Negative Hyperselection of Patients with HER2$^+$ and *RAS* Wild-Type Metastatic Colorectal Cancer Receiving Dual HER2 Blockade: the PRESSING-HER2 Study

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ABSTRACT

Purpose: To demonstrate the negative prognostic impact of a panel of genomic alterations (PRESSING-HER2 panel) and lack of *HER2* amplification by next-generation sequencing (NGS) in patients with HER2$^+$, *RAS* wild-type metastatic colorectal cancer receiving dual HER2 blockade.

Experimental Design: The PRESSING-HER2 panel of *HER2* mutations/rearrangements and RTK/MAPK mutations/amplifications was assessed by NGS. *HER2* amplification was confirmed by NGS if copy-number variation (CNV) was ≥ 6. With a case–control design, hypothesizing 30% and 5% PRESSING-HER2 positivity in resistant [progression-free survival (PFS) <4 months and no RECIST response] versus sensitive cohorts, respectively, 35 patients were needed per group.

Results: PRESSING-HER2 alterations included *HER2* mutations/rearrangements, *EGFR* amplification, and *BRAF* mutations and had a prevalence of 27% (9/33) and 3% (1/35) in resistant versus sensitive patients ($P = 0.005$) and 63% predictive accuracy. Overall, *HER2* nonamplified status by NGS had 10% prevalence. Median PFS and overall survival (OS) were worse in PRESSING-HER2$^+$ versus negative (2.2 vs. 5.3 months, $P < 0.001$; 5.4 vs. 14.9 months, $P = 0.001$) and in *HER2* nonamplified versus amplified (1.6 vs. 5.2 months, $P < 0.001$; 7.4 vs. 12.4 months, $P = 0.157$). These results were confirmed in multivariable analyses [PRESSING-HER2 positivity: PFS HR = 3.06, 95% confidence interval (CI), 1.40–6.69, $P = 0.005$; OS HR = 2.93, 95% CI, 1.32–6.48, $P = 0.007$]. Combining PRESSING-HER2 and *HER2* CNV increased the predictive accuracy to 75%.

Conclusions: PRESSING-HER2 panel and *HER2* nonamplified status by NGS warrant validation as potential predictive markers in this setting.

See related commentary by Raghav et al., p. 260

Introduction

Several phase II nonrandomized trials have shown promising activity of dual HER2 blockade in pretreated patients with HER2$^+$ metastatic colorectal cancer and established the role of HER2 as a clinically actionable target (1–5). On the basis of these results, trastuzumab plus lapatinib or pertuzumab regimens have been included in the National Comprehensive Cancer Network guidelines and trastuzumab plus tucatinib has been recently granted accelerated approval by the FDA. However, a substantial proportion of patients enrolled in clinical trials did not benefit from these targeted strategies, as the rate of early disease progression is 25% to 41% and the median progression-free survival (PFS) ranges from 2.9 to 8.2 months according to the specific regimen and the adopted molecular selection criteria (2, 3, 5, 6). The biological bases of primary resistance to dual HER2 targeting in patients with HER2$^+$ metastatic colorectal cancer are poorly characterized. The cooccurrence of other oncogenic drivers is mostly represented by *RAS* comutations, reported in around 17% of patients (7). Because *(K) RAS* mutations have been clearly associated with extremely poor outcomes after dual HER2 blockade, the most recent trials restricted the enrolment to patients with *RAS* wild-type (WT) status (2, 6).

Tissue and circulating tumor DNA (ctDNA) exploratory analyses of phase II trials suggested that additional uncommon alterations in receptor tyrosine kinase (RTK)/MAPK pathway may bypass therapeutic HER2 blockade. Therefore, a paradigm of negative selection beyond *RAS* status is needed to potentially improve the precision of HER2-targeting strategies. In addition, tissue or ctDNA *HER2* copy-number variation (CNV) assessed by RT-PCR or next-generation

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Translational Relevance

The presence of a panel of candidate genomic resistance alterations (PRESSING-HER2 panel including *HER2* mutations/rearrangements and mutations/amplifications in *RTK/MAPK* genes) and *HER2* nonamplified status [*HER2* copy-number variation (CNV) <6] assessed by means of NGS predicts primary resistance to dual HER2 blockade in HER2$^+$ metastatic colorectal cancer. Thus, comprehensive genomic profiling may improve the negative selection for HER2 targeting, and the negative predictive value of PRESSING-HER2 and *HER2* CNV warrants validation in ongoing randomized controlled trials. In patients with low predicted sensitivity to anti-HER2 targeted strategies, alternative options such as antibody–drug conjugates may bypass the genomic mechanisms of resistance. Finally, the use of NGS to select tumors without genomic codrivers of resistance and with confirmed *HER2* amplification may identify patients with HER2 addiction who may benefit from chemo-free targeted strategies.

sequencing (NGS) has been associated with the outcomes of dual HER2 blockade (1, 3, 6). In fact, the level of *HER2* amplification in tumor cells may be a surrogate of HER2 addiction and predict the sensitivity to trastuzumab-based regimens. Of note, comprehensive genomic profiling (CGP) may concomitantly identify the potential drivers of primary resistance and *HER2* CNV.

Drawing from these considerations, we hypothesized that genomic-based hyper-selection may refine the prognostic stratification of patients receiving dual HER2 blockade. To this aim, we conducted a multinational effort aimed at investigating the prognostic performance of a panel of rare genomic drivers of primary resistance (i.e., the PRESSING-HER2 panel) and *HER2* CNV, both assessed by NGS, in patients with HER2$^+$ and *RAS* WT metastatic colorectal cancer receiving dual HER2 blockade.

Materials and Methods

Patient population

Patients with HER2$^+$, *RAS* WT, and microsatellite stable metastatic colorectal cancer treated with trastuzumab-based dual HER2 blockade were retrieved from three different screening sources (Supplementary Table S1): a prospective observational study in Italy and Spain, the MSK-IMPACT dataset, and the TRIUMPH trial (3, 8–10). Additional inclusion criteria were: availability of CGP data, Eastern Cooperative Oncology Group (ECOG) Performance Status (PS) ≤ 2, at least one measurable lesion according to RECIST v1.1, at least one prior treatment line for metastatic disease and written informed consent to study participation. HER2 positivity was defined by: (i) HER2 IHC 3$^+$ in ≥ 10% of cells or HER2 IHC 2$^+$ and *HER2*/CEP17 ratio ≥ 2 by ISH according to previously reported criteria (1); or (ii) presence of *HER2* amplification detected by NGS and defined by *HER2* CNV ≥6. Response assessment was performed according to RECIST v1.1 and CT scans were performed every 8 ± 1 weeks. Primary resistance to dual HER2 blockade was defined by PFS <4 months and best response stable disease (SD) or progressive disease (PD), whereas sensitivity was defined by PFS ≥4 months regardless of RECIST response, that is, SD, partial response (PR - including unconfirmed PR) or complete response (CR). The study was approved by the Institutional Review Board of the Fondazione IRCCS Istituto Nazionale dei Tumori di Milano (INT 117/15) and was conducted in accordance with the ethical principles for medical research involving human subjects adopted in the Declaration of Helsinki.

CGP

CGP was performed in archival formalin-fixed paraffin-embedded tumor tissue obtained prior to the start of anti-HER2 therapy. The PRESSING-HER2 panel grouped genomic alterations with sound clinical and biological rationale as driver of primary resistance to anti-HER2 blockade and included: (i) *HER2* on-target alterations, that is, *HER2* pathogenic mutations or rearrangements shown to drive resistance; (ii) off-target alterations, that is, mutations/amplifications in RTK/MAPK genes: *EGFR*, *MET*, or *KRAS* coamplifications; *BRAF* class 1 and 2 mutations or *PIK3CA* exon 20 mutations. Patients receiving trastuzumab plus pertuzumab were considered as PRESSING-HER2$^+$ if harboring *HER2* mutations in the tyrosine kinase domain and established as resistant to trastuzumab plus pertuzumab in the TAPUR trial, such as: L755S, R678Q, L755-T759, D769H, D769Y, V777L, P780ins, V842I, R896C, or *HER2* pathogenic fusions (4, 11). Regarding patients receiving trastuzumab plus a tyrosine kinase inhibitor (TKI), *HER2* mutations were included in the PRESSING-HER2 panel according to the robust literature data on their role as driver of resistance to the specific TKI used (12–15). Regarding *HER2* CNV assessed by NGS, tumors were reclassified as nonamplified if *HER2* CNV was <6 despite HER2 positivity initially detected by IHC +/− ISH.

Statistical analysis

The study was designed as a multicenter, case–control study based on a translational hypothesis of primary resistance to dual HER2 inhibition. The independent collection of cases (primary resistant group) and controls (sensitive group) with one control per case was planned. In the TRIUMPH trial Nakamura reported the presence of three driver resistance mechanisms out of 7 patients with PD as best response to trastuzumab and pertuzumab (42%) as assessed by archival tissue NGS (3). Therefore, we hypothesized that a more conservative prevalence threshold of 30% would better apply to the present definition of primary resistance. Therefore, hypothesizing a prevalence of PRESSING-HER2 alterations equal to 30% and 5% among cases and controls, respectively, 35 patients per group were needed to reject the null hypothesis of equally prevalent alterations, with α and β errors of 0.05 and 0.20. An uncorrected χ^2 statistic was used to compare the prevalence of alterations in the PRESSING-HER2 panel and in other alterations between resistant and sensitive patients. PFS was defined as the time from the start of dual anti-HER2 treatment to disease progression or death from any cause. Overall survival (OS) was defined as the time from the start of dual anti-HER2 treatment to death from any cause or last follow-up for alive patients. The Kaplan–Meier estimator and Cox proportional hazards regression were used for survival analysis using the *survival*, *survminer*, and *survMisc* packages of the R software (version 3.5.0) and R Studio (version 2022.07.2). In Cox proportional hazards regression models, all the covariates associated with PFS and OS in the univariable analyses with a $P < 0.05$ were included in the multivariable model. $P < 0.05$ was considered statistically significant. *HER2* CNV was modeled by means of 3-knots natural cubic splines (using the splines package) to assess flexible fit and to check for nonlinearity.

Data availability

The data generated during and/or analyzed during this study are available within the article and its Supplementary Files. Genomic data, including CNVs, were pulled from other sources and can be requested

to the sources detailed in Supplementary Table S1; scientific agreements would be required, and the corresponding author can provide the report ID numbers upon reasonable request.

Results

Study population

The study flowchart is depicted in Supplementary Fig. S1. The final study population included 33 cases and 35 controls. HER2 positivity was identified by IHC +/− ISH in 54 (79%) cases and by NGS alone in 14 (21%). Among the screened patients, no RECIST PR were observed in those with PFS <4 months. *HER2* amplification was confirmed by NGS in 43 of 45 patients with IHC 3+ and only in 4 of 9 with IHC 2+, with overall prevalence of *HER2* nonamplified samples of 10%. **Table 1** summarizes the patient and disease characteristics, overall and according to the status of primary resistance versus sensitivity. No statistically significant differences in terms of the main baseline variables were observed between the two resistant and sensitive cohorts, except for ECOG PS. In fact, the prevalence of ECOG PS 1–2 was 64% versus 17% ($P < 0.001$) in resistant versus sensitive groups. Eighteen patients (27%) had received prior anti-EGFR–based therapy, without significant differences in resistant versus sensitive groups (24% vs. 29%; $P = 0.786$). Trastuzumab was given in combination with pertuzumab in 44 (65%) patients, or with the TKI lapatinib, tucatinib, or neratinib in 24 (35%). In the overall population, at a median follow-up of 18.7 months (IQR, 13.4–27.3), the median PFS was 4.1 months (95% CI, 2.8–5.5), and the median OS was 12.2 months (95% CI, 9.9–16.4), as shown in Supplementary Fig. S2.

Table 1. Patients and disease baseline characteristics, overall and according to primary resistance vs. sensitivity to dual HER2 blockade.

Characteristics	Overall study population (*N* = 68) *N* (%)	Resistant patients (*n* = 33) *n* (%)	Sensitive patients (*n* = 35) *n* (%)	*P*
Age (years)	—	—	—	>0.999
<70	55 (81)	27 (82)	28 (80)	—
≥70	13 (19)	6 (18)	7 (20)	—
Sex	—	—	—	0.811
Female	38 (56)	19 (58)	19 (54)	—
Male	30 (44)	14 (42)	16 (46)	—
ECOG PS	—	—	—	<0.001
0	41 (60)	12 (36)	29 (83)	—
1–2	27 (40)	21 (64)	6 (17)	—
Primary tumor location	—	—	—	0.349
Right colon	4 (6)	3 (9)	1 (3)	—
Left colon/Rectum	64 (94)	30 (91)	34 (97)	—
Primary tumor resection	—	—	—	>0.999
Yes	14 (21)	26 (79)	28 (80)	—
No	54 (79)	7 (21)	7 (20)	—
Prior adjuvant chemotherapy	—	—	—	0.806
Yes	26 (38)	21 (64)	21 (60)	—
No	42 (62)	12 (36)	14 (40)	—
Metastatic sites (*N*)	—	—	—	0.580
1	17 (25)	7 (21)	10 (29)	—
>1	51 (75)	26 (79)	25 (71)	—
Prior exposure to anti-EGFR	—	—	—	0.786
Yes	18 (27)	8 (24)	10 (29)	—
No	50 (73)	25 (76)	25 (71)	—
Prior treatment lines (*N*)	—	—	—	0.341
1–2	31 (46)	13 (39)	18 (51)	—
≥3	37 (54)	20 (61)	17 (49)	—
Anti-HER2 therapy	—	—	—	0.079
mAbs	44 (65)	25 (76)	19 (54)	—
TKI plus trastuzumab	24 (35)	8 (24)	16 (46)	—

Abbreviations: ECOG PS, Eastern Cooperative Oncology Group performance status; mAb, monoclonal antibody; TKI, tyrosine-kinase inhibitor.

CGP results

The genomic profiles per single patient in the two cohorts of resistant versus sensitive tumors are depicted in the heat map in **Fig. 1**. PRESSING-HER2 panel alterations were detected in 10 (15%) patients and were mutually exclusive of each other. The following alterations were detected: *HER2* pathogenic mutations occurring in the protein tyrosine and serine/threonine kinase domain (i.e., V777L, V842I, D769Y, and V777_Gly778insGSP) in five tumor samples, *HER2* rearrangements in two samples, *EGFR* coamplification in one sample, and *BRAF* class 1 or 2 mutations (i.e., V600E and G469A) in two samples. No *MET* or *KRAS* coamplifications were detected. A significantly higher frequency of PRESSING-HER2 alterations was found in resistant (9 of 33, 27%) versus sensitive patients (1 of 35, 3% - $P = 0.005$). The accuracy of the PRESSING-HER2 panel for predicting the status of primary resistance was 63%. The individual features and treatment outcomes of patients with PRESSING-HER2$^+$ tumors are detailed in Supplementary Table S2. Regarding patients with *HER2*-mutated tumors, 4 received trastuzumab plus pertuzumab, whereas 3 received trastuzumab plus a TKI. Among these, a patient treated with trastuzumab plus lapatinib harbored the lapatinib-resistant V482I mutation and it was therefore considered PRESSING-HER2$^+$ (16). The remaining patients were considered *a priori* as PRESSING-HER2$^-$, despite being clinically resistant in our dataset: the first received trastuzumab plus lapatinib and harbored the lapatinib-sensitive H878Y mutation (17); the second received trastuzumab plus neratinib and harbored the neratinib-sensitive D769Y mutation (15). Two patients with *HER2*-rearranged tumors received trastuzumab plus pertuzumab; the first harbored the *GRB7-HER2* fusion (*HER2* CNV 198) and was in the resistant cohort, while the second patient with *WIPF2-HER2* (*HER2* CNV 163) fusion was classified as sensitive, but had only a 4.4-month lasting SD. The median *HER2* CNV was 34.5 (IQR, 18.7–78.5) in the overall population, without significant differences in such value according to the three NGS assays used. Notably, median *HER2* CNV was 23.0 (IQR, 9.0–41.4) versus 68.5 (IQR, 33.4–105.0) in the resistant versus sensitive cohort ($P < 0.001$; Supplementary Fig. S3). On the other hand, median CNV was 31.7 (IQR, 13.2–63.2) in PRESSING-HER2$^+$ versus 35 (IQR, 19.0–78.5) in PRESSING-HER2$^-$ tumors ($P = 0.489$); *HER2* CNV <6 was found in 20% (2 of 10) of PRESSING-HER2$^+$ versus 9% of PRESSING-HER2$^-$ tumors (5 of 58; $P = 0.272$). When combining the presence of PRESSING-HER2 with lack of *HER2* amplification by NGS (*HER2* CNV <6), the predictive accuracy of primary resistance was 70%. In an exploratory analysis restricting the sensitive group to patients with CR/PR as RECIST best response, a significantly higher frequency of PRESSING-HER2 alterations was found in resistant (9 of 33, 27%) versus sensitive patients (0 of 19, 0%; $P = 0.018$).

Prognostic role of PRESSING-HER2 panel and *HER2* CNV

Patients with PRESSING-HER2$^+$ tumors had significantly worse PFS and OS compared with those with PRESSING-HER2$^-$ status

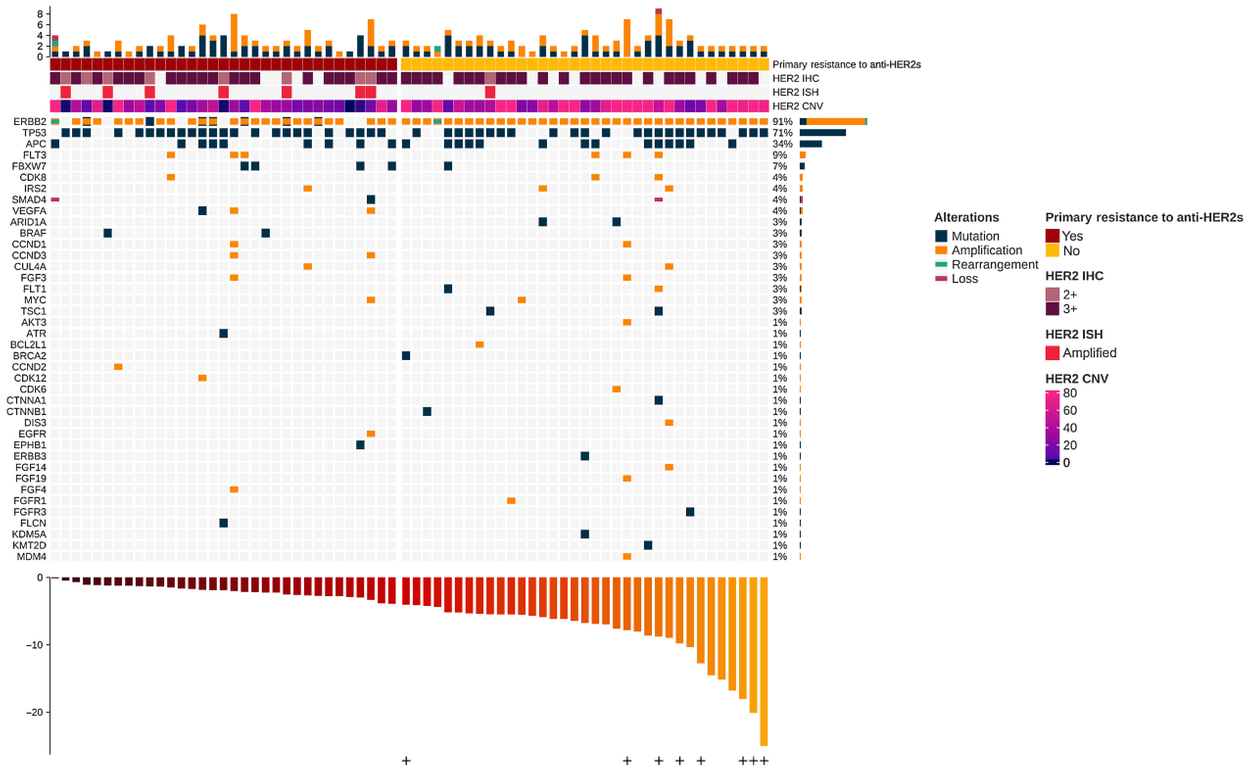

Figure 1.

Heat map showing the genomic profiles according to the primary resistance status. Patients in the two groups were ordered according to the length of individual PFS. CNV, copy number variation.

(median PFS: 2.2 vs. 5.3 months; HR, 3.76; 95% CI, 1.78–7.95; $P < 0.001$; median OS: 5.4 vs. 14.9 months; HR, 3.61; 95% CI, 1.66–7.82; $P = 0.001$; **Fig. 2A** and **B**). Regarding *HER* CNV as a continuous variable, a linear effect on the log hazard function was observed for PFS (P for nonlinearity = 0.142) and OS (P for nonlinearity = 0.884) as shown in Supplementary Fig. S4. Compared with patients with *HER2*-amplified status confirmed by NGS (*HER2* CNV ≥6), patients with *HER2* nonamplified tumors (*HER2* CNV <6) had significantly worse PFS (median PFS: 1.6 vs. 5.2 months; HR, 4.63; 95% CI, 1.97–10.92; $P < 0.001$) and nonsignificantly inferior OS (median OS, 7.4 vs. 12.4 months; HR, 1.87; 95% CI, 0.79–4.46; $P = 0.157$), as shown in **Fig. 2C** and **D**. In the multivariable models (**Table 2**), the presence of PRESSING-HER2 alterations was significantly associated with both PFS and OS (adjusted HR for PFS = 3.06, 95% CI, 1.40–6.69, $P = 0.005$; adjusted HR for OS = 2.93, 95% CI, 1.32–6.48, $P = 0.007$), whereas *HER2* nonamplified status by NGS was significantly associated only with PFS (adjusted HR, 3.89; 95% CI, 1.60–9.49; $P = 0.002$), but not with OS (HR, 1.87; 95% CI, 0.79–4.46; $P = 0.157$). Notably, ECOG PS was independently associated with both PFS and OS.

In the combined assessment of PRESSING-HER2 panel and *HER2* amplification assessed by NGS, patients with PRESSING-HER2$^+$ and/or *HER2* nonamplified tumors had significantly worse PFS and OS compared with patients without PRESSING-HER2 alterations and *HER2* CNV ≥6 (median PFS: 2.1 vs. 5.5 months; HR = 4.70, 95% CI, 2.35–9.41; $P < 0.001$; median OS = 6.7 vs. 14.9 months, HR = 2.69, 95% CI, 1.38–5.23; $P = 0.004$; **Fig. 3A** and **B**).

Discussion

In nonrandomized trials, dual HER2 blockade showed promising activity in patients with previously treated HER2$^+$ metastatic colorectal cancer. However, the recently reported SWOG S1613 randomized phase II trial failed to show the superiority of a trastuzumab plus pertuzumab chemo-free regimen compared with irinotecan plus cetuximab as second- or third-line treatment in patients with HER2$^+$, *RAS* and *BRAF* WT disease (18). Although these results may have been influenced by the small sample size and the lack of prior irinotecan exposure in about half of the patients, this study confirms that a nonnegligible proportion of patients do not derive benefit from anti-HER2–targeted therapy. Therefore, it is critical to identify the determinants of treatment resistance in this molecularly selected subgroup of patients characterized by high degree of genomic heterogeneity. Because of the low prevalence of each candidate resistance alteration and the current lack of large randomized clinical trials with an anti-HER2–free arm, a formal evaluation of the negative predictive role of these mechanisms individually is not currently feasible. In the attempt to partially overcome these limitations, we investigated the role of a genomic panel that groups together several uncommon biomarkers of primary resistance in a case–control study on the basis of a formal *a priori* statistical hypothesis. We found that genomic alterations included in the PRESSING-HER2 panel were significantly enriched in patients exhibiting primary resistance to dual HER2 inhibition and were independently associated with inferior PFS and OS in multivariable analyses.

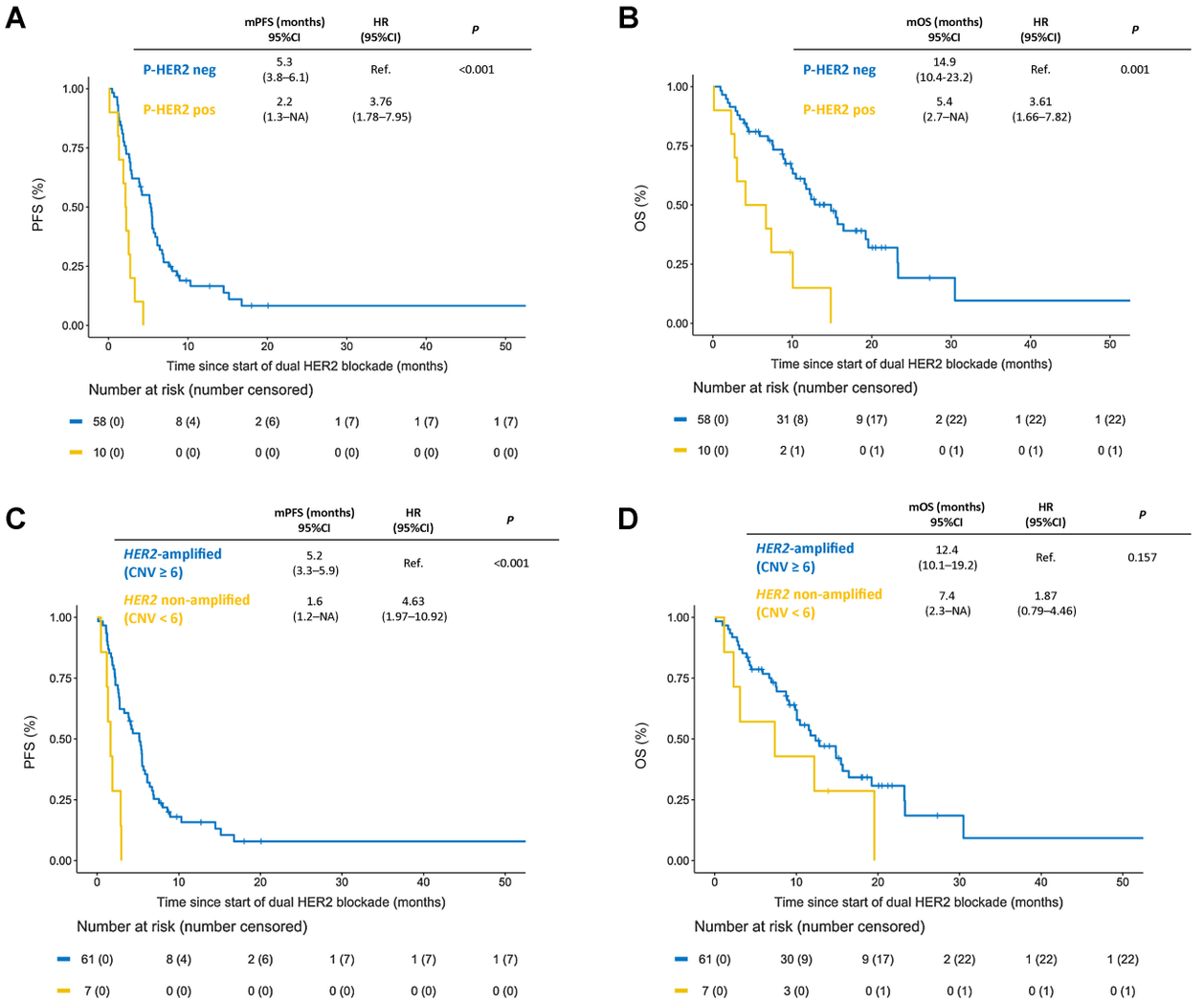

Figure 2.

Kaplan–Meier curves for PFS (**A** and **C**) and OS (**B** and **D**) according to the presence of PRESSING-HER2 status (positive vs. negative) and *HER2* amplification status by NGS (*HER2* CNV <6 vs. ≥6). mOS, median overall survival; mPFS, median progression-free survival; P-HER2, PRESSING-HER2 panel.

Expectedly, all patients bearing HER2 off-target alterations (RTK/MAPK pathway) were resistant irrespective of the specific anti-HER2 regimen with trastuzumab plus either pertuzumab or a TKI. Among patients with HER2 on-target alterations, those with *HER2* activating mutations treated with trastuzumab plus pertuzumab were expectedly resistant, whereas patients treated with a TKI were classified as PRESSING-HER2 positive or negative based on the available preclinical and/or clinical literature data regarding the specific agent (14, 15). Indeed, there is evidence indicating that several *HER2* activating mutations in colorectal cancer (S310F, L755S, V777L, V842I, and L866M) are efficiently targeted by tucatinib or neratinib plus trastuzumab, whereas the binding of lapatinib might be impaired in presence of V842I and L755S; refs. 15, 16, 19). Although all patients with *HER2* mutations in our dataset were in the resistant cohort irrespective of initial classification as PRESSING-HER2 positive or negative, conclusive data regarding the predictive impact of individual *HER2* mutations in the context of *HER2*-amplified tumors are lacking. The same is true for *HER2* rearrangements: in fact, gene fusions may cause constitutive kinase activity and resistance to extracellular domain–targeting agents (11, 20). Of note, similarly to the reported occurrence of *EGFR* fusions in *EGFR*-amplified metastatic colorectal cancer, *HER2* rearrangements occurred in *HER2* "hyperamplified" tumors with very high CNV (9).

A nonnegligible proportion (10%) of patients in our dataset did not show *HER2* amplification by NGS despite HER2 positivity having been previously detected by standard IHC ± ISH. The lack of *HER2* amplification by NGS may be a consequence of spatial heterogeneity of *HER2* amplification and may thus mirror a low level of HER2 addiction. Accordingly, we showed that patients with *HER2* nonamplified tumors by NGS had poorer outcomes, although the low number of patients in this subgroup may have prevented significant OS results. Interestingly, most patients with *HER2* nonamplified tumors by NGS did not have concomitant PRESSING-HER2 alterations, and therefore the combined assessment of the two biomarkers increased the

Table 2. Cox proportional hazards regression models for PFS and OS in the entire study population.

	PFS				OS			
	Univariable models		Multivariable model		Univariable models		Multivariable model	
Characteristics	**HR (95% CI)**	***P***	**HR (95% CI)**	***P***	**HR (95% CI)**	***P***	**HR (95% CI)**	***P***
Age (years)	—	0.412	—	—	—	0.813	—	—
<70	Ref	—	—	—	Ref	—	—	—
≥70	1.31 (0.69–2.48)	—	—	—	1.09 (0.52–2.28)	—	—	—
Sex	—	0.798	—	—	—	0.508	—	—
Female	Ref	—	—	—	Ref	—	—	—
Male	0.93 (0.56–1.56)	—	—	—	0.81 (0.44–1.50)	—	—	—
ECOG PS	—	**0.001**	—	**0.026**	—	**<0.001**	—	**<0.001**
0	Ref	—	Ref	—	Ref	—	Ref	—
≥1	2.39 (1.42–4.03)	—	1.88 (1.08–3.29)	—	3.32 (1.82–6.06)	—	3.04 (1.65–5.59)	—
Primary tumor location	—	0.247	—	—	—	0.630	—	—
Right	Ref	—	—	—	Ref	—	—	—
Left colon/Rectum	0.54 (0.19–1.52)	—	—	—	0.75 (0.23–2.43)	—	—	—
Primary tumor resection	—	0.498	—	—	—	0.453	—	—
No	Ref	—	—	—	Ref	—	—	—
Yes	0.80 (0.41–1.54)	—	—	—	1.31 (0.64–2.69)	—	—	—
Adjuvant CT	—	0.449	—	—	—	0.560	—	—
No	Ref	—	—	—	Ref	—	—	—
Yes	1.22 (0.73–2.06)	—	—	—	0.83 (0.44–1.56)	—	—	—
Metastatic sites (*N*)	—	0.487	—	—	—	0.218	—	—
1	Ref	—	—	—	Ref	—	—	—
>1	1.23 (0.68–2.22)	—	—	—	1.62 (0.75–3.51)	—	—	—
Prior treatment lines	—	0.288	—	—	—	0.065	—	—
1–2	Ref	—	—	—	Ref	—	—	—
≥3	1.32 (0.79–2.20)	—	—	—	1.79 (0.96–3.31)	—	—	—
PRESSING-HER2	—	**<0.001**	—	**0.005**	—	**<0.001**	—	**0.007**
Negative	Ref	—	Ref	—	Ref	—	Ref	—
Positive	3.49 (1.74–6.99)	—	3.06 (1.40–6.69)	—	3.79 (1.78–8.07)	—	2.93 (1.32–6.48)	—
HER CNV	—	**<0.001**	—	**0.002**	—	0.157	—	—
≥6	Ref	—	Ref	—	Ref	—	—	—
<6	4.63 (1.97–10.92)	—	3.89 (1.60–9.49)	—	1.87 (0.79–4.46)	—	—	—

Abbreviations: CT, chemotherapy; Ref, reference.

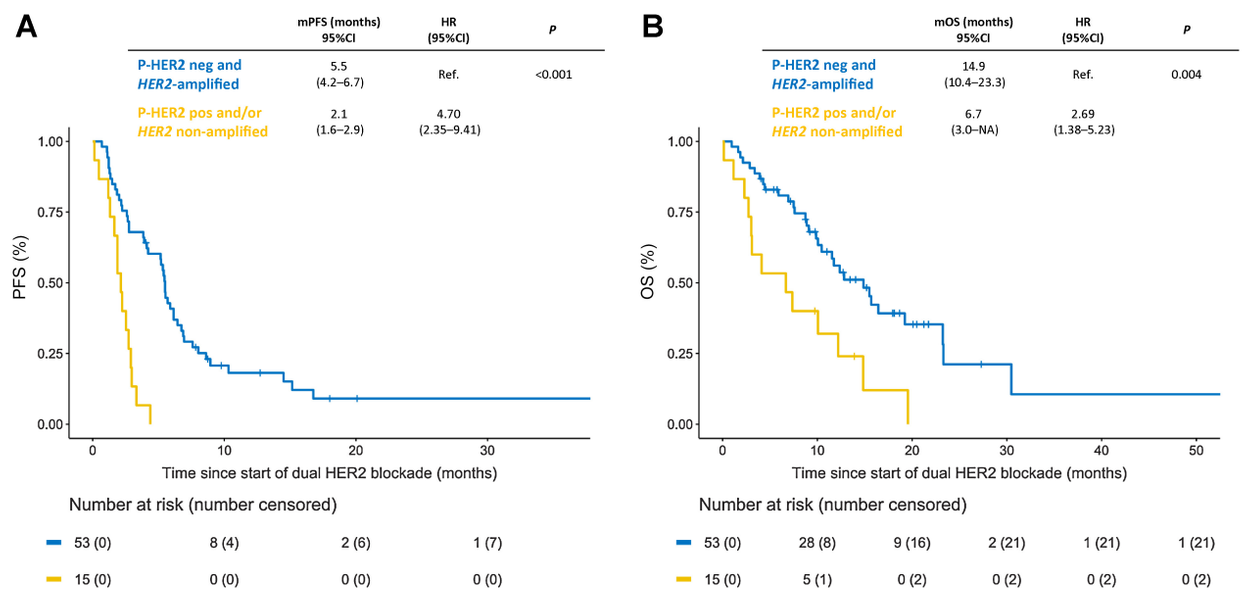

Figure 3.
Kaplan–Meier curves for PFS (**A**) and OS (**B**) according to the combined assessment of PRESSING-HER2 and *HER2* amplification status by NGS (PRESSING-HER2$^-$ and HER2 amplified vs. PRESSING-HER2$^+$ and/or *HER2* nonamplified). mOS, median overall survival; mPFS, median progression-free survival; P-HER2, PRESSING-HER2 panel.

predictive accuracy and the prognostic stratification of the survival outcomes.

Herein, poorer ECOG PS was significantly associated with survival, regardless of PRESSING-HER2 alterations or *HER2* nonamplified status by NGS. These data strengthen the need of implementing HER2 blockade in earlier treatment lines, ideally before the potential occurrence of ECOG PS decline in the chemorefractory setting.

Collectively, our data suggest that CGP may improve the negative selection for anti-HER2 therapy by the concomitant assessment of resistance alterations and *HER2* CNV. However, the definitive demonstration of the clinical usefulness of GCP in this setting should derive from the validation of the negative predictive role of the above discussed biomarkers in the context of randomized clinical trials with an anti-HER2–free arm, such as the ongoing MOUNTAINEER-3 (21). Moreover, our study was focused on primary resistance to anti-HER2 therapy, but *HER2* CNV as measured by CGP may also allow to identify *HER2* hyperamplified tumors with exceptional benefit from dual HER2 blockade, as suggested by previous translational analyses of clinical trials (3, 6, 18).

Consistent with the experience of EGFR blockade, patients with *PIK3CA* mutations or right-sided primary tumor had inferior outcomes on trastuzumab plus pertuzumab in the MyPathway trial, that included $HER2^+$ patients regardless of *RAS* mutational status (2). However, because both *PIK3CA* mutations and right sidedness are associated with *RAS* mutations in metastatic colorectal cancer, their prognostic role in patients with $HER2^+$ and *RAS* WT tumors is largely undetermined (22). After the exclusion of *RAS* mutated samples in our dataset, we did not report any *PIK3CA* exon 20 mutation and only 4 (6%) right-sided tumors. Thus, we could not draw any conclusion on the prognostic impact of primary tumor sidedness in the setting of HER2 inhibition. Similarly, $BRAF^{V600E}$ mutations have a clear-cut role in mediating resistance to RTK pathway blockade strategies. However, only individual patients with $BRAF^{V600E}$ metastatic colorectal cancer and resistant disease have been reported in two clinical trials with dual HER2 blockade, and our results seem to confirm the negative prognostic role of cooccurring *BRAF* alterations (2, 3).

Notably, we previously showed that the antibody–drug conjugate trastuzumab deruxtecan (T-DXd) may bypass specific drivers of primary resistance (such as *KRAS* amplification) in *HER2*-amplified gastric cancer models treated with several anti-HER2 combinations (23). More importantly, the exploratory analyses of the DESTINY-CRC-01 trial showed a potentially retained activity of this agent in patients with *RAS* mutations or *HER2* CNV-low status in ctDNA (24). Therefore, T-DXd may be regarded as a "smart chemotherapy" option and may be active irrespective of the presence of genomic resistance alterations. Our results suggest that treatment decisions and sequencing of the available anti-HER2 options may consider the results of CGP, but its potential role in driving treatment choices should be interpreted with caution. More preclinical/translational data are needed to comprehensively investigate sensitivity to different HER2 targeted therapies, and additional trials or real-world evidence are necessary.

Our study has several limitations. First, we acknowledge that the definition of sensitive patients by PFS ≥4 months and no PD as best RECIST response (especially those not achieving PR/CR) may have led to the inclusion of patients with indolent disease rather than truly anti-HER2s sensitive in this group. However, in this hard-to-treat heavily preteated patient population, even a short-lasting disease stabilization may be considered as a sign of treatment efficacy. Moreover, we showed that the discriminative capability of the PRESSING-HER2 panel was preserved after excluding patients with SD as their best response in the sensitive cohort.

Then, while we cannot conclusively demonstrate the predictive role of the biomarkers tested, the use of a case–control study design allowed us to closely mirror the setting of a predictive validation. Second, the reproducibility of *HER2* CNV assessment may have been negatively influenced by the heterogeneity of the NGS assays. It should be also kept in mind that NGS may underestimate the *HER2* CNV because of the stromal dilution as compared with standard morphologic assays such as ISH (25). Finally, a substantial proportion of patients with primary resistance did not display any PRESSING-HER2 alteration nor low *HER2* CNV by tissue NGS. As a matter of fact, heterogeneity of genomic profiles as well as nongenomic resistance may account for primary resistance to the dual HER2 blockade (24, 26). Therefore, liquid biopsy may further improve the stratification of patients' outcomes, as previously shown in the TRIUMPH and HERACLES studies (1, 3).

In conclusion, a panel of genomic on/off target resistance alterations and the lack of *HER2* amplification as assessed by NGS may be useful to predict primary resistance to dual HER2 blockade in patients with $HER2^+$ and *RAS* WT metastatic colorectal cancer. CGP may allow physicians to refine patients' selection through negative hyperselection beyond *RAS* mutational status applied to the context of HER2 overexpression/amplification as a positive predictive biomarker.

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Note

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