





Pertuzumab and trastuzumab emtansine in patients with HER2-amplified metastatic colorectal cancer: the phase II HERACLES-B trial



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ABSTRACT

Background HER2 is a therapeutic target for metastatic colorectal cancer (mCRC), as demonstrated in the pivotal HERACLES-A (HER2 Amplification for Colo-rectal cancer Enhanced Stratification) trial with trastuzumab and lapatinib. The aim of HERACLES-B trial is to assess the efficacy of the combination of pertuzumab and trastuzumab-emtansine (T-DM1) in this setting.

Methods HERACLES-B was a single-arm, phase II trial, in patients with histologically confirmed *RAS/BRAF* wild-type and HER2+ mCRC refractory to standard treatments. HER2 positivity was assessed by immunohistochemistry and in situ hybridisation according to HERACLES criteria. Patients were treated with pertuzumab (840 mg intravenous load followed by 420 mg intravenous every 3 weeks) and T-DM1 (3.6 mg/kg every 3 weeks) until disease progression or toxicity. Primary and secondary end points were objective response rate (ORR) and progression-free survival (PFS). With a Fleming/Hern design (H_0 =ORR 10%; α =0.05; power=0.85), 7/30 responses were required to demonstrate an ORR \geq 30% (H_1).

Results Thirty-one patients, 48% with \geq 4 lines of previous therapies, were treated and evaluable. ORR was 9.7% (95% CI: 0 to 28) and stable disease (SD) 67.7% (95% CI: 50 to 85). OR/SD \geq 4 months was associated with higher HER2 immunohistochemistry score (3+ vs 2+) (p =0.03). Median PFS was 4.1 months (95% CI: 3.6 to 5.9). Drug-related grade (G) 3 adverse events were observed in two patients (thrombocytopenia); $G \leq 2$ AE in 84% of cycles (n =296), mainly nausea and fatigue.

Conclusions HERACLES-B trial did not reach its primary end point of ORR; however, based on high disease control, PFS similar to other anti-HER2 regimens, and low toxicity, pertuzumab in combination with T-DM1 can be considered for HER2+mCRC as a potential therapeutic resource.

Trial registration number 2012-002128-33 and NCT03225937.

Key questions

What is already known about this subject?

► HER2 is a therapeutic target for metastatic colorectal cancer (mCRC), as demonstrated in the pivotal HERACLES-A trial with trastuzumab and lapatinib.

What does this study add?

► The HERACLES-B trial assessed the efficacy of a HER2-targeted combination of pertuzumab and trastuzumab-emtansine (T-DM1) in chemorefractory mCRC, showing a 9.7% overall response rate that was below the threshold selected as end point for success of the study.
► At the same time, it has been observed a high disease control (77.4%), and a PFS of 4.1 months that is similar to other anti-HER2 regimens.

How might this impact on clinical practice?

► The combination of pertuzumab and T-DM1 does not appear to induce meaningful tumour shrinkage as compared with other HER2-targeted options; however, based on high disease control, PFS and low toxicity, it can be considered for HER2+ mCRC as a potential alternative therapeutic resource.

INTRODUCTION

Precision medicine in solid tumours was essentially born with the game changing discovery that the gene encoding HER2, the human epidermal growth factor receptor 2 (*ERBB2* formerly known as *HER2/neu*), was amplified and overexpressed in approximately 20% of patients with newly diagnosed breast cancers.¹ Thereafter, deregulated HER2 became the target of several approved cancer drugs and anti-HER2 treatments turned out to be a phenomenal success story in breast cancer.² Tumour-driving *ERBB2*

alterations have next been confirmed in many tumours types, including gastric, biliary, lung, ovary, and also in approximately 5% of colorectal cancer (CRCs).^{3–5} Our group tested several combinations of anti-HER2 drugs in randomised preclinical trials of patient-derived HER2+ CRC xenografts (HER2-PDXs), and elected to test first in the clinic a vertical HER2 blockade regimen consisting of trastuzumab combined with lapatinib, a dual HER1/HER2 tyrosine kinase inhibitor⁶ in our first trial, named HERACLES-A, in which patients selection was performed by stringent CRC-specific HER2+ pathology criteria.^{4 7} Results in the first 27 *KRAS* exon 2 wild-type patients showcased a 30% ORR, including two complete responses, one still without evidence of disease after 7 years, and an excellent median survival (10 months) considering the heavily pretreated population^{7 8}

Leveraging liquid biopsies and rapid postmortem autopsy, we uncovered mechanisms of resistance to therapeutic HER2 blockade and suggested how to optimise patient's selection. First, we showed that plasma and tissue *ERBB2* copy number positively correlates, the former distributing with a cut-off value highly predictive for clinical response.⁹ We then reported that resistance to anti-HER2 therapy is associated with *KRAS* mutations, *BRAF* amplification and other molecular alterations already known to sustain resistance in breast cancer.¹⁰

Results of HERACLES-A trial led to the inclusion of trastuzumab and lapatinib regimen in the 2019 NCCN Guidelines for mCRC and triggered clinical research to optimise of anti-HER2 regimens in this setting.^{11 12} To this aim, based on the encouraging efficacy in breast cancer,^{13 14} we studied in preclinical models of PDX a 'targeted chemotherapy' precision approach combining the HER2/HER3 dimerisation inhibitor pertuzumab¹⁵ with trastuzumab emtansine (T-DM1), and subsequently

designed the HERACLES-B trial, evaluating this combination of drugs in patients with HER2+ mCRC.

METHODS

Preclinical colorectal cancer xenograft

Tumour implantation and expansion were performed as previously described.¹⁶ After engraftment in NOD-SCID (Nonobese diabetic-severe combined immunodeficiency) mice, established tumours (average volume 400 mm³) were treated with either single-agent or combination of pertuzumab (Roche Genentech) 20 mg/kg intraperitoneal, once weekly; T-DM1 (Roche Genentech), 10 mg/kg intravenous, once weekly and lapatinib (Carbosynth) 100 mg/kg by oral gavage, daily. Tumour size was evaluated once weekly by calliper measurements and the approximate volume of the mass was calculated using the formula $4/3\pi \cdot (d/2)^2 \cdot D/2$, where d is the minor tumour axis and D is the major tumour axis.

Study design and patients

HERACLES-B is a multicentre, open-label, phase II trial done at five academic centres in Italy (online supplemental appendix 1). Eligible patients were 18 years or older and had a histologically confirmed diagnosis of mCRC with *RAS* (*KRAS* exons 2, 3, 4; *NRAS* exons 2, 3, 4) wild-type status and HER2 positivity as defined by the CRC-specific HERACLES diagnostic criteria.⁴ Participants must had at least one measurable lesion, as defined by the Response Criteria Evaluation in Solid Tumours (RECIST) V.1.1; an Eastern Cooperative Oncology Group performance status score of 0 or 1 and adequate haematological, renal and hepatobiliary functions. Another major inclusion criterion was progression while on treatment or within 6 months from treatment with approved standard drugs

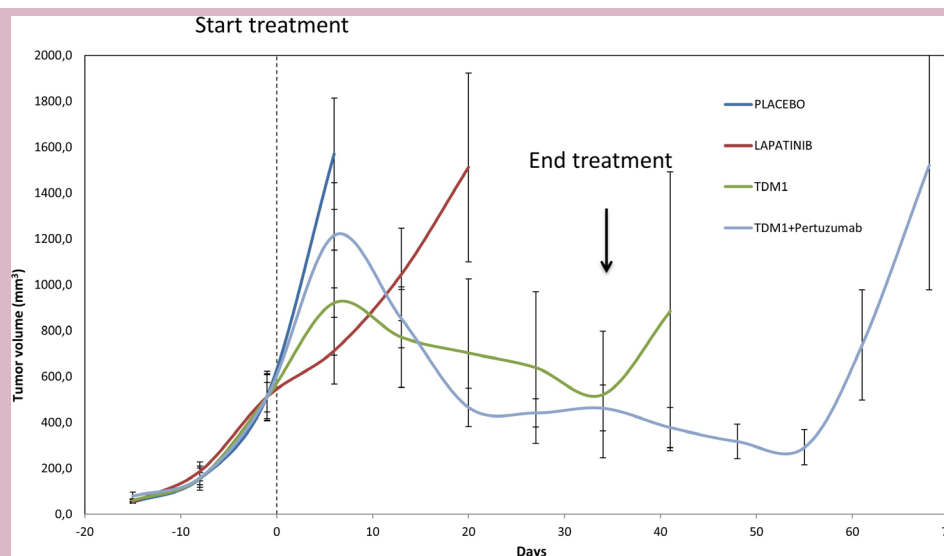


Figure 1 Efficacy in preclinical trials of pertuzumab and T-DM1 in patient-derived HER2+ CRC xenografts (HER2-PDXs). Tumour growth curves in tumour graft cohorts from individual patients with *ERBB2* amplification treated with placebo or indicated HER2-targeted treatments (n=6 mice per group), showing long-lasting growth inhibition achieved with pertuzumab and T-DM1. TDM1: 10 mg/kg once weekly; pertuzumab: 20 mg/kg once weekly; lapatinib: 100 mg/kg/day.

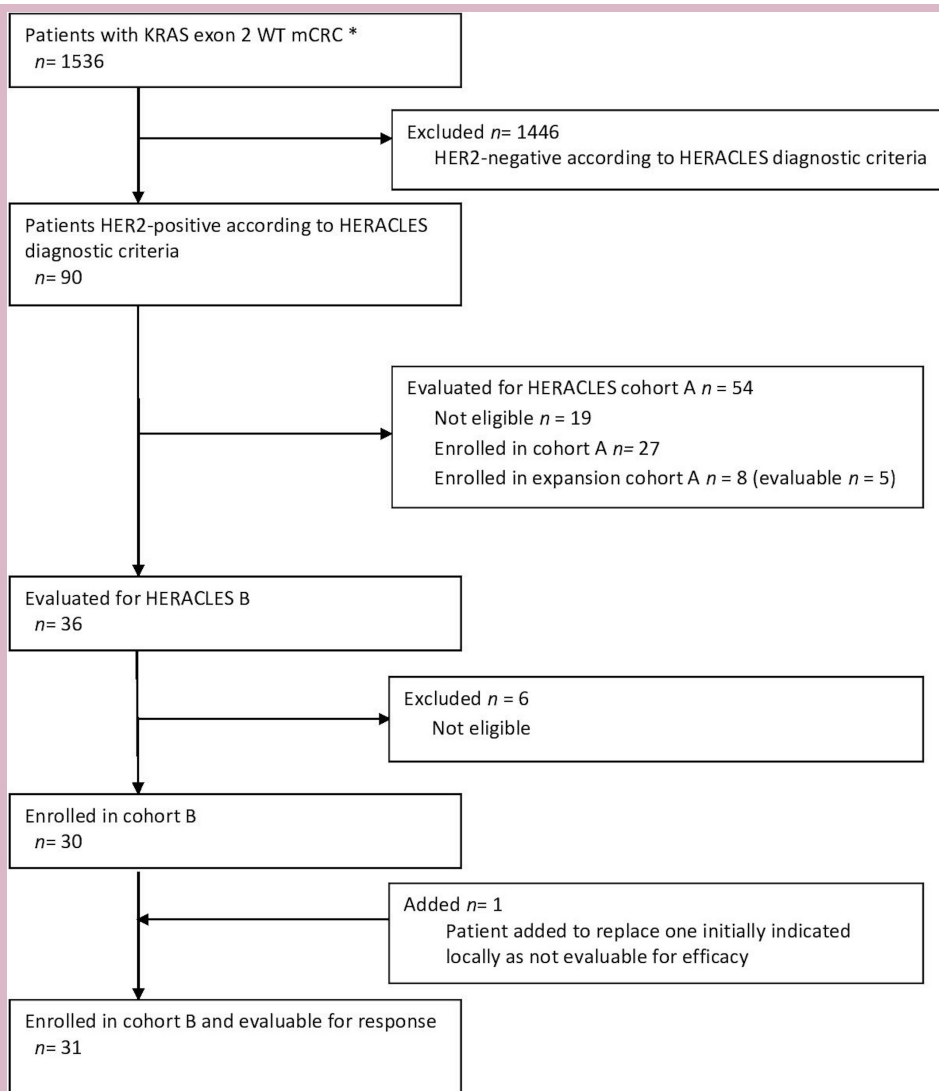


Figure 2 Consolidated Standards of Reporting Trials diagram of HERACLES-B trial. Between August 2012 and March 2018, 1536 patients with KRAS exon 2 and BRAF wild type (WT) (from 15 March 2016 exons 2, 3, 4 KRAS and NRAS and BRAF WT) were screened by immunohistochemistry (IHC) and in situ hybridisation (ISH) as per HERACLES criteria. Ninety patients (5.9%) had a HER2+ tumour. HERACLES cohort A comprised overall 35 patients (27 original cohort plus 8 expansion cohort), of whom 32 were evaluable for response.⁸ Enrolment in cohort B (HERACLES B trial) started in August 2016, data were collected until March 2019 and final analysis and centralised radiological revision were completed by 30 July 2019. One patient signed consent but developed rapid disease progression and was locally indicated as not evaluable for efficacy. The patient was duly substituted as per protocol. However, the centralised revision of the treatment history subsequently revealed instead that patient was fully evaluable for both safety and efficacy as for protocol since two full cycles of treatment were delivered. For this reason, all analyses have been performed on 31 rather than 30 patients as originally intended. *From 15 March 2016 RAS WT (KRAS, NRAS exon 2, 3, 4).

for mCRC (fluoropyrimidines, oxaliplatin, irinotecan, containing regimens, with or without anti-angiogenic or anti-EGFR antibodies).

Procedures

Patients enrolled received pertuzumab 840 mg intravenous loading dose on the first day (D1) of cycle 1, followed by 420 mg intravenous on D1 of each subsequent three weekly cycle and T-DM1 3.6 mg/kg intravenous on D1 of each 3 weeks cycle. Treatment was continued until disease progression, occurrence of an adverse event requiring treatment cessation, withdrawal of consent or investigator

decision to terminate treatment. Tumour assessments were evaluated at each centre in accordance with RECIST V.1.1 at baseline, at 6 weeks and every 9 weeks thereafter until progression. All tumour assessments were reviewed centrally by two radiologists (DR, AV) masked to outcomes using mintLesion V.3.1 software to collect, store and guide the revision of the imaging results. The imaging review protocol and tumour assessment reconciliation report are included in online supplemental appendix 2. Toxicities and safety were assessed on the basis of the Common Terminology Criteria for Adverse Events (CTCAE) V.4.0.

Table 1 Baseline characteristics

	Patients given pertuzumab and T-DM1 (n=31)
Age in years	60 (50.5–68.5)
Sex	
Men	24 (77%)
Women	7 (23%)
ECOG performance status 0–1	31 (100%)
HER2 status by IHC and FISH	
IHC 3+	25 (80%)
IHC 2+	6 (20%)
FISH +	31 (100%)
Site of primary tumour	
Rectum	12 (39%)
Colon	19 (61%)
Proximal*	2 (10%)
Distal†	17 (90%)
Metastatic disease in multiple sites	24 (77%)
Metastatic site	
Lung	25 (80%)
Liver	24 (77%)
Lymphnodes	7 (24%)
Others sites	13 (42%)
Number of previous lines of therapy	3 (3–5)
Patients with ≥4 previous lines of therapy	15 (48%)
Previous anti-angiogenesis treatment	26 (83%)
Previous therapy with panitumumab or cetuximab	27 (87%)

Data are n (%) or median (IQR).

*Located in caecum, ascending colon, liver flexure and transverse colon.

†Located in splenic flexure, descending and sigmoid colon.

ECOG, Eastern Cooperative Oncology Group; FISH, fluorescence in situ hybridisation; IHC, immunohistochemistry.

No dose reduction was allowed for pertuzumab, while T-DM1 was permitted to be reduced to 2.4 mg/kg intravenous per cycle; patients who needed further dose reductions were withdrawn from the study. Patients were screened for HER2 positivity at participating institutions on the most recently available formalin-fixed paraffin-embedded (FFPE) tumour sample and confirmed at the central pathology laboratory at Niguarda Cancer Center (Milan, Italy) as previously described.⁴ Next-generation sequencing (NGS)-based molecular analyses (Foundation Medicine—Roche) of tumour tissue were performed on available FFPE specimens.

Outcomes

The primary objective was to define the antitumour activity of pertuzumab plus T-DM1 in patients with

chemorefractory HER2+ mCRC. The primary end point was ORR and secondary end points were progression-free survival (PFS) and safety. Translational exploratory end points investigated association of tumour biomarkers and tumour response/resistance.

Statistical analysis

The sample size was determined according to the Fleming and Hern single stage design. For the primary objective, 30 patients were needed with a power of 85% and a one-sided α of 0.05, to test the null hypothesis that the proportion of patients achieving an objective response to the pertuzumab plus T-DM1 combination would be $\leq 10\%$ versus the alternative hypothesis that the proportion of patients achieving an objective response would be 30% or more. Under these conditions, seven objective responses were needed to declare the study positive. Response was assessed in the intention-to-treat population. Time-to-event variables were estimated with the Kaplan-Meier product-limit method and p values for differences between curves were calculated with the log-rank test. We considered p values of 0.05 or less to show significance; Fisher's exact test was used for subgroup comparisons of categorical variables. Analyses were conducted with SAS V.9.2 and Stata V.12.

RESULTS

Preclinical evaluation in HER2+ CRC PDXs of the combination of pertuzumab and T-DM1 demonstrated inhibition of tumour growth and, interestingly, this activity was long-lasting, as it was maintained for several weeks after treatment discontinuation (figure 1). These results prompted us to launch the HERACLES-B trial as part of the HERACLES initiative and following HERACLES-A cohort. Between August 2012 and March 2018, 1536 patients with *KRAS* exon 2 and *BRAF* wild type (from 15 March 2016 exons 2, 3 and 4 *KRAS*, exons 2, 3 and 4 *NRAS* and exon 15 *BRAF* wild type) were screened by immunohistochemistry (IHC) and in situ hybridisation (ISH)⁴ within the HERACLES programme. Enrolment in cohort B (HERACLES B trial) started in August 2016, data were collected until March 2019 and final analysis and centralised radiological revision were completed by 30 July 2019. The Consolidated Standards of Reporting Trials flow diagram is reported in figure 2.

The majority of IHC HER2 diagnostic samples (80%) in eligible patients scored 3+ for HER2 at IHC. Fluorescence in situ hybridisation was performed in all the specimens confirming gene amplification. NGS in available FFPE specimens was also performed in 20/30 (67%) patients and confirmed *ERBB2* amplification and *RAS* wild-type status in all samples. Most patients had colon tumour location, metastatic disease in multiple sites and almost half (48%) had been treated with at least four lines of chemotherapy including bevacizumab, aflibercept or regorafenib (table 1). Twenty-six patients (83%) had previously received therapy with an anti-EGFR antibody.

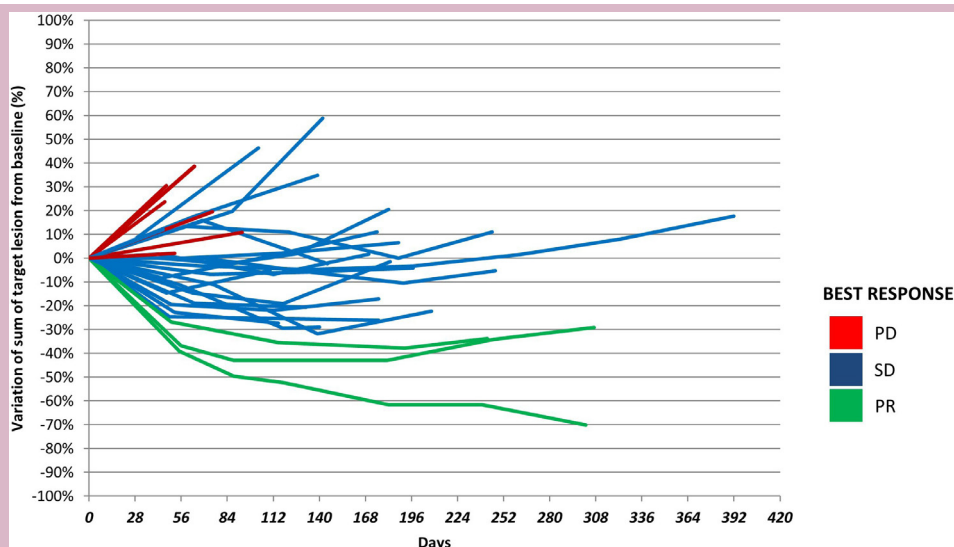


Figure 3 Dynamic of response in patients in HERACLES-B trial. Individual lines represent for each patient the percentage of change from treatment start (day 0) to the day of objective disease progression. Red lines are for patients with progressive disease (PD), blue lines for patients with stable disease (SD) and green lines for patients who achieved a partial response (PR). Pt number 122053 rapidly progress after two cycle of pertuzumab and trastuzumab-emtansine and was not represented in the spaghetti plot.

Sensitivity to panitumumab or cetuximab was assessable in four cases, according to stringent criteria excluding responses to chemotherapy/anti-EGFR combinations,⁷ and none had achieved a previous objective response.

At the time of data cut-off median follow-up was 7.7 months (IQR 6.8 months). Partial response was achieved in 3 patients, for an ORR of 9.7% (95% CI: 0 to 28); 21 patients (67.7% (95% CI: 50 to 85)) achieved disease stabilisation, accounting for a disease control rate of 77.4%. Median PFS was 4.1 months (95% CI: 3.6 to 5.9) (online supplemental figure 1A). A higher HER2 IHC score (3+ vs 2+) was associated with better PFS: of the 31 patients included, 25 patients with tumours displaying a 3+ IHC score had a PFS of 5.7 months, while the PFS of patients with tumour scoring IHC 2+ was 1.9 months (HR 0.20 (95% CI: 0.07 to 0.56), $p=0.0008$) (online supplemental figure 1B). Higher HER2 IHC score (3+ vs 2+) was also associated with objective response and long-lasting disease stabilisation (≥ 4 months), $p=0.03$). Six patients remained stable for further 8 months from the time of data cut-off, and three of them up to 10 months later (figure 3).

Table 2 shows treatment-related adverse events that occurred in at least 1% of patients or all that were CTCAE grade 3 or worse. Treatment-related adverse events \leq grade 2 were reported in 84% of cycles (N=296) while grade 3 adverse event occurred only in two patients. No grade 4 or 5 adverse events were reported. The most common all-grade adverse events were fatigue (14 (18%) patients), hyperbilirubinaemia (7 (9%) patients), thrombocytopenia (6 (8%) patients), pruritus (6 (8%) patients), nausea/vomiting (6 (8%) patients) and muscular pain (6 (8%) patients). The only grade 3 treatment adverse event was thrombocytopenia occurring in two patients.

Only 2/31 patients required T-DM1 dose reduction with an overall dose intensity of 97.9%. For pertuzumab, no dose reduction was allowed as per protocol; incidentally, we report one patient that was overdosed receiving 138% of the intended pertuzumab dose, without toxicity.

DISCUSSION

The combination of pertuzumab and T-DM1 in HERACLES-B resulted in a 9.7% ORR, thus unexpectedly not reaching the predetermined trial end point $\geq 30\%$. Nevertheless, the vast majority (77.4%) of patients achieved disease control. Interestingly, although with all the caveats of comparing a time-dependent secondary end point across different phase II trials, the median PFS in HERACLES-B was in line with those reported in two other trials of dual anti-HER2 blockade in *KRAS* wild-type HER2+ mCRC: trastuzumab plus lapatinib in HERACLES-A and trastuzumab plus pertuzumab in MyPathway (4.1 vs 4.2 vs 5.3 months, respectively).^{7 17}

The antitumour effect observed, predominantly consisting of growth inhibition rather than shrinkage, is in line with observations in our preclinical models, in which the outcome was a durable stalling of tumour growth—persisting also after therapy suspension—rather than tumour regression. T-DM1 combines trastuzumab with DM1, a taxane-like cytotoxic agent that inhibits microtubule polymerisation and was specifically designed to minimise systemic toxicity through selective cytotoxic drug delivery to tumour cells.¹⁸ In mCRC, taxanes have failed to induce significant objective responses.^{19–21} Present results of HERACLES-B trial are also consistent with those of two multihistology basket trials for *ERBB2* amplified cancers^{22 23} according to which T-DM1

**Table 2** Adverse events

	Grades 1–2	Grade 3
Laboratory		
Thrombocytopaenia	6 (8%)	2
Hyperbilirubinaemia	7 (9%)	0
ALT increase	2 (3%)	0
Anaemia	2 (3%)	0
Neutropenia	2 (3%)	0
Metabolic and nutritional		
Anorexia	4 (5%)	0
Fatigue	14 (18%)	0
Dermatological		
Conjunctivitis	1 (1%)	0
Dermatitis	5 (6%)	0
Pruritus	6 (8%)	0
Gastrointestinal		
Abdominal pain	2 (3%)	0
Diarrhoea	2 (3%)	0
Nausea/Vomiting	6 (8%)	0
Pain		
Muscular pain	6 (8%)	0
Nervous		
Dysgeusia	3 (4%)	0
Limbs paraesthesia	1 (1%)	0
Bleeding		
Epistaxis	3 (4%)	0
Respiratory		
Cough	2 (3%)	0
Fever		
Fever	2 (3%)	0
Cardiovascular		
Hypertension	1 (1%)	0

Data are n (%). Treatment-related adverse events are reported if they occurred in at least 1% of patients or were of Common Terminology Criteria for Adverse Events grade 3 or worse. All patients who received at least one dose of drug are included (n=31). No grade 4 or 5 adverse events occurred. ALT, alanine aminotransferase.

monotherapy in mCRC did not engender objective responses but did induce some prolonged (≥ 6 months) disease stabilisations.²³ Good disease control in the presence of suboptimal induction of tumour shrinkage could also be explained by pharmacodynamic considerations. By using T-DM1 instead of trastuzumab, 30% less of the anti-HER2 antibody moiety was delivered during the first weeks of treatment due to the different schedule and the presence of the cytotoxic payload. The lower bioavailability of trastuzumab is likely counterweighed by the long-lasting therapeutic effect of T-DM1; indeed, the median PFS was comparable to that documented for

patients who responded to anti-HER2 combination therapies with tumour regression, and in preclinical models the growth inhibitory activity of T-DM1 plus pertuzumab was maintained for weeks after therapy discontinuation. Whether this durable impact is ascribable to the biological cooperation between antibody-mediated inactivation of HER2 and the cytotoxic component, and how pertuzumab contributes to cancer cytostasis and prolonged PFS, remains to be established. Finally, no other major clinical or molecular factors seem to have negatively influenced the therapeutic efficacy in HERACLES-B as compared with HERACLES-A regimen, since patients in the former were less pretreated (48% of cases received 4 lines of previous lines vs a median of 5 in HERACLES A) and distribution of HER2 IHC scoring was even more favourable (fractions of 3+/2+ were 81%/19% vs 74%/26%).

The HERACLES-B regimen exhibited a very good toxicity profile, with drug-related grade 3 adverse events in only two patients (thrombocytopaenia) and grade ≤ 2 events in 84% of cycles (N=296), mainly nausea and fatigue, also potentially suggesting a subefficacious dosage. The 9.7% ORR achieved by HERACLES-B regimen, lower than the 30% in HERACLES-A, should not dampen the interest for target-treating HER2+ mCRC; on the contrary, it underlines that several effective therapeutic options are at hand, including trastuzumab-based combinations with either small molecules tyrosine kinase inhibitors⁷ or monoclonal antibody, such as pertuzumab aimed, at different epitopes.^{17–24} Interestingly, recent data of the HER2+ mCRC arm of the Targeted Agent and Profiling Utilisation Registry study showed despite a higher ORR (25%) with the combination of trastuzumab and pertuzumab,²⁵ as data of the *KRAS* wild-type cohort of the MyPathway trial, a similar PFS of 4.3 months, once again indicating that a maintained disease control may be indicative of the antitumour potential of a HER2 therapeutic blockade. Other emerging therapeutic strategies include the development of more potent anti-HER2 agents,²⁶ such as bispecific antibodies which can bind two non-overlapping epitopes on HER2 or engage immune mediators together with HER2 binding, and novel antibody-drug conjugates such as trastuzumab deruxtecan.²⁷ The latter agent delivers through the HER2 receptor a topoisomerase I inhibitor payload, and positive results have been recently reported for mCRC in the DESTINY CRC01 trial.^{27–28}

In conclusion, the HERACLES-B trial did not reach its primary end point of ORR; however, this anti-HER2 regimen provided remarkable rates of sustained disease control at the price of little toxicity. Therefore, this treatment can be regarded as a viable therapeutic option for patients with HER2+ mCRC with low tumour burden disease not requiring substantial shrinkage, especially in the context of sequential lines of HER2-targeted therapy.^{29–30}

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Contributors AS-B and SS were involved in the study conception and design, provision of study materials or patient recruitment, data analysis and interpretation, manuscript writing and manuscript approval. SM, AB and LT were involved in the study conception and design, data analysis and interpretation, manuscript writing and manuscript approval. CM was involved in collection and assembly of data, data analysis and interpretation, project management, manuscript writing and manuscript approval. FT was involved in provision of study materials or patient recruitment, manuscript writing and manuscript approval. SL, EF, FL, FB, VZ, FC, AAr, AAm and KB were involved in provision of study materials or patient recruitment, and manuscript approval. EV, EB and EG were involved in generation of molecular data, manuscript writing and manuscript approval. SG was involved in data management, collection and assembly of data, manuscript writing and manuscript approval. VT was involved in statistical analysis, manuscript writing and manuscript approval. AV, DR and GC involved in radiological analyses, collection and assembly of data and manuscript approval. AS provided overall study support and contributed to tissue specimen pathology analysis and manuscript approval.

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Competing interests AS-B is advisory board member for Amgen, Bayer, Sanofi and Servier. SL reports consulting or advisory roles in Amgen, Bayer, Merck, Lilly and Servier; speakers' bureau roles at Lilly, Roche and BMS, and research funding from Amgen and Merck. VZ is advisory board member for Bristol-Myers Squibb and Merck; speakers' bureau for AstraZeneca and Lilly; reports personal fees from Bayer, Roche, Servier. FC reported receipt of honoraria or consultation fees for speaker, consultancy or advisory roles at Amgen, Bayer, Bristol-Myers Squibb, Celgene, Merck Serono, Pfizer, Roche, Servier; direct research funding as the principal investigator for institutional research projects from Amgen, Bayer, Merck Serono, Roche, Ipsen; institutional financial interests, financial support for clinical trials or contracted research from Merck Serono, Roche, Symphogen, Array; leadership Positions in Professional Societies (non-financial interests) including ESMO past president, and president of the Associazione Italiana Oncologia Toracica. AAr reports grants and personal fees from BMS, personal fees from MSD, personal fees from Eli-Lilly, personal fees from Boehringer, personal fees from Pfizer, grants from Celgene, grants and personal fees from Roche, outside the submitted work. AAm is advisory Board from Amgen, Roche, Bayer. LT reports grants from Symphogen, grants from Servier, grants from Merus, grants from Pfizer, grants from Menarini, personal fees from AstraZeneca, personal fees from Merck KGaA, personal fees from Eli Lilly, outside the submitted work. SS is advisory board member for Amgen, Bayer, BMS, CheckmAb, Clovis, Daiichi-Sankyo, Merck, Roche-Genentech, Seattle Genetics.

Patient consent for publication Not required.

Ethics approval All animal procedures were approved by the Ethical Commission of the Candiolo Cancer Institute and by the Italian Ministry of Health (authorisation 806/2016-PR). All patients provided written informed consent. The study was done in accordance with the principles of the Declaration of Helsinki and the International Conference on Harmonization and Good Clinical Practice guidelines. The institutional review boards of the participating centres approved the study procedures.

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REFERENCES

- King C, Kraus M, Aaronson S. Amplification of a novel v-erbB-related gene in a human mammary carcinoma. *Science* 1985;229:974-6.
- Hayes DF. HER2 and Breast Cancer - A Phenomenal Success Story. *N Engl J Med* 2019;381:1284-6.
- Ross JS, Wang K, Gay L, et al. New routes to targeted therapy of intrahepatic cholangiocarcinomas revealed by next-generation sequencing. *Oncologist* 2014;19:235-42.
- 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.
- Siena S, Sartore-Bianchi A, Marsoni S, et al. Targeting the human epidermal growth factor receptor 2 (HER2) oncogene in colorectal cancer. *Ann Oncol* 2018;29:1108-19.
- Bertotti A, Migliardi G, Galimi F, et al. A molecularly annotated platform of patient-derived xenografts ("xenopatient") identifies HER2 as an effective therapeutic target in cetuximab-resistant colorectal cancer. *Cancer Discov* 2011;1:508-23.
- 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;17:738-46.
- Sartore-Bianchi A, Lonardi S, Aglietta M, et al. Central nervous system as possible site of relapse in ErbB2-positive metastatic colorectal cancer: long-term results of treatment with trastuzumab and lapatinib. *JAMA Oncol* 2020. doi:10.1001/jamaoncol.2020.0571. [Epub ahead of print: 23 Apr 2020].
- 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.
- Siravegna G, Lazzari L, Crisafulli G, et al. Radiologic and genomic evolution of individual metastases during HER2 blockade in colorectal cancer. *Cancer Cell* 2018;34:148-62.
- Strickler JH, Zemla T, Ou F-S, 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:v200.
- Yoshino T, Siena S, Dalal R, et al. A multicenter, multicohort, phase II study of trastuzumab deruxtecan (DS-8201a) in subjects with HER2-expressing metastatic colorectal cancer. *Ann Oncol* 2018;29:ix45.
- Perez EA, Barrios C, Eiermann W, et al. Trastuzumab emtansine with or without pertuzumab versus trastuzumab plus taxane for human epidermal growth factor receptor 2-positive, advanced breast cancer: primary results from the phase III MARIANNE study. *J Clin Oncol* 2017;35:141-8.
- Miller KD, Diéras V, Harbeck N, et al. Phase IIA trial of trastuzumab emtansine with pertuzumab for patients with human epidermal growth factor receptor 2-positive, locally advanced, or metastatic breast cancer. *J Clin Oncol* 2014;32:1437-44.



- 15 Yarden Y, Sliwkowski MX. Untangling the ErbB signalling network. *Nat Rev Mol Cell Biol* 2001;2:127–37.
- 16 Zanella ER, Galimi F, Sassi F, *et al.* Igf2 is an actionable target that identifies a distinct subpopulation of colorectal cancer patients with marginal response to anti-EGFR therapies. *Sci Transl Med* 2015;7:272ra12.
- 17 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;20:518–30.
- 18 Perez EA, Barrios C, Eiermann W, *et al.* Trastuzumab emtansine with or without pertuzumab versus trastuzumab with taxane for human epidermal growth factor receptor 2-positive advanced breast cancer: final results from MARIANNE. *Cancer* 2019;125:3974–84.
- 19 Taguchi T. [An early phase II clinical study of RP56976 (docetaxel) in patients with cancer of the gastrointestinal tract]. *Gan To Kagaku Ryoho* 1994;21:2431–7.
- 20 Pazdur R, Lassere Y, Soh LT, *et al.* Phase II trial of docetaxel (Taxotere) in metastatic colorectal carcinoma. *Ann Oncol* 1994;5:468–70.
- 21 Swanton C, Tomlinson I, Downward J. Chromosomal instability, colorectal cancer and taxane resistance. *Cell Cycle* 2006;5:818–23.
- 22 BT L, Makker V, Buonocore DJ, *et al.* A multi-histology basket trial of ado-trastuzumab emtansine in patients with HER2 amplified cancers. *J Clin Oncol* 2018;36:2502
- 23 Jhaveri KL, Wang XV, Makker V, *et al.* Ado-trastuzumab emtansine (T-DM1) in patients with HER2-amplified tumors excluding breast and gastric/gastroesophageal junction (GEJ) adenocarcinomas: results from the NCI-MATCH trial (EAY131) subprotocol Q. *Ann Oncol* 2019;30:1821–30.
- 24 Nakamura Y, Okamoto W, Kato T, *et al.* Triumph: primary efficacy of a phase II trial of trastuzumab (T) and pertuzumab (P) in patients (PTS) with metastatic colorectal cancer (mCRC) with HER2 (ErbB2) amplification (AMP) in tumour tissue or circulating tumour DNA (ctDNA): a GOZILA sub-study. *Ann Oncol* 2019;30:v199–200.
- 25 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. *JCO* 2020;38:132.
- 26 Oh D-Y, Bang Y-J. HER2-targeted therapies - a role beyond breast cancer. *Nat Rev Clin Oncol* 2020;17:33–48.
- 27 Ogitani Y, Aida T, Hagihara K, *et al.* DS-8201a, a novel HER2-Targeting ADC with a novel DNA topoisomerase I inhibitor, demonstrates a promising antitumor efficacy with differentiation from T-DM1. *Clin Cancer Res* 2016;22:5097–108.
- 28 Siena S, Di Bartolomeo M, Raghav KPS, *et al.* A phase II, multicenter, open-label study of trastuzumab deruxtecan (T-DXd; DS-8201) in patients (PTS) with HER2-expressing metastatic colorectal cancer (mCRC): DESTINY-CRC01. *JCO* 2020;38:4000.
- 29 Martinelli E, Troiani T, Sforza V, *et al.* Sequential HER2 blockade as effective therapy in chemorefractory, HER2 gene-amplified, Ras wild-type, metastatic colorectal cancer: learning from a clinical case. *ESMO Open* 2018;3:e000299.
- 30 Siena S, Bardelli A, Sartore-Bianchi A, *et al.* HER2 amplification as a 'molecular bait' for trastuzumab-emtansine (T-DM1) precision chemotherapy to overcome anti-HER2 resistance in HER2 positive metastatic colorectal cancer: The HERACLES-RESCUE trial. *J Clin Oncol* 2016;34:TPS774