Biomarkers of Primary Resistance to Trastuzumab in HER2-Positive Metastatic Gastric Cancer Patients: the AMNESIA Case-Control Study

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1 Medical Oncology Department, Fondazione IRCCS Istituto Nazionale dei Tumori, Milan, Italy.
2 Department of Pathology and Laboratory Medicine, Fondazione IRCCS Istituto Nazionale dei Tumori, Milan, Italy.
3 University of Torino, Department of Oncology and Candiolo Cancer Institute, FPO-IRCCS, Candiolo, Italy.
4 Department of Oncology, ULSS8 Berica, San Bortolo General Hospital, Vicenza, Italy.
5 Division of Medical Oncology, Department of Clinical and Experimental Medicine and Surgery, University of Campania 'Luigi Vanvitelli', School of Medicine, Naples, Italy.
6 Department of Oncology, ASST Ospedale di Cremona, Cremona, Italy.
7 Department of Oncology, University and General Hospital, Udine, Italy.
8 Medical Oncology Unit, Azienda Ospedaliero-Universitaria Careggi, Florence, Italy.
9 Molecular Targeting Unit, Department of Research, Fondazione IRCCS Istituto Nazionale dei Tumori, Milan, Italy.
10Oncology and Haemato-Oncology Department, University of Milan, Universit□a degli Studi di Milano, Milan, Italy.
11Unit of Medical Oncology 2, Azienda OspedalieraUniversitaria Pisana, Pisa, Italy.

Corresponding Author: Filippo Pietrantonio, Fondazione IRCCS Istituto Nazionale dei Tumori, Via Venezian, 1, 20133 Milan, Italy. Phone: 39-02-23903807; Fax: 39-02-23902149; E-mail: filippo.pietrantonio@istitutotumori.mi.it

Abstract

Purpose: Refining the selection of HER2-positive metastatic gastric cancer patient candidates for trastuzumab is a challenge of precision oncology. Preclinical studies have suggested several genomic mechanisms of primary resistance, leading to activation of tyrosine kinase receptors other than HER2 or downstream signaling pathways.

Experimental Design: We carried out this multicenter, prospective, case-control study to demonstrate the negative predictive impact of a panel of candidate genomic alterations (AMNESIA panel), including EGFR/MET/KRAS/PI3K/PTEN mutations and EGFR/MET/KRAS amplifications. Hypothesizing a prevalence of candidate alterations of 30% and 0% in resistant and sensitive HER2-positive patients, respectively, 20 patients per group were needed.

Results: AMNESIA panel alterations were significantly more frequent in resistant (11 of 20, 55%) as compared with sensitive (0% of 17) patients (P < 0.001), and in HER2 IHC 2þ (7 of 13, 53.8%) than 3þ (4 of 24, 16.7%) tumors (P ¼ 0.028). Patients with tumors bearing no candidate alterations had a significantly longer median progression-free [5.2 vs. 2.6 months; HR, 0.34; 95% confidence interval (CI), 0.07–0.48; P ¼ 0.001] and overall survival (16.1 vs. 7.6 months; HR, 0.38; 95% CI, 0.09–0.75; P ¼ 0.015). The predictive accuracy of the AMNESIA panel and HER2 IHC was 76% and 65%, respectively. The predictive accuracy of the combined evaluation of the AMNESIA panel and HER2 IHC was 84%.

Conclusions: Our panel of candidate genomic alterations may be clinically useful to predict primary resistance to trastuzumab in patients with HER2-positive metastatic gastric cancer and should be further validated with the aim of molecularly stratifying HER2-addicted cancers for the development of novel treatment strategies.

Introduction

Long-term survival of patients with gastric cancer is still unsatisfactory following standard treatments (1). HER2 overexpression/amplification is found in less than 20% of cases, but frequent heterogeneity of HER2 as "leading" oncogenic driver poses fundamental clinical challenges (2). In metastatic GC (mGC), all major guidelines currently recommend HER2 testing to guide patients' selection for trastuzumab treatment (3), because the addition of trastuzumab to doublet chemotherapy significantly improved all endpoints of overall response rate, progression-free survival (PFS), and overall survival (OS) in the pivotal ToGA trial (4). However, the unsatisfactory absolute gain of median PFS highlights the crucial issue of primary and acquired resistance to anti-HER2 therapies. In the targeted therapy field, the validation of predictive biomarkers allowed to refine the risk/benefit ratio of several treatments. In HER2-positive mGC, predictors of greater benefit from trastuzumab have been proposed (HER2 IHC 3þ vs. 2þ or HER2/ CEP17 ratio > vs. < 4.7; refs. 4, 5), but at present, they cannot be used to restrict the label of trastuzumab, which is still the only approved targeted agent in the front-line setting. On the other doi: 10.1158/1078-0432.CCR-17-2781 2017 American Association for Cancer Research. Clinical Cancer Research www.aacrjournals.org OF1 Cancer Research. Downloaded from clincancerres.aacrjournals.org on February 23, 2018. © 2017 American Association for Published OnlineFirst December 5, 2017; DOI: 10.1158/1078-0432.CCR-17-2781 side, an immediately transferrable line of research may be focused on coexisting oncogenic drivers that may be primed as negative predictors of trastuzumab benefit and, potentially, exploited as therapeutic cotargets. Several candidate alterations (EGFR and/or MET coamplifications, KRAS alterations including point mutations and amplifications, EGFR/MET/HER3/ PI3K/PTEN mutations) may possibly co-occur with HER2 amplification and putatively confer trastuzumab resistance (6). However, there is still a lack of studies investigating the correlation of such candidate mechanisms of resistance with clinical outcomes following trastuzumab-based treatment. Moreover, the clinical investigation of trastuzumab primary resistance has been halted for several years by the unique availability of small and uncontrolled retrospective series (7, 8), the confounding effects of the combined chemotherapy, and the heterogeneity and multiplicity of the candidate genomic mechanisms (9)—precluding any concrete chance of formal validation. Deepening our knowledge on the correlation of the molecular landscape of HER2-positive mGC with treatment outcome data is crucial to develop future treatment strategies taking into account both the status of HER2 addiction and the coexistence of other genomic dependencies (10). Given the above-mentioned challenges and in order to improve the chance of success, we carried out the multicenter, prospective case-control AMNESIA-1 study, aimed at exploring a panel of candidate genomic alterations (AMNESIA panel) in patients with HER2-positive mGC eligible for first-line treatment with trastuzumab and chemotherapy.

Materials and Methods

The AMNESIA-1 study was approved by the Institutional Review Board of Fondazione IRCCS Istituto Nazionale dei Tumori (Milan, Italy), and all patients signed a written-informed consent.

Patient population

Patients with HER2-positive metastatic gastric or gastroesophageal junction cancers were included in two cohorts of trastuzumab-resistant versus -sensitive patients. As shown in Supplementary Fig. S1, patients with resistant tumors were those experiencing progressive disease (PD) according to RECIST 1.1 criteria at the first CT scan reassessment during trastuzumab plus cisplatin/ fluoropyrimidine chemotherapy, or stable disease lasting 4 months after the starting of treatment. Patients with sensitive tumors were those initially achieving a RECIST partial/complete response and subsequently developing PD at least 3 months after the last chemotherapy dose (i.e., after at least 3 months of maintenance treatment with trastuzumab alone). This definition of sensitivity corresponds to a PFS of at least 7 months, and this cutoff was selected based on the median PFS of 6.7 months achieved in the trastuzumab arm of the ToGA trial, allowing us to significantly limit the confounding effects of potential longterm benefit from chemotherapy in trastuzumab-refractory patients. Other inclusion criteria were at least one measurable lesion according to RECIST 1.1 and one radiological reassessment, duration of trastuzumab-containing treatment of at least 9 weeks, and no early interruption due to unacceptable toxicity or other reasons including early death. The following baseline characteristics were

collected: age, sex, Eastern Cooperative Oncology Group performance status (ECOG PS), primary tumor location, Lauren histotype, number of metastatic sites, prior resection of the primary tumor, time to metastases (synchronous vs. metachronous). HER2-positive status was assessed as per international guidelines, and all samples were centrally reviewed for histotype and HER2 status (11).

Molecular analyses

Candidate genomic alterations assessed in the AMNESIA panel were selected based on available preclinical/clinical insights (6, 7, 9, 10, 12) and included EGFR/MET/KRAS/PI3K/PTEN mutations and EGFR/MET/KRAS amplifications. Formalin-fixed paraffin-embedded archival tumor tissue blocks obtained prior to any treatment were used for the purpose of this study. Mutational data were obtained via next-generation sequencing (NGS) using the Ion AmpliSeq Cancer Hotspot Panel v2 (Life Technologies) with the Ion-Torrent Personal Genome Machine platform (Life Technologies) as previously described (13, 14) and detailed in Supplementary Methods. MET and HER2 gene statuses were assessed by bright-field dual-color SISH analysis performed as already reported (14). The EGFR gene status was assessed by bright-field dual-color SISH. The Colorado scoring system (15–17) was adopted to classify samples into ISH strata according to the frequency of cells with each EGFR gene copy number and referred to the chromosome 7 centromere. EGFR ISH-negative cases had no or low genomic gain for EGFR gene copy number (disomy, low trisomy, high trisomy, and low polysomy), whereas the distinction between high polysomy and gene amplification was defined by the presence of gene clusters only in EGFR-amplified cases. Quantitative PCR experiments for estimation of KRAS copynumber variations were performed in triplicates using a Human TaqMan Copy Number Assay for KRAS (assay ID: hs06936191_cn), a Human TaqMan Copy Number Assay for GREB1 (assay ID: hs01738470_cn), and the TaqMan Copy Number Reference Assay RNase P (assay ID: 4316831), Applied Biosystems. PCR runs were performed with ABI Prism 7900HT (Applied Biosystems) using 80 ng total DNA as a template for each reaction. Samples were scored positive when a twofold increase was observed with both the references in two different reactions.

Statistical design and analyses

AMNESIA was a prospective, multicentre, case-control study based on a translational hypothesis. Cases (resistant patients) and controls (sensitive patients) with one control per case were included. Hypothesizing a prevalence of candidate alterations of the AMNESIA panel equal to 0% and 30% among controls and cases, respectively, 20 cases and 20 controls were required to be able to reject the null hypothesis of equally prevalent alterations, with alpha and beta errors of 0.05 and 0.20, respectively. An uncorrected c2 test was adopted to compare the prevalence of alterations in the AMNESIA panel between resistant and sensitive patients. The impact of alterations included in the AMNESIA panel and of HER2 status on PFS and OS was investigated. PFS was defined as the time from the beginning of the trastuzumab-containing treatment to the radiologic evidence of disease progression or last follow-up. OS was defined as the time from the beginning of the trastuzumab-containing treatment to death or last follow-up. PFS and OS analyses were determined according to the Kaplan–Meier method, and survival curves were compared using the log-rank test. The predictive accuracy of proposed assessments was calculated as the sum of true-positive and true-negative observations relative to the total number of observations.

Results

Study population The study flowchart is depicted in Fig. 1. Following prescreening of 65 patients starting trastuzumab-based treatment, the final study population included 20 resistant (cases) and 17 sensitive patients (controls) treated at five Italian institutions between December 2011 and August 2016. Inclusion of further controls was stopped after the primary endpoint was met. As shown in Table 1, no significant imbalance of main patient- and tumor-related variables was found in resistant and sensitive patients' groups, except for a slightly higher proportion of HER2 IHC 2þ cancers among resistant (50%) versus sensitive (18%) patients (P ¼ 0.08). At a median follow-up of 19.3 months, median PFS was 2.3 and 9.5 months among resistant and sensitive patients' groups, whereas median OS was 11.1 and 23.3 months, respectively.

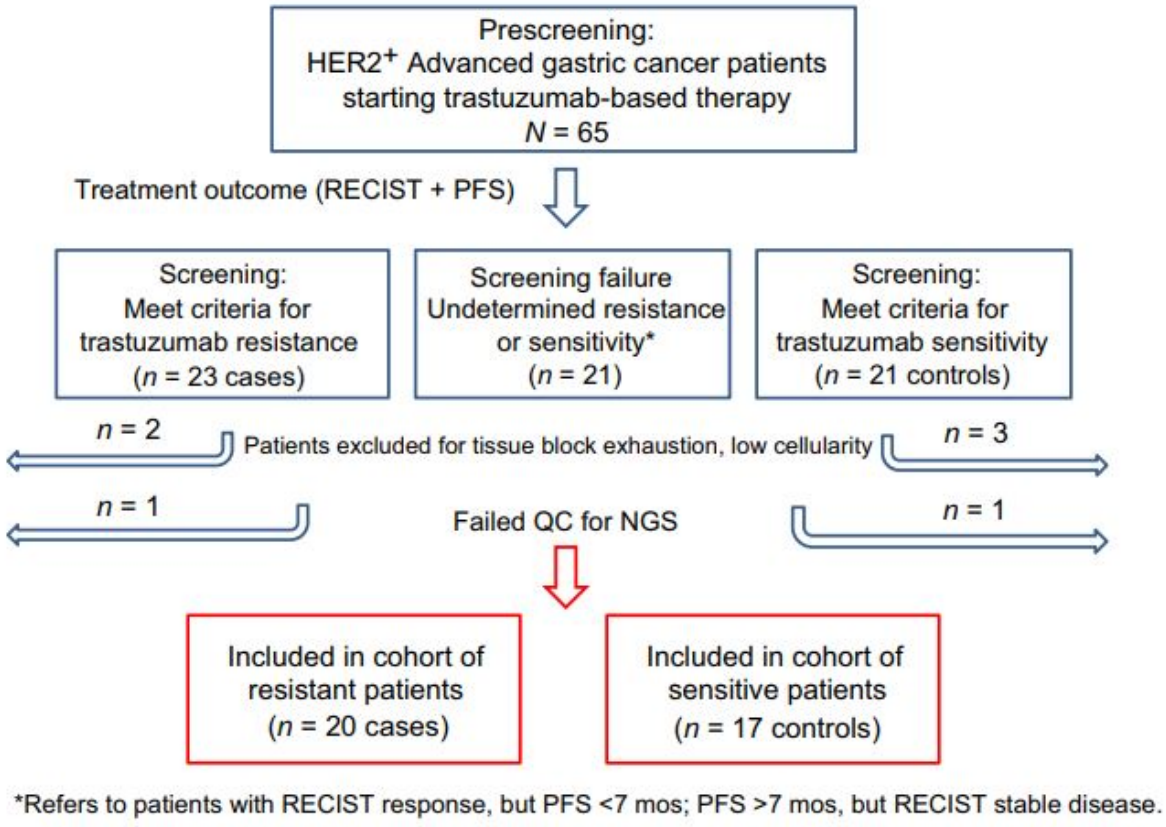


Fig.1 Study flowchart depicting the steps necessary for patients' inclusion in the case-control study.

AMNESIA panel alterations are enriched among resistant patients

Results of molecular analyses performed on the study population are summarized in Table 2 and depicted in Fig. 2. The primary endpoint of AMNESIA-1 study was met: in fact, the frequency of candidate alterations included in the AMNESIA panel was 55% (11 of 20) for resistant patients, as compared with 0% of sensitive ones (P < 0.001). Of note, two resistant patients carried two co-occurring alterations (MET N375S mutation þ KRAS amplification and MET R988C mutation þ MET amplification). PI3K mutations were the most frequently found mutations in resistant patients (three cases, 15%), whereas no EGFR and PTEN mutations were detected. Overall, based on our findings, the 55% of resistant cases was associated to the presence of a candidate molecular alteration, and the relative contribution of each alteration in the AMNESIA panel to primary resistance to trastuzumab is shown in Fig. 3. Other mutations found by means of NGS in all analyzed samples are detailed in Supplementary Table S1. In particular, we detected SRC V414M and ALK C1182Y substitutions in two different resistant patients without other putative resistance mechanisms, whereas a BRAF V600E mutation was found concomitantly to KRAS A146V. Patients with tumors bearing no candidate alterations had a significantly longer median PFS when compared with positive ones [5.2 vs. 2.6 months; HR, 0.34; 95% confidence interval (CI), 0.07–0.48; P ¼ 0.001; Fig. 4A]. The same results were observed in terms of median OS (16.1 vs. 7.6 months; HR, 0.38; 95% CI, 0.09– 0.75; P ¼ 0.015; Fig. 4B).

Table 1. Patients demographics and disease characteristics in the overall population and in resistant versus sensitive groups

Variables	Overall population $N = 37$ (%)	Resistant group $N = 20$ (%)	Sensitive group $N = 17$ (%)	P^a
Age				
Median	67	69	56	
Range	34–87	43–87	34–84	0.133
Gender				
Male	25 (68)	14 (70)	11 (65)	0.729
Female	12 (32)	6 (30)	6 (35)	
ECOG PS				
0	23 (62)	11 (55)	12 (71)	0.330
1–2	14 (38)	9 (45)	5 (29)	
Primary tumor location				
Cardias	17 (46)	11 (55)	6 (35)	0.482
Fundum/body	13 (35)	6 (30)	7 (41)	
Antrum	7 (19)	3 (15)	4 (24)	
Number of metastatic sites				
1	18 (49)	7 (35)	11 (65)	0.071
>1	19 (51)	13 (65)	6 (35)	
Primary tumor resection				
Yes	8 (22)	6 (30)	2 (12)	0.246
No	29 (78)	14 (70)	15 (88)	
Time to metastases				
Synchronous	28 (76)	14 (70)	14 (82)	0.463
Metachronous	9 (24)	6 (30)	3 (18)	
Histotype				
Intestinal type	33 (89)	17 (85)	16 (94)	0.609
Diffuse type	4 (11)	3 (15)	1 (6)	
Grading				
1–2	7 (19)	5 (25)	2 (12)	0.381
3	15 (41)	7 (35)	8 (47)	
NA	15 (41)	8 (40)	7 (41)	
HER2 IHC score				
2+	14 (38)	10 (50)	4 (24)	0.100
3+	23 (62)	10 (50)	13 (76)	

[a]Comparison between resistant and sensitive groups was performed by two-sided Fisher exact test, χ^2 test, or Mann–Whitney test as appropriate.

Predictive accuracy of HER2 immunohistochemistry, AMNESIA panel, or both

Candidate alterations of the AMNESIA panel were found in 53.8% (7 of 13) of HER2 IHC 2þ tumors versus 16.7% (4 of 24) of HER2 IHC 3þ ones (P¼0.028). Among AMNESIA panel–negative patients (n ¼ 26), 19 had HER2 IHC 3þ and 7 IHC 2þ tumors. Six patients (32%) in 3þ subgroup were resistant versus 5 (71%) in 2þ (P ¼ 0.095). Consistently, AMNESIA panel–negative patients with HER2 IHC 2þ tumors had a nonsignificant shorter median PFS when compared with HER2 3þ (5.2 vs. 7.1 months; HR, 0.73; 95% CI, 0.29–1.85; P ¼ 0.123; Supplementary Fig. S2). OS analysis was limited by the low number of events. The predictive accuracy of HER2 expression and AMNESIA panel assessment with regard to treatment outcome were 65% and 76%, respectively, whereas it reached the value of 84% when evaluating HER2 expression in AMNESIA panel–negative cases.

Table 2. Prevalence of candidate genomic alterations of the AMNESIA panel in samples from resistant (cases) versus sensitive (controls) patients' groups

Genomic alterations	Resistant group $N = 20$	Sensitive group $N = 17$
MET amplification	2	0
MET mutations	2 (N375S and R988C[a])	0
EGFR amplification	2	0
EGFR mutations	0	0
KRAS amplification	2	0
KRAS mutations	2 (G13D and A146V)	0
BRAF mutations	1 (V600E)	0
PIK3CA mutations	3 (N345T, H1047R, and H1047L)	0
Patients with candidate alterations	11[b]	0

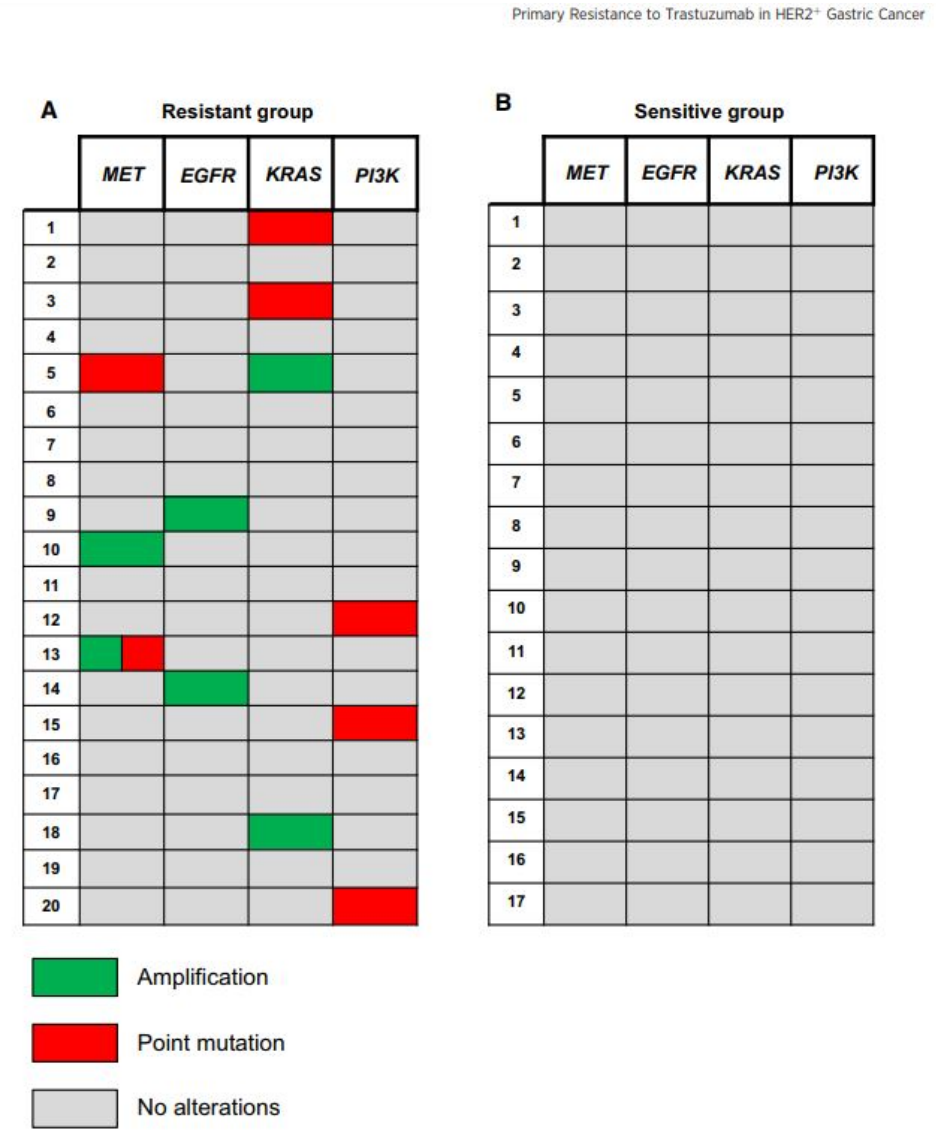
[a]The R988C mutation was reported in CLINVAR database with conflicting interpretations of pathogenicity, although it was not reported in COSMIC database.

[b]Three resistant patients carried two co-occurring alterations: KRAS A146V mutation + BRAF V600E mutation, MET N375S mutation + *KRAS* amplification, and MET R988C mutation + *MET* amplification.

Discussion

HER2 is the only validated biomarker routinely tested in mGC, because trastuzumab in combination with doublet platinum-based chemotherapy is approved for patients with IHC 3þ or 2þ/ISHþ tumors based on a post hoc analysis of the ToGA trial (4). Even if trastuzumab has significantly improved the outcomes of HER2-positive patients with mGC, primary and acquired resistance to treatment are relevant challenges narrowing its therapeutic index. In HER2-positive breast cancer, the molecular

mechanisms of trastuzumab resistance have been extensively investigated (18, 19). However, none of them is validated for clinical use, preventing a drug label restriction that might have saved costs and toxicities after patients' molecular selection. This disappointing path has substantially discouraged such a clinical research field in mGC, and the available literature regarding this topic is scant and relatively recent (9). However, HER2-positive gastric and breast cancers are different diseases for several reasons. From a biological point of view, HER2 positivity is more heterogeneous in gastric cancer at both intra- and interlesional level (20), and HER2 amplification may also be coupled with other coexisting oncogenic drivers (6). Of note, in silico studies and analyses carried out in retrospective patients' series showed that oncogene amplifications are not mutually exclusive as it was thought initially (21), but HER2 gene may be coamplified with EGFR or MET (6, 12, 22). Receptor coamplifications and several other putative mechanisms of primary resistance to trastuzumab—such as PI3K/PTEN and MET/HER3 mutations—have been found in the samples of patients with HER2-positive primary gastric cancer and were preclinically validated (6). Therefore, HER2-addicted gastric cancers might be easily identified not only by the presence of an intense and relatively more homogeneous HER2 staining, but also by the concomitant absence of other relevant oncogenic drivers. From a clinical point of view, HER2 addiction entails a potential long-lasting benefit from trastuzumab, whereas primary resistance to "pure" HER2 targeting only leaves potential sensitivity to chemotherapy alone. This spectrum may explain the relatively limited median OS absolute gain conferred by trastuzumab in both metastatic HER2-positive breast and gastric cancers (4, 23).



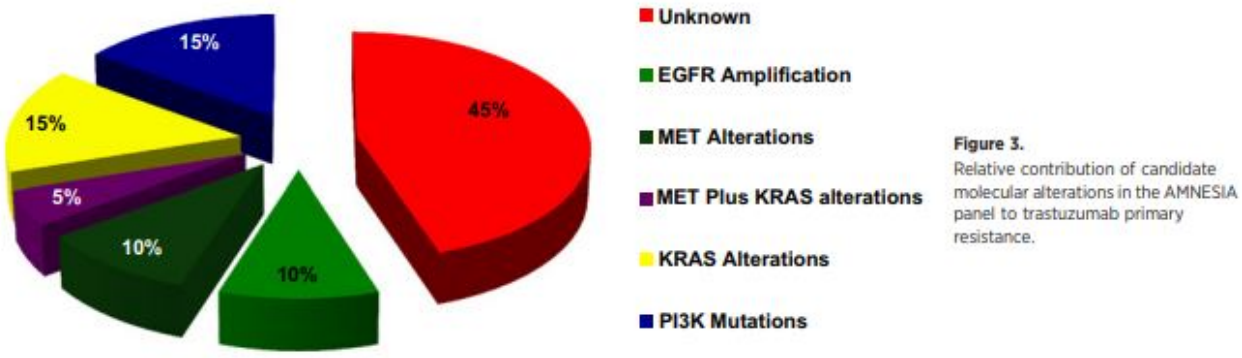


Figure 3.
Relative contribution of candidate molecular alterations in the AMNESIA panel to trastuzumab primary resistance.

Several reasons may explain the clinical challenges faced in validating biomarkers of trastuzumab resistance: first, because trastuzumab is administered in association with chemotherapy and such combination is markedly effective, a relevant proportion of mechanisms of primary resistance may be masked by chemotherapy. Second, even if the best chance of validation could be provided by controlled clinical trials, the availability of tumor samples from industry-sponsored studies is often limited. Finally, there is still a lack of hypothesis-generating studies aimed at correlating—at least retrospectively—the presence of putative resistance alterations with outcomes of patients with mGC receiving trastuzumab-based treatment. Drawing from these considerations and based on our experience on rare resistance mechanisms beyond RAS and BRAF in patients with metastatic colorectal cancer receiving anti-EGFRs (24), we designed the present case-control study, AMNESIA-1. The strength of our results relies on: (1) the simultaneous assessment of multiple resistance mechanisms with an individual low frequency, but reaching 55% when combined together as "AMNESIA panel." This approach may provide a greater chance of validating genomic signatures as opposed to attempts of investigating just one biomarker at a time; (2) the proper patients' clinical selection by adopting restrictive criteria for defining primary resistance versus clear sensitivity based on the combined assessment of RECIST response and time to progression (Supplementary Fig. S1), in order to overcome the challenge represented by the potential activity of chemotherapy; and (3) the case-control study design based on a predefined statistical hypothesis. Even if AMNESIA panel genomic alterations are enriched in HER2 IHC 2þ cancers, we show that the combined assessment of the AMNESIA panel and HER2 IHC reaches the highest predictive accuracy (84%) for identifying patients with mGC with trastuzumab primary resistance. In patients with AMNESIA panel–negative cancers, HER2 2þ status is still associated with treatment resistance/worse outcomes, even if the low number of evaluable patients does not allow to reach statistically significant results. Interestingly, with the exception of KRAS alterations, the majority of primary resistance mechanisms may be potentially cotargeted by adding to trastuzumab other agents such as PI3K, EGFR/panHER, or MET inhibitors. Finally, in the resistant patients' subgroup, we found two mutations that were mutually exclusive with other resistance mechanisms and should be further preclinically validated: SRC V414M and ALK C1182Y. Accordingly, SRC was described as a central hub of several resistance pathways in HER2-positive breast cancers treated with trastuzumab (25) and may be targeted by selective inhibitors such as dasatinib, whereas ALK mutations may be targeted by several tyrosine kinase inhibitors. Our study has some obvious limitations. First, other rare genomic (such as HER3 mutations) or even nongenomic mechanisms of primary resistance may have been missed by the AMNESIA panel. Second, the lack of a control group untreated with trastuzumab does not allow to draw any definitive conclusion on the predictive value of the AMNESIA panel. The translational relevance of our results relies on the chance of validating the negative predictive impact of the AMNESIA panel could by means of post hoc translational analyses of phase III trials reporting negative results of anti-HER2 agents other than trastuzumab such as lapatinib (26, 27), TDM-1 (28) and pertuzumab (29).

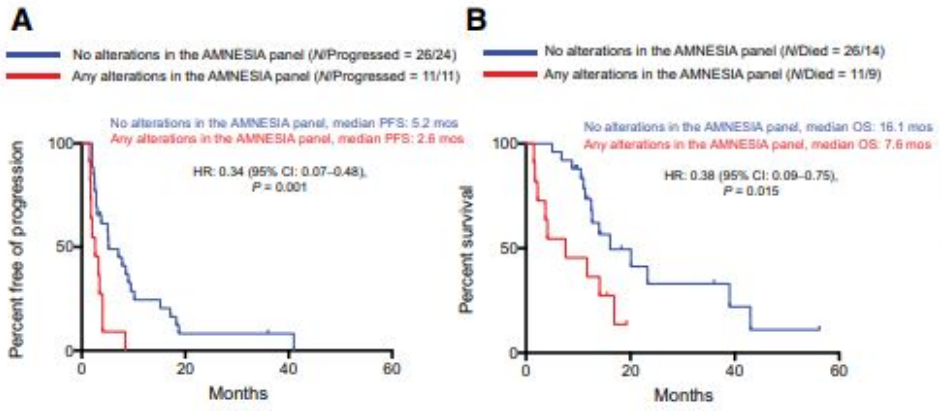


Figure 4.
Kaplan–Meier estimates of PFS (**A**) and OS (**B**) according to the presence (red line) or absence (blue line) of AMNESIA panel alterations in the study population.

We emphasize that a better molecular selection of patients with HER2 addiction might have turned such trials into positive ones. With specific regard to trastuzumab, a randomized discontinuation trial aimed at assessing the noninferiority of no versus yes trastuzumab maintenance could prospectively validate our resistance biomarkers. However, because targeted strategies overcoming trastuzumab resistance have been mechanistically validated and clinically reported (6, 12), we believe that a different strategy based on exploiting the individual resistance mechanisms as therapeutic cotargets will be the most clinically useful in improving patients' life expectancy. Specifically, an umbrella trial could assess the value of adding other biological agents to trastuzumab as personalized salvage treatment in primarily resistant patients according to the specific tumor resistance mechanism. Finally, future trials might use novel molecular stratification criteria to refine patients' selection, with the goal of improving the efficacy of HER2 blockade strategies and, hopefully, avoiding the use of chemotherapy in a subset of molecularly selected patients.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed. Authors' Contributions Conception and design: F. Pietrantonio, F. de Braud Development of methodology: F. Pietrantonio, G. Aprile, F. Perrone, A. Pellegrinelli, G. Pruneri Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): F. Pietrantonio, G. Fucà, F. Morano, A. Gloghini, S. Corso, G. Aprile, F. De Vita, E. Tamborini, G. Tomasello, A.V. Gualeni, E. Ongaro, E. Giommoni, S. Corallo, M. Prisciandaro, M. Antista, A. Pellegrinelli, L. Castagnoli, S.M. Pupa, G. Pruneri, S. Giordano, M. Di Bartolomeo Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): F. Pietrantonio, G. Fucà, F. Perrone, E. Tamborini, A. Busico, E. Giommoni, A. Pellegrinelli, G. Pruneri, C. Cremolini Writing, review, and/or revision of the manuscript: F. Pietrantonio, G. Fucà, F. Morano, A. Gloghini, S. Corso, G. Aprile, F. Perrone, E. Tamborini, G. Tomasello, A.V. Gualeni, C.C. Volpi, S. Corallo, M. Prisciandaro, M. Antista, A. Pellegrinelli, G. Pruneri, F. de Braud, S. Giordano, C. Cremolini, M. Di Bartolomeo Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases): F. Pietrantonio, C.C. Volpi, S. Corallo, M. Prisciandaro Study supervision: F. Pietrantonio, M.M. Laterza Other (interpretation of bright field in situ hybridization assays): A. Gloghini Other (bright-field in situ hybridization experiments managed patients): A.V. Gualeni

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References

1. Torre LA, Bray F, Siegel RL, Ferlay J, LortetTieulent J, Jemal A. Global cancer statistics, 2012. CA Cancer J Clin 2015;65:87–108.

2. Cancer Genome Atlas Research Network. Comprehensive molecular characterization of gastric adenocarcinoma. Nature 2014;513: 202–9.

3. Bartley AN, Washington MK, Colasacco C, Ventura CB, Ismailaet N. HER2 testing and clinical decision making in gastroesophageal adenocarcinoma: guideline from the College of American Pathologists, American Society for Clinical Pathology, and the American Society of Clinical Oncology. J Clin Oncol 2017;35:446–64.

4. Bang YJ, Van Cutsem E, Feyereislova A, Chung HC, Shen L, Sawaki A, et al. Trastuzumab in combination with chemotherapy versus chemotherapy alone for treatment of HER-2 positive advanced gastric or gastro-oesophageal junction cancer (ToGA): a phase 3, open-label, randomized controlled trial. Lancet 2010;376:687–97.

5. Gomez-Martin C, Plaza JC, Pazo-Cid R, Salud A, Pons F, Fonseca P, et al. Level of HER2 gene amplification predicts response and overall survival in HER2-positive advanced gastric cancer treated with trastuzumab. J Clin Oncol 2013;31:4445–52.

6. Kim J, Fox C, Peng S, Pusung M, Pectasides E, Matthee E, et al. Preexisting oncogenic events impact trastuzumab sensitivity in ERBB2 amplified gastroesophageal adenocarcinoma. J Clin Invest 2014;124:5145–58.

7. Deguchi Y, Okabe H, Oshima N, Hisamori S, Minamiguchi S, Muto M, et al. PTEN loss is associated with a poor response to trastuzumab in HER2- overexpressing gastroesophageal adenocarcinoma. Gastric Cancer 2017; 20:416–27.

8. Takahashi N, Furuta K, Taniguchi H, Sasaki Y, Shoji H, Honma Y, et al. Serum level of hepatocyte growth factor is a novel marker of predicting the outcome and resistance to the treatment with trastuzumab in HER2- positive patients with metastatic gastric cancer. Oncotarget 2016;26; 4925–38.

9. Lee JY, Hong M, Kim ST, Park SH, Kang WK, Kim KM, et al. The impact of concomitant genomic alterations on treatment outcome for trastuzumab therapy in HER2-positive gastric cancer. Sci Rep 201519;5:9289.

10. Lordick F, Janjigian YY. Clinical impact of tumour biology in the management of gastroesophageal cancer. Nat Rev Clin Oncol 2016;13:348–60.

11. Ruschoff J, Hanna W, Bilous M, Hofmann M, Osamura RY, Penault-Llorca € F, et al. HER2 testing in gastric cancer: a practical approach. Mod Pathol 2012;25:637–50.

12. Kwak EL, Ahronian LG, Siravegna G, Mussolin B, Godfrey JT, Clark JW, et al. Molecular heterogeneity and receptor coamplification drive resistance to targeted therapy in MET-amplified esophagogastric cancer. Cancer Discov 2015;5:1271–81.

13. Pietrantonio F, Perrone F, Mennitto A, Gleeson EM, Milione M, Tamborini E, et al. Toward the molecular dissection of peritoneal pseudomyxoma. Ann Oncol 2016;27:2097–103.

14. Pietrantonio F, Vernieri C, Siravegna G, Mennitto A, Berenato R, Perrone F, et al. Heterogeneity of acquired resistance to Anti-EGFR monoclonal antibodies in patients with metastatic colorectal cancer. Clin Cancer Res 2017;23:2414–22.

15. Cappuzzo F, Hirsch FR, Rossi E, Bartolini S, Ceresoli GL, Bemis L, et al. Epidermal growth factor receptor gene and protein and gefitinib sensitivity in non – small-cell lung cancer. J Natl Cancer Inst 2005;97:643–55.

16. Varella-Garcia M, Diebold J, Eberhard DA, Geenen K, Hirschmann A, Kockx M, et al. EGFR fluorescence in situ hybridisation assay: guidelines for application to non-small-cell lung cancer. J Clin Pathol 2009;62:970–7.

17. Sartore-Bianchi A, Fieuws S, Veronese S, Moroni M, Personeni N, Frattini M, et al. Standardisation of EGFR FISH in colorectal cancer: results of an international interlaboratory reproducibility ring study. J Clin Pathol 2012;65:218–23.

18. Berns K, Horlings HM, Hennessy BT, Madiredjo M, Hijmans EM, Beelen K, et al. A functional genetic approach identifies the PI3K pathway as a major determinant of trastuzumab resistance in breast cancer. Cancer Cell 2007;12:395–402.

19. Stemke-Hale K, Gonzalez-Angulo AM, Lluch A, Neve RM, Kuo WL, Davies M, et al. An integrative genomic and proteomic analysis of PIK3CA, PTEN, and AKT mutations in breast cancer. Cancer Res 2008;68:6084–91.

20. Pietrantonio F, Caporale M, Morano F, Scartozzi M, Gloghini A, De Vita F, et al. HER2 loss in HER2-positive gastric or gastroesophageal cancer after trastuzumab therapy: Implication for further clinical research. Int J Cancer 2016;139:2859–64.

21. Deng N, Goh LK, Wang H, Das K, Tao J, Tan IB, et al. A comprehensive survey of genomic alterations in gastric cancer reveals systematic patterns of molecular exclusivity and co-occurrence among distinct therapeutic targets. Gut 2012;61:673–84.

22. Ha SY, Lee J, Jang J, Hong JY, Do IG, Park SH, et al. HER2-positive gastric cancer with concomitant MET and/or EGFR overexpression: a distinct subset of patients for dual inhibition therapy. Int J Cancer 2015;136: 1629–35.

23. Slamon DJ, Leyland-Jones B, Shak S, Fuchs H, Paton V, Bajamonde A, et al. Use of chemotherapy plus a monoclonal antibody against HER2 for metastatic breast cancer that overexpresses HER2. N Engl J Med 2001; 344:783–92.

24. Cremolini C, Morano F, Moretto R, Berenato R, Tamborini E, Perrone F, et al. Negative hyper-selection of metastatic colorectal cancer patients for anti-EGFR monoclonal antibodies: the PRESSING case-control study. Ann Oncol 2017;28:3009–14.

25. Zhang S, Huang WC, Li P, Guo H, Poh SB, Brady SW, et al. Combating trastuzumab resistance by targeting SRC, a common node downstream of multiple resistance pathways. Nat Med 2011;17:461–9.

26. Hecht JR, Bang YJ, Qin SK, Chung HC, Xu JM, Park JO, et al. Lapatinib in combination with capecitabine plus oxaliplatin in human epidermal growth factor receptor 2-positive advanced or metastatic gastric, esophageal, or gastroesophageal adenocarcinoma: TRIO-013/LOGiC–A randomized phase III trial. J Clin Oncol 2016;34:443–51.

27. Satoh T, Xu RH, Chung HC, Sun GP, Doi T, Xu JM, et al. Lapatinib plus paclitaxel versus paclitaxel alone in the second-line treatment of HER2- amplified advanced gastric cancer in Asian populations: TyTAN–a randomized, phase III study. J Clin Oncol 2014;32:2039–49.

28. Thuss-Patience PC, Shah MA, Ohtsu A, Van Cutsem E, Ajani JA, Castro H, et al. Trastuzumab emtansine versus taxane use for previously treated HER2-positive locally advanced or metastatic gastric or gastro-oesophageal junction adenocarcinoma (GATSBY): an international randomised, open-label, adaptive, phase 2/3 study. Lancet Oncol 2017;18:640–653.

29. Tabernero J, Hoff P, Shen L, Ohtsu A, Shah M, Cheng K, et al. Pertuzumab (P) þ trastuzumab (H) þ chemotherapy (CT) for HER2-positive metastatic gastric or gastro-oesophageal junction cancer (mGC/GEJC): final analysis of a phase III study (JACOB). Ann Oncol 2017;28:616O.