B were diarrhea (52.3%), fatigue (29.1%) and nausea (18.6%), most of which were grades 1 and 2 (Supplementary Table 2). The most common tucatinib-related TEAEs of grade 3 or greater were diarrhea (2.3%) and elevated alanine aminotransferase (2.3%).

TEAEs after crossover to tucatinib plus trastuzumab among the patients in Cohort C are shown in Supplementary Table 3. TEAEs occurred in 82.1% of patients in Cohort C. Most were low-grade (50.0%); 32.1% of patients had grade 3 TEAEs. The most common grade 3 TEAE was elevated aspartate aminotransferase (10.7%). No grade 4 or grade 5 TEAEs occurred.

Exploratory analyses

Among the 84 patients in Cohorts A + B, 70 tumor tissue specimens were available for central testing by immunohistochemistry (IHC)/fluorescence in situ hybridization (FISH), of which 50 also had

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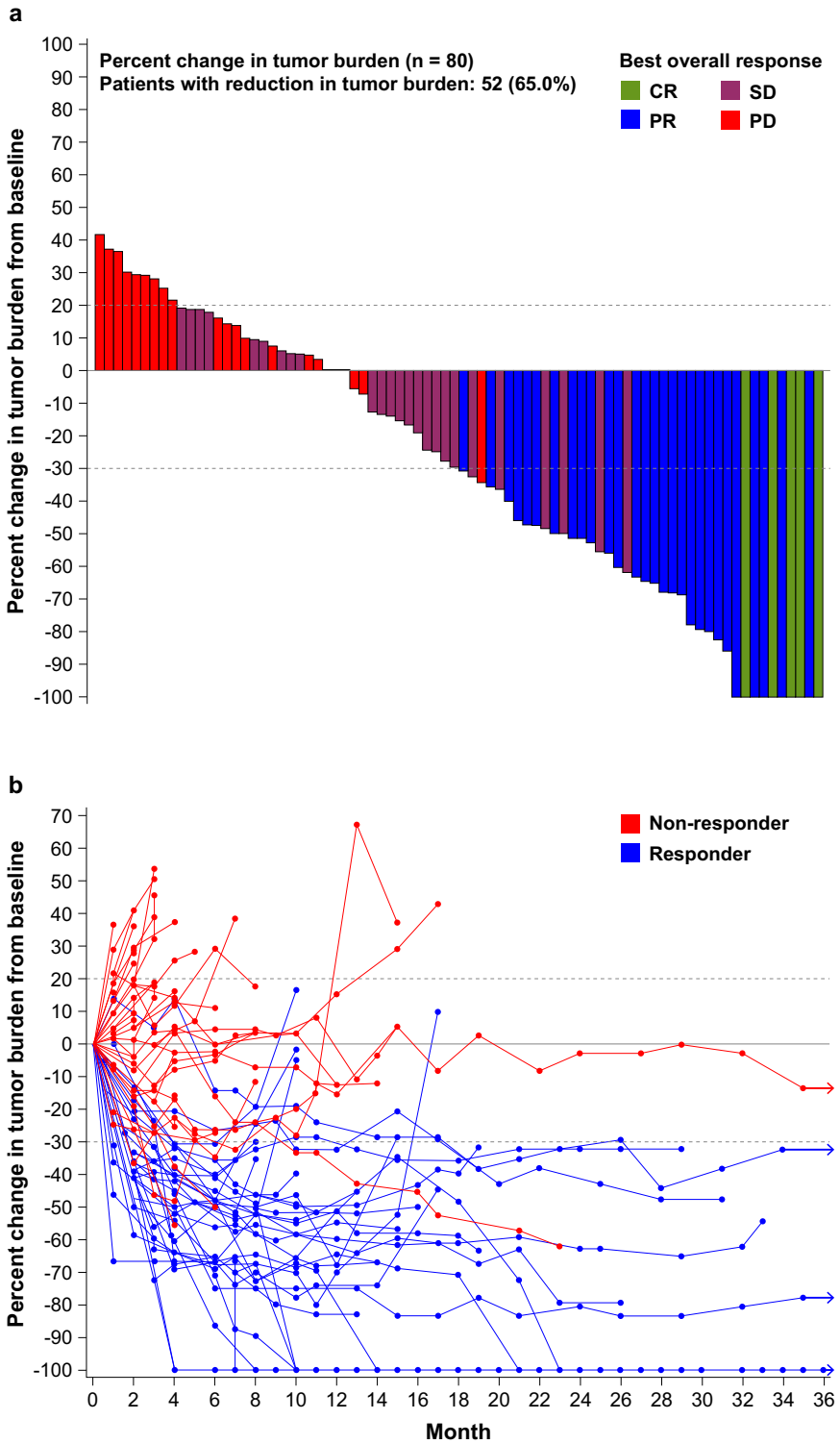


Fig. 3 | Tumor activity of tucatinib plus trastuzumab in patients with locally assessed HER2+ mCRC. BICR blinded independent central review, CR complete response, PD progressive disease, PR partial response, RECIST Response Evaluation Criteria in Solid Tumors version 1.1, SD stable disease. **a** Waterfall plot showing the maximum percentage reduction in the sum of diameter of target lesions from baseline to the best postbaseline lesion measurements ($n = 80$); each column representing a patient is colored according to best overall response per RECIST v1.1 per BICR. The line at 20% indicates progressive disease. The line at −30% indicates the threshold for a partial response. **b** Spider plot showing percent change in the sum of diameter of target lesions from baseline per BICR according to the duration of treatment in responders to treatment and nonresponders. Data up to 36 months are included. Arrows denote treatment duration beyond 36 months.

evaluable results by tissue-based next-generation sequencing (NGS); 75 blood specimens were available for circulating tumor DNA (ctDNA) NGS analysis (Fig. 1b). Across the 3 HER2 testing methods, the greatest agreement was between the tissue-based assays of IHC/FISH and NGS (93.8%; 95% CI: 82.8–98.7%) (Supplementary Table 4). There was moderate agreement between IHC/FISH and blood-based NGS (83.9%; 95% CI: 72.3–92.0%) and tissue-based and blood-based NGS (81.0%; 95% CI: 65.9–91.4%). Across the 3 testing methods, the cORR for

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Table 1 | Safety summary for Cohorts A + B

n (%)	Cohorts A + B (*n* = 86)			
Any TEAE	82 (95.3)			
Grade 1	12 (14.0)			
Grade 2	35 (40.7)			
Grade 3	29 (33.7)			
Grade 4	6 (7.0)[a]			
Grade 5	0			
Any serious TEAE	20 (23.3)			
TEAE leading to discontinuation of any study treatment	5 (5.8)			
Discontinued tucatinib	5 (5.8)			
Discontinued trastuzumab	3 (3.5)			
TEAE leading to tucatinib dose modification	25 (29.1)			
Dose held	23 (26.7)			
Dose reduced	9 (10.5)			
Common TEAEs ≥ 15%	Total	Grade 1	Grade 2	Grade 3
Diarrhea	57 (66.3)	42 (48.8)	12 (14.0)	3 (3.5)
Fatigue	38 (44.2)	27 (31.4)	9 (10.5)	2 (2.3)
Nausea	30 (34.9)	24 (27.9)	6 (7.0)	0
Infusion-related reaction	18 (20.9)	8 (9.3)	10 (11.6)	0
Pyrexia	18 (20.9)	11 (12.8)	7 (8.1)	0
Back pain	17 (19.8)	9 (10.5)	6 (7.0)	2 (2.3)
Decreased appetite	17 (19.8)	9 (10.5)	8 (9.3)	0
Dermatitis acneiform	17 (19.8)	12 (14.0)	5 (5.8)	0
Arthralgia	16 (18.6)	13 (15.1)	2 (2.3)	1 (1.2)
Chills	16 (18.6)	8 (9.3)	7 (8.1)	1 (1.2)
Cough	15 (17.4)	12 (14.0)	3 (3.5)	0
Hypertension	15 (17.4)	1 (1.2)	8 (9.3)	6 (7.0)
Vomiting	14 (16.3)	12 (14.0)	2 (2.3)	0
Abdominal pain	13 (15.1)	6 (7.0)	4 (4.7)	3 (3.5)

TEAE treatment-emergent adverse event.
[a]Grade 4 TEAEs in >1 patient included alanine aminotransferase increase in 2 patients (2.3%), aspartate aminotransferase increase in 2 patients (2.3%), and COVID-19 pneumonia in 2 patients (2.3%).

patients with HER2+ disease ranged from 41.7% to 50.0%; DOR was 16.6 months across all 3 assays (Table 2). Patients with HER2+ status consistently had higher cORR than those with HER2-negative status, as determined by any of the assays. Median PFS ranged from 8.1 to 10.9 months among patients identified as having HER2+ disease by any of the testing methods.

The 3 testing methods were also compared when analyzing treatment response by clinicopathologic subgroups (Supplementary Fig. 1). Patients with left-sided primary tumors had a numerically higher cORR than those with all other primary sites, but the number of patients with non-left-sided disease was low. Response rates were also numerically higher for patients with 1 or 2 prior lines of therapy than those with ≥3 prior lines of therapy. Treatment response was similar regardless of prior exposure to anti-epidermal growth factor receptor (anti-EGFR) therapy. Overall, treatment responses across patients with different clinicopathologic features were generally consistent across central HER2 testing methods.

The impact of baseline *ERBB2* copy number and *ERBB2*:centromere of chromosome 17 (CEN17) ratio, detected by FISH, on cORR and DOR, is shown in Fig. 4. The cORR for all patients with evaluable FISH results ($N = 65$) was 41.5% (95% CI: 29.4–54.4%). In patients with *ERBB2* copy number > 9.45 ($n = 57$), cORR was 45.6% (95% CI: 32.4–59.3%), whereas it was 12.5% (95% CI: 0.3–52.7%) in those with *ERBB2* copy number ≤ 9.45 ($n = 8$). In patients with *ERBB2*:CEN17 ratio > 5 ($n = 50$), cORR was 48.0% (95% CI: 33.7–62.6%), whereas in those with *ERBB2*:CEN17 ratio ≤ 5 ($n = 15$), cORR was 20.0% (95% CI: 4.3–48.1%). Although response rates were numerically lower (12.5% to 20.0%) in patients with lower *ERBB2* copy number and *ERBB2*:CEN17 ratio, some responded to treatment, and 95% CIs were wide.

Further exploratory biomarker analyses were conducted on the subset of patients with at least 1 central HER2 assay indicating HER2+ status. This subset had 45 patients with evaluable results by tissue-based NGS. Among these patients, 22 (48.9%) were responders with a median DOR of 16.6 months (Fig. 5a). Co-occurring biomarker alterations detected in tumor tissue at baseline that reached the 5-patient threshold for analysis included amplifications of 2 genes and mutations in 10 genes. There was no clear association of co-occurring tumor biomarker alterations with treatment response. Across all biomarker alterations found with tissue-based NGS, the overall population cORR was within the 95% CI for each alteration. Responders were observed among all of the biomarker alterations except for patients with *PIK3CG* mutations; in this small subset ($n = 5$), none were responders, but the tissue-based NGS overall population cORR was within the 95% CI of the cORR in patients with this mutated *PIK3CG*.

For the subset of patients with at least 1 central HER2 assay indicating HER2+ status, there were 68 patients with evaluable results by blood-based NGS. Among these patients, 29 (42.6%) were responders with a DOR of 15.2 months (Fig. 5b). Co-occurring biomarker alterations detected in at least 5 patients included amplifications of 6 genes and mutations in 10 genes. There was no clear association of co-occurring gene alterations with treatment response. Responders were observed among all of the biomarker alterations.

Figure 5c displays acquired alterations from ctDNA in selected genes and loss of *ERBB2* amplification. Compared with baseline specimens, many patients ($n = 30$) acquired multiple heterogeneous tumor biomarker alterations by the time of disease progression or end of treatment. Of 30 patients with *ERBB2* amplification detected at baseline by blood-based NGS, 5 patients (17%) had loss of *ERBB2* amplification. The most common acquired tumor biomarker alterations were found in the *KRAS/NRAS*, *PIK3CA* and receptor tyrosine kinase genes (*EGFR, FGFR1, FGFR2, MET*).

Discussion

In this final analysis of the MOUNTAINEER trial, with approximately 16 additional months of follow-up than the primary analysis, tucatinib plus trastuzumab showed a cORR of 39.3% and a median DOR lasting 15.2 months. Median PFS by BICR was 8.1 months and median OS was 23.9 months. The majority of TEAEs were low-grade (1–2) and manageable with standard-of-care interventions. No new safety signals were identified. Few patients discontinued treatment due to AEs. No treatment-emergent deaths occurred. These findings are consistent with the primary analysis and show that tucatinib plus trastuzumab is clinically active and well tolerated in patients with chemotherapy-refractory, HER2+, *RAS* wild-type mCRC. This patient population has significant unmet need; before the 2023 FDA approval of the tucatinib and trastuzumab regimen, the only treatment options available had low efficacy, with overall response rates of 1% to 4%[8].

Compared with other HER2-targeted therapies in combination with trastuzumab[2–5], the durability of response observed with tucatinib and trastuzumab is particularly noteworthy. In the long-term follow-up (82 months) of the HERACLES-A trial, lapatinib and trastuzumab showed a response rate of 28% in 32 patients (1 patient had a complete response maintained for 7 years)[3]. Reported only in the first analysis of the HERACLES-A trial, median DOR was 9.5 months[2]. In the subset of the MyPathway basket study of patients with treatment-refractory HER2+ mCRC treated with pertuzumab and trastuzumab ($n = 57$), treatment response was observed in 32% of patients, median DOR was

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Table 2 | Treatment response stratified by central HER2 status testing method

	IHC/FISH		Tissue-based NGS		Blood-based NGS	
	Positive (IHC3+ or IHC2+/FISH+) (*n* = 60)	Negative (*n* = 10)	Detected (*n* = 44)	Not Detected (*n* = 6)	Detected (*n* = 59)	Not Detected (*n* = 16)
Tumor response, *n* (%)						
CR	5 (8.3)	0	3 (6.8)	0	3 (5.1)	1 (6.3)
PR	20 (33.3)	1 (10.0)	19 (43.2)	0	22 (37.3)	3 (18.8)
SD[a]	21 (35.0)	4 (40.0)	15 (34.1)	2 (33.3)	18 (30.5)	7 (43.8)
PD	13 (21.7)	5 (50.0)	7 (15.9)	4 (66.7)	15 (25.4)	4 (25.0)
NA	1 (1.7)	0	0	0	1 (1.7)	1 (6.3)
cORR,[b] *n* (%) (95% CI)	25 (41.7) (29.1–55.1)	1 (10.0) (0.3–44.5)	22 (50.0) (34.6–65.4)	0 (0–45.9)	25 (42.4) (29.6–55.9)	4 (25.0) (7.3–52.4)
Median DOR,[b] months (95% CI)	16.6 (11.4–25.5)	–	16.6 (10.6 – 18.8)	–	16.6 (8.3–18.8)	15.2 (11.4–NE)
Median PFS,[b] months (95% CI)	10.1 (4.2–14.5)	2.8 (1.2–6.3)	10.9 (6.8–20.0)	2.1 (1.3–NE)	8.1 (3.1–10.3)	6.3 (2.0–25.5)

CI confidence interval, *cORR* confirmed objective response rate, *CR* complete response, *DOR* duration of response, *FISH* fluorescence in situ hybridization, *IHC* immunohistochemistry, *NA* not available, *NE* not estimable, *NGS* next-generation sequencing, *PD* progressive disease, *PFS* progression-free survival, *PR* partial response, *SD* stable disease.
[a]Includes non-CR/non-PD.
[b]Confirmed disease response and progression were assessed according to Response Evaluation Criteria in Solid Tumors version 1.1 by blinded independent central review.

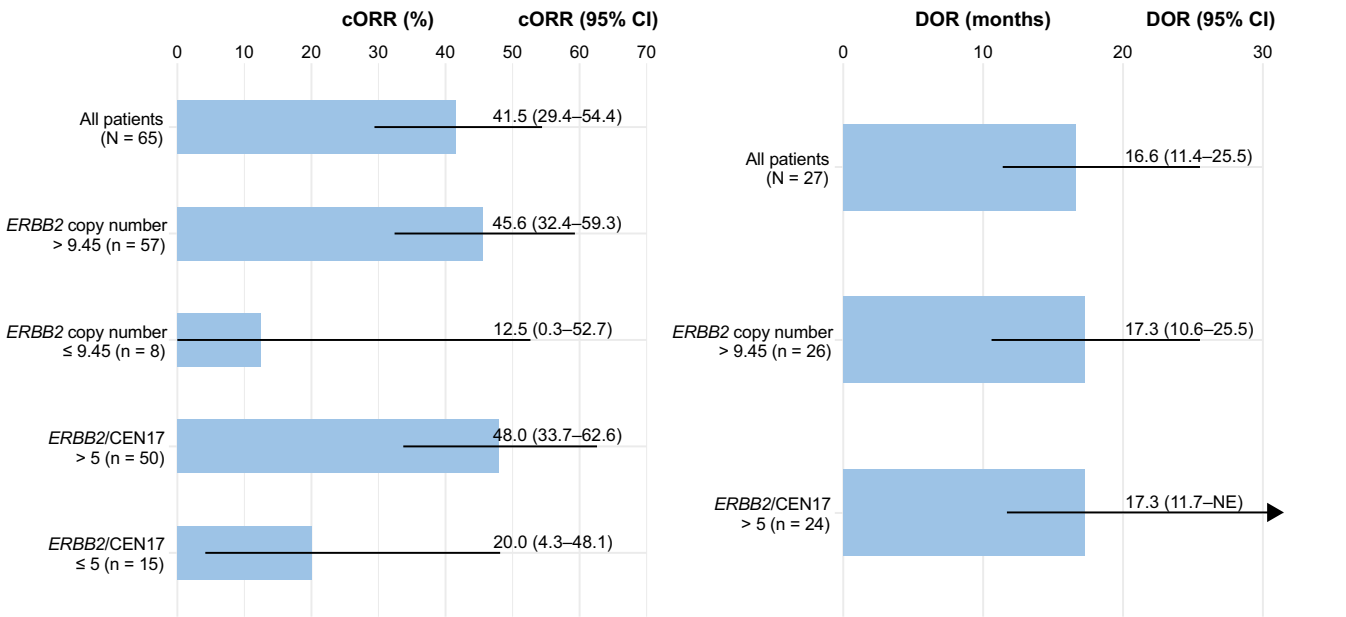


Fig. 4 | Tissue FISH assay: impact of baseline *ERBB2* copy number and *ERBB2*:CEN17 ratio on cORR and DOR. BICR blinded independent central review, CEN17 centromere of chromosome 17, CI confidence interva, cORR confirmed objective response rate, DOR duration of response, FISH fluorescence in situ hybridization, NE not estimable, RECIST Response Evaluation Criteria in Solid Tumors version 1.1. cORR (horizontal axis, left side) is the proportion of patients with complete response and partial response according to RECIST v1.1 per BICR; error bars represent 95% CIs calculated using the Clopper-Pearson exact method. DOR (horizontal axis, right side) estimated using Kaplan–Meier method and reported as median in months with corresponding 95% CI (error bars) estimated with log-log transformation. DOR subgroups not shown had < 5 responders and analyses were not performed.

5.9 months, with 4 patients having a response lasting >12 months[4]. In the TRIUMPH trial, pertuzumab and trastuzumab had a cORR of 30% in those with HER2+ disease detected by tissue-based NGS (*n* = 27) and a cORR of 28% in those with blood-based NGS-detected HER2+ disease (*n* = 25); median DORs were 12.1 months and 8.1 months, respectively[5].

Trastuzumab deruxtecan, an antibody-drug conjugate with a topoisomerase inhibitor payload, is another therapy that received FDA approval in April 2024 for the treatment of patients with unresectable or metastatic HER2+ (IHC3+) solid tumors who have received prior systemic treatment[9,10]. In the DESTINY-CRC02 phase 2 trial, trastuzumab deruxtecan demonstrated a cORR of 37.8% in 82 patients with HER2+ mCRC; median DOR was 5.5 months[11]. In patients with IHC3+ HER2 status (*n* = 64), the response rate was 46.9%, while it was only 5.6% (1 of 18 patients) in those who had IHC2+/ISH+ tumors[11]. In the primary analysis of the MOUNTAINEER trial, the cORR was 46.7% in patients who had IHC3+ tumors and were treated with tucatinib and trastuzumab, whereas it was 20.0% (3 of 15 patients) in those with IHC2+/FISH+ status[6]. These findings indicate that dual HER2 inhibition is of important clinical value and that patients who are IHC2+/FISH+ may derive meaningful clinical benefit with tucatinib and trastuzumab. Notable important differences in the populations of these trials include that approximately 40% of patients who were HER2+ received

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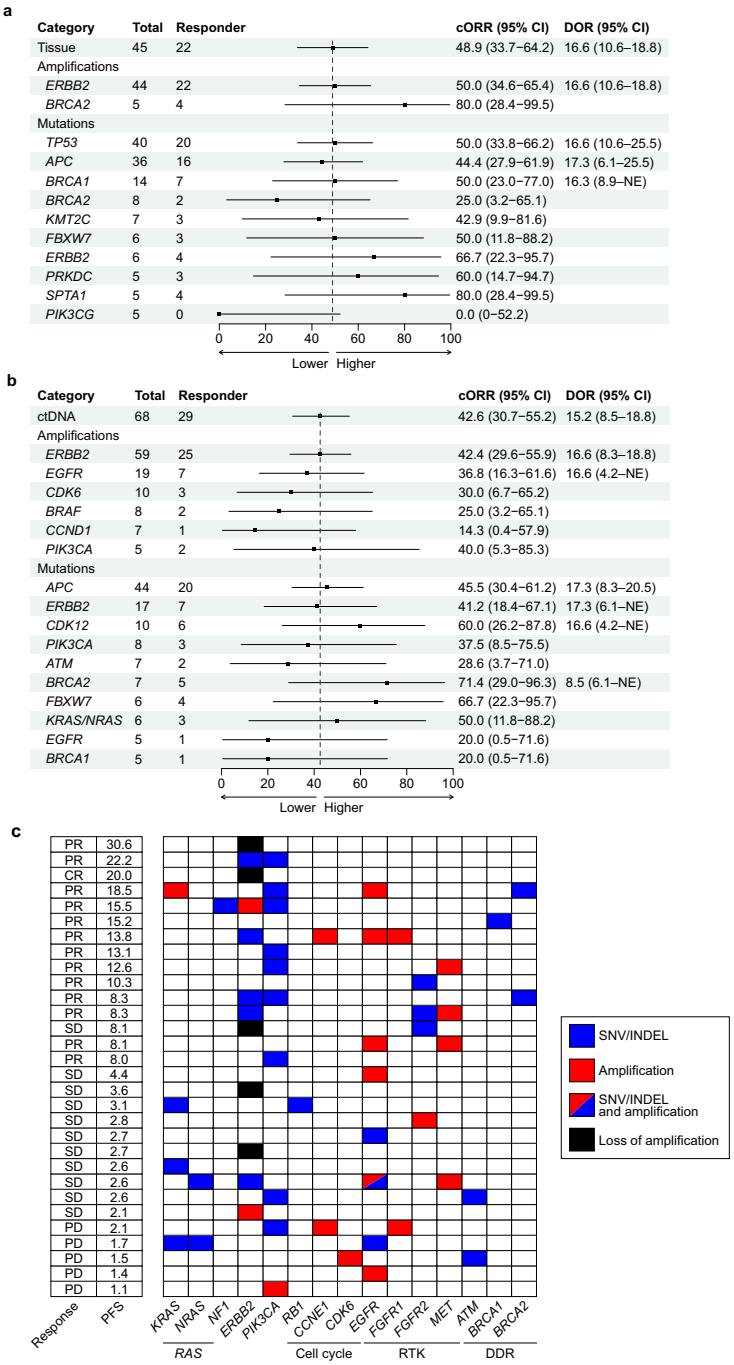


Fig. 5 | Biomarker analyses by treatment response (Cohorts A + B). BICR blinded independent central review, ctDNA circulating tumor DNA, CI confidence interval, cORR confirmed objective response rate, CR complete response, DDR DNA damage response, DOR duration of response, FISH fluorescence in situ hybridization, IHC immunohistochemistry, NE not evaluable, NGS next generation sequencing, PD progressive disease, PFS progression-free survival, PR partial response, RAS rat sarcoma, RECIST Response Evaluation Criteria in Solid Tumors version 1.1, RTK receptor tyrosine kinase, SD stable disease, SNV/INDEL single nucleotide variation/insertion or deletion. **a** Forest plot of cORR (proportion of patients with CR + PR according to RECIST v1.1 per BICR; error bars represent 95% CIs calculated using the Clopper-Pearson exact method) in patients that were HER2+ by at least 1 central assay and had baseline evaluable results by tissue-based NGS ($n = 45$). DOR estimated using Kaplan–Meier method and reported as median in months with corresponding 95% CI (error bars) estimated with log-log transformation. DOR was only calculated for groups in which there were ≥5 responders. **b** Forest plot of cORR (proportion of patients with CR + PR according to RECIST v1.1 per BICR; error bars represent 95% CIs calculated using the Clopper-Pearson exact method) in patients that were HER2+ by at least 1 central assay with baseline evaluable results by blood-based NGS ($n = 68$). DOR estimated using Kaplan–Meier method and reported as median in months with corresponding 95% CI (error bars) estimated with log-log transformation. DOR was only calculated for groups in which there were ≥5 responders. Error bars represent 95% CIs. **c** Acquired tumor alterations or loss of *ERBB2* amplification at the time of progression or end of treatment in patients with evaluable results ($n = 34$) by blood-based NGS and treatment response for selected genes. Four patients showed no gains in displayed genes and no *ERBB2* loss and are not represented; tumor biomarker alterations in the remaining 30 patients are shown. Note that 30/34 showed *ERBB2* amplification at screening and there was loss of *ERBB2* in 5/30. A single blue or red/blue box can represent multiple SNV/INDEL detections in the same gene. Mutation and amplification gains are shown; loss of amplification was only analyzed for *ERBB2*.

prior anti-HER2 therapy, and not all patients were *RAS* wild-type in the DESTINY-CRC02 trial[11], both of which were exclusion criteria in the MOUNTAINEER trial[6]. Thus, comparisons of efficacy outcomes with the DESTINY-CRC02 trial, as with those of the other aforementioned trials of dual HER2-targeted combination therapies, especially for such heavily pretreated populations, should be interpreted with caution. Future comparative trials, alongside real-world evidence studies, will be valuable for differentiating the clinical benefits of these treatments.

We observed a relatively high level of concordance (agreement ranging from 81.0% to 93.8%) in the cross-platform comparison of HER2 status determined by centrally conducted IHC/FISH, tissue- and blood-based NGS assays, with the greatest agreement observed between the tissue-based assays. Blood-based NGS testing likely has limited sensitivity to detect HER2+ in patients with low tumor burden or low ctDNA shedding into the circulation[12]. In patients where HER2+ is not detected by blood-based NGS analysis, reflex testing to a tissue-based assay should be considered, especially in heavily pretreated patients, to ensure that all patients who may benefit from tucatinib are comprehensively identified. In this study, the efficacy of tucatinib and trastuzumab was similar across all 3 central HER2 testing methods and to the overall final analysis, which was also apparent across the clinicopathologic subgroups evaluated. Our findings support using both tissue and blood-based methods to identify HER2+ mCRC. These results highlight the importance of developing a testing algorithm specific for HER2+ disease in mCRC for routine use in clinical trials and real-world practice. In a retrospective analysis of the MOUNTAINEER trial, 100% concordance was found among tissue specimens from patients tested according to breast and gastric HER2 status testing algorithms, indicating either algorithm may be potentially used to identify patients with HER2 + mCRC[13]. However, because HER2 expression, staining patterns, and *ERBB2* amplification vary across tumor types and within CRC[14,15], a consensus on the best practices for HER2 testing and interpretation in the context of mCRC is warranted for uniformity of reporting across studies. We also found that *ERBB2* gene copy number and *ERBB2*:CEN17 ratio above defined thresholds[2,16] at baseline were associated with an increased response rate to tucatinib and trastuzumab. Considering the small sample sizes in our analyses, further study is warranted to determine if *ERBB2* gene copy number and/or increased *ERBB2*:CEN17 ratio may have utility as additional biomarkers for defining HER2 status and treatment decision-making in the setting of mCRC.

In both baseline tissue- and blood-based NGS analyses, we did not find that co-occurring *ERBB2* mutations (tissue-based cORR: 66.7% [4/6]; blood-based cORR: 41.2% [7/17]) were associated with primary resistance to tucatinib and trastuzumab. Co-occurring mutations or amplifications in other genes also did not clearly affect response rate, possibly resulting from the small sample sizes for most biomarker alterations. In the few cases with larger sample sizes, the observed cORR was very similar to the overall population cORR. This was also seen in the blood-based NGS analysis. In total, there was no clear evidence of a tumor biomarker alteration profile that either promoted or abrogated treatment response to tucatinib and trastuzumab, and further research in larger populations of patients with HER2+ mCRC is needed.

At the time of progression or end of treatment, analyses of ctDNA showed that most patients acquired multiple heterogeneous potential resistance alterations, the most common being in *KRAS/NRAS*, *PIK3CA*, and genes that encode receptor tyrosine kinases. In some cases, the acquired biomarker alterations may be actionable. For example, acquired *MET* amplification ($n = 4$) is a biomarker of poor prognosis in gastric and breast cancers[17,18], and some investigational therapies have shown significant clinical activity against *MET*-amplified advanced solid tumors[19]. Notably, *ERBB2* amplification remained in most patients; only 5 of 30 patients had amplification loss with variable treatment responses, indicating that *ERBB2* downregulation does not appear to be a frequent result of tucatinib and trastuzumab treatment. This may have implications for the treatment sequencing of tucatinib and trastuzumab treatment versus other HER2-targeted agents in treatment plans.

This study was an open-label design and without the power for a formal comparative study, constraining any across-trial efficacy comparisons with other HER2-targeted therapies. Secondly, because central testing was not required for eligibility in the MOUNTAINEER trial, some HER2-negative patients by central testing were enrolled in this study based on locally determined HER2 status. Finally, not all patients had blood and/or tissue specimens available for the biomarker analyses.

The findings from this final analysis of the MOUNTAINEER trial are consistent with the primary analysis demonstrating that tucatinib plus trastuzumab is clinically active and well tolerated in patients with HER2+, *RAS* wild-type mCRC. This further strengthens the clinical rationale for dual HER2 inhibition. Tucatinib and trastuzumab showed durable and clinically meaningful efficacy that can be achieved with a dual HER2-targeted chemotherapy-free strategy. Efficacy was observed irrespective of central HER2+ testing methods and heterogeneous tumor biomarker profiles. This analysis supports further investigation of tucatinib in combination with trastuzumab in earlier lines of therapy. The phase 3 MOUNTAINEER-03 trial (NCT05253651) has been initiated towards this goal. It will compare the efficacy and safety of tucatinib plus trastuzumab in combination with 5-fluorouracil, leucovorin, and oxaliplatin (mFOLFOX6) with standard-of-care chemotherapy in the first-line treatment of HER2+ mCRC.

Methods

Study design and participants

MOUNTAINEER was a multicenter, open-label, phase 2, interventional clinical trial conducted at 56 hospitals and clinics in 5 countries (USA, France, Italy, Belgium, and Spain; MOUNTAINEER study sites and investigators provided in Supplementary Table 5).

Study design, inclusion and exclusion criteria, and the methodology of the MOUNTAINEER trial have been previously reported[6]. Briefly, key inclusion criteria were ≥18 years of age, HER2+, *RAS* wild-type, unresectable or metastatic CRC previously treated with fluoropyrimidines, oxaliplatin, irinotecan, an antivascular endothelial growth factor (VEGF) monoclonal antibody, and an anti-programmed death ligand 1 (PD-L1) or anti-PD-1 monoclonal antibody if the tumor had mismatch repair deficient proteins or had high microsatellite instability. HER2 positivity was determined locally by ≥1 positive tissue-based HER2 test, defined as having a score of 3+ by IHC or 2+ by IHC plus amplification by FISH or chromogenic in situ hybridization, or amplification by tissue-based NGS. Key exclusion criteria were previous treatment with HER2-directed therapies and a left ventricular ejection fraction of less than 50% documented within 28 days before treatment. The MOUNTAINEER trial began with a single Cohort A; after an expansion amendment after an interim analysis of antitumor activity[20], Cohorts B and C were added.

Treatment interventions

Patients in Cohort A were assigned to receive tucatinib (300 mg orally twice daily) in combination with trastuzumab (8 mg/kg IV as the initial loading dose, then 6 mg/kg every 21 days) with no randomization. Patients were randomly assigned to Cohorts B and C in a 4:3 manner. Patients in Cohort B received tucatinib in combination with trastuzumab as in Cohort A, while those in Cohort C received tucatinib monotherapy (300 mg orally twice daily). Patients treated with tucatinib plus trastuzumab continued treatment until evidence of radiographic or clinical progression, unacceptable toxicity, or death. Patients in Cohort C were allowed to crossover to receive trastuzumab in combination with tucatinib if they had radiographic tumor

progression at any time point or if they had not had a complete or partial response per investigator assessment by 12 weeks.

Endpoints

The primary endpoint of this study was cORR, defined as the proportion of patients with confirmed complete response or partial response, per BICR according to the Response Evaluation Criteria in Solid Tumors (RECIST; version 1.1), in Cohorts A + B. Secondary endpoints for Cohorts A + B included DOR, defined as the time from the first confirmed objective response until progressive disease, assessed per BICR, or death from any cause, whichever occurred first, ORR per BICR at 12 weeks, PFS, defined as the time from start of study treatment (Cohort A) or randomization (Cohort B) to progressive disease, assessed per BICR, or death from any cause, whichever occurred first, and OS. In Cohort C, cORR and DOR per BICR were also assessed after crossover. Safety endpoints for all study cohorts included frequency and severity of TEAEs, serious AEs, deaths due to an AE, treatment modifications and discontinuations, laboratory abnormalities, and vital signs. AEs were defined according to the Medical Dictionary for Regulatory Activities (version 24.0) and the National Cancer Institute Common Terminology Criteria for AEs (version 4.03). The efficacy and safety endpoints evaluated remained the same in the final analysis. Biomarker analyses were exploratory.

Exploratory analyses of HER2 testing methods with treatment response rates

Archival or fresh patient tumor tissue and/or blood specimens were submitted when available to a sponsor-designated central laboratory for confirmatory HER2 testing, which was done retrospectively by three different methods: IHC/FISH, tissue-based NGS and/or blood-based NGS (ie, analysis of ctDNA). An FDA-approved or CE-marked HER2 IHC test was used for tissue specimens following the package insert's interpretational manual for breast cancer. IHC staining was performed using the Roche Pathway anti-HER-2/neu (4B5) rabbit monoclonal antibody on the Ventana Benchmark Ultra (Ventana Medical Systems, Tucson, AZ, USA). For FISH, Dako HER2 IQFISH pharmDX (Dako North America, Carpinteria, CA, USA) was used per package insert instructions.

HER2 assay agreement

HER2 status, defined as the final HER2 overexpression/amplification status, could be either positive, negative, or not determined. For central IHC and FISH testing, HER2 status was determined as follows: HER2+ defined as IHC2+ with FISH amplification or IHC3+ with any FISH result; HER2-negative defined as IHC0, IHC1, or IHC2+ without FISH amplification; and HER2 not determined as IHC2+ without evaluable FISH results.

Exploratory biomarker analyses

ERBB2 copy number and *ERBB2*:CEN17 ratio were determined with FISH using the cutoff points for HER2 positivity by *ERBB2* copy number (>9.45 vs ≤9.45) and *ERBB2*:CEN17 ratio (>5 vs ≤5) were as previously used[2,16]. cORR and DOR per BICR were determined for the patient subgroups stratified by these definitions of HER2+ disease.

Tissue- and blood-based NGS analyses were performed to interrogate the amplification and mutation status of a panel of oncogenes and tumor suppressor genes associated with tumor growth, survival and resistance to targeted therapeutics. Tissue-based NGS determination of amplifications and sequence alterations were from internal pipelines used by PGDx (elio Tissue Complete; Supplementary Table 6). Blood-based NGS analyses of amplifications and sequence alterations (mutations and insertions/deletions) were from the standard analysis pipelines used by Guardant (Guardant360 CDx Test; Supplementary Table 7). For exploratory biomarker analyses, the patients with any positive central HER2 test were used as the analysis set. cORR was only examined for gene alterations found in ≥5 patients to limit spurious findings. Similarly, DOR was only calculated for gene or other biomarker alterations with ≥5 patients with complete and/or partial responses. For blood-based NGS analyses, *TP53* was excluded due to concerns with contamination by background clonal hematopoiesis of indeterminate potential (CHIP). Synonymous mutations were excluded except those indicated as potentially affecting transcript splicing. cORR and DOR were stratified by patients with identified preexisting or acquired biomarker amplifications/mutations.

Statistical analyses

Reported cORRs represent the proportion of patients with confirmed complete response or partial response in the study cohort or subgroup (eg, testing method subset, clinicopathologic subgroup, etc.). Two-sided 95% CIs were calculated using the Clopper-Pearson exact method. The Kaplan–Meier method was used to estimate DOR, PFS, and OS. The complementary log-log transformation was used to estimate the associated 95% CIs. Safety results were summarized with descriptive statistics. Pairwise comparisons across HER2 testing methods were conducted using data from patients who had results (eg, positive or negative) from both assays. Results are reported as percent agreement with 95% binomial CIs calculated using the Clopper-Pearson exact method. All analyses except those of biomarkers 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).

The trial protocol and statistical analysis plan are available in the Supplementary information.

Ethics statement

The study was conducted in accordance with regulatory requirements and International Conference on Harmonization Good Clinical Practice guidelines. All patients provided written informed consent and were not compensated for their participation. Institutional review boards and ethics committees approved the protocol at each participating study site. The trial is registered with ClinicalTrials.gov (NCT03043313).

Reporting summary

Further information on research design is available in the Nature Portfolio Reporting Summary linked to this article.

Data availability

Upon request, and subject to review, Pfizer will provide the data that support the findings of this study. The protocol and statistical analysis plan are available in the Supplementary information. Pfizer may also provide access to the related individual deidentified participant data. The deidentified participant data will be made available to qualified researchers whose proposals meet the research criteria and other conditions, and for which an exception does not apply, via a secure portal. Due to patient privacy/lack of consent, sequencing data and source data are not available with the article but upon request, and subject to review, may be made accessible via the Vivli platform as per below. Qualified researchers' proposals are submitted via the Vivli platform. To obtain Pfizer data for analysis on the Vivli platform, data requestors must complete a data request on the Vivli platform. Pfizer will review the request using an internal committee, composed of Pfizer colleagues who are responsible for the asset program, statistician, and data sharing experts. All those receiving access to data will be required to enter into a Data Use Agreement (DUA). Upon execution of the DUA, Pfizer will upload anonymized data into the Vivli platform for use by researchers. See https://www.pfizer.com/science/clinical-trials/trial-data-and-results/data-requests for more information on legitimate research purposes and reasons that would preclude granting access in response to a data request.

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Competing interests

J.H.S. reported being in a consultant or advisory role for Abbvie, Astellas, AstraZeneca, Bayer, Beigene, Daiichi-Sankyo, Eli Lilly, GE Healthcare, GSK, Johnson & Johnson, Jazz Pharmaceuticals, Merck, Natera, Pfizer, Roche/Genentech, Regeneron, Sanofi, Taiho, Takeda, Xilio Therapeutics; stock options from Triumvira Immunologics; research funding/contracted research from Abbvie, Amgen, AStar D3, Bayer, Beigene, Curegenix, Daiichi-Sankyo, Eli Lilly, Erasca, GSK, Leap Therapeutics, Novartis, Pfizer, Quanta Therapeutics, Revolution Medicines, and Roche/Genentech. A.C. reported grants paid to their institution by Seagen, GlaxoSmithKline, Inspira (previously RGenix); receiving consultancy and advisory fees from Bayer, Merck, Seagen, GlaxoSmithKline, Janssen, and G1 Therapeutics. S.S. reported participating on advisory boards for Agenus, AstraZeneca, Bayer, Bristol Myers Squibb, CheckmAb, Daiichi-Sankyo, Guardant Health, Menarini, Merck, Novartis, Pierre-Fabre, Roche-Genentech, and Seagen. T.A. reported receiving honoraria from Bristol Myers Squibb, GlaxoSmithKline, Merck & Co. Inc., Merck Serono, Roche, Sanofi, Seagen,

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Additional information

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