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

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

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Summary

Background

In metastatic colorectal cancer (mCRC), actionable genetic lesions represent opportunities for new therapies. We previously found that dual HER2 blockade with trastuzumab and lapatinib, but not treatment with either drug alone, led to remarkable inhibition of tumour growth in patient-derived tumourgrafts of *HER2*-amplified mCRC. We then conducted a diagnostic study in 256 CRC cases to define CRC-specific criteria for assessment of HER2 positivity by immunohistochemistry (IHC) and in situ hybridization (ISH). The ensuing diagnostic algorithm was utilised to screen HER2-positive tumours for therapeutic targeting in patients in the HERACLES phase 2 trial.

Methods

The open-label, controlled phase 2 HERACLES trial was conducted at four Italian academic centres. Adult patients with *KRAS* exon 2 (codons 12 and 13) wild-type and HER2-positive mCRC refractory to standard of care (including cetuximab or panitumumab), an Eastern Cooperative Oncology Group performance status of 0 or 1, and at least one measurable lesion were enrolled in the study. HER2 positivity in tumour samples was defined by IHC and fluorescence in situ hybridization (FISH) according to previously validated CRC-specific diagnostic criteria. Patients received trastuzumab i.v. at 4 mg/kg loading dose followed by 2 mg/kg weekly, and lapatinib p.o. at 1000 mg daily, until evidence of disease progression. The primary endpoint was the objective response rate, which was assessed by independent central review in the intention-to-treat population. Secondary endpoints were progression-free survival and safety. Safety was evaluated in any patient who received at least one dose of treatment. At the final analysis for the primary objective, six objective responses out of 27 patients were necessary to declare the study positive with a power of at least 85% at a one-sided alpha level of 0.05. This trial is registered with EudraCT, number 2012-002128-33.

Findings

Between August 27, 2012, and May 15, 2015, 914 patients with *KRAS* exon 2 (codons 12 and 13) wild-type mCRCs were screened and 46 (5%) were found to harbour HER2-positive tumours. Of these, 27 were eligible for the HERACLES trial. All were fully evaluable for response. Enrolment was closed on May 15, 2015. At the time of data cut-off on October 15, 2015, with a median follow-up of 94 weeks (IQR 51-127), eight (30%, 95% CI 14-50) of 27 patients achieved an objective response, with one (4%, 95% CI -3-11) of eight achieving a complete response, and seven (26%, 95% CI 9-43) a partial response; 12 (44%, 95% CI 25-63) of 27 patients had stable disease (SD). Toxicity was generally limited to grade 1-2, with

six (22%) of 27 patients experiencing grade-3 side effects: fatigue in four patients, skin rash in one patient, and elevated bilirubin levels in one patient. No drug-related serious adverse events were observed.

Interpretation

The rationally designed combination of trastuzumab and lapatinib is effective and well tolerated in treatment-refractory mCRC patients harbouring HER2-positive tumours. Our results provide preliminary evidence for the activity of this combination in clinical practice.

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INTRODUCTION

Relevant therapeutic advances have been achieved by targeting oncogenic drivers of tumour onset and progression in several human malignancies[1]. Colorectal cancer (CRC) stands as an exception. Although the genomic landscape of CRC has been extensively explored[2,3], the knowledge of clinically actionable genetic abnormalities in this tumour type remains limited. Pharmacologic blockade of the epidermal growth factor receptor (EGFR) with specific monoclonal antibodies, namely, cetuximab and panitumumab, represents the mainstay of tumour targeted therapy for metastatic CRC (mCRC)[4]. However, the *EGFR* gene is seldom activated by mutations or amplifications[2,3,5]. Treatment with cetuximab or panitumumab, in monotherapy or in combination with a chemotherapy back-bone, has resulted in limited clinical benefit when applied to molecularly unselected mCRC patients[6]. More substantial response and survival improvements have been obtained by excluding patients with tumours harbouring *KRAS*, *NRAS,* or *BRAF* mutations from EGFR-targeted therapy[4]. Such alterations, which constitutively activate typical EGFR downstream transducers, have been shown to trigger substitute survival pathways that bypass therapeutic blockade of EGFR signalling, thus abating the efficacy of anti-EGFR antibodies ("primary resistance")[7].

Building on these premises and with the aim of developing novel targeted therapies for mCRC, we sought to uncover pharmacologically actionable mechanisms of primary resistance to anti-EGFR antibodies. To this objective we deployed a large colony of patient-derived CRC tumourgrafts that were molecularly profiled for several genetic parameters and concomitantly propagated in recipient mice for therapeutic studies[3,8,9]. By doing so, we detected amplification of the *HER2* gene in a fraction of CRC tumourgrafts that proved to be resistant to cetuximab and did not harbour mutations in *KRAS*, *NRAS* or *BRAF*[3,8,9].

To discover novel therapeutic options in this genotype-specific CRC subpopulation, mice bearing *HER2*-amplified, cetuximab-resistant tumourgrafts were treated with different HER2-targeted therapies, alone or in combination. In these preclinical models, monotherapy with either HER2 tyrosine kinase inhibitors or anti-HER2 antibodies was poorly effective[8,9]. Conversely, the combination of an antibody (pertuzumab or trastuzumab) and a tyrosine kinase inhibitor (lapatinib) led to durable tumour shrinkage[8,9]. While the pertuzumab-lapatinib combination is not clinically licensed and knowledge of its toxicity profile is incomplete, the trastuzumab-lapatinib combination is a standard therapy for HER2-positive breast cancer[10]. In light of these considerations, we reasoned that HER2 could represent a valuable therapeutic target in patients with *KRAS* wild-type mCRC resistant to anti-EGFR antibodies, and selected the combination of trastuzumab and lapatinib as a treatment modality for clinical investigation. HERACLES (*HER2* Amplification for Colo-RectaL Cancer Enhanced Stratification) is an independent phase 2 trial to assess the efficacy of trastuzumab and lapatinib in patients with HER2-positive, *KRAS* exon 2 (codons 12 and 13) wild-type mCRC, after failure of standard therapies. The HERACLES trial was preceded by a diagnostic study in 256 CRC cases, which was aimed at defining CRC-specific criteria for assessment of HER2 positivity by immunohistochemistry (IHC) and in situ hybridization (HERACLES Diagnostic Criteria)[11]. The ensuing diagnostic algorithm was utilised in the present study.

METHODS

Study design and patients

HERACLES (EudraCT 2012-002128-33) is an academic, non-profit, open-label trial conducted with a stage 1 design[12] in four Italian cancer centres (Appendix p 1). The study,

designed and analysed by the investigators, was sponsored by the Istituto di Candiolo FPO-IRCCS. The protocol is available at this link. Eligible patients had an histologically confirmed diagnosis of metastatic colorectal cancer with *KRAS* exon 2 (codons 12 and 13) wild-type status and HER2 positivity, as defined according to the CRC-specific HERACLES Diagnostic Criteria (tumours with 2+/3+ HER2 score in more than 50% of cells by IHC or with a HER2:CEP17 ratio higher than 2 in more than 50% of cells by FISH)[11]. At least one measurable lesion, as defined by Response Criteria Evaluation in Solid Tumours (RECIST, version 1.1), was required. Other major inclusion criteria were: an Eastern Cooperative Oncology Group (ECOG) performance status of 0 or 1; age 18 years or older; progression while on treatment or within six months from treatment with approved standard therapies for mCRC (fluropyrimidines, oxaliplatin, irinotecan, cetuximab or panitumumab containing regimens; anti-angiogenic regimens); adequate haematological, renal, and hepatobiliary functions. The study was conducted in accordance with the principles of the Declaration of Helsinki and the International Conference on Harmonization and Good Clinical Practice guidelines. Institutional review boards of all participating centres approved the study procedures. All patients provided written informed consent. Detailed inclusion and exclusion criteria are found in appendix p 2.

Procedures

Trastuzumab was administered i.v. at 4 mg/kg loading dose followed by 2 mg/kg weekly, and lapatinib p.o. at 1000 mg daily. Trastuzumab was manufactured by Roche Pharma AG, Grenzach-Wyhlen, Germany; lapatinib was manufactured by Glaxo Wellcome Operation, Hertfordshire, UK. No dose reduction was allowed for trastuzumab. The dose of lapatinib was permitted to be reduced to 750 mg/day; patients requiring further dose reductions had to be

taken off study. Dose reductions were made because of adverse events. Guidance on how to manage the adverse events and the appropriate dose reductions are provided in the protocol. Treatment was continued until disease progression, the occurrence of an adverse event requiring treatment cessation, withdrawal of consent, or investigators decision to terminate treatment. Tumour assessments were performed at each centre by local radiologists within 2 weeks before treatment start and were repeated every 8 weeks according to RECIST version 1.1. All patients underwent tumour imaging including CT of chest, abdomen or pelvis, and MRI brain scans when clinically indicated according to protocol. Tumour assessments were performed at baseline and every eight weeks thereafter until progression. All tumour assessments of each patient were reviewed centrally by two radiologists (D. R., A. V.) who read the CT scans blinded using the mintLesion™ software to collect, store, and guide the revision of the imaging results. The imaging review protocol and tumour assessment re-conciliation report are included in appendix p 4. Safety was continuously assessed and graded according to the Common Terminology Criteria for Adverse Events (CTCAE), version 4.0. Laboratory testing (haematology, serum chemistry, urine analysis and electrocardiograms) was conducted at baseline (days -14 to 0), at day 1 of every cycle, and at the end of treatment. LVEF was assessed at baseline (days -14 to 0), every three months thereafter, and at the end of treatment. Treatment compliance was calculated in each patient according to the sum of actual dose received by cycle against the total planned dose. Response to prior anti-EGFR treatment was classified according to the algorithm in appendix p 10. Dose intensity of previous chemotherapy was not collected. Rather, total time on treatment (defined by adding, for each patient, the net time on treatment for each prior regimen) was calculated as a proxy of cumulative exposure to earlier lines treatment.

Patients were screened for HER2 positivity at participating institutions. HER2 protein

expression was assessed by IHC on the most recently available formalin-fixed paraffin-embedded tumour sample of each screened patient according to the HERACLES Diagnostic Criteria reported in appendix p 11. Screening-positive samples were re-tested at the central pathology laboratory at Niguarda Cancer Centre, Milan, Italy for HER2 protein expression and gene amplification by FISH, as previously described[11]. In addition, *HER2* gene copy number (GCN) was assessed by quantitative polymerase-chain reaction (qPCR), as described in appendix p 12.

Outcomes

The primary objective was to determine the anti-tumour activity of trastuzumab and lapatinib in patients with HER2-positive mCRC. The primary endpoint was the objective response rate. Secondary endpoints were progression-free survival and safety. Translational exploratory objectives were aimed at studying the molecular determinants of response and resistance to study treatment and included molecular characterisation of solid and liquid biopsies in tumour and blood samples. Part of these studies will be reported separately.

Statistical analyses

For the primary objectives, we determined that we would need to enrol 27 patients in order to achieve a power of at least 85% to test the null hypothesis that the rate of response to the lapatinib-trastuzumab combination would be 10% or less, versus the alternative hypothesis that the response rate would be 30% or more, at a one-sided alpha level of 0·05. Six objective responses were necessary to declare the study positive. Time-to-event variables were

estimated using the Kaplan-Meier product-limit method and the significance of differences between curves was determined using the log-rank test. Results were considered significant when p values were 0·05 or less; Fisher's exact test was used for subgroup comparisons of categorical variables. For the translational exploratory objectives, receiver operating characteristic (ROC) curve analysis, calculated by a non-parametric method[13], was used to assess HER2 protein expression intensity by IHC and *HER2* GCN variation by qPCR as potential classifiers. To quantify the discriminatory accuracy of the IHC score and the GCN variation as predictors of efficacy outcomes, we used Harrell C statistics Area under the curve analysis[14] and a recursive partitioning method for detecting the best cut-off points[15]. Analyses were performed with the use of SAS statistical software, version 9.2 (SAS Institute) and Stata version 12.

Role of the Funding Sources

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data. The corresponding author had final responsibility for the decision to submit for publication.

RESULTS

Patients

Nine hundred and fourteen patients with *KRAS* exon 2 (codons 12 and 13) wild type mCRC were screened and 46 (5%) had HER2-positive tumours. Of these, 27 were eligible for the HERACLES trial (Study CONSORT in appendix p 13). From August 27, 2012, to May 15, 2015, all 27 patients were accrued, with data lock on October 15, 2015. The assessment of HER2 status was conducted centrally for 20 (74%) of 27 samples and locally, with central re-testing, for the remaining seven cases. Concordance was 71% (5/7). Details are included in appendix p 14. Tested samples were derived from primary tumours in 10 (59%) of 27 patients and from metastatic lesions in the remaining 17. Paired HER2 assessments in the primary tumour and distant metastases, conducted on three available cases, showed full concordance for HER2 expression score. In one case, FISH was also performed: the analysis showed a similar percentage of cells with *HER2* amplification (95%) in matched primary and metastatic samples; the extent of amplification was higher in the metastatic lesion (HER2:CEP17 ratio in primary sample = 2·64; liver metastasis sample = 10). As detailed in **Table 1,** the vast majority of the patient population had extensive metastatic disease and distal colon tumours. Patients were heavily pre-treated: 20 (74%) of 27 had received at least four prior regimens (median 5; range 2-11), including bevacizumab, regorafenib or aflibercept, and all had been previously

treated with EGFR-targeted antibodies. Notably, none of the 15 patients (56%) out of the 27 evaluable for response to anti-EGFR therapy achieved an objective response to either cetuximab or panitumumab (appendix p 15).

The average total time on prior treatment, available for 135 of the 136 prior regimens administered to the 27 enrolled patients, was 20 months (median 20 months). Time on treatment differed according to primary tumour site (**Table 1**), in particular for patients with proximal colon localization (average 16 months, median 15 months).

Efficacy

Efficacy results are summarised in **Table 2**. Among the 27 enrolled patients, one (4%) had a complete response (CR), seven (26%) had a partial response (PR), and 12 (44%) had stable disease (SD). Therefore, the primary endpoint was met, with an overall objective response rate of 30% (95% confidence interval [CI] 14 to 50). In the 12 patients with SD, stabilisation lasted 16 weeks or longer in eight patients, including one patient with a PR (33% tumour shrinkage) that was not confirmed due to refusal to undergo a confirmatory CT scan. Overall, the rate of disease control (CR+PR+SD lasting more than 16 weeks) was 59% (95% CI, 39 to 78). Two (7%) of 27 patients had clinically detected tumour progression prior to first radiological assessment at the eighth week. Among the 25 patients evaluated for radiological response, the vast majority (84%, 21/25) experienced tumour shrinkage (**Figure 1A**). Seven (88%) out of eight patients who achieved objective response had tumours with a 3+ HER2 score by IHC, which was paralleled by a high *HER2* GCN as assessed by FISH and qPCR (appendix p 16); among the patients with disease stabilisation or progression, only 10 (59%) out of 17 had 3+ HER2-positive tumours.

The median time to best objective response was 8 weeks (95% CI, 3 to 16); the median duration of response was 38 weeks (95% CI, 24 to 94+); three patients continued to respond by the time of data cut-off at 5, 11, and 21 months after treatment initiation (**Figure 1B**). Among the 22 patients with radiological evidence of progressive disease, 11 (50%) had developed new lesions; in seven (64%) of these 11 patients, the original target and non-target lesions maintained response.

At a median follow-up of 94 weeks (IQR 51-127), the median progression-free survival time was 21 weeks (95% CI, 16 to 32) (appendix p 17). At 6 months after treatment initiation, nine (33%) of 27 patients did not show signs of disease progression and were censored for survival analyses. The median overall survival was 46 weeks (95% CI, 33 to 68), with all the censored patients alive at the time of data cut-off, and 12 (45%) of 27 patients still alive at one year (appendix p 17).

Safety and treatment compliance

All treatment-related adverse events occurring in $\geq$5% of patients or of CTCAE grade $\geq$3 are reported in **Table 3.** The most common toxicities in the 27 enrolled patients were: diarrhoea (21 [78%]), rash (13 [48%]), fatigue (13 [48%]), paronychia (9 [33%]), and conjunctivitis (5 [19%]). There were no treatment-related grade 4 and 5 adverse events. Only six (22%) of 27 patients experienced grade 3 adverse events: fatigue (four patients), skin rash (one patient), and elevated bilirubin levels (one patient). No treatment-related cardiotoxicity was observed. No patients interrupted treatment due to toxicity, nor was treatment withdrawal requested in any case. Lapatinib dose was reduced in three (11%) of 27 patients (in two cases for diarrhoea, in one case for rash). Overall more than 90% of patients received the planned dosage.

Molecular Determinants of Response

A recursive partitioning estimate of the area under the ROC curve identified a discriminatory *HER2* GCN cut-off value of 9·45, as assessed by qPCR, for optimal segregation of responders versus non responders (appendix p 18). The median progression-free survival of patients harbouring tumours at or above this cut-off value was 29 weeks (CI 95%, 19-43), as compared to a median progression-free survival of 16 weeks (CI 95%, 3 to 17) for patients with tumours scoring below this threshold (p= 0·0001) (**Figure 2).** Patients with *HER2* GCN levels higher than the ROC-based cut-off had a 33% higher probability of response than patients with a lower GCN [HR 0·67 (CI 95%, 0·6-0·8)]. None of the nine patients with a GCN below 9·45 had an objective response, while all eight responders had GCNs above that value (44% [95% CI, 22 to 79] p=0·02).

Discussion

The results of this phase 2 study show that the combination of trastuzumab and lapatinib is active in HER2-positive mCRC patients refractory to chemotherapy and anti-EGFR antibodies. At the time of this analysis, 30% of patients achieved an objective response according to RECIST 1.1. Responses were long-lasting, with a median duration of 38 weeks, and the combination was well tolerated, with predominant grade 1 or 2 toxicities.

HER2-positive mCRC patients, identifiable as those harbouring tumours with HER2 positivity by CRC specific criteria[11], account for 5% of individuals with *KRAS* exon 2 wild type tumours. To our knowledge, this genetically defined subpopulation is the first showing consistent sensitivity to pharmacologic blockade of a specific oncogenic product in mCRC. In the few

cases where the comparison was possible, we found concordance for HER2 positivity in the primary tumour and the matched metastatic lesion, in line with previous findings in a larger retrospective series of 44 paired samples[11]. These results point to HER2 gene amplification/protein overexpression as an early molecular alteration that persists during tumour progression.

Until now, the clinical application of targeted therapies in mCRC subgroups classified according to the tumour molecular status has been confined to the exclusion of patients with undruggable *KRAS* and *NRAS* mutations from therapy with panitumumab or cetuximab. Molecular alterations with potential therapeutic actionability, such as those involving *BRAF*, *MET, MMR* genes[4] and rare *NTRK*[16] and *ALK* gene fusions[17], are still under investigation.

The deployment of HER2-targeted drugs in mCRC is not without precedent. Earlier clinical trials evaluating the efficacy of adding trastuzumab to standard chemotherapy were prematurely closed because of inconclusive results, attributable to lack of stringent molecular parameters for patient selection and of CRC-specific diagnostic criteria for HER2 positivity[18,19]. Anecdotal reports have described a short-lived response to single-agent trastuzumab in one patient with a rectal tumour displaying high-grade *HER2* amplification[20], as well as responses to a combination of trastuzumab (or pertuzumab) and standard chemotherapy in two patients with HER2-positive distal colon cancers[21]. Diverging from such previous evidence, the HERACLES study capitalised on two propaedeutic pieces of information that led to more knowledgeable diagnostic and therapeutic decisions. First, we established HERACLES diagnostic criteria for reliable assessment of HER2 positivity in CRC by adaptation of existing protocols and collegial revision of a training set of 256 archival samples, followed by a validation step in a clinical cohort of additional 830 patients[11]. Second,

we utilised the therapeutic regimen that proved to be the most effective, among different options, according to preclinical testing of several HER2 antagonists in *HER2*-amplified patient-derived mCRC tumourgrafts[8,9]. Descriptive therapeutic results have been consolidated by mechanistic investigation in cell lines and patient tumourgrafts; in particular, it was found that the combinatorial mode of action of trastuzumab and lapatinib relies on the ability of trastuzumab to prevent paradoxical phosphorylation of HER3, which occurs during prolonged lapatinib treatment as a consequence of compensatory *HER3* transcriptional upregulation[9].

The objective response and disease control rates observed in this study (of 30% [8/27] and 59% [16/27], respectively) were achieved in patients who had been heavily pre-treated. These results are particularly meaningful when benchmarked against response rates achievable with licensed third-line therapies such as the multikinase inhibitor regorafenib[22] and the trifluridine-tipiracil combination TAS 102[23]. Data are even more compelling when compared to the 15-20% objective response rate that is commonly observed in earlier lines of therapy in patients with *KRAS* wild-type mCRC treated with anti-EGFR antibodies[24]. In this regard, it should be noted that none of the patients enrolled in our study who were evaluable for response to anti-EGFR therapy obtained an objective response to either cetuximab or panitumumab. These prospective findings corroborate independent retrospective analyses highlighting an association between *HER2* amplification and resistance to anti-EGFR antibodies[8,25,26]. Therefore, considering the predictably limited efficacy of panitumumab or cetuximab, one can envisage anticipation of dual HER2-targeted therapeutic blockade before EGFR inhibitors in the salvage setting and also in less advanced contexts. Notably, most of the treated patients (85% [23/27]) had tumours in their distal colon or rectum; this information confirms previous studies[27] and might prove valuable to instruct enrichment strategies for HER2-positive CRC.

On the basis of *post-hoc* analysis of molecular determinants of therapeutic sensitivity, progression-free survival appeared to be influenced by the magnitude of gene amplification. Using the discriminatory cut-off of 9·45 gene copy number identified by the ROC analysis of progression-free survival, also the responses proved to be significantly enriched in tumours displaying higher *HER2* gene copy number. The fact that intense *HER2* amplification correlated with treatment efficacy is in line with the notion that tumours can be dependent on relentless oncogenic signalling, as the one triggered by abnormally high *HER2* gene dosage, for their growth and survival[28]. Interestingly, high HER2 expression has been found to correlate with neo-adjuvant response to the combination of lapatinib and trastuzumab also in HER2-positive breast cancer[29]. In contrast, the value of HER2 gene amplification/protein expression in predicting sensitivity to HER2-targeting agents remains controversial in advanced breast and gastric cancers, likely because the therapeutic outcome is blurred by concomitant chemotherapy [30,31]. Albeit manifest, in the HERACLES trial the association between strong *HER2* amplification and objective response is not absolute and univocal. In two patients, tumours proved to be refractory in the face of an elevated gene copy number, and three further patients with tumours exhibiting more than 10 gene copies experienced disease stabilisation rather than regression. This intrinsic resistance is common to many other targeted therapies in genetically defined tumour contexts and is probably driven by concurrent cytoprotective cues that safeguard cancer cells against HER2 inactivation by conveying substitute survival signals.

According to an extensive genetic profiling of independent sample sets, and different from that described for EGFR, mutations in components of the RAS pathway are unlikely to account for primary resistance to HER2 blockade in *HER2*-amplified mCRC[3]. Indeed, *HER2* amplification is mutually exclusive with alterations in *KRAS*, *NRAS* and *BRAF* in two large

retrospective cohorts of advanced colorectal cancers patients[2,8]. However, it is worth noting that such data were obtained in samples from patients who, in their vast majority, were cetuximab- or panitumumab-naïve. At present, we cannot anticipate whether previous anti-EGFR treatment in the HERACLES cohort might have selected for subclones harbouring mutations in the RAS pathway. A preliminary analysis of extended *RAS* mutation in baseline (pre-treatment) plasma samples by digital droplet PCR (ddPCR) revealed a relatively pervasive BRAF V600E mutation (31% fractional abundance) in one patient experiencing progression as best response. A second patient experiencing stabilisation of disease (lasting 4 months) as best response harboured a tumour with a subclonal KRAS A146T mutation (0.8% fractional abundance). We are now in the process of extending the ddPCR analysis to plasma samples longitudinally collected over the course of the therapy, as well as performing next generation sequencing analysis on the original tissue samples and in biopsies obtained at progression. It is expected that unveiling the genomic landscape and the signalling network of *HER2*-amplified cases will provide the foundation to understanding tumour heterogeneity, clonal evolution, and resistance to HER2-directed therapies as a means to inform future therapeutic decisions.

In conclusion, we introduce *HER2* amplification as a clinically relevant genetic alteration in mCRC. This alteration can be screened with established diagnostic tools, is successfully actionable at the therapeutic level, and is found in patients at the non-negligible frequency of 3-5%, similar to that of other mutant targets for which licensed drugs are effective (for example in lung cancer[32]). Results of the HERACLES study have been achieved through a precision oncology program that stemmed from preclinical findings in pertinent *in vivo* models[8], was strengthened by a rigorous methodological effort for molecular diagnosis[11], and finally resulted in the present new therapy for CRC patients with HER2-positive tumours.

RESEARCH IN CONTEXT

Evidence before this study

This study was initiated based on preclinical data indicating that patient-derived tumourgrafts of metastatic colorectal cancers harbouring amplification of the *HER2* oncogene responded with massive tumour regression to the combination of trastuzumab and lapatinib, but not to either therapy alone. Additionally, we did a PubMed search on Feb 7, 2012, without date or language restrictions, using the search terms “trastuzumab, lapatinib and colorectal cancer”. We also searched abstracts from the American Society of Clinical Oncology and the European Society for Medical Oncology. No trials were found that examined the combination of trastuzumab and lapatinib in colorectal cancer patients. This combination had been shown to be well tolerated and active in patients with HER2-positive mammary tumours.

Added value of this study

To our knowledge, this is the first trial to assess the combination of trastuzumab and lapatinib in patients with HER2-positive colorectal carcinoma. Our findings document that the combination of trastuzumab and lapatinib is active, in the absence of any chemotherapy back bone, in heavily pre-treated patients with metastatic disease. Additionally, our results indicate that the extent of *HER2* gene copy number and HER2 protein expression may associate with better response to treatment. Finally, this combination appears to be more active when compared with response rates obtained with other therapeutic modalities in heavily pre-treated patients.

Implications of all the available evidence

The results of this study could change the day-to-day clinical care management of patients

with advanced HER2-positive colorectal cancer. Our findings could lead to the use of trastuzumab and lapatinib in earlier lines of treatment, with a possibility of chemotherapy-free regimens, for patients with HER2-positive tumours. A phase 3 trial is warranted for more definitive data.

Author Contribution

ASB, and SS were involved in the study conception and design, provision of study materials or patients, data analysis and interpretation, manuscript writing, and manuscript approval. SM 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. AA, KB, FB, FC, ID, FL, SL, EM, LP, PR, TT, and VZ were involved in provision of study materials or patients, manuscript writing, and manuscript approval. AC, CL, GS, EV, and SV were involved in generation of molecular data, manuscript writing, and manuscript approval. SG and GM, were 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. AB and AB were involved in the study conception and design, manuscript writing, and manuscript approval. DR and AV were involved in radiology assessment and manuscript writing, and manuscript approval.

Declaration of interest statement

ASB received personal fees from Amgen, Bayer, Lilly and Roche, outside the submitted work; DR was a lecturer for G.E. Healthcare, outside the submitted work, and has an issued patent with Springer s.r.l. LT received grants from AIRC - Associazione Italiana per la Ricerca sul Cancro, during the conduct of the study, and grants from Merus B.V, outside the submitted

work. PMC reports grants from AIRC - Associazione Italiana per la Ricerca sul Cancro, during the conduct of the study; grants and personal fees from Metheresis Translational Research, personal fees from Pieris, Molecular Partners, Samsung, Janssen Pharmaceutica NV, outside the submitted work; In addition reports two issued patent: “Anti-met monoclonal antibody, fragments and vectors thereof, for the treatment of tumors and corresponding products”, and “MET inhibitors for enhancing radiotherapy efficacy”. SS received personal fees from Amgen, Roche, Bayer, Sanofi, Merck, Merus, Ignyta, Eli Lilly, and Novartis outside the submitted work. The other authors declared no conflicts of interest.

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Figure legends:

Figure 1 Radiographic response

Panel A shows the best tumour response of patients treated with lapatinib and trastuzumab. The bars indicate best percent change in the target tumour burden from baseline. Two patients progressed before the first restaging, so the tumour response was unknown. The dashed line indicates a 30% reduction from baseline. The crosses below individual bars denote patients who were responding at the time of data cut-off. The red and blue bars denote patients harbouring tumours with a HER2 3+ and HER2 2+ score, respectively.

Panel B shows the dynamics of response in 25 patients with HER2 positive tumours who received lapatinib and trastuzumab and were evaluated by CT scan until disease

progression. For each patient, individual lines represent the percent change in target tumour burden from treatment start (day 0) to the day of objective disease progression, based on serial assessment every 8 weeks. The red and blue lines denote patients harbouring tumours with a HER2 3+ and HER2 2+ score, respectively. The dashed lines indicate a 30% reduction (black) or a 20% increase (red) from baseline. Crosses below individual bars denote patients who were responding at the time of data cut-off.

Figure 2: Progression free survival

The Kaplan-Meyer curves show the efficacy estimated progression-free survival in the 27 patients treated with lapatinib and trastuzumab. Progression-free survival was defined as the time from administration of the first dose of trastuzumab and lapatinib to objective disease progression. Data from three patients, who remained in follow-up for progression-free survival at the time of data cut-off, were censored. The red solid line denotes patients with tumours displaying high-grade *HER2* amplification (*HER2* gene copy number $\geq$9.45). The blue dashed line denotes patients with tumours displaying *HER2* gene copy number <9.45.

Table 1. Baseline characteristics of the 27 patients enrolled		
Demography		
Median age in years (range)	62	(39-86)
Males / Females - no. (%)	23 / 4	(85 / 15)
Clinical variables		
ECOG performance status 0-1 – no. (%)	27	(100)
HER2 protein expression by IHC 3+ / 2+ - no. (%)	20 / 7	(74 / 26)
Site of primary: Colon / Rectum - no. (%):	20 / 7	(74 / 26)
Proximal[1] / Distal[2] colon tumors - no. (%):	4 / 16	(20 / 80)
Metastatic disease in multiple sites - no. (%)	26	(96)
Prior treatments		
Median number of prior lines of therapy - no. (range)	5	(2-11)
Patients with ≥4 prior lines of therapy - no. (%)	20	(74)
Prior antiangiogenesis treatment - no. (%)	20	(74)
Prior therapy with panitumumab or cetuximab - no. (%)	27	(100)
Patients evaluable for sensitivity to panitumumab or cetuximab[3] - no. (%)	15 / 27	(56)
Previous response to panitumumab or cetuximab - no. (%)	0 / 15	(0)
Average time on prior treatment[4]		
Total - months (median)	20	(20)
By primary site: Proximal / Distal / Rectum - months (median)	16 / 20 / 24	(15 / 19 / 23)

[1]Proximal tumours are those located in caecum, ascending colon, liver flexure, and transverse colon.
[2]Distal tumours are those located in splenic flexure, descending colon, and sigma.
[3]Definition of eligibility to assessment of sensitivity to panitumumab or cetuximab are reported in Supplementary table 2A.
[4]Information available from 135/136 total prior regimens (treatment holiday excluded).

Table 2. Efficacy		
Type of response		
Complete response - no. (%; 95%CI)	1	(4; -3 - 11)
Partial response - no. (%; 95%CI)	7	(26; 10 - 43)
Stable Disease ≥ 16 weeks[1] - no. (%; 95%CI)	8	(30; 13 - 47)
Stable Disease < 16 weeks - no. (%; 95%CI)	4	(14; 1 - 27)
Objective response rate (95%CI) -%	30	(14-50)
Disease Control rate[2] (95%CI) -%	59	(39-78)
Median duration of response (range) - weeks	38	(24-94+)
Median time to response (range) - weeks	8	(3-16)

[1]including one unconfirmed partial response according to RECIST 1.1.

[2]Defined as: complete + partial responses + stable disease >16 weeks.

Table 3. Safety*

Event	Grades 1-2	Grade 3
	no. of patients (%)	
Gastrointestinal		
Diarrhoea	21 (78)	0
Abdominal pain	4 (15)	0
Nausea	3 (11)	0
Vomiting	3 (11)	0
Dermatologic		
Rash	12 (44)	1 (4)
Dry skin	8 (30)	0
Dermatitis	3 (11)	0
Nail disorder	3 (11)	0
Pruritus	3 (11)	0
Erythema	2 (7)	0
Folliculitis	2 (7)	0
Metabolism and nutrition disorder		
Fatigue	9 (33)	4 (14)
Anorexia	2 (7)	0
Paronychia	9 (33)	0
Conjunctivitis	5 (19)	0
Hand and Foot syndrome	2 (7)	0
Blood bilirubin increase	0	1 (4)

*Included are treatment related adverse events incurring in ≥5% of patients or of CTCAE Grade≥3. All 27 patients were included in the analysis. No grade 4 or 5 toxicities were observed.

Figure 1. **Radiographic response**

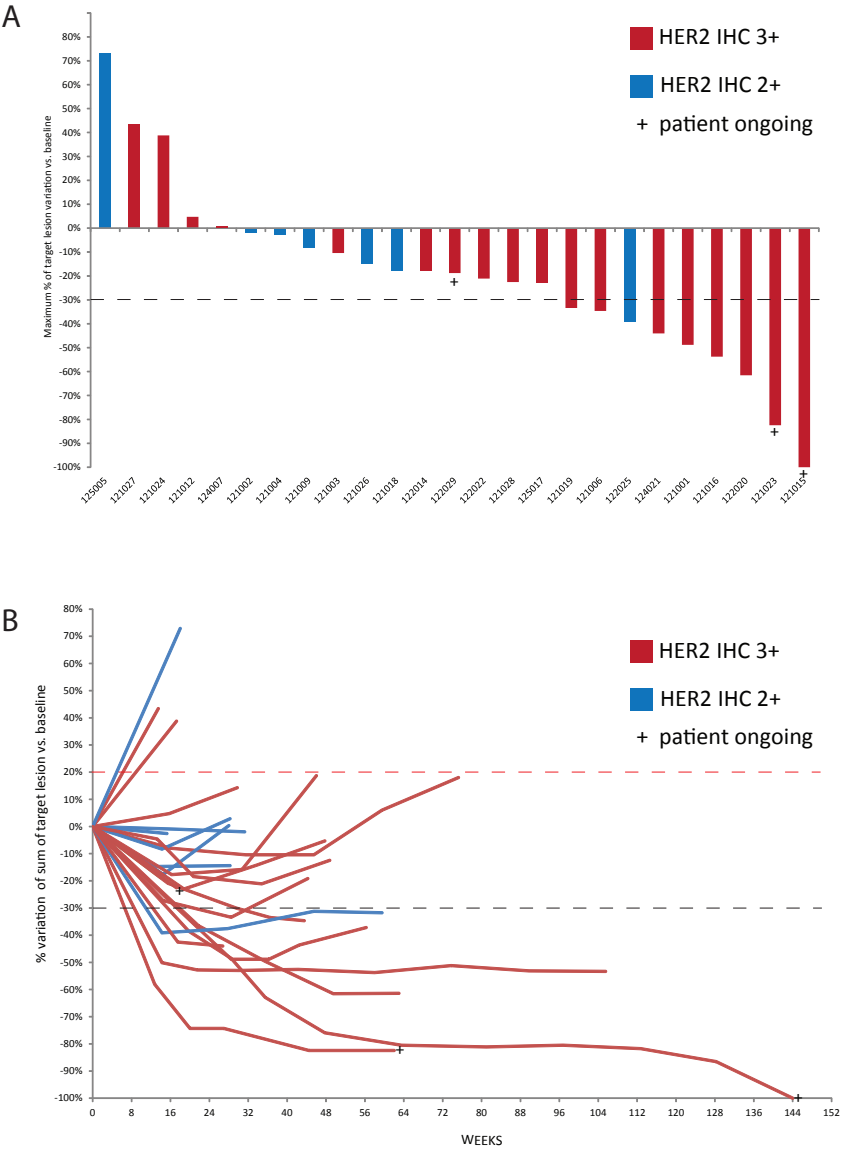
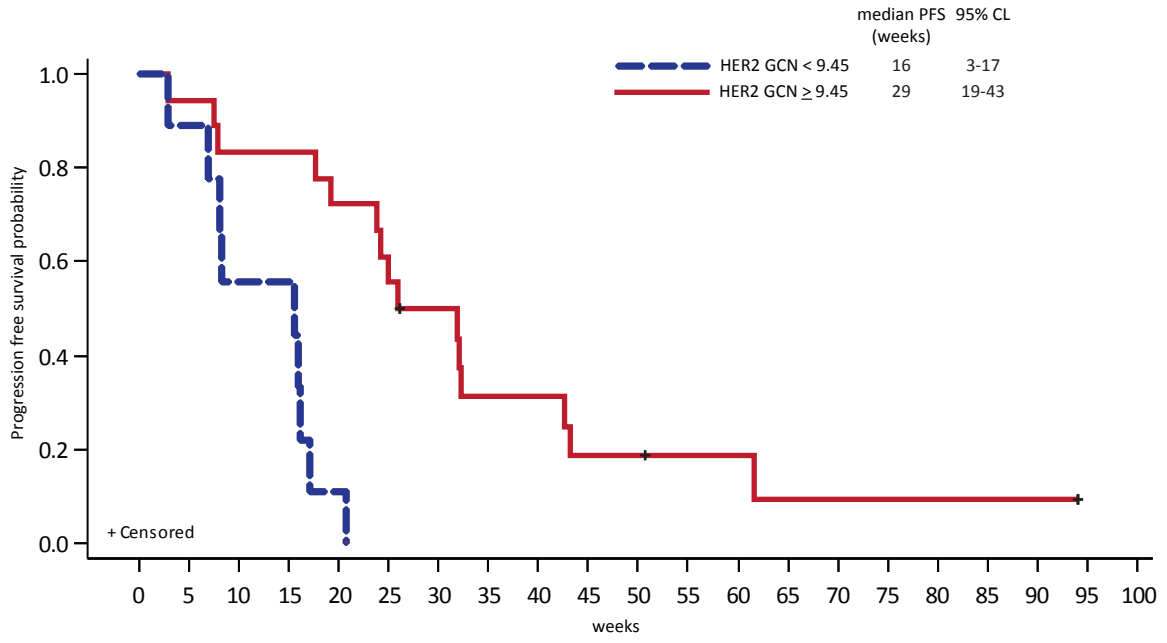


Figure 2. **Progression free survival time by HER2 gene copy number variation**



at risk:

HER2 GCN ≥ 9.45	18	17	15	15	13	11	8	5	5	3	3	2	2	1	1	1	1	1	1	0
HER2 GCN < 9.45	9	8	5	5	1	0														