Tucatinib plus trastuzumab for HER2-positive metastatic colorectal cancer (MOUNTAINEER): A multicentre, open-label, phase 2 study

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Summary

Background

HER2 is an actionable target in metastatic colorectal cancer (mCRC). The activity of tucatinib plus trastuzumab in patients with HER2-positive mCRC was assessed.

Methods

MOUNTAINEER is a global, open-label, phase 2 study conducted at 56 sites in 5 countries in patients with chemotherapy-refractory, HER2-positive *RAS* wild-type mCRC receiving tucatinib (300mg PO BID) plus intravenous trastuzumab (8mg/kg initially then 6mg/kg Q3W). The study consisted of one cohort treated with tucatinib plus trastuzumab (cohort A) and then expanded to randomized cohorts treated with tucatinib plus trastuzumab (cohort B) or tucatinib monotherapy (cohort C). Randomisation was performed using Interactive Web Response Systems with a 4:3 ratio, stratified by primary tumour location. Full analysis set (FAS) consisted of patients with HER2-positive disease who received study treatment. Primary endpoint was confirmed objective response rate (cORR) per blinded independent central review (BICR) for cohorts A+B. was assessed in patients. This trial is registered with ClinicalTrials.gov, NCT03043313.

Findings

From August 8, 2017, to September 22, 2021, 45 patients in cohort A, 41 in B, and 31 in C were enroled. Median age was 56·0 years; 57·9% were male, and 77·2% were White. Demographics were similar across all cohorts. As of March 28, 2022, in 84 patients from cohorts A+B in FAS,

cORR per BICR was 38·1% (3 complete and 29 partial responses; 95% CI, 27·7-49·3). The most common adverse events for tucatinib plus trastuzumab was diarrhoea (55/86; 64·0%). The most common grade ≥3 event was hypertension (6/86; 7·0%). Three (3·5%) patients had treatment-related serious events. No deaths occurred because of adverse events.

Interpretation

Tucatinib plus trastuzumab demonstrated clinically meaningful antitumour activity and favorable tolerability. This treatment is the first FDA-approved anti-HER2 regimen for mCRC and represents an important new treatment option for chemotherapy-refractory HER2-positive mCRC.

Funding

Seagen Inc. and Merck & Co.

Introduction

Colorectal cancer is a leading cause of cancer death worldwide.[1] Approximately 20% of patients with colorectal cancer have metastatic disease at diagnosis, and up to 40% of patients initially diagnosed with locoregional colorectal cancer develop recurrent disease.[2,3] For patients with metastatic colorectal cancer refractory to current chemotherapy-based standard-of-care regimens, treatment options generally have limited efficacy.[4-7] Therapies that exploit actionable biomarkers may offer favorable tolerability and improved treatment outcomes.[4,5,8]

Human epidermal growth factor receptor 2 (HER2) is encoded by the *ERBB2* gene, and overexpression of HER2 (HER2-positive) occurs because of *ERBB2* gene amplification. HER2 is an important oncogenic driver in breast and gastric cancers and has emerged as a target in colorectal cancer.[8] Approximately 3% to 5% of patients with metastatic colorectal cancer have HER2-positive tumours,[4,5,9] but the incidence is higher for those with *RAS* wild-type tumours.[4,5]

Extensive preclinical studies have examined the activity of anti-HER2 agents in cell lines and patient-derived xenograft models of colorectal cancer.[10-12] These studies demonstrate that a single anti-HER2 agent is much less effective for inhibiting tumour growth than dual-HER2 inhibition. Based on these data, HER2-targeting strategies in metastatic colorectal cancer have primarily focused on combining anti-HER2 agents. Trastuzumab with lapatinib or pertuzumab has demonstrated clinically meaningful antitumour activity in patients with HER2-positive metastatic colorectal cancer. [9,13-16] Additionally, the antibody-drug conjugate trastuzumab deruxtecan has demonstrated clinical activity.[17] These clinical findings have led to inclusion of

trastuzumab-based doublets or antibody-drug conjugates as treatment options for HER2-positive metastatic colorectal cancer in established guidelines.[4,5]

Tucatinib is an oral, potent tyrosine kinase inhibitor highly selective for HER2.[18] Tucatinib is approved in combination with trastuzumab and capecitabine for HER2-positive metastatic breast cancer in multiple regions and is being studied in other HER2-positive cancers. Preclinical work has examined the activity of tucatinib and trastuzumab individually and in combination in HER2-positive tumour models, including colorectal cancer.[12] Consistent with other HER2-directed agents, tucatinib and trastuzumab resulted in greater activity in combination than individually.

The MOUNTAINEER study (NCT03043313) evaluated antitumour activity and safety of the combination of tucatinib plus trastuzumab in patients with HER2-positive metastatic colorectal cancer refractory to standard-of-care chemotherapy.

Methods

Study design and participants

MOUNTAINEER is an open-label phase 2 study conducted at 56 sites in the United States, France, Italy, Belgium, and Spain. Eligible patients were ≥18 years of age with HER2-positive, *RAS* wild-type, unresectable/metastatic colorectal cancer previously treated with fluoropyrimidines, oxaliplatin, irinotecan, an anti-vascular endothelial growth factor monoclonal

antibody, and an anti-programmed cell death ligand 1 or anti-programmed cell death protein 1 monoclonal antibody if the tumour had deficient mismatch repair proteins or was microsatellite instability -high. Prior use of anti-epidermal growth factor receptor antibodies was not required. Patients had measurable disease defined by Response Evaluation Criteria in Solid Tumours (RECIST, version 1·1)[19] and an Eastern Cooperative Oncology Group Performance Status (ECOG PS) score of ≤2. Patients were excluded if they had received prior HER2-directed therapies or had a left ventricular ejection fraction <50% documented ≤28 days before treatment. A complete list of eligibility criteria is provided in the protocol (appendix p 21).

The study began as an investigator-sponsored study on Jun 23, 2017, and consisted of a single cohort (cohort A) in which patients were treated with tucatinib plus trastuzumab. Study sponsorship was transferred to Seagen Inc. on Sep 17, 2019 following an interim analysis of antitumour activity.[20] Changes to support a regulatory approval included more patients treated with tucatinib plus trastuzumab (cohort B), allowing a more precise estimation of the antitumour activity and safety of the combination therapy. As an exploratory objective, cohort C was added to describe the antitumour activity and safety of tucatinib as a single agent in the same population. Patients were randomly assigned 4:3 via Interactive Web Response Systems to receive either tucatinib plus trastuzumab (cohort B) or tucatinib monotherapy (cohort C; Figure 1), respectively. Patients were stratified by left-sided primary versus all other primary sites (transverse, right-sided, and multiple primaries). No blinding or masking was performed.

HER2 status of tumour tissue was determined at a local Clinical Laboratory Improvement Amendments certified or International Organization for Standardization accredited laboratory. Eligible patients had ≥1 positive HER2 tests, defined by a score of 3+ by immunohistochemistry (IHC), 2+ by IHC plus amplification by in situ hybridisation (ISH), or amplification by next-generation sequencing (NGS).

The study was conducted in accordance with regulatory requirements and International Conference on Harmonisation Good Clinical Practice guidelines. All patients were provided written informed consent. The protocol was approved by institutional review boards and ethics committees at each participating study site.

Procedures

Patients were treated with either tucatinib (300 mg orally twice daily) plus trastuzumab (8 mg/kg intravenously as initial loading dose, then 6 mg/kg every 21 days) or tucatinib monotherapy (300 mg orally twice daily). Patients treated with tucatinib plus trastuzumab continued treatment until evidence of radiographic or clinical progression. Patients deriving clinical benefit per investigator assessment could continue treatment through radiographic progression. Patients assigned to receive tucatinib monotherapy were allowed to receive trastuzumab in addition if they experienced radiographic tumour progression at any time point or had not achieved a complete or partial response (ie, stable disease) per investigator assessment by 12 weeks. The 12-week timepoint was chosen based on time-to-response observations from the interim results[20] of cohort A.

Contrast-enhanced computed tomography or magnetic resonance imaging scans were conducted at baseline and every 9 weeks (±2 weeks) for cohort A and at baseline and every 6 weeks (±1 week) for cohorts B and C until disease progression, death, withdrawal of consent, study closure, or alternative therapy. There were no restrictions on subsequent therapies that a patient could receive after discontinuing the study. Patients in cohort C had a new baseline disease assessment before receiving trastuzumab in addition to tucatinib. Disease response and progression were assessed according to RECIST version 1·1 per blinded independent central review (BICR) and investigator. For an exploratory endpoint, patient tumour tissue was submitted to a central laboratory for retrospective confirmatory HER2 testing. An FDA-approved or CE-marked HER2 IHC test was used, following the package insert's interpretational manual for breast cancer. The IHC staining was performed using Ventana Benchmark Ultra. For FISH, Dako HER2 IQFISH pharmDX was used per package insert instructions. No instruments were used for automated processing for FISH.

Safety was assessed based on the incidence of adverse events, defined according to the *Medical Dictionary for Regulatory Activities* and the National Cancer Institute Common Terminology Criteria for Adverse Events (version 4·03). Changes in vital signs, laboratory results, ECOG PS scores, and cardiac ejection fraction results were assessed. Cardiac ejection fraction was assessed by using a multigated acquisition scan or echocardiogram.

Outcomes

The primary endpoint was confirmed objective response rate (defined as the proportion of patients with confirmed complete response or partial response per BICR, with confirmation of response required ≥4 weeks from the first documentation of response) in patients who had HER2-positive disease and treated with tucatinib plus trastuzumab. Secondary endpoints for patients treated with tucatinib plus trastuzumab included duration of response (defined as the time from first confirmed objective response until progressive disease, assessed per BICR, or death from any cause, whichever occurred first), objective response rate per BICR at 12 weeks, progression-free survival (defined as the time from start of study treatment [cohort A] or randomisation [cohort B] to progressive disease, assessed per BICR, or death from any cause, whichever occurred first), and overall survival (defined as the time from start of study treatment or randomisation to death from any cause). Exploratory endpoints included subgroup analyses for the confirmed objective response rate, and the change from baseline in assessments of the European Organisation for Research and Treatment of Cancer Quality-of-Life 30-item core questionnaire (EORTC QLQ-C30) for patients in cohort B. Other exploratory endpoints, such as pharmacokinetics, biomarkers, and health resource utilization will be reported elsewhere.

Objective response rate per BICR by 12 weeks was a secondary endpoint for patients treated with tucatinib monotherapy. . Exploratory endpoints include progression-free survival and overall survival for patients treated with tucatinib monotherapy and confirmed objective response rate per BICR after addition of trastuzumab. Safety endpoints included frequency and severity of treatment-emergent adverse events, serious adverse events, deaths due to events, treatment discontinuations, laboratory abnormalities, and vital signs and were assessed in all patients who received study treatment.

Statistical analysis

Approximately 80 patients were to be treated with tucatinib plus trastuzumab, which would provide a 95% CI within 12% of the expected overall response rate of 40%. Since approximately 40 patients were enroled in cohort A at the time of the study's expansion, 40 additional patients were to be enroled in cohort B. For cohort C, approximately 30 patients were to be treated with tucatinib, which would provide a 95% CI within 8% of the expected overall response rate of 10%. Sample size calculations were performed using EAST, version 6·0. The primary analysis was to be performed after all treated patients were followed for ≥6 months or discontinued from the study. For the primary endpoint of confirmed objective response rate, point estimate and exact two-sided 95% CI were calculated using the Clopper–Pearson method.

The Kaplan-Meier method was used to estimate the secondary endpoints of duration of response, progression-free survival, and overall survival. The complimentary log-log transformation was used to estimate the associated 95% CIs. Antitumour activity analyses were performed for all patients who received any amount of study treatment and had HER2-positive tumours defined by ≥1 protocol-required local tests. The reduction of tumour size was assessed in those with baseline and postbaseline target lesion measurements. Patients missing the postbaseline measurements were treated as nonresponders. Antitumour activity assessed by investigators (objective response rate, duration of response, and progression-free survival) was prespecified, and the difference between the BICR and investigator assessments were to be summarised descriptively. The prespecified subgroup analyses of confirmed response rate were age, baseline ECOG performance score, primary site of disease, and geographic region. Subgroup analysis by *BRAF* mutation status was not feasible due to limited overlap with HER2-positive tumours.

Safety analyses were performed for all patients who received any amount of study treatment. There were no formal statistical comparisons between patients treated with tucatinib plus trastuzumab and patients treated with tucatinib monotherapy. All endpoints were prespecified except disease control rate; confirmed objective response per investigator; and subgroup analyses for the confirmed objective response rate stratified by HER2 IHC and prior anti-epidermal factor receptor (EGFR) therapy. Disease control rate for both cohorts A+B and cohort C was a post hoc analysis, defined as the proportion of patients with confirmed response or stable disease. All analyses were done using SAS version 9·3 or higher. This study is registered with ClinicalTrials.gov, NCT03043313.

Role of the funding source

This study began as an investigator-sponsored study and was transferred to Seagen Inc. on September 17, 2019. In November of 2019, the study was expanded. The study was funded by Seagen Inc. in collaboration with Merck Sharp & Dohme LLC, a subsidiary of Merck & Co., Inc. Seagen Inc. participated in the study design, data collection, data analysis, data interpretation, and writing of the manuscript.

Results

From August 8, 2017, to September 22, 2021, a total of 117 patients were enroled (Figure 1), and 114 patients had locally assessed HER2-positive disease and received treatment. Demographic and disease characteristics at baseline are presented in Tables 1 and appendix p 7. For the overall patient population, the median age was 56·0 years; 57·9% were male, and 77·2% were White.

The demographics were similar across all cohorts (appendix p 7). Liver metastases were reported in 69 (60·5%) of 114 patients and lung metastases in 79 (69·3%) of 114 patients. Patients had received a median of three prior regimens of systemic therapy in any setting (interquartile range [IQR], 2·0-3·0). As of data cutoff (March 28, 2022), the overall study median duration of follow-up was 16·3 months (IQR, 10·8-28·2).

Patients were enroled into the study with ≥1 HER2-positive local test results. Sixty-nine patients had HER2 amplification detected by tissue-based NGS, 46 had IHC 3+, and 36 had ISH amplification. Local HER2 testing results by assay methods used to determine eligibility are summarized in appendix p 9.

At data cutoff, 86 patients assigned to receive tucatinib plus trastuzumab had received ≥1 dose of study treatment, of whom 84 were HER2 positive per protocol-defined testing criteria. Two patients were excluded from the antitumour activity analysis because of negative local HER2 test results. Thirty patients had received ≥1 dose of tucatinib monotherapy (Figure 1).

The confirmed objective response rate per BICR for 84 patients treated with tucatinib plus trastuzumab was 38·1% (95% CI, 27·7-49·3; Table 2); 3 (3·6%) of 84 patients had a complete response and 29 (34·5%) had a partial response. This was consistent with the confirmed objective response rate by investigator assessment (42·9% [95% CI, 32·1-54·1]; 2 [2·4%] complete responses and 34 [40·5%] partial responses). Reduction from baseline in target lesion size was observed in 65·0% of patients (52 of 80 patients; Figure 2). In addition, the response

rates were consistent across prespecified subgroups (appendix p 4). As part of a post hoc analysis, the response rate was 36·4% (95% CI, 22·4-52·2) in patients who received prior anti-EGFR therapies and 40·0% (95% CI, 24·9-56·7) in those who did not.

Tissue samples were available for retrospective central testing in 70 of 84 patients treated with tucatinib plus trastuzumab. Objective response rates (post hoc analyses) were 46·7% (21 of 45 patients; 95% CI, 31·7-62·1) in patients with IHC 3+ tumours, 20·0% (3 of 15 patients; 95% CI, 4·3-48·1) in patients with IHC 2+/ISH+ tumours, and 10·0% (1 of 10 patients; 95% CI, 0·3-4·5) in patients with HER2-negative tumours (appendix p 10).

The median time to a confirmed response was 2·1 months (IQR, 2·0-3·6), and the objective response rate by 12 weeks of treatment was 28·6% (95% CI, 19·2-39·5; appendix p 11). The median duration of response per BICR was 12·4 months (IQR, 8·3-25·5) and per investigator assessment was 15·4 months (IQR, 6·2-not achieved). The median progression-free survival per BICR was 8·2 months (95% CI, 4·2-10·3; Figure 3A), with 59 (70·2%) events of 84 patients at data cutoff. The median progression-free survival per investigator assessment was 7·0 months (95% CI, 4·3-9·7). The median overall survival was 24·1 months (95% CI, 20·3-36·7; Figure 3B), with 38 (45·2%) events of 84 patients at data cutoff.

At data cutoff, 65 (77·4%) of 84 patients were off treatment. Thirty-eight (58·5%) off-treatment patients received a subsequent, systemic, anticancer therapy, including 18 (27·7%) patients who

received subsequent HER2-directed therapy. The full list of subsequent therapies is presented in appendix p 12.

The safety summary is presented in appendix p 13. The median duration of treatment was 6·9 months (IQR, 2·8-15·1) for tucatinib, and 6·7 months (IQR, 2·8-15·2) for trastuzumab. The most frequent treatment-emergent adverse events (≥20%) were diarrhoea in 55 (64·0%) of 86 patients, fatigue in 38 (44·2%) patients, nausea in 30 (34·9%) patients, and infusion-related reaction in 18 (20·9%) patients. The most common grade ≥3 adverse event was hypertension. Treatment-emergent adverse events are summarized in Table 3 and appendix p 14. Serious treatment-emergent events were observed in 19 (22·1%) of 86 patients; 3 (3·5%) of 86 patients had serious events that were treatment-related (appendix p 15). Adverse events leading to tucatinib discontinuation occurred in five (5·8%) of 86 patients and included alanine aminotransferase (ALT) increase in two (2·3%) patients, COVID-19 pneumonia in one (1·2%) patient, cholangitis in one (1·2%) patient, and fatigue in one (1·2%) patient. No deaths were attributed to adverse events.

Events of diarrhoea were grade 1 in 43 (50·0%) of 86 patients or grade 2 in nine (10·5%) and manageable with standard supportive care. Events of grade 3 diarrhoea occurred in three (3·5%) of 86 patients. No event of grade ≥4 diarrhoea was reported, and no patient discontinued treatment because of diarrhoea. Diarrhoea leading to tucatinib dose reductions occurred in two (2·3%) of 86 patients and tucatinib dose holds in three (3·5%).

Elevations in ALT, aspartate aminotransferase (AST), and total bilirubin occurred in five (5·8%) patients, five (5·8%) of 86 patients, and one (1·2%) patient, respectively. These events were generally reversible, asymptomatic, and manageable with dose modifications. Hepatotoxicity leading to tucatinib dose reductions occurred in three (3·5%) of 86 patients and dose holds in four (4·7%). No Hy's Law cases were identified.

Hypertension was observed in 15 (17·4%) of 86 patients. All events occurred in patients with history of hypertension or elevated blood pressure at baseline. Hypertension leading to tucatinib dose hold occurred in one (1.2%) of 86 patients, and no tucatinib dose reductions or discontinuations due to hypertension were reported. Decreased asymptomatic left ventricular ejection fraction leading to tucatinib dose modification or discontinuation occurred in two (2·3%) of 86 patients.

Thirty-seven patients treated with tucatinib plus trastuzumab in cohort B were assessed for patient-reported outcomes. The mean changes from baseline during the treatment period for EORTC QLQ-C30 generally remained stable across all scales, including global health status/quality of life (appendix p 5) and functional domains.

By 12 weeks, the objective response rate per BICR for 30 patients treated with tucatinib monotherapy was 3·3% (95% CI, 0·1-17·2; appendix p 15), with one patient with partial response. As only one patient treated with tucatinib monotherapy responded to the treatment and this patient was censored by the data cutoff date, the duration of response is not included in this

report. Reduction from baseline in target lesion size was observed in 13 (46·4%) of 28 patients (appendix p 6). After addition of trastuzumab, the confirmed objective response rate per BICR was 17·9% (95% CI, 6·1-36·9; appendix p 16). The median progression-free survival for patients treated with tucatinib monotherapy is not included in this report, as 18 (60·0%) of 30 patients have crossed over to receive trastuzumab without documented progression and were censored. The median overall survival for patients treated with tucatinib monotherapy and tucatinib plus trastuzumab post-monotherapy was 21·1 months (95% CI, 18·6-not achieved; appendix p 17).

The safety summary is presented in appendix p 18. The median duration of tucatinib treatment was 2·8 months (IQR, 1·5-2·9). The most frequent treatment-emergent adverse events (≥20%) were diarrhoea in ten (33·3%) of 30 patients, abdominal pain in six (20·0%), and fatigue in six (20·0%), and all were grade 1 or 2 (Table 4). The most common adverse events of grade ≥3 were ALT increase and AST increase, both in two (6·7%) of 30 patients. No adverse events led to tucatinib discontinuation or death. Among patients who crossed over from tucatinib monotherapy to receive trastuzumab in addition to tucatinib, adverse events leading to tucatinib discontinuations occurred in two (7·1%) of 28 patients and included ALT increase in one (3·6%) and AST increase in one (3·6%). One (3·3%) of 30 patients treated with tucatinib monotherapy had a treatment-related serious adverse event (overdose; appendix p 20). No tucatinib-related serious adverse events were reported for patients who crossed over from tucatinib monotherapy to additional receive trastuzumab. Two (7·1%) of 28 patients had trastuzumab-related serious events, one (3·6%) cardiac failure and one (3·6%) ejection fraction decreased. No deaths were attributed to adverse events.

Discussion

In patients with HER2-positive and *RAS* wild-type metastatic colorectal cancer, the combination of tucatinib and trastuzumab demonstrated clinically meaningful and durable clinical activity with favorable tolerability and safety. The confirmed objective response rate per BICR was 38·1%, with a median response duration of 12·4 months. Notably, the median progression-free survival was 8.2 months, and the median overall survival was 24·1 months. The regimen was well tolerated; only five patients (5·8%) discontinued the treatment because of adverse events, and there were no deaths due to adverse events. Diarrhoea was the most commonly observed adverse event but was mostly low-grade and manageable with standard-of-care interventions. No patients discontinued treatment because of diarrhoea. Of note, anti-diarrhoeal prophylaxis was not required per protocol. The most common reason for treatment discontinuation was disease progression, highlighting the need to better understand and treat mechanisms of acquired resistance.

Overall, quality of life was also not adversely impacted by the regimen. Consistent with data for tolerability and quality of life, the majority (58·5%) of patients at the time of progression were able to receive subsequent anticancer therapies, and 27·7% received an additional anti-HER2 therapy. The ability of patients to remain fit enough to receive subsequent treatments may have contributed to the encouraging overall survival.

Treatment of colorectal cancer continues to evolve with the emergence of biomarker-driven strategies, including promising results from HER2-targeted therapies.[9,13-17,21] To our knowledge,

MOUNTAINEER is the largest prospective study to date to evaluate a chemotherapy-free HER2-targeted regimen in patients with unresectable or metastatic HER2-positive colorectal cancer. [9,13-17,21] Dual-HER2 inhibition with trastuzumab-based combinations have been studied extensively, and studies have consistently reported objective response rates of approximately 10% to 32%.[9,13-16,21] Acknowledging the differences in study designs, the confirmed objective response rate by BICR, progression-free survival, and duration of response achieved with tucatinib plus trastuzumab in MOUNTAINEER compares favorably with the previous observations,[9,13-16,21] including trastuzumab deruxtecan, which yielded numerically comparable objective response rate as tucatinib and trastuzumab [17] These promising antitumour activity and safety outcomes have resulted in tucatinib plus trastuzumab being the first treatment option approved by the United States Food and Drug Administration and recommended by established guidelines for patients with chemotherapy-refractory HER2-positive, *RAS* wild-type metastatic colorectal cancer.

The literature describing single-agent activity of HER2-directed therapies in HER2-positive metastatic colorectal cancer is sparse and primarily limited to individual case reports. This is due to the limited activity of HER2-directed monotherapy observed in preclinical studies and the positive clinical data from studies utilizing dual HER2 inhibition. To our knowledge, MOUNTAINEER is the first study to evaluate a single, non-cytotoxic agent targeting the HER2 pathway in this population. Based on preclinical observations,[10-12] trastuzumab monotherapy in patients with HER2-positive metastatic colorectal cancer was expected to be ineffective. Inclusion of trastuzumab-based doublets in established treatment guidelines made further

exploration of monotherapy activity infeasible. However, the activity of tucatinib monotherapy in colorectal cancer was unknown and therefore explored in the MOUNTAINEER study.

Consistent with the preclinical observations for trastuzumab monotherapy,[10-12] the objective response rate with tucatinib monotherapy was lower, even though disease stabilization was observed in most patients. Radiographic responses increased after the addition of trastuzumab in patients treated with tucatinib monotherapy; however, dual-HER2 inhibition at the initiation of study treatment achieved a more robust, durable reduction in tumour burden, suggesting that concurrent initiation of dual-HER2 inhibition is necessary to maximize the clinical benefit. The enhanced activity observed with combination of tucatinib plus trastuzumab is likely the result of complementary effects on inhibition of HER2 signaling within the tumour cell and stimulation of the host immune response. Trastuzumab is thought to induce the innate immune effector function through Fc receptor interactions,[22] and with tucatinib, it may lead to more innate immune activation by driving the immune effector cell infiltration to the tumour and priming T cells to tumour cell antigens.

Although HER2 has emerged as an actionable target for colorectal cancer,[9,13-17,21] testing for HER2 overexpression and amplification is underutilized.[4,5,23] Multiple testing modalities have been used successfully to identify HER2-positive diseases, [9,13-17,21, 23-25] and this real-world testing approach was utilized in the MOUNTAINEER study. Results presented add to the growing body of evidence that HER2-directed therapies should be utilized in this patient

population.[9,13-17,21] The need for HER2 testing by clinicians is important to inform treatment decisions and optimize outcomes for patients with HER2-positive metastatic colorectal cancer.

A post hoc analysis showed that response rates were numerically higher in patients with IHC 3+ tumours compared with those with IHC 2+/ISH+ tumours, which is consistent with results from other anti-HER2 regimens in metastatic colorectal cancer.[9,17] Interpretation of these results is limited because not all patients had tissue samples available for retrospective testing and multiple local testing methods were used to determine HER2 positivity. While the use of multiple tests reflects real-world clinical practice,[23] some enroled patients had negative HER2 results per central testing. This may have led to an underestimation of the antitumour response, compared with response per more standardized testing criteria.

Limitations of MOUNTAINEER include the open-label design and the lack of power for a formal comparative study. There was no subgroup analysis stratified by *BRAF* mutation status because there is limited overlap between HER2-positive and *BRAF*-mutated tumours.[8] Additionally, imaging intervals changed when the study was expanded to allow patients treated with tucatinib monotherapy to receive trastuzumab earlier if a response was not achieved. The increase in imaging frequency had the potential to document disease progression sooner, which may have affected the secondary endpoint of progression-free survival. Finally, a somewhat higher response rate may have been observed for tucatinib monotherapy if the patients were treated for longer than 12 weeks before addition of trastuzumab.

As a follow-up to the promising results of MOUNTAINEER, tucatinib plus trastuzumab in metastatic colorectal cancer is being further investigated in MOUNTAINEER-03 (NCT05253651), an ongoing, randomised, global, phase 3 study that compares the efficacy of tucatinib, trastuzumab, and mFOLFOX6 with standard of care in treatment-naïve patients with HER2-positive metastatic colorectal cancer.

In conclusion, tucatinib plus trastuzumab was well tolerated with durable and clinically meaningful antitumour activity in patients with chemotherapy-refractory, HER2-positive metastatic colorectal cancer, representing a new standard treatment option.

Contributors

All authors were involved in the conception or design of the study, the interpretation of the data, the drafting and revision of the manuscript for publication, and the approval of the final manuscript. All authors had full access to all the data in the study and participated in the decision to submit for publication. JS, AC, SS, TA, KN, EC, CW, AP, JH, ALC, CF, AK, PK, H-JL, KC, EE, DB, CC, FS, and TB-S were involved in data collection. WF was involved in data analysis. JHS, MS, and WF verified the data.

Declaration of interests

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Data sharing

De-identified patient-level trial data that underlie the results reported in this publication will be made available on a case-by-case basis to researchers who provide a methodologically sound proposal. Additional documentation may also be made available. Data availability will begin after approval of the qualified request and end 30 days after receipt of datasets. All requests can be submitted to CTDR@seagen.com and will be reviewed by an internal review committee.

Please note that the data sharing policy of this clinical study's sponsor, Seagen Inc., requires all requests for clinical trial data be reviewed to determine the qualification of the specific request. This policy is available at https://www.seagen.com/healthcare-professionals/clinical-data-requests and is aligned with BIO's Principles on Clinical Trial Data Sharing (available at https://www.bio.org/blogs/principles-clinical-trial-data-sharing-reaffirm-commitment).

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Research in context

Evidence before this study

This study was initiated based on observations from preclinical data utilizing patient-derived xenograft models of colorectal cancer, showing that the tyrosine kinase inhibitor tucatinib in combination with trastuzumab resulted in greater antitumour activity compared with the individual agents, with possible synergistic activity. A search was conducted on February 1, 2022, in Embase and Medline via the OvidSP platform for 2012 to current without language restriction. We searched for clinical studies investigating HER2-directed therapies in patients with HER2-positive metastatic colorectal cancer. Search concepts included [“metastatic colorectal cancer”] and [“HER2,” or “HER-2,”, or HER2-positive, or “ERBB2”] and [“trastuzumab,” "HERCEPTIN," “trastuzumab emtansine,” "Kadcyla," or “trastuzumab deruxtecan”]. A separate search was conducted on February 4, 2022, to include “neratinib” or “lapatinib". We found single-arm, phase 2 studies of human epidermal growth factor receptor 2 (HER2)-directed strategies as a single-agent antibody-drug conjugate (DESTINY-CRC01) or dual combinations of antibody and/or tyrosine kinase inhibitors (HERACLES-A, HERACLES-B, MyPathway, TRIUMPH, and TAPUR) in pre-treated patients with HER2-positive metastatic colorectal cancer.

Response rates reported from HERACLES-A, MyPathway, TRIUMPH, TAPUR, and DESTINY-CRC01 led to the recommendation in established guidelines for dual-HER2 inhibition and trastuzumab deruxtecan in the treatment of HER2-amplified, *RAS* wild-type metastatic colorectal cancer in second-line treatment or as initial therapy for patients who are not candidates

for intensive therapy. These studies provided proof-of-concept evidence that targeting HER2 with is feasible and a rational approach to the treatment of patients with HER-positive metastatic colorectal cancer.

Added value of this study

To our knowledge, MOUNTAINEER is the largest prospective study to date that has evaluated chemotherapy-free HER2-targeted therapy in patients with unresectable or metastatic HER2-positive colorectal cancer. The combination of tucatinib plus trastuzumab resulted in clinically meaningful and durable responses in chemotherapy-refractory patients. Treatment was tolerable with few discontinuations and no deaths due to adverse events. Most patients that discontinued treatment could receive additional therapies, including HER2-directed agents after progression.

The MOUNTAINEER study additionally provided clinical insight regarding the activity of tucatinib monotherapy in this patient population. The protocol required previous exposure to standard chemotherapy backbones, including oxaliplatin and irinotecan, and failure or intolerance of the last therapy before the study. The percentage of disease stabilization supports the monotherapy contribution of tucatinib to the dual-therapy regimen. The robust antitumour activity observed in patients that started on the doublet confirms that both tucatinib and trastuzumab are necessary to achieve maximum benefit in this population.

Implications of all the available evidence

The results from previous reports support the use of a chemotherapy-free, dual HER2-targeted regimen for patients with HER2-positive metastatic colorectal cancer. The findings from the combination, monotherapy, and crossover cohorts of MOUNTAINEER further strengthen the clinical rationale for dual-HER2 inhibition. Tucatinib plus trastuzumab showed that encouraging activity with tolerable side effects can be achieved with a dual-HER2 targeted strategy, regardless of the heterogeneous methods for confirming HER2-positive status. The results led to the approval of tucatinib in combination with trastuzumab in patients with previously treated HER2-positive, *RAS* wild-type by the FDA. These results warrant further investigation of tucatinib in combination with trastuzumab in earlier lines of therapy. An ongoing phase 3 study will compare the efficacy and safety of this regimen with standard-of-care chemotherapy in treatment-naïve patients with HER2-positive metastatic colorectal cancer.

References

1 Ferlay J, Colombet M, Soerjomataram I, et al. Estimating the global cancer incidence and mortality in 2018: GLOBOCAN sources and methods. *Int J Cancer* 2019; **144:** 1941–53.

2 National Cancer Institute: Cancer Stat Facts: Colorectal Cancer. 2019. https://seer.cancer.gov/statfacts/html/colorect.html (accessed August 11, 2022).

3 Kahi CJ, Boland CR, Dominitz JA, et al. Colonoscopy surveillance after colorectal cancer resection: Recommendations of the US Multi-Society Task Force on Colorectal Cancer. *Gastroenterology* 2016; **150:** 758–768.e11.

4 Benson AB, Venook AP, Al-Hawary MM, et al. Colon Cancer, Version 2·2021, NCCN Clinical Practice Guidelines in Oncology. *J Natl Compr Canc Netw* 2021; **19:** 329–59.

5 Benson AB, Venook AP, Al-Hawary MM, et al. NCCN Guidelines Insights: Rectal Cancer, Version 6·2020. *J Natl Compr Canc Netw* 2020; **18:** 806–815.

6 Grothey A, Van Cutsem E, Sobrero A, et al. Regorafenib monotherapy for previously treated metastatic colorectal cancer (CORRECT): an international, multicentre, randomised, placebo-controlled, phase 3 trial. *The Lancet* 2013; **381:** 303–12.

7 Mayer RJ, Van Cutsem E, Falcone A, et al. Randomized Trial of TAS-102 for Refractory Metastatic Colorectal Cancer. *N Engl J Med* 2015; **372:** 1909–19.

8 Ross JS, Fakih M, Ali SM, et al. Targeting HER2 in colorectal cancer: The landscape of amplification and short variant mutations in ERBB2 and ERBB3. *Cancer* 2018; **124:**1358–1373.

9 Sartore-Bianchi A, Trusolino L, Martino C, et al. Dual-targeted therapy with trastuzumab and lapatinib in treatment-refractory, KRAS codon 12/13 wild-type, HER2-positive metastatic

colorectal cancer (HERACLES): a proof-of-concept, multicentre, open-label, phase 2 trial. *Lancet Oncol* 2016; **17:** 738–74.

10 Bertotti A, Migliardi G, Galimi F, et al. A molecularly annotated platform of patient-derived xenografts ("xenopatients") identifies HER2 as an effective therapeutic target in cetuximab-resistant colorectal cancer. *Cancer Discov* 2011; **1:** 508–23.

11 Leto SM, Sassi F, Catalano I, et al. Sustained inhibition of HER3 and EGFR is necessary to induce regression of HER2-amplified gastrointestinal carcinomas. *Clin Cancer Res* 2015; **21**: 5519-31.

12 Kulukian A, Lee P, Taylor J, et al. Preclinical Activity of HER2-Selective Tyrosine Kinase Inhibitor Tucatinib as a Single Agent or in Combination with Trastuzumab or Docetaxel in Solid Tumor Models. Mol Cancer Ther 2020; **19**: 976–987.

13 Tosi F, Sartore-Bianchi A, Lonardi S, et al. Long-term Clinical Outcome of Trastuzumab and Lapatinib for HER2-Positive Metastatic Colorectal Cancer. *Clin Colorectal Cancer* 2020; **19:** 256–262.

14 Meric-Bernstam F, Hurwitz H, Raghav KPS, et al. Pertuzumab plus trastuzumab for HER2-amplified metastatic colorectal cancer (MyPathway): an updated report from a multicentre, open-label, phase 2a, multiple basket study. *Lancet Oncol* 2019; **20:** 518–530.

15 Nakamura Y, Okamoto W, Kato T, et al. Circulating tumor DNA-guided treatment with pertuzumab plus trastuzumab for *HER2*-amplified metastatic colorectal cancer: a phase 2 trial. *Nat Med* 2021; **27:**1899–1903.

16 Gupta R, Garrett-Mayer E, Halabi S, et al. Pertuzumab plus trastuzumab (P+T) in patients (pts) with colorectal cancer (CRC) with ERBB2 amplification or overexpression: results from the TAPUR study. *J Clin Oncol* 2020; **38:** 132 (abstr).

17 Siena S, Di Bartolomeo M, Raghav K, et al. Trastuzumab deruxtecan (DS-8201) in patients with HER2-expressing metastatic colorectal cancer (DESTINY-CRC01): a multicentre, open-label, phase 2 trial. Lancet Oncol 2021; **22**: 779–789.

18 Pheneger T, Bouhana K, Anderson D, et al. In vitro and in vivo activity of ARRY-380: a potent, small molecule inhibitor of ErbB2. *Cancer Res* 2009; **69:** 1795 (abstr).

19 Eisenhauer EA, Therasse P, Bogaerts J, et al. New response evaluation criteria in solid tumours: revised RECIST guideline (version 1·1). *Eur J Cancer* 2009; **45:** 228–47.

20 Strickler JH, Zemla T, Ou F, et al. Trastuzumab and tucatinib for the treatment of HER2 amplified metastatic colorectal cancer (mCRC): Initial results from the MOUNTAINEER trial. *Ann Oncol* 2019; **30** (suppl_5): v198–v252.

21 Sartore-Bianchi A, Lonardi S, Martino C, et al. Pertuzumab and trastuzumab emtansine in patients with HER2-amplified metastatic colorectal cancer: the phase II HERACLES-B trial. *ESMO Open* 2020; **5:** e000911.

22 Bianchini G, Gianni L. The immune system and response to HER2-targeted treatment in breast cancer. *Lancet Oncol* 2014; **15:** e58–e68.

23 Strickler JH, Yoshino T, Graham RP, Siena S, Bekaii-Saab T. Diagnosis and Treatment of ERBB2-Positive Metastatic Colorectal Cancer: A Review. *JAMA Oncol* 2022; **8:** 760–769.

24 Valtorta E, Martino C, Sartore-Bianchi A, et al. Assessment of a HER2 scoring system for colorectal cancer: results from a validation study. *Mod Pathol* 2015; **28:** 1481–91.

25 Siravegna G, Sartore-Bianchi A, Nagy RJ, et al. Plasma HER2 (ERBB2) Copy Number Predicts Response to HER2-targeted Therapy in Metastatic Colorectal Cancer. *Clin Cancer Res* 2019; **25:** 3046–53.

Figure legends

Figure 1. Study profile

*The clinical progression resulted in death.

†All patients who discontinued treatment or remained on treatment have received tucatinib plus trastuzumab.

Figure 2. Antitumour activity in patients treated with tucatinib plus trastuzumab

Shown are the maximum percentage changes in the sum of the diameters of target lesions per blinded independent central review for all patients treated with the tucatinib-combination therapy who had baseline and postbaseline target lesion measurements. Four patients who did not have these measurements were excluded. Of 80 patients with baseline and postbaseline measurements, 52 (65·0%) had a reduction in tumour burden, and 19 (marked with asterisks) were still on treatment at data cutoff. Six patients had 100% reductions and a best overall confirmed response of partial response due to nontarget lesions that had not completely resolved. Similarly, four patients with greater than 30% reduction were classified as having stable disease based on failure to confirm the response due to progression. The upper dashed horizontal line indicates a 20% increase in tumour size, and the lower dashed line indicates a 30% decrease in tumour size. CR=complete response. PD=progressive disease. PR=partial response. SD=stable disease.

Figure 3. Kaplan-Meier estimates of progression-free survival and overall survival in patients treated with tucatinib plus trastuzumab

Panel A shows the Kaplan-Meier estimates of progression-free survival assessed by blinded independent central review. The number of events refers to events of disease progression or death. The dashed vertical lines indicate landmark time points (6 and 12 months). Panel B shows the Kaplan-Meier estimates of overall survival. The dashed vertical lines indicate landmark time points (12 and 24 months). The tick marks indicate censored data for both panels.