

ORIGINAL ARTICLE



## AGAPP: efficacy of first-line cisplatin, 5-fluorouracil with afatinib in inoperable gastric and gastroesophageal junction carcinomas. A Hellenic Cooperative Oncology Group study

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### ABSTRACT

**Purpose:** Gastric cancer is the fifth most common neoplasm worldwide with high rates of mortality. Afatinib, a low molecular, irreversible potent inhibitor of ErbB trans-membrane receptor family, has shown promising results according to preclinical and phase I clinical trial data when combined with chemotherapy. We aimed at evaluating the safety and efficacy of the combination of cisplatin, 5FU with afatinib in molecularly unselected patients with advanced gastric cancer.

**Methods:** Patients with locally advanced or metastatic gastric/gastroesophageal junction adenocarcinoma received first line combination therapy of cisplatin, 5FU and afatinib every 21 days, followed by afatinib maintenance monotherapy. The primary endpoint was the Objective Response Rate (ORR); secondary endpoints included Overall Survival (OS), Progression Free Survival (PFS) and the safety profile. Unplanned exploratory analysis of HER2 and tumor mutational profile was performed.

**Results:** Among 55 patients (ITT population) enrolled, 19 (34.5%) achieved an objective tumor response; stable disease was observed in 16 patients (29.1%) and progressive disease in 10 patients (18.2%). The ORR in the per protocol population (PP) was 42.9%. Within a median follow-up of 56 months, the median PFS and OS in the ITT population was 5.0 and 8.7 months, respectively. Seven of the 47 HER2 informative cases carried HER2 positive tumors while *TP53*, *BRCA2* and *SMAD4* were the most frequently mutated genes. The most common toxicities were neutrophil count and white blood cell decrease occurring in 56.4% of patients, followed by anemia (50.9%), hyperglycemia (40%), and diarrhea (38.2%).

**Conclusions:** The combination of cisplatin/5FU with afatinib did not surpass the benchmarks of efficacy of the contemporary therapeutic regimens that are being applied for the treatment of patients with advanced gastric cancer. However, the observed efficacy and the improved safety profile support that our administration schedule may be further investigated to overcome toxicity problems when integrating afatinib to cytotoxic chemotherapy.

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Gastric cancer; afatinib; chemotherapy; combination; anti-EGFR TKI

### Introduction

Gastric cancer is the fifth most common neoplasm worldwide with over one million new cases reported in 2018, and the third most fatal cancer [1]. Surgical removal remains the only curative modality for patients with localized disease, but

fewer than 30% of patients with gastric cancer are diagnosed with a localized, operable tumor [2,3].

In patients with advanced stages of the disease, the administration of palliative cytotoxic chemotherapy may control the tumor's symptoms, enhance quality of life and

prolong survival, but it does not provide a cure [4]. Targeted therapies, notably against the angiogenic and HER2 pathways, have been incorporated in current management with a moderately beneficial impact on the patient's outcome [4,5].

Gastric cancer shows an overexpression of the transmembrane receptors EGFR and HER2 in 50–80% and 10–22% of cases, respectively [6–8]. Preclinical and retrospective clinical data have associated the overexpression of these *HER* pathway oncoproteins with cell proliferation, angiogenesis, invasion and metastasis, a higher T and N disease stage, and a poor prognosis in patients with gastric cancer [9,10]. In the ToGA phase III trial, the addition of trastuzumab to cisplatin fluoropyrimidine therapy resulted in a clinically and statistically significant improvement of median overall survival (OS) from 11 to 13 months in patients with HER2+ gastric cancer [11].

Afatinib is a potent, low-molecular weight, inhibitor of ErbB family transmembrane receptors with irreversible intracellular tyrosine kinase inhibiting activity against all HER family proteins (ErbB1/EGFR, ErbB2/HER2, and ErbB4/HER4), apart from HER3, which lacks an intracellular domain. Afatinib is connected through a homopolar bond and strongly inhibits the phosphorylation of tyrosine residues in the cytoplasmic department of the receptors [12,13]. data from breast cancer xenografts show that it has antitumor activity in xenografts that overexpress HER2 and are either sensitive or resistant to trastuzumab as well as in xenografts that express high levels of EGFR. Afatinib's activity results in the arrest of tumor growth, including the growth of gastric tumors [14–16]. In addition, afatinib arrests the growth of neoplastic cell lines carrying activating or resistant mutations of the *EGFR* gene, as well as the EGFRvIII protein [17]. At tolerated doses it induced an almost complete regression of subcutaneously implanted human malignant tumors that overexpressed EGFR and HER2 receptors [16,18,19]. The long half-life of afatinib (>35 h) allows for its administration once daily [19], as well as for the investigation of novel dosage schemes, which permit short, 24–48 h pauses ('weekend breaks'), providing a better toxicity profile without a significant decrease in plasma levels.

Preclinical and phase I clinical trial data have shown a synergistic cytotoxic activity of afatinib combined with platinum derivatives, taxanes and fluoropyrimidines, especially following chemotherapy [20]. Moreover, preclinical experiments suggested that a 48-hour lead-in of cytotoxic chemotherapy before afatinib administration leads to synchronization of neoplastic cells in the active cell cycle and to a more effective arrest of tumor growth based on HER inhibition [16,21]. Based on the available data, we aimed at investigating the administration of cisplatin plus 5FU chemotherapy in combination with afatinib in patients with advanced gastric-gastroesophageal cancer.

## Patient and methods

This prospective, single arm phase II trial enrolled previously untreated patients with histological or cytological diagnosis of gastric and/or gastroesophageal junction adenocarcinoma/

carcinoma. Main inclusion criteria were the diagnosis of advanced inoperable or metastatic disease measurable under RECIST 1.1, age of at least 18 years old, adequate marrow, liver, and renal function, left ventricular ejection fraction  $\geq 50\%$  (ECHO/MUGA) and Eastern Cooperative Oncology Group performance status (PS ECOG) 0–1. Main exclusion criteria were previous systemic first line therapy, previous administration of EGFR/HER TKI or other experimental agent, presence of uncontrolled brain metastases, spinal cord compression or carcinomatous meningitis. In addition, patients with uncontrollable hypertension or significant heart conditions that have occurred within 12 months before the start of the study treatment (myocardial infarction, serious or unstable angina pectoris, aortic-coronary or peripheral bypass surgery, symptomatic heart failure, vascular stroke, transient ischemic attack or pulmonary embolism) were also excluded. Finally, patients with atrial fibrillation of any grade could not be enrolled in the study.

The study was conducted in accordance with the Declaration of Helsinki. The clinical protocol was approved by the participating hospital Institutional Review Boards and by the National Organization for Medicines. The trial was registered with the ClinicalTrials.gov identifier: NCT01743365 and with EudraCT (EudraCT No.: 2011-006198-25). All patients provided written informed consent for the study and for provision of biological material for future translational research studies. *Post hoc* translational analysis of HER2 (A.B) and tumor mutational profile (K.P) was performed along with pathology reports reassessment (M.B).

## Treatment

The modified regimen of cisplatin, 5FU and afatinib (mCisFU-A) was administered every 21 days. Cisplatin was administered at 75 mg/m<sup>2</sup> diluted in 1 liter of normal saline solution (0.9% N/S) over 60–120 min on day 1, with rigorous saline-based intravenous hydration. 5-Fluorouracil (5FU) was infused intravenously over 24 h at a daily dose of 750 mg/m<sup>2</sup> for a total of 96 h (days 1–4) every 21 days. Afatinib was administered orally at a daily dosage of 40 mg between 08:00 and 09:00 a.m., at least 1 h before breakfast. Afatinib administration started on day 3 of each therapy cycle, having been preceded by cisplatin infusion on day 1 and 5FU infusion on days 1 and 2. The once-daily oral administration of afatinib continued throughout the week with intervals during weekends ('Weekday-on – Weekend-off'). Therefore, in each 21-day therapy cycle the administration of afatinib took place once daily on days 3–5 of week 1, days 1–5 of week 2, and days 1–5 of week 3. The total daily dosage of 40 mg was administered independently of the patient's body surface area. If vomiting occurred after administration, the patient was not given a replacement dose.

In the absence of disease progression or significant toxicity, six cycles of mCisFU-A were administered followed by afatinib monotherapy at a daily dosage of 40 mg according to the 'Weekday-on – Weekend-off' schema. The afatinib maintenance therapy continued until the emergence of disease progression, significant toxicity, or withdrawal of

patient's consent. All adverse events (AE) were classified according to the NCI CTC 4.0 grading scale. CT-based imaging was applied every 8 weeks during the administration of mCisFU-A, and every 12 weeks in the maintenance phase with afatinib monotherapy.

### Tissue processing

Formalin-Fixed Paraffin-Embedded (FFPE) tumor blocks, corresponding to either biopsy or surgical specimens, were available for 42 patients with advanced gastric and gastroesophageal junction carcinomas, treated with cisplatin and 5-fluorouracil combination chemotherapy together with afatinib as a first-line therapy.

FFPE processing and Next-Generation Sequencing (NGS) was performed at the Laboratory of Molecular Oncology (LMO), Hellenic Foundation for Cancer Research (HeFCR)/Aristotle University of Thessaloniki. H&E Histological review, macro-dissection from whole sections and DNA extraction was accomplished as before [22]. Out of the 42 available FFPE, two had <5% tumor cell content (TCC) and were excluded from analysis. DNA concentration  $\geq 2$  ng/ $\mu$ l was a prerequisite for NGS analysis. Tumor DNA samples from 34 patients were processed for genotyping; of these 23 had TCC  $\geq 50\%$  (mean 58%; median 60%; range 10–100) (Supplemental Figure 1).

### Targeted NGS tumor genotyping

We performed targeted NGS with a previously published custom panel, targeting TCGA mutations in 59 genes frequently altered in colorectal but also gastro-esophageal carcinomas [23–26]. Genotyping and data retrieval was accomplished in an Ion Proton Sequencer as previously described [23]. Further quality filtering excluded samples if <50% of amplicons had >100 reads; mean depth <185 $\times$ ; uniformity <65%; number of variants <40 and variants if  $p > .0001$ ; amplicon reads < 100; position and variant coverage < 100 and <40 and  $\pm$  strand bias <10% for each; non-annotated; G-stretches; variant allele frequency (VAF) < 5%. Based on the above, 3274 out of the 4170 variants (78.5%) were eligible and distributed among 31 of the 34 tumors with median mean depth 2304 (mean: 2246; min–max: 189–6483); Median uniformity 89% (mean: 86%; Min–max: 67–94%); Median number of variants 75 (mean: 106; Min–max: 46–380). Eligible variants were called mutations if amino acid or splice site changing with either no reported minor allele frequency (MAF) or with MAF <0.1% according to 5000 Exomes database if annotated SNPs, and considered clonal with variant allele frequency (VAF) >25% as before [23,27] and thus, subclonal otherwise (VAF < 25%) and pathogenic according to ClinVar database.

### Statistical analysis

The primary endpoint was the evaluation of the Objective Response Rate (ORR), defined as the percentage of patients with a confirmed complete response (CR) or partial response (PR) as the best response, according to RECIST 1.1 criteria.

Secondary endpoints included Overall Survival (OS), Progression-Free Survival (PFS) and the safety profile. Based on Simon's two-stage optimal design, assuming that the expected ORR would be 45% and the minimum acceptable 30%, with type I and II errors of 10% and 20%, respectively, 20 patients were enrolled in the first stage of the study. Seven responders were observed (as planned) and 35 additional patients were enrolled leading to a total sample size of 55 patients. All enrolled patients were included in the analysis on an intent-to-treat (ITT) basis.

ORR, PFS and OS were estimated separately in the ITT population and in the subgroup of patients with gastric adenocarcinoma/carcinoma who received at least one full cycle of the study treatment, and had an initial tumor assessment (Per Protocol, PP population,  $N=42$ ). The safety profile was assessed in the safety population consisting of all patients who received at least one dose of the study drugs ( $N=55$ ). In an unplanned exploratory analysis, we described the tumors' mutational profile and the effect of HER2 and other molecular characteristics on the endpoints of interest. The data cutoff for the analysis was 25th October 2019. Statistical significance was set at a two-sided  $p < .05$ . Statistical analyses were performed using the Statistical Analyses Software (SAS; version 9.3, SAS Institute Inc., Cary, NC).

## Results

### Patient and tumor characteristics

Fifty-five patients were enrolled between February 2013 and September 2016. Two patients were ineligible. One of them had a misdiagnosed gastric lymphoma but received four cycles of mCisFU-A. Another patient completed the six cycles of mCisFU-A and 53 cycles of maintenance therapy, however, upon revision of the case, the malignant peritoneal metastatic deposits constituted non-measurable disease at baseline. Selected patient and tumor characteristics are summarized in Table 1. Information on HER2 status was retrieved from the histology reports of the local hospitals for 25 patients (45.5%), whereas for 22 patients (40%) HER2 status was assessed centrally at the Department of Pathology of the Ioannina University Hospital (by A.B). Seven cases carried HER2 positive tumors (14.9% of informative; 12.7% of the total cohort).

Overall, 549 mutations were detected in 25 of the 31 patients with NGS informative tumors (80.6%). Of them, 247 were clonal (45%) and found in all mutated tumors. *TP53* was the most frequently mutated gene (61.3% of informative cases; 76% of mutated), followed by *BRCA2* (35.5% of informative cases; 44% of mutated) and *SMAD4* (29% of informative cases; 36% of mutated). *RAS* mutations were identified in seven patients (22.6% of informative; 28% of mutated), whereas *PIK3CA* and *EGFR* mutations were found in five patients, respectively (16.1% of informative; 20% of mutated). Forty pathogenic mutations were detected in 17 patients (54.8% of informative; 68% of mutated). It is of note that eight tumors (25.8% of informative; 32% of mutated) were hypermutated with mutations >10 genes and multiple

Table 1. Selected patient and tumor characteristics at study entry.

|                                         | Total<br>(N = 55) |
|-----------------------------------------|-------------------|
| <b>Age</b>                              |                   |
| Median (min, max)                       | 64.3 (20.2, 77.4) |
|                                         | <b>N (%)</b>      |
| ≤65                                     | 30 (54.5)         |
| >65                                     | 25 (45.5)         |
| <b>Sex</b>                              |                   |
| Man                                     | 36 (65.5)         |
| Woman                                   | 19 (34.5)         |
| <b>Histology grade</b>                  |                   |
| I                                       | 3 (5.5)           |
| II                                      | 22 (40)           |
| III                                     | 30 (54.6)         |
| <b>Histology type (LAUREN)</b>          |                   |
| Diffuse type carcinoma                  | 11 (20)           |
| Intermediate                            | 14 (25.5)         |
| Intestinal type carcinoma               | 25 (45.5)         |
| Mixed carcinoma                         | 5 (9.1)           |
| <b>Histology type (WHO)<sup>a</sup></b> |                   |
| Tubular                                 | 39 (70.9)         |
| Poorly cohesive                         | 11 (20)           |
| Mixed                                   | 5 (9.1)           |
| <b>PS</b>                               |                   |
| 0                                       | 35 (63.6)         |
| 1                                       | 20 (36.4)         |
| <b>Primary site</b>                     |                   |
| Stomach                                 | 42 (76.4)         |
| Gastroesophageal junction/low esophagus | 13 (23.6)         |
| <b>H. pylori infection</b>              |                   |
| No                                      | 45 (81.8)         |
| Yes                                     | 5 (9.1)           |
| Unknown                                 | 5 (9.1)           |

PS: performance status.

<sup>a</sup>Board EbtWCoTE [41].

mutations in Homologous Recombination Repair (HRR)/DNA damage Repair (DDR)- and Mismatch Repair (MMR)-associated genes. Mutations in these tumors accounted for 90.3% of the total number of mutations (Figure 1).

### Treatment exposure

All 55 patients received at least one dose of cisplatin and 5FU, while two patients (3.6%), who only received one cycle of cytotoxic therapy, did not take afatinib. One of them presented with disseminated intravascular coagulation on day 2, discontinued study treatment due to progression and died of disease approximately 2.5 months later. The other patient discontinued treatment due to noncompliance upon physician's decision. Three more patients did not complete afatinib treatment in the first cycle and discontinued all study treatment after that. Among them, one patient discontinued treatment due to grade 4 stroke, while another patient experienced grade 1 gastrointestinal pain during treatment and withdrew her consent. In one additional patient, treatment was discontinued upon physician's decision due to patient's poor clinical performance.

Patients received 230 cycles (median 5) of mCisFU-A and the median relative dose intensities administered were 0.97 for 5FU, 0.96 for cisplatin and 0.94 for afatinib. Twenty-two patients (40%) completed six cycles of mCisFU-A. Eighteen patients received 117 cycles of afatinib (median 2; range 1–53) as maintenance treatment. Thirty-three patients (60%) discontinued mCisFUA due to death (one patient, 3.0%),

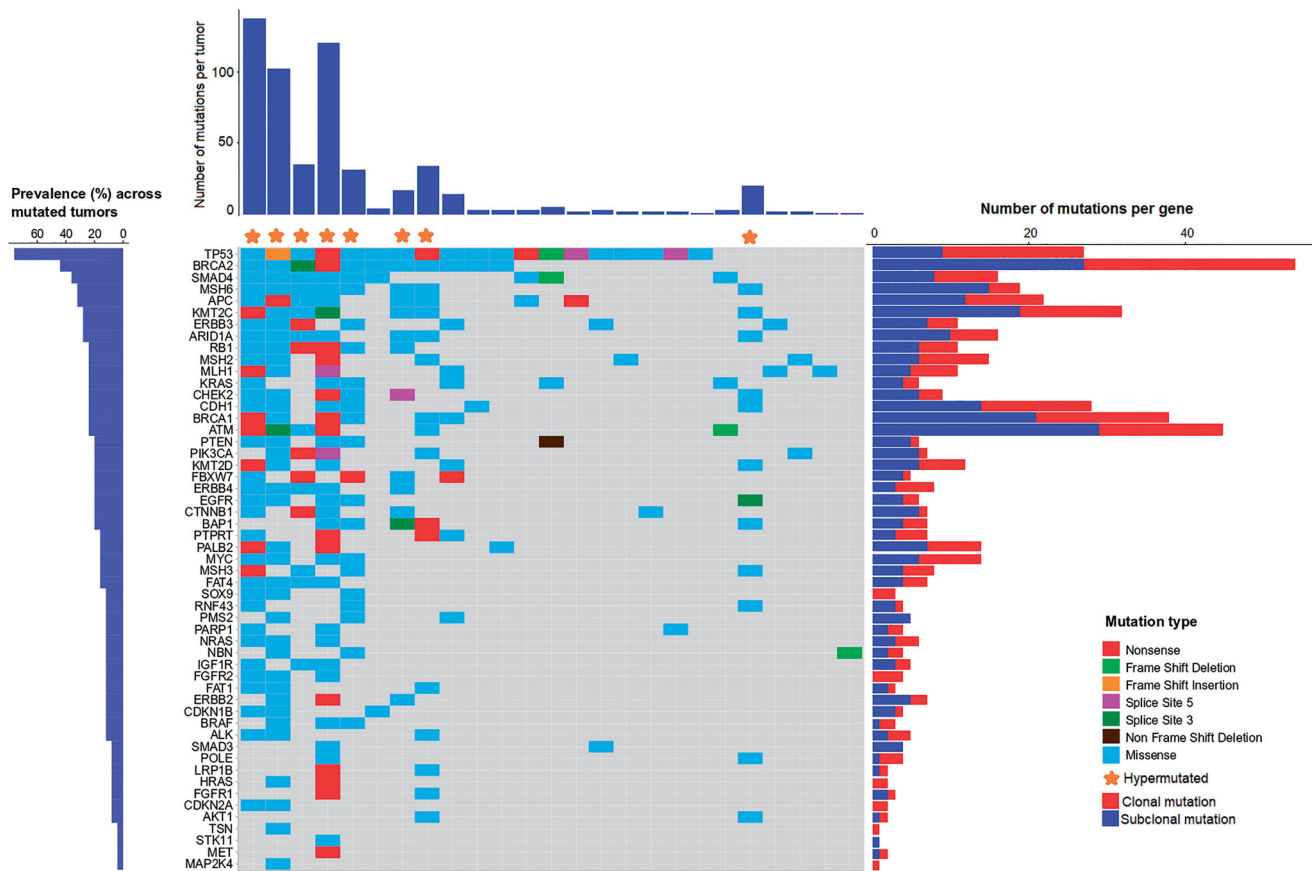


Figure 1. Map showing the distribution of mutations and mutation patterns per gene per tumor.

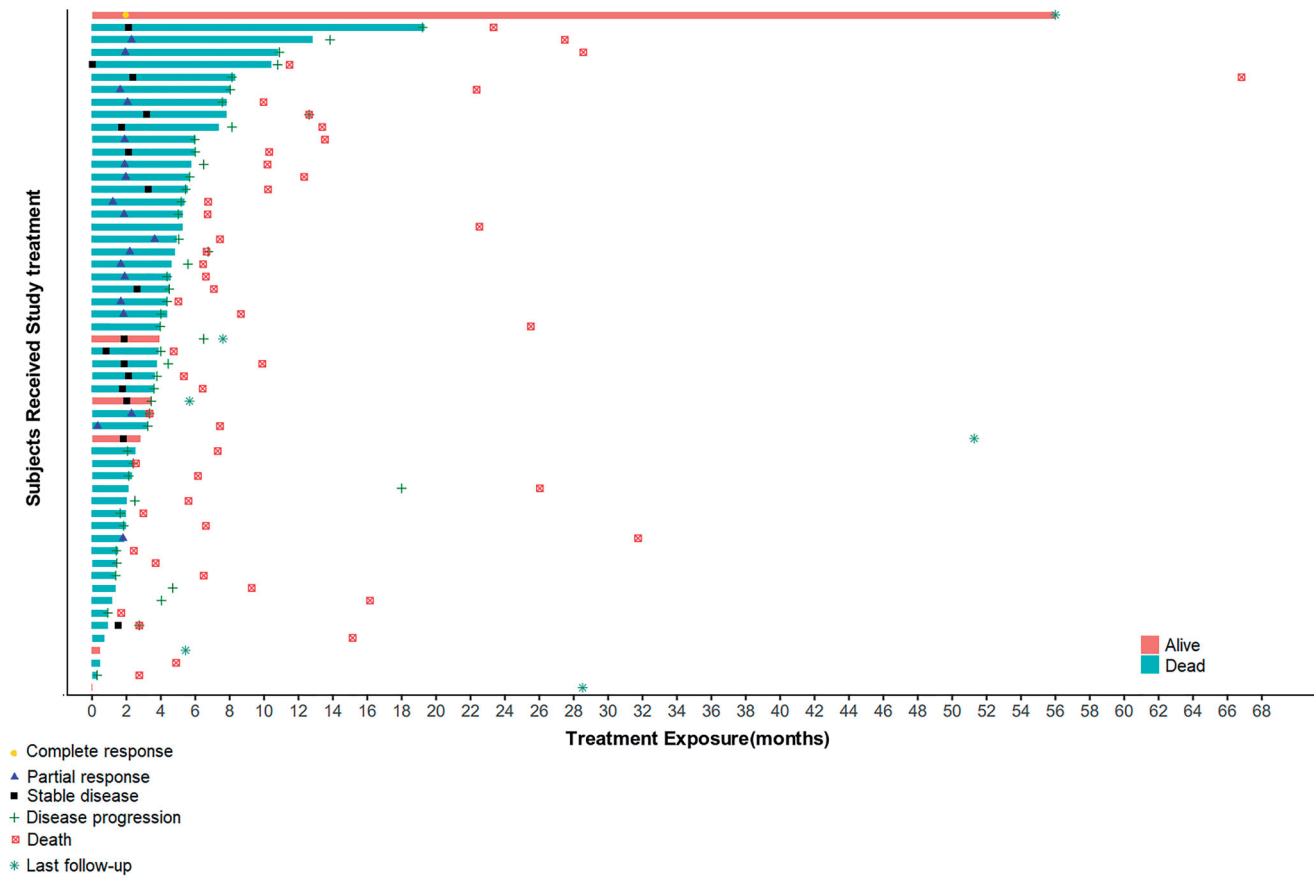


Figure 2. Swimmer plot showing the time to response for the ITT population.

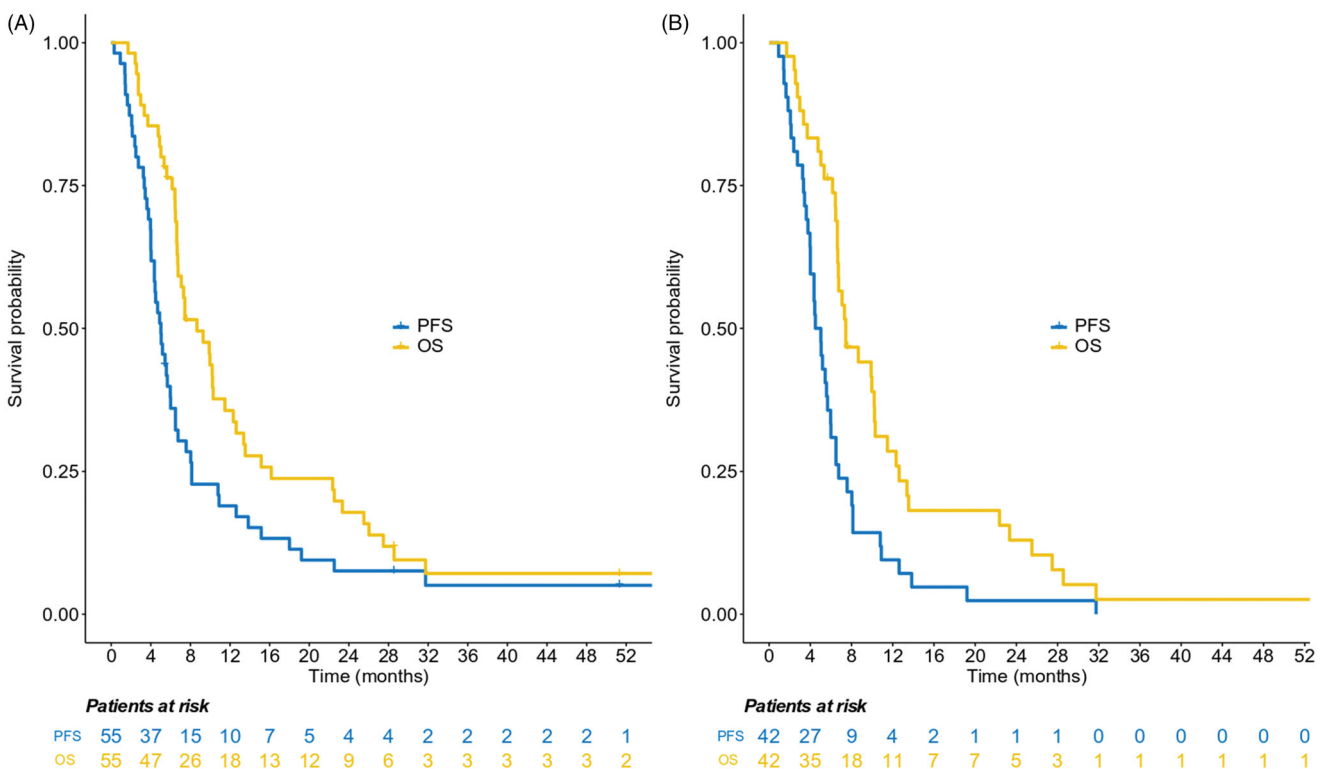


Figure 3. Kaplan-Meier curves with respect to PFS and OS for the (A) ITT and (B) PP population.

**Table 2.** Adverse events observed in more than 10% of the study population.

| Preferred term                 | Grade 1–2 |          |       | Grade 3–4 |          |       |
|--------------------------------|-----------|----------|-------|-----------|----------|-------|
|                                | N of evts | N of pts | % pts | N of evts | N of pts | % pts |
| Anemia                         | 21        | 21       | 38.18 | 7         | 7        | 12.73 |
| Diarrhea                       | 18        | 18       | 32.73 | 3         | 3        | 5.45  |
| Mucositis oral                 | 12        | 12       | 21.82 | 1         | 1        | 1.82  |
| Nausea                         | 15        | 15       | 27.27 | 0         | 0        | 0.00  |
| Vomiting                       | 18        | 18       | 32.73 | 2         | 2        | 3.64  |
| Edema limbs                    | 7         | 7        | 12.73 | 0         | 0        | 0.00  |
| Fatigue                        | 10        | 10       | 18.18 | 0         | 0        | 0.00  |
| Alkaline phosphatase increased | 7         | 7        | 12.73 | 3         | 3        | 5.45  |
| Creatinine increased           | 16        | 16       | 29.09 | 0         | 0        | 0.00  |
| Neutrophil count decreased     | 16        | 16       | 29.09 | 15        | 15       | 27.27 |
| Platelet count decreased       | 11        | 11       | 20.00 | 1         | 1        | 1.82  |
| White blood cell decreased     | 27        | 27       | 49.09 | 4         | 4        | 7.27  |
| Hyperglycemia                  | 20        | 20       | 36.36 | 2         | 2        | 3.64  |
| Hypoalbuminemia                | 16        | 16       | 29.09 | 0         | 0        | 0.00  |
| Hypocalcemia                   | 16        | 16       | 29.09 | 1         | 1        | 1.82  |
| Hypokalemia                    | 11        | 11       | 20.00 | 6         | 6        | 10.91 |
| Hyponatremia                   | 12        | 12       | 21.82 | 3         | 3        | 5.45  |
| Peripheral sensory neuropathy  | 7         | 7        | 12.73 | 0         | 0        | 0.00  |
| Rash acneiform                 | 11        | 11       | 20.00 | 0         | 0        | 0.00  |
| Rash maculo-papular            | 9         | 9        | 16.36 | 0         | 0        | 0.00  |

Evts: events; pts: patients.

non-fatal adverse events (four patients, 12.1%), physician's decision (three patients, 9.1%), disease progression (16 patients, 48.5%), informed consent withdrawal (seven patients, 21.2%), and other reasons (two patients, 6.1%).

### Treatment efficacy

In the ITT population, the ORR was 34.5% (95%CI 22.2–48.6) (18 patients with PR and one with CR) according to the local investigators' assessment. Stable disease was observed in 16 patients (29.1%) and progressive disease in 10 (18.2%). Nine patients who discontinued treatment prior to evaluation and one additional patient who underwent gastrectomy were not evaluated for tumor response. The ORR in the PP population was 42.9% (95%CI 27.7–59.0) (Figure 2). Of the seven patients with HER2 positive tumors, four were evaluable for response and three of them had PR (75% of evaluable; 42.9% of HER2 positive), whereas 13 of the 34 response evaluable patients (38.2%) with HER2 negative disease (32.5% of HER2 negative patients) achieved an objective response.

Eleven of the 31 patients with NGS informative tumors achieved an objective response and eight of them (72.7%) carried at least one mutation. Seven patients with OR harbored mutations in the *TP53* gene, three in *BRCA2*, whereas none of the 11 patients with an objective response carried mutations in the *PIK3CA* gene. *EGFR* and *RAS* (*KRAS*) mutations were identified in only one of the 11 patients with an objective response (one in each category).

At a median follow-up of 56 months (95%CI 28.5—not reached), 51 PFS events had occurred and 49 patients had died. Forty-four patients died of their disease, two by heart failure, while no further information on cause of death was available for the other three patients. The median PFS and OS in the ITT population were 5.0 (95%CI 4.0–6.0) and 8.7 months (95%CI 6.7–11.5), respectively (Figure 3(A)). In the PP population, the median PFS and OS was 4.8 months (95%CI 4.0–5.7) and 7.4 months (95%CI 6.6–10.2) (Figure 3(B)). All seven patients with HER2-positive disease died

within 5.6 and 26 months since study entry. The median PFS and OS for patients with HER2-negative tumors was 5.0 (95%CI 4.0–6.5) and 10.2 months (95%CI 6.7–13.4), respectively. The median PFS and OS for patients with mutated tumors were 4.9 months (95%CI 3.4–8.1) and 9.9 months (95%CI 6.2–13.4), respectively.

A trend toward shorter PFS and OS was observed for tumors in the gastroesophageal junction/low esophagus compared to gastric tumors (Supplemental Table 1, Supplemental Table 2).

### Safety

Overall, 554 AEs were reported in 54 patients (98.2%) with no toxic death (Table 2) and 148 of them were related to afatinib treatment; 12 during maintenance therapy (Supplemental Table 3). Twenty-seven patients (49.1%) experienced 59 serious adverse events. One patient experienced grade 4 Steven's Johnson syndrome after the completion of six cycles of mCisFUA, which led to discontinuation of maintenance therapy. The patient died of disease 20 days after the diagnosis of Steven's Johnson syndrome.

### Discussion

Gastric cancer is a heterogeneous disease that is currently morphologically classified in two classes, the diffuse and the intestinal histologic variants, each with different epidemiology, etiology, pathogenesis, and molecular abnormalities [28]. The EGFR family consists of four transmembrane cell receptors (HER1 or EGFR1, HER2, HER3, and HER4) that can form homodimers or heterodimers leading to the activation of transcription factors and to gene transcription that promotes cell proliferation, invasion and metastasis, apoptosis inhibition and neo-angiogenesis [29]. In 15–25% of gastric cancer cases, a neoplastic overexpression of HER2 protein (immunohistochemical expression 2+ or 3+) and in 7–15%

of cases a *HER2* gene amplification has been reported [30–32].

Patients with advanced gastric cancer are treated at present with combined chemotherapy based on platinum derivatives, based on trials showing a longer survival with improved quality of life with chemotherapy than with supportive treatment, [33–36]. In addition, the results of the ToGA trial established the combination of trastuzumab plus cisplatin and a fluoropyrimidine as the treatment of choice for patients with *HER2*-positive advanced gastric or gastroesophageal cancers based on the improvement of median OS [11].

Afatinib has been evaluated in combination with cytotoxic agents in cell-culture and xenograft models to assess antitumor activity. Experiments regarding concomitant administration of afatinib with doxorubicin, docetaxel, 5FU, and cisplatin have shown the presence of hyper-cumulative cytotoxic activity [16]. This activity has been observed in the presence of activating *EGFR* mutations and *HER2* gene amplification, as well as in other molecular disorders of these receptors.

Based on these preclinical and clinical data, we aimed at investigating the activity of the cisplatin, 5FU, and afatinib combination as a first-line treatment in patients with advanced, inoperable or metastatic gastric/gastroesophageal cancer. According to the results of the study, the overall response rate in the ITT population was 34.5% while in the per protocol population it was estimated at 42.9% failing to surpass the expected limit of 45% as designed. With a median follow-up of 56 months the median PFS and OS in the ITT population were 5.0 and 8.7 months, respectively, failing to improve the one-year benchmark of median OS accomplished by combination chemotherapy, thus indicating a modest activity of the regimen.

The treatment regimen was well tolerated overall, with the majority of reported adverse events being grade 1 or 2. Hematologic toxicity was the main adverse event as expected, followed by hyperglycemia and diarrhea. Gastrointestinal disorders as well as skin manifestation were also reported among the common adverse events. The safety profile of mCisFUA was significantly improved over prior attempts to combine afatinib with cytotoxic chemotherapy, indicating that the weekday-on, weekend-off schedule may be used as a pilot for further attempts to integrate afatinib into modern cytotoxic chemotherapy regimens [20].

Out of the 47 available specimens in our study, seven cases of *HER2*-positive tumors were identified representing 12.7% of the total cohort, consonant with the standard estimates of *HER2* positivity in gastric cancers. Further molecular profiling of the tumor specimens with the application of NGS was carried out in 31 informative tumors. *TP53* was the most frequently mutated gene, followed by *BRCA2* and *SMAD4*. *RAS* mutations were identified in seven cases whereas *PI3KCA* and *EGFR* mutations were found in five patients.

Of the 16 patients in our study who achieved an objective response, three carried *HER2*-positive tumors, while all the patients with *HER2*-positive tumors died within 5.6 and 26 months after study entry. None of the patients harboring

*PI3KCA* mutation achieved an objective response, contrary to one patient carrying *RAS* and one with *EGFR* mutations, respectively. In addition, molecular profiling with the application of NGS revealed eight cases of hypermutated tumors carrying alterations in more than 10 genes, particularly in *HRR/DDR*- and *MMR*-associated genes, thus accounting for most of the mutations identified in our study population. Two of those patients achieved an objective response and four had stable disease.

The underlying mechanisms of resistance may be responsible for the modest results in our study, which are in keeping with other failed phase III trial attempts at adding anti-*EGFR* agents to combination chemotherapy. In REAL 3, the addition of panitumumab to chemotherapy did not increase OS of patients with advanced or metastatic esophagogastric carcinoma which was the primary endpoint of the study [37]. Another attempt to incorporate anti-*EGFR* in patients with gastric carcinomas was EXPAND, where no benefit was seen from the addition of cetuximab to cytotoxic chemotherapy [38].

The role of *MET* has been studied in gastric cell lines that were resistant to afatinib. In particular, in afatinib-resistant, *MET*-amplified Hs746T cells, the sensitivity of the gastric cell lines to afatinib was restored after *MET* knockdown, confirming the amplification of *MET* as a possible underlying mechanism of resistance of gastric cancer cells to afatinib administration [39]. In a phase II study by Sanchez-Vega *et al.*, patients with a diagnosis of *HER2*-positive metastatic gastric or gastroesophageal adenocarcinoma who had progressed after at least one line of systemic therapy containing trastuzumab were treated with afatinib monotherapy or with the combination of afatinib and trastuzumab. The results were poor regarding the response rate and progression-free survival while molecular analysis of the tumor specimens identified *KRAS*, *PI3KCA*, and *NF1* mutations as predictors of rapid disease progression. In contrast, patients who harbored tumors that co-expressed *EGFR* and *HER2* benefited from afatinib [40]. Finally, an alternative interpretation of these data could be that *EGFR* signaling may not constitute a crucial, ‘addicted’ pathway for cancer survival in gastric tumors or at least is a pathway easily bypassed, when inhibited, by the malignant signal transduction.

The limitations of our trial include the small sample size and the absence of randomization to a chemotherapy-only arm, which probably would have led to the identification of patient subgroups with an increased benefit from the

combination or possible mechanisms of resistance to afatinib. Definitive results regarding the association of afatinib and the molecular signature of the tumors cannot be extracted due to the small sample size limitation.

## Conclusions

In our study, the combination of cisplatin/5FU with afatinib did not surpass the benchmarks of efficacy of the contemporary therapeutic regimens that are being applied for the treatment of patients with advanced gastric cancer.

However, the observed efficacy and the improved safety profile support that our weekday-on, weekend-off afatinib administration schedule may be further investigated in order to overcome problems with toxicity when integrating afatinib into cytotoxic chemotherapy. Finally, large-scale analyses for the identification of molecular subgroups of gastric tumors amenable to EGFR inhibition should be prospectively validated in order to confirm or refute the added value from EGFR TKIs in this lethal disease.

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## Ethical approval

The study was conducted in accordance with the Declaration of Helsinki. The clinical protocol was approved by the participating hospital Institutional Review Boards and by the National Organization for Medicines. The trial was registered with the ClinicalTrials.gov identifier: NCT01743365 and with EudraCT (EudraCT No.: 2011-006198-25).

## Consent to participate

All patients provided written informed consent for the study and for provision of biological material for future translational research studies.

## Disclosure statement

ES: Advisory Board of Merck, MSD, Astra-Zeneca, Roche, Amgen and Genesis

GA: Advisory Boards: Novartis, BMS, Roche Hellas, Astra Zeneca, Sanofi, Amgen, Genesis Pharma, Merck, Pfizer

AP: Consultation Fees: Amgen, Merck Serono, Roche, BMS, Astra Zeneca, MSD. Honoraria: Amgen, Merck Serono, Roche, BMS, Astra Zeneca, MSD, Research funds: BMS, Kura

TM: Advisory Role: Roche, Genesis Pharmaceuticals, Novartis, Honoraria: Roche, Merck, Gilead Sciences, Astra-Zeneca, BMS, Travel: Merck, Pfizer, MSD, Sanofi

DP: Advisory Role: Roche, MSD, Astellas. Honoraria: Roche, MSD, Astellas

GF: Advisory Board: Pfizer, Novartis and Roche. Honoraria: Astra Zeneca. Stock ownership: ARIAD, GENPREX, Daiichi Sankyo, RFL Holdings, FORMYCON.

GP: Advisory Role: Roche, Amgen, Merck, Astra-Zeneca, BMS, MSD, Lilly. Research Funding: Boehringer, Merck, Amgen, Astra-Zeneca, Roche, Enorasis, BMS, Lilly. Institutional financial support for clinical trials or contracted research: Boehringer, Merck, Amgen, Astra-Zeneca.

The rest of the authors declare no conflict of interest.

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## Data availability statement

All data are available upon reasonable request.

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