



Original research



Trastuzumab plus pertuzumab for *HER2*-amplified advanced colorectal cancer: Results from the drug rediscovery protocol (DRUP)

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ABSTRACT

Background: In 2–5% of patients with colorectal cancer (CRC), human epidermal growth factor 2 (*HER2*) is amplified or overexpressed. Despite prior evidence that anti-*HER2* therapy confers clinical benefit (CB) in one-third of these patients, it is not approved for this indication in Europe. In the Drug Rediscovery Protocol (DRUP), patients are treated with off-label drugs based on their molecular profile. Here, we present the results of the cohort ‘trastuzumab/pertuzumab for treatment-refractory patients with *RAS/BRAF*-wild-type *HER2* amplified metastatic CRC (*HER2*+mCRC)’.

Methods: Patients with progressive treatment-refractory *RAS/BRAF*-wild-type *HER2*+mCRC with measurable disease were included for trastuzumab plus pertuzumab treatment. Primary endpoints of DRUP are CB (defined as confirmed objective response (OR) or stable disease (SD) ≥ 16 weeks) and safety. Patients were enrolled using a Simon-like 2-stage model, with 8 patients in stage 1 and 24 patients in stage 2 if at least 1/8 patients had CB. To identify biomarkers for response, whole genome sequencing (WGS) was performed on pre-treatment biopsies.

Results: CB was observed in 11/24 evaluable patients (46%) with *HER2*+mCRC, seven patients achieved an OR (29%). Median duration of response was 8.4 months. Patients had undergone a median of 3 prior treatment lines. Median progression-free survival and overall survival were 4.3 months (95% CI 1.9–10.3) and 8.2 months (95% CI 7.2–14.7), respectively. No unexpected toxicities were observed. WGS provided potential explanations for resistance in 3/10 patients without CB, for whom WGS was available.

Conclusions: The results of this study confirm a clinically significant benefit of trastuzumab plus pertuzumab treatment in patients with *HER2*+mCRC.

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1. Introduction

Colorectal cancer (CRC) constitutes 10% of global cancer diagnosis and cancer-related deaths annually. [1] At the time of diagnosis, 20% of patients have metastatic CRC (mCRC), with a 5-year survival rate of less than 20%. [2] Recent advancements in genomic technology with large-scale molecular profiling of tumours have led to new treatment opportunities for these patients.

In approximately 2–5% of patients with CRC, overexpression or amplification of human epidermal growth factor 2 (*HER2*) is observed, with a higher incidence in left-sided colon and primary rectal *RAS/BRAF*-wild-type (*RAS/BRAF*_{wt}) tumours. [3–5] Currently, patients with left-sided *RAS/BRAF*_{wt} tumours are treated with anti-EGFR therapy (i.e., panitumumab or cetuximab) with or without combination chemotherapy and/or anti-VEGF therapy. [6] However, accumulating evidence suggests that presence of *HER2* amplification or overexpression is associated with resistance to anti-EGFR therapy. [7,8] *ERBB2* is a well-known oncogene, which is successfully targeted in breast, gastric and oesophageal cancers. Recent reports indicate that patients with *HER2*-positive mCRC (*HER2*+mCRC) also benefit from anti-*HER2* therapy. [9–16] Consequently, in January 2023, the Food and Drug Administration (FDA) approved tucatinib in combination with trastuzumab for *RAS*_{wt} *HER2*+mCRC that has progressed following fluoropyrimidine-, oxaliplatin- and irinotecan based chemotherapy. [16] Based on the TRIUMPH trial, there is regulatory approval from the Ministry of Health, Labour and Welfare for trastuzumab plus pertuzumab treatment in patients with *HER2*+mCRC in Japan. [17,18].

Despite promising data, targeted therapy for patients with *HER2*+mCRC is not available in Europe. This is mainly due to the challenges of obtaining regulatory approval based on single-arm studies in small subgroups of patients, such as those with *HER*+mCRC. However, there is a clear unmet need to get anti-*HER2* agents available for these patients as a standard of care treatment option. Here, we describe treatment outcomes of a Drug Rediscovery Protocol (DRUP, NCT02925234) cohort in which patients with treatment-refractory *HER2*+mCRC were treated with trastuzumab plus pertuzumab to strengthen the evidence that this is a clinically relevant treatment option for this patient group.

Within DRUP study, patients with advanced cancer who have exhausted all standard-of-care treatment options are treated based on their tumour molecular profile with registered targeted treatments outside their labeled indications. [19] This study aims to describe anti-tumour activity and toxicity of these commercially available targeted therapies used outside of their approved indications. Additionally, to perform an explorative biomarker analysis, whole genome sequencing (WGS) is performed on pre-treatment biopsies.

2. Methods

The general study design, and eligibility criteria of DRUP (ClinicalTrials.gov, number: NCT02925234) have been reported previously [19].

2.1. Study population

Eligible patients had progressed on all standard mCRC treatment lines. *HER2* positivity was defined as at least seven copies of *HER2*, as estimated by panel-based Next Generation Sequencing (NGS), WGS or In Situ Hybridization in either the primary tumour or a metastatic lesion. Patients had measurable disease according to Response Evaluation Criteria in Solid Tumours version 1.1 (RECISTv1.1) and an Eastern Cooperative Oncology Group Performance status of 0–2. [20] Additionally, patients had normal organ and bone marrow function, including a left ventricular ejection fraction above 50%, measured within four weeks prior to start of study treatment.

Patients with pathogenic mutations in *KRAS*, *NRAS* or *BRAF* were

excluded. Other exclusion criteria are summarized in [Supplementary Table 1](#).

Patients were considered evaluable for the primary endpoint analysis if at least two treatment administrations were completed. Non-evaluable patients were replaced and excluded from efficacy- and biomarker analysis.

2.2. Treatment and study endpoints

Patients received intravenous trastuzumab (initial loading dose of 8 mg/kg body weight, followed by a maintenance dose of 6 mg/kg body weight) combined with pertuzumab (initial loading dose of 840 mg, followed by a maintenance dose of 420 mg) every three weeks until disease progression or intolerable side effects. Tumour assessments initially occurred every 9 weeks from treatment initiation. If study treatment continued beyond 3 response evaluations (i.e., 27 weeks), subsequent evaluations were performed at the end of every 4th treatment cycle (i.e., every 12 weeks). Safety was assessed by the frequency of treatment-related grade ≥ 3 adverse events (AEs) and serious adverse events (SAEs) within 30 days after the last dose of study medication, graded according to CTCAE v4.03.

Primary endpoints were clinical benefit (CB), defined as confirmed complete or partial response (CR/PR) or stable disease (SD) for at least 16 weeks, according to RECISTv1.1 (measured at least twice, at least 28 days apart), and safety. Secondary endpoints included: objective response rate (ORR, defined as PR or CR), duration of response (DoR), progression-free survival (PFS), overall survival (OS) and Growth Modulation Index (GMI) as defined by Von Hoff. [21] The GMI was defined as the ratio of PFS to the time to progression (TTP) on last systemic therapy that the patient received before participating in DRUP. Sequencing-success rate of pre-treatment biopsies and biomarker analyses using WGS on pre-treatment biopsies formed exploratory endpoints.

2.3. WGS on pre-treatment biopsies and biomarker analysis

A new fresh frozen tumour biopsy specimen (obtained ≤ 2 months before inclusion, and without any anti-cancer therapy within that period) was mandatory prior to treatment. WGS was performed on pre-treatment biopsies by Hartwig Medical Foundation [22], as well as germline DNA analysis on a 10-ml blood sample to determine the background variation of the germline DNA of the patient. WGS data were analyzed using a high-quality bioinformatics pipeline, and genomic drivers were identified using PURPLE, as described previously. [23] To distinguish functionally relevant driver mutations from passenger events, the threshold for high driver probability, driver likelihood > 0.8 , was applied. For each patient a summarizing report of all relevant findings was created, including information on tumour purity, ploidy, somatic variants, copy number variations, mutational load and more complex genomic features such as gene fusions, COSMIC mutational signatures, microsatellite (in-)stability and homologous repair deficiency. [24].

2.4. Statistical analysis

DRUP cohorts were monitored using a Simon-like two-stage “admissible” plan to identify cohorts with evidence of activity [25]. The null hypothesis and alternative hypothesis to be tested are defined as a clinical benefit rate (CBR) of $\leq 10\%$ versus $\geq 30\%$. This monitoring rule has 85% power to reject the null hypothesis of a CBR 10% when the true CBR is 30%, with a one-sided alpha error rate of 7.8%. Exact 95% confidence intervals (CIs) were calculated using the Clopper-Pearson method. All statistical analysis were performed using R version 4.0.3 (<https://www.R-project.org>). Descriptive statistics summarized patient characteristics, AEs and tumour responses. Kaplan-Meier methods were used to estimate PFS (from start treatment to progression or death from

any cause, whichever came first, and censoring patients alive without progression at data cut-off) and OS (calculated from the first day of treatment administration to the date of death from any cause, censoring patients who were alive at follow up).

3. Results

3.1. Patients

Twenty-seven patients with *HER2*+mCRC were enrolled and treated from October 2016 to February 2023 in 13 hospitals in the Netherlands that participated in DRUP. All patients were included for baseline characteristics and safety analyses. Of these patients, three patients were not evaluable for the primary endpoint per protocol. (Supplementary Figure 1).

Baseline characteristics of the 27 treated patients are presented in Table 1. Median age was 60 years (range, 31–81) and 56% of patients were men (n = 15). The majority (n = 24, 88%) had left-sided tumours.

Table 1

Baseline characteristics of the 27 patients with *HER2*+mCRC treated with trastuzumab + pertuzumab in DRUP.

Characteristics	No. of patients (%)
Median age, years (range)	60[31–81]
Sex	
Male	N = 15 (56%)
Female	N = 12 (44%)
WHO performance status	
WHO 0	N = 9 (33%)
WHO 1	N = 15 (56%)
WHO 2	N = 3 (11%)
Prior treatments	
No. of previous systemic therapy lines	
1	N = 4 (15%)
2	N = 2 (8%)
≥ 3	N = 21 (78%)
Prior capecitabine	N = 26 (96%)
Prior oxaliplatin	N = 25 (93%)
Prior fluoropyrimidine	N = 9 (33%)
Prior bevacizumab	N = 18 (67%)
Prior irinotecan	N = 21 (78%)
Prior anti-EGFR therapy	N = 18 (67%)
Prior trifluridine/tiparacil	N = 7 (26%)
Tumour characteristics	
Tumour sidedness	
Left-sided	N = 24 (88%)
Right-sided	N = 3 (12%)
Primary tumour in situ	
Yes	N = 8 (30%)
No	N = 19 (70%)
Liver/peritoneal involvement	
Yes	N = 22 (82%)
No	N = 5 (18%)
Molecular profile	
<i>RAS</i> / <i>RAF</i> wt	N = 26 (96%)
<i>KRAS</i> mutation	N = 1 (4%)
MSS	N = 23 (85%)
MSI	N = 0
Unknown	N = 4 (15%)
<i>HER2</i> testing before entering DRUP	
Tissue	
Primary tumour	N = 14 (52%)
Metastatic lesion	N = 8 (30%)
Unknown	N = 5 (18%)
Method	
ISH	N = 15 (56%)
NGS/WGS	N = 12 (44%)

Abbreviations: *HER2*, human epidermal growth factor receptor 2; mCRC, metastatic colorectal cancer; DRUP, drug rediscovery protocol; WHO, World Health Organization; No, number; *EGFR*, epidermal growth factor receptor; *RAS*/*RAF*wt, *RAS*/*RAF* wild-type; MSS, microsatellite stable; MSI, microsatellite instable; ISH, in situ hybridization; NGS, next generating sequencing; WGS, whole genome sequencing

Seventy-eight percent received three or more prior systemic treatment lines. Baseline characteristics were comparable between patients with and without CB, as shown in Supplementary Table 1.

3.2. Clinical benefit and survival

At data cut-off in August 2023, five patients were alive and followed for a median of 6.3 months (IQR 5.5 – 9.1). Among 24 evaluable patients, 46% (n = 11) demonstrated CB upon treatment with trastuzumab plus pertuzumab. ORR was 29% (n = 7); all patients achieved a PR. Fig. 1, a waterfall plot, illustrates the greatest changes in sum of target lesions for each patient. The median PFS and OS were 4.3 months (95% CI 1.9 – 10.3) and 8.2 months (95% CI 7.2 – 14.7), respectively (Fig. 2A and B). The swimmers plot in Fig. 3 displays the time on treatment for the patients with a median of 15.1 weeks (95% CI 6.0 – 24.7). The median DoR for patients with an OR was 8.4 months (95% CI 5.4 – not reached). All responders had a DoR of at least 3 months, with 5 out of 7 patients achieving a 6-month DoR and 2 out of 6 patients reaching a 1-year DoR. The median GMI was 1.20 (range: 0.24 – 11.19). Eleven patients (46%) had a GMI > 1.33, and 13 patients (54%) had a GMI < 1.33. The main reason for treatment discontinuation was disease progression (n = 21, 81%). Three patients discontinued treatment due to symptomatic deterioration (12%), one patient due to toxicity (4%), and one patient died during study treatment (4%). One patient was still on study treatment (4%).

3.2.1. Safety

Overall, trastuzumab plus pertuzumab was well tolerated without any new safety signals. A total of 27 grade ≥ 3 AEs occurred in 16 patients, of which 6 were possibly, probably or definitely related to study treatment. A total of 15 SAEs were reported in 10 patients, of which 2 were related to study treatment. One patient with a PR died (grade 5 SAE) from an unknown cause, but deemed unrelated to study treatment by the treating physician. Table 2 lists all treatment-related grade ≥ 3 AEs and observed SAEs.

3.2.2. Baseline biopsies and WGS results

All evaluable patients underwent a mandatory pre-treatment biopsy obtained from metastatic lesions: liver (n = 15), lung (n = 4), lymph

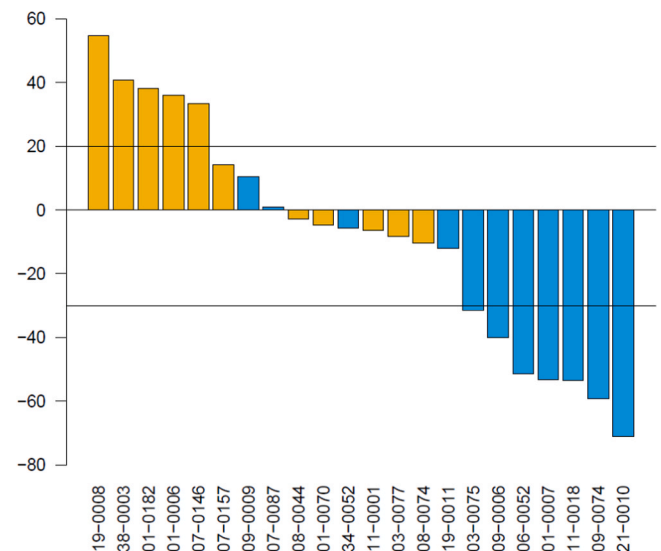
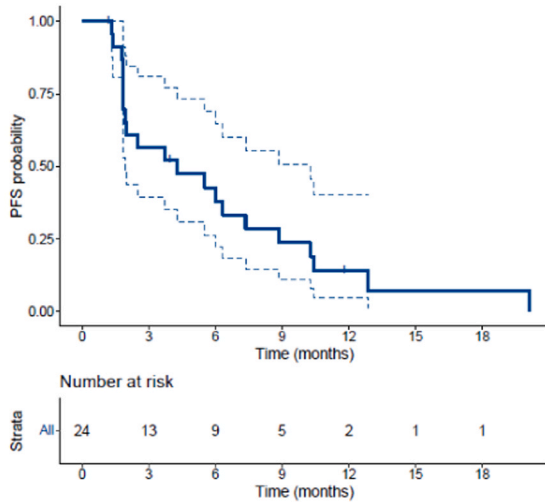


Fig. 1. Waterfall plot of best response. Best percentage of change in target lesion size from baseline for patients that are evaluable for clinical benefit and that had RECISTv1.1 measurements at baseline and at least one later time point. Patients with clinical benefit are depicted in blue, patients without clinical benefit in orange. Horizontal lines indicate the boundaries for partial response and progressive disease.

A. Progression free survival



B. Overall survival

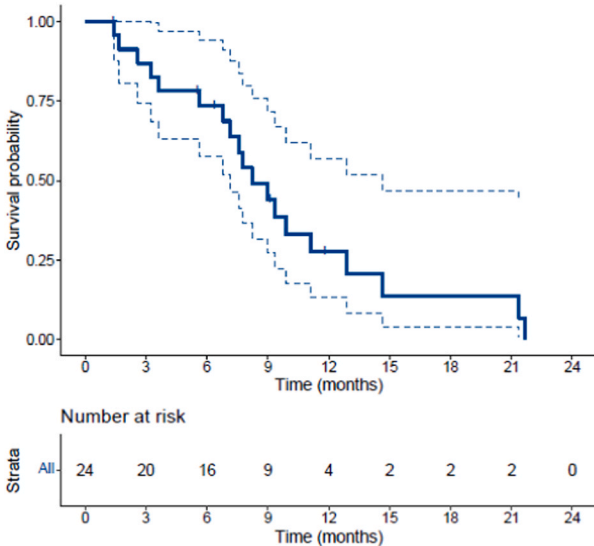


Fig. 2. Progression free survival and overall survival curves. Kaplan-Meier curves for estimated progression free survival (3A) and overall survival (3B), with 95% confidence intervals (dashed lines). A. Progression free survival. B. Overall survival.

node (n = 3), bone (n = 1) and vaginal lesion (n = 1). Seventeen biopsies (71%) were successfully sequenced; 5 patients with PR, 2 patients with SD and 10 patients without CB. The remaining seven biopsies were unsuitable for sequencing due to a low tumour cell percentage. All potential drivers detected by WGS are depicted in Fig. 4. The most frequently identified concomitant potential drivers were mutations in *TP53* and *APC*. The median *HER2* copy number was 141 (range 26 – 268) and 73.5 (range 0 – 310) in patients with and without CB, respectively. No correlation between *HER2* copy number and CB was observed (Supplementary Figure 2A). Furthermore, there was no association between *HER2* copy number and PFS or OS (Supplementary Figure 2B). Interestingly, there was a trend towards a significant difference in the median number of potential driver alterations between patients with or without CB (4 versus 5.5; p = 0.052). All patients with CB had four or fewer other potential driver mutations (Supplementary Figure 2C). In addition, it was found that patients with four or fewer other potential driver alterations had a significant longer PFS

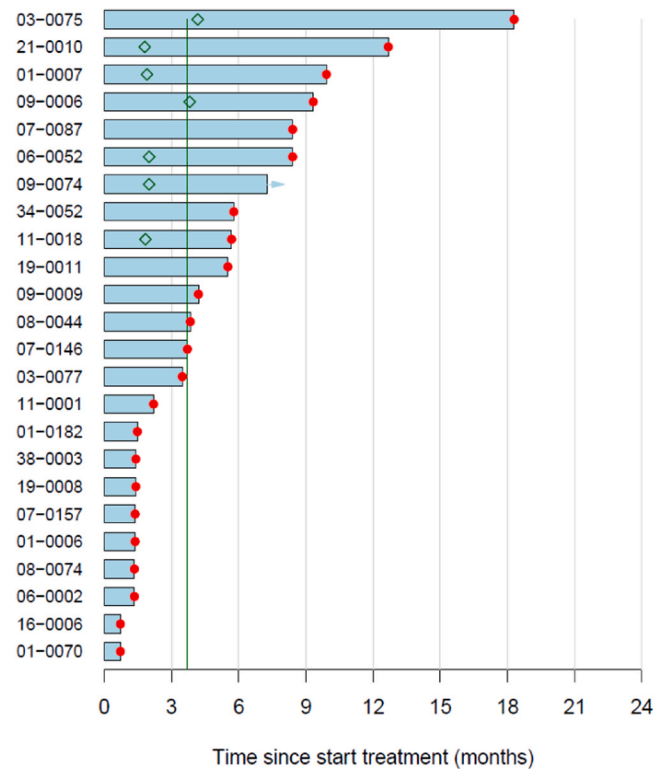


Fig. 3. swimmers plot of time on treatment. The swimmers plot depicts the time on treatment for the 24 patients that received at least two treatment cycles. The green line corresponds to 16 weeks. End of treatment is marked with a red dot, partial response is indicated with a green diamond at the time of first appearance.

Table 2
Adverse events.

	Grade 1 / 2	Grade 3	Grade 4	Grade 5
Treatment related grade ≥ 3 AE's				
Diarrhea		1		
Erythema multiform		1		
Hypertension		1		
Infusion related reaction	1			
Left ventricular dysfunction		1		
Mucosal inflammation		1		
All reported SAE's (in bold SAE's possible, probable or definite related to treatment)				
Abdominal pain		2		
Arthralgia		1		
Cerebral edema			1	
COPD exacerbation		1		
Death				1
Diarrhea		2		
Dyspnea		1		
Infusion related reaction	1			
Fever		1		
Rectal hemorrhage	1			
Rectal obstruction			1	
Tumor associated fever		1		
Vaginal hemorrhage	1			

All treatment related grade ≥ 3 AE's and observed SAE's. Abbreviations: AE's; adverse events, SAE's; serious adverse events; COPD, chronic obstructive pulmonary disease

(p = 0.0056) and OS (p = 0.05) compared to patients with more than four other potential driver mutations (Supplementary Figure 2D). Of the patients with a higher than median *HER2* copy number (i.e. 90 copies) and no benefit from treatment with trastuzumab plus pertuzumab (n = 4), three patients had more than four potential other driver

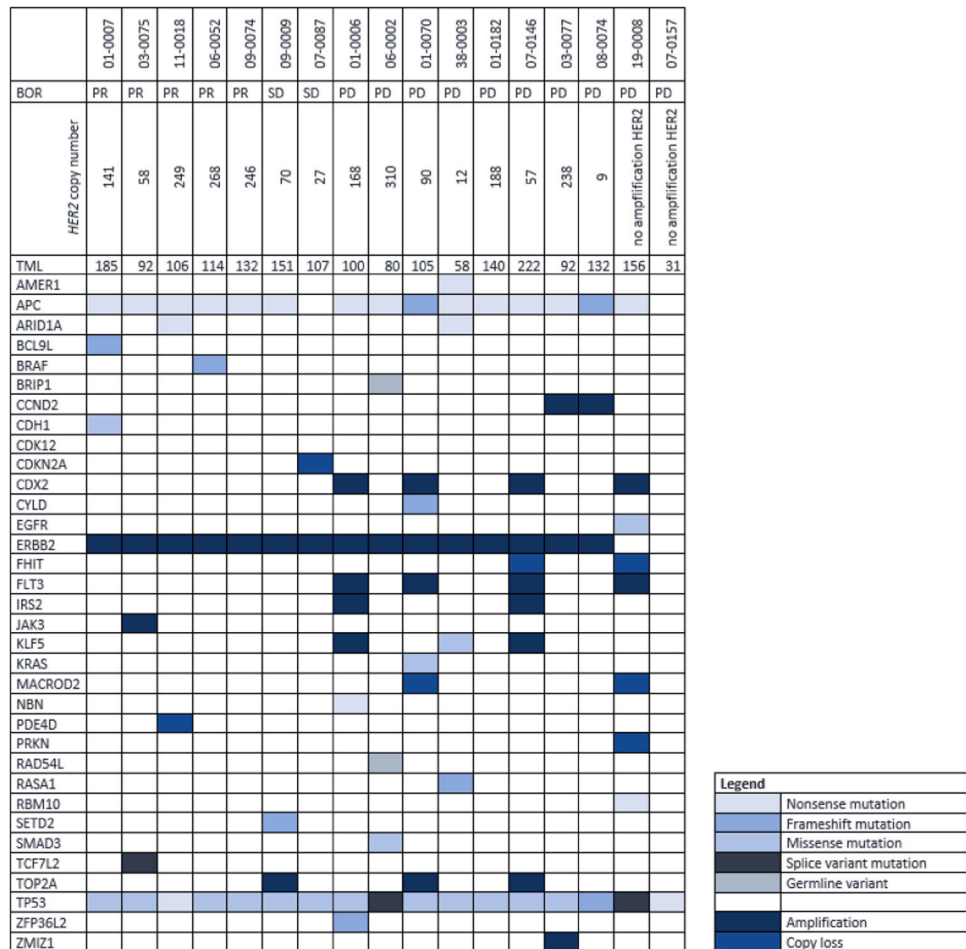


Fig. 4. Co-occurrence of potential drivers as detected by WGS. For all patients the best overall response according to RECISTv1.1, copy numbers of *HER2* amplification, mutational load and detected potential drivers are shown. Abbreviations: BOR, best overall response; TML, tumor mutational load; PR, partial response; SD, stable disease; PD, progressive disease; WGS, whole genome sequencing.

mutations. Genomic data were available for 10 patients without CB. For three patients, a reasonable hypothesis for non-responsiveness could be formulated. For two patients, WGS could not confirm the inclusion target (i.e. *HER2* amplification as assessed by FISH) and in one patient, WGS revealed a *KRAS* mutation (p.Gly12Cys). In the other seven patients, WGS did not reveal any known resistance mechanisms for therapy. However, in four patients without CB, amplification of *FLT3* was found, also activating RTK/RAS-pathway.

4. Discussion

In the current DRUP-cohort, heavily pre-treated patients with *HER2*+mCRC were administered trastuzumab plus pertuzumab. Forty-seven percent of patients with *HER2*+mCRC achieved CB from trastuzumab plus pertuzumab treatment, with an ORR of 29% and median PFS and OS of 4.3 months and 8.2 months, respectively. *HER2* amplification is observed in 2–5% of patients with mCRC, and despite accumulating evidence demonstrating the clinically significant benefits of anti-*HER2* therapy, approval for this treatment regimen is still lacking in Europe.

At present, four single arm studies have evaluated the combination trastuzumab plus pertuzumab in the treatment of patients with *HER2*+mCRC [10, 13, 17]. Results from these trials were highly comparable and are summarized in Supplementary Table 2. Despite the majority of patients in these trials being heavily pre-treated (defined as having received three or more previous systemic therapy lines), the reported ORRs were notably high (25–32%). This is especially significant when compared to the ORR of approved anti-EGFR therapy (15–20%) or

trifluridine/tipiracil (2%) in third line. [26,27] The median OS for trifluridine/tipiracil in selected patients with treatment-refractory CRC was reported at 5.3 – 15.2 months. [28] Nevertheless, the median OS for trastuzumab plus pertuzumab in patients with *HER2*+mCRC ranged between 8.2 – 15.0 months, based on the results of TRIUMPH, MyPathway, TAPUR and DRUP. In addition to efficacy data, all trials collected safety data, with none reporting unexpected toxicities. Moreover, in our study, all observed AEs that were possibly, probably or definitely related were consistent with the drug label, including diarrhea, erythema multiform, infusion reactions and left ventricular dysfunction.

More anti-*HER2* treatment regimens have demonstrated promising results in patients with *HER2*+mCRC. [9–15] In the HERACLES-A trial, 27 patients with treatment-refractory *HER2*+mCRC received trastuzumab in combination with lapatinib, a tyrosine kinase inhibitor. The trial reported an ORR of 30%. [11] Conversely, the HERACLES-B single-arm phase 2 trial, treating patients with treatment-refractory *HER2*+mCRC with pertuzumab plus trastuzumab-emtansine (T-DM1, an ADC), reported a lower ORR of 9.7%, but favourable SD percentage of 67.7%. [12] The FDA approval for tucatinib in combination with trastuzumab was based on the MOUNTAINEER trial, where the efficacy of tucatinib in combination with trastuzumab was evaluated in 84 patients with *HER2*+mCRC. [14] The trial reported an ORR of 38% and a median DoR of 12.4 months. In DESTINY-CRC01, patients with *HER2*+mCRC were treated with trastuzumab deruxtecan (T-DXd), an antibody-drug conjugate targeting *HER2*, resulting in a high ORR 45.3%. [15].

This accumulated data demonstrated that about 1/3 of patients with

treatment refractory *HER2* +mCRC will benefit from anti-*HER2* therapy. The National Comprehensive Cancer Network (NCCN) already recommends already testing for *HER2* amplification in patients with mCRC and considers this targeted therapy as a relevant option for patients with *HER2* +mCRC. [29].

Crucial next steps for advancing anti-*HER2* treatment strategies in *HER2* +mCRC involve the development of biomarkers to enhance patient selection and ultimately raise the CBR. Evidence indicates that molecular alterations in *RAS*, *PIK3CA* and *BRAF* are linked to resistance to anti-*HER2* therapy. [10,30]. Consequently, while patients with *RAS* and *BRAF* alterations should not undergo anti-*HER2* therapy alone, they might be able to benefit from T-DXd as reported in the DESTINY-CRC01 trial. [15,31] Biomarker analyses of our cohort revealed hypotheses for treatment resistance in three patients without CB: one patient had a co-mutation in *KRAS*, and in two patients we could not confirm *HER2*-amplification, likely due to tumour heterogeneity. However, it is known that *HER2* status conversion is a concern in about one-third of patients with breast cancer [32], which may also apply to patients with CRC. Apart from *HER2* status conversion, *HER2* amplification could emerge during anti-EGFR therapy. [6] In four other patients without CB, we identified *FLT3* amplification, possibly acquired during anti-EGFR therapy and potentially associated with resistance by activating *RTK/RAS* pathway. [33,34] However, to our knowledge there are no preclinical data available supporting an association between the presence of *FLT3* amplification and resistance to anti-*HER2* therapy. Interestingly, all patients with more than four potential co-driver mutations failed on trastuzumab plus pertuzumab treatment in this trial, suggesting that targeting *HER2* alone is insufficient to inhibit tumour growth. It might be that combining multiple targeting therapies could overcome resistance in these patients. Therefore, it is crucial to conduct molecular testing just before initiating anti-*HER2* therapy to confirm *HER2* positivity and identify possible resistance mechanisms. In certain cases, especially in patients with known *HER2* amplification, ctDNA could potentially be used for confirmation to avoid an invasive biopsy. [17] The MyPathway study suggests that patients with higher-than-median copy number had higher response rates compared to those with lower copy number. In this cohort, no association between *HER2* copy number and CB, PFS or OS was found, potentially due to the small sample size. Interestingly, patients showing the best response to treatment had undergone fewer prior treatments compared to patients with PD, indicating that anti-*HER2* might be more effective early in the trajectory of their disease. Currently, the MOUNTAINEER-03 trial is ongoing to investigate whether anti-*HER2* therapy is more effective than standard of care in patients with *HER2* +mCRC in the first line (ClinicalTrials.gov, number: NCT05253651).

Limitations of this study are the absence of randomization and a control group and a relatively small patient cohorts. Nevertheless, we believe that these results are valuable for the growing body of evidence that anti-*HER2* therapy is effective in patients with *HER2* +mCRC, paving the way for approval for anti-*HER2* therapy in these patients in Europe. Further research is needed to identify biomarkers for better patient selection and to determine timing of anti-*HER2* testing and therapy.

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CRedit authorship contribution statement

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Conceptualization. **Vincent van der Noort:** Writing – review & editing, Formal analysis. **Hans Gelderblom:** Writing – review & editing, Supervision, Methodology, Conceptualization. **Soemeya F. Haj Mohammad:** Writing – review & editing, Project administration, Investigation, Data curation. **Derk-Jan A. de Groot:** Writing – review & editing, Resources. **Elske C. Gootjes:** Writing – review & editing, Resources. **Anne M.L. Jansen:** Writing – review & editing, Formal analysis. **Wendy W.J. de Leng:** Writing – review & editing, Formal analysis. **Liselot B.J. Valkenburg-van Iersel:** Writing – review & editing, Resources. **Karlijn Verkerk:** Writing – review & editing, Project administration, Investigation, Data curation. **Jeanine M.L. Roodhart:** Writing – review & editing, Resources. **Birgit S. Geurts:** Writing – review & editing, Project administration, Investigation, Data curation. **Theo van Voorthuizen:** Writing – review & editing, Resources. **Laurien J. Zeverijn:** Writing – review & editing, Project administration, Investigation, Data curation. **Emile D. Kerver:** Writing – review & editing, Resources. **Ilse A.C. Spiekman:** Writing – original draft, Visualization, Validation, Project administration, Investigation, Data curation. **Henk M.W. Verheul:** Writing – review & editing, Supervision, Methodology, Conceptualization.

Declaration of Competing Interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests. **Derk-Jan A. de Groot** received funding for research grants from Siemens and Hoffman-La Roche. All outside the submitted work and all money had been received by the UMCG.

Jeanine M.L. Roodhardt is a member of Advisory boards from Bayer, BMS, Merck-Serono, Pierre Fabre, Servier, GSK and Amgen (all paid to institution), received research funding from BMS, Pierre Fabre, Servier, Cleara, Xilis, DoMore diagnostics and HUB organoids B.V. (all paid to institution) and is a Board Member of Foundation Hubrecht Organoid Biobank.

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Emile E. Voest is founder and current member of the supervisory board of the Hartwig Medical Foundation, independent non-executive director of Sanofi, co-founder of Mosaic Therapeutics, and a board member and founder of the Center for Personalized Cancer Treatment. He has received clinical study grants from Amgen, AstraZenica, BI, BMS, Clovis, Eli Lilly, GSK, Ipsen, MSD, Novartis, Pfizer, Roche and Sanofi, all paid to the Netherlands Cancer Institute. The **other authors** declare no conflicts of interests.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at [doi:10.1016/j.ejca.2024.113988](https://doi.org/10.1016/j.ejca.2024.113988).

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