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ORIGINAL PAPER

# Phase II study of panitumumab combined with capecitabine and oxaliplatin as first-line treatment in metastatic colorectal cancer patients: clinical results including extended tumor genotyping

George Papaxoinis[1] · Vassiliki Kotoula[2,3] · Eleni Giannoulatou[4,5] · Georgia-Angeliki Koliou[6] · Vasilios Karavasilis[7] · Sotirios Lakis[3] · Andreas Koureas[8] · Mattheos Bobos[3] · Elpida Chalaralambous[3] · Emily Daskalaki[2] · Kyriakos Chatzopoulos[3] · George Tsironis[9] · Elisavet Pazarli[2] · Sofia Chrisafi[3] · Epaminontas Samantas[10] · Ioannis G. Kaklamanos[11] · Ioannis Varthalitis[12] · Athina Konstantara[13] · Konstantinos N. Syrigos[14] · George Pentheroudakis[15] · Dimitrios Pectasides[1] · George Fountzilas[3,16]

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## Abstract

This clinical trial assessed the efficacy and toxicity of panitumumab combined with oxaliplatin and capecitabine as first-line treatment in *KRAS* exon 2 wild-type metastatic colorectal cancer (mCRC) patients. Patients with exon 2 *KRAS* wild-type mCRC received panitumumab 9 mg/Kg, oxaliplatin 130 mg/m$^2$, and capecitabine 2000 mg/m$^2$ repeated every 3 weeks. The primary endpoint was objective response rate (ORR, minimum 42 responses). We retrospectively assessed mutations in genes implicated in CRC with massively parallel sequencing; ERBB2 and EGFR amplification with fluorescence in situ hybridization, and tumor-infiltrating lymphocyte density. Among 78 patients enrolled, 45 (57.7%) completed 6 cycles. Most common grade 3–4 toxicities were skin rash (19.2%), diarrhea (18%), and neuropathy (6.4%). Among 5 (6.4%) potentially treatment-related deaths, 2 (2.6%) were characterized toxic. Objective response occurred in 43 (55.1%) of the patients (complete 6.4% and partial response 48.7%; stable 17.9% and progressive disease 7.7%), while 3.8% were non-evaluable and 15% discontinued their treatment early. Additional mutations in *KRAS*/*NRAS*/*BRAF* were found in 11/62 assessable (18%) tumors. After 51 months median follow-up, median progression-free (PFS) was 8.1 and overall survival 20.2 months, independently of *KRAS*/*NRAS*/*BRAF* or PI3K-pathway mutation status. Patients with *TP53* mutations ($n=34$; 55%), as well as those with left colon primary tumors ($n=66$; 85%), had significantly better PFS, also confirmed in multivariate analysis. Although the clinical trial met its primary endpoint, according to the current standards, the efficacy and tolerability of the drug combination are considered insufficient. Extended genotyping yielded interesting results regarding the significance of *TP53* mutations. ClinicalTrials.gov identifier: NCT01215539, Registration date: Sep 29, 2010.

**Keywords** Metastatic colorectal cancer · Panitumumab · Tumor sidedness · *TP53* · Next generation sequencing

## Background

Colorectal cancer (CRC) is the third most common malignancy in the western world. A significant proportion of patients is diagnosed with metastatic (stage IV) disease, while even patients with locoregional disease relapse after surgical resection, independent of (neo)adjuvant treatment. Systemic treatment for metastatic CRC (mCRC) has significantly evolved during the last two decades, with the incorporation of many novel antineoplastic agents which helped to extend median life expectancy from approximately 9 months in the era of fluoropyrimidine monotherapy, to more than 24 months in recent years [1].

George Papaxoinis and Vassiliki Kotoula authors contributed equally to this study.

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✉ George Papaxoinis
georgexoinis@gmail.com; hecogoff@otenet.gr

Extended author information available on the last page of the article

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Capecitabine is an oral fluoropyrimidine analog, which has shown evidence of safety and activity in patients with mCRC and as adjuvant treatment in locoregional disease. Capecitabine-based chemotherapy has been the preferred treatment because it does not require the placement of central intravenous access devices. However, it is usually associated with more diarrhea and hand-foot syndrome compared to 5-fluorouracil (5-FU) slow infusion. Due to these side effects, the combination of capecitabine with anti-epidermal growth factor receptor (EGFR) agents, also well known to cause skin toxicity and diarrhea, has shown significant limitations [2].

The anti-EGFR monoclonal antibodies (mAbs) have shown significant activity as first- or second-line treatment in patients with advanced RAS wild-type colorectal cancer, and thus have been approved in combination with FOLFOX or FOLFIRI. In contrast, the activity and toxicity of their combination with capecitabine-based regimens remained doubtful until recent years [2]. Since then many potential biomarkers of response to anti-EGFR mAbs have been investigated, the most interesting being RAS-BRAF and PI3K-AKT pathway genetic alterations, tumor sidedness [3], and the development of skin rash as a complication of treatment [4]. Also, *TP53* is frequently mutated in CRC and its function might interfere with the PI3K-AKT pathway [5].

The aim of the present study was to assess the activity and toxicity profile of panitumumab combined with oxaliplatin and capecitabine (CAPOX) as first-line systemic treatment in patients with advanced colorectal adenocarcinoma.

## Methods

### Patients

This multicenter prospective single-arm phase II trial enrolled patients with stage IV CRC, previously untreated for metastatic disease. Patients were enrolled from October 13, 2010 until September 10, 2013. Main inclusion criteria were histologically or cytologically documented mCRC, measurable disease according to RECIST1.1 criteria and absence of mutations in codons 12 and 13 of the KRAS oncogene, according to the European Medicines Association recommendations for panitumumab administration before 2013 [6]. Also, patients should have been ≥ 18 years of age with Eastern Cooperative Oncology Group performance status (PS ECOG) 0–2 and adequate bone marrow, liver, and renal function. Main exclusion criteria were a history of adjuvant or neoadjuvant chemotherapy in the last 6 months before enrollment and history of major surgery or radiotherapy in the last 30 days before accrual.

### Treatment

Patients received panitumumab 9 mg/kg day 1, followed by oxaliplatin 130 mg/m$^2$ day 1 and capecitabine 1000 mg/m$^2$ days 1–14 repeated every 21 days for at least 6 cycles or until progression of disease or until unacceptable toxicity.

All adverse events were classified according to the NCI CTC 3.0 grading scale. Panitumumab doses were modified according to a specific algorithm (Supplementary Fig. S1, Online Resource 1). The next cycle was not administered unless the granulocyte number was ≥ 1500/mm$^3$, platelet number ≥ 100,000/mm$^3$, and all non-hematological toxicities resolved to grade ≤ 1. Next cycle could be deferred for a maximum of 6 weeks. The doses of capecitabine that had been omitted were not given at a later point. Prophylactic administration of G-CSF was not allowed.

### Evaluation of disease

Disease evaluation was carried out every 6 weeks until the 18th week during treatment then every 3 months thereafter by CT scans of the chest, abdomen, and pelvis until disease progression. MRI or bone scan was allowed when indicated. Objective response assessment was performed according to the RECIST 1.1 criteria by the investigators. A central radiology review of the imaging material was done retrospectively by AK.

### Prospective and retrospective tumor genotyping

Tumor genotyping was applied centrally at the Laboratory of Molecular Oncology (Hellenic Foundation for Cancer Research/Aristotle University of Thessaloniki, Thessaloniki, Greece) for patient selection. In total, tumors from 209 patients were screened, out of which 91 had KRAS mutant tumors (45.3%); the majority were primary tumors (166; 79.4%). Routinely processed formalin-fixed paraffin-embedded (FFPE) tumors were submitted for histology review and assessment of tumor cell content (TCC%), macrodissection where needed, DNA extraction with standard methods [QIAamp® DNA Mini kit (Qiagen, Hilden, Germany)], and testing for codon 12 and 13 KRAS mutations with qPCR and Sanger sequencing according to validated protocols [7].

In order to observe whether additional tumor genotype characteristics interfered with patient response to panitumumab, we retrospectively interrogated the same tumor DNA samples with massively parallel sequencing (NGS) targeting coding regions in CRC-related genes [8–10] in an Ion Torrent Proton Sequencer (Thermo-Fisher Scientific/Life Technologies, Paisley, UK). The Ampliseq panel IAD47763_31 (Supplementary Table S1, Online

Resource 2) targeted regions of interest in 51 RNA-coding and 6 non RNA-coding genes spanning a total sequence of 47.9 Kb. Samples were eligible for library construction if they had ≥ 2ngDNA/ul [Qubit fluorometer measurements (Thermo-Fisher Scientific)]. This criterion was fulfilled for 64 out of 78 prospectively tested tumor DNA samples, which were processed for NGS. We also sequenced with the same panel peripheral blood DNA available for 52 patients in the study and a series of 30 prospectively screened KRAS mutation positive samples from patients that were not enrolled in the study, as a control for the orthogonal validation of NGS results. Thus, the total number of samples examined with NGS was 146.

Samples were excluded from analysis if mean depth was < 250 and/or if < 10 eligible variants were returned. Variants were obtained from Ion Reporter v.4 and were considered eligible for analysis if $p < 0.0001$ (software quality metric including FDR); no GC-stretches (semiconductor sequencing artifacts); in worst cases, if coverage for the variant allele was at least 40 for positions covered at least 100 times. For comparisons between blood and tumor samples, reads of all amplicons and variant positions were compared; failed amplicons and positions were excluded from comparisons [67 out of 663 (10%) eligible variant pairs].

FFPE sample mean/median (range) values for mean read depth and variant number were 872/612 (range 211–3638) and 31/32 (range 11–87), respectively; mean/median (range) values for blood samples were for mean depth 270/180 (range 198–1263) and for variant number 24/25 (range 10–42). Sixty-two out of the 64 study tumors, 43/52 blood samples, and 28/30 control tumors yielded informative NGS data and were considered for analysis.

Variants were called mutations if amino acid changing with Minor Allelic Frequency < 0.1% (< 0.001) and if splice site changing. TCC was ≥ 50% in 75% of the samples allowing for clonal mutations to be considered for variant allelic frequencies (VAF) > 25% [11] in these cases. All classic exon 2 KRAS mutations prospectively assessed with qPCR and Sanger sequencing were validated with NGS (28/28 informative tumors in the control group) supporting the robustness of the method.

Study tumors with available tissue material were also examined at the chromosomal level for EGFR and HER2 gene status with fluorescent in situ hybridization (FISH) that was applied on 5-um-thick tissue microarray (in-house TMAs, 1-mm cores, 2 cores per tumor) or on whole sections. For all probes, sequential (5 planes at 1.0 um) digital images were captured using the Plan Apo VC 100×/1.40 oil objective (Nikon, Japan) using specific filters for each probe. The resulting images were reconstructed using specifically developed software for cytogenetics (XCyto-Gen, ALPHELYS, Plaisir, France). For the evaluation of all probes, 40 non-overlapping nuclei from the invasive part of the tumor were randomly selected, according to morphological criteria using DAPI staining, and scored (ET). EGFR and HER2 gene amplification were interpreted as previously described for CRC [12]. In addition, cut-offs for increased CEN copies were assessed on 20 normal specimens and were calculated as normal mean CEN signal counts plus 3XSD, as previously suggested for assessing chromosomal instability [13]; increased CEN17 copies were called for > 3.22 and CEN7 for > 3.36 mean signal counts per tumor.

## Assessment of tumor-infiltrating lymphocytes (TILs)

TILs were assessed retrospectively on FFPE tumor whole sections and were evaluated both in the neoplastic epithelium and in the stroma as a percentage to the number of neoplastic and stromal cells accordingly (modified from [14, 15]. Morphologic evaluation was performed in optic microscope high power fields (HPF, area 0.237 $mm^2$) and involved measurements in at least 20 HPFs (excluding biopsy material) upon tissue availability avoiding areas of necrosis. The average percentage was recorded. Where possible (i.e., adequate material from surgically resected primary carcinomas), the percentage of TILs in the stroma of the invasive front was also recorded.

## Statistical analysis

The primary endpoint of the study was to assess the objective response rate (ORR) with the study treatment, while secondary endpoints were progression-free survival (PFS), overall survival (OS), and the toxicity profile. The sample size estimation was based on the primary endpoint. According to the Simon two-stage optimal design, considering an expected ORR of at least 60% and a minimum accepted ORR of 45%, 26 patients needed to be enrolled in the first stage. If at least 13 responses were recorded, then the trial would proceed to the second stage. A total sample size of 77 patients with at least 42 objective responses would be required to ensure an 80% power at the 5% level of significance.

Continuous variables were presented as medians with the corresponding range and categorical variables as frequencies with the respective percentages. $\chi^2$ or Fisher's exact test (where appropriate) were used for comparisons between categorical variables.

Two different statistical methods were examined for the estimation of the normal cut-off in order to define chromosomal instability. Both methods relied on the upper limit of the confidence interval using the confidence interval around the mean and the inverse beta function, respectively [16]. However, none of these methods proved to be reliable enough to be considered as biologically applicable for this study. Therefore, normal cut-offs for our study were

calculated based on the mean and standard deviation of each signal pattern.

OS was measured from the date of study entry to the date of patient's death (event) or last contact. Surviving patients were censored at the date of their last contact. PFS was measured from the date of study entry to documented recurrence (event), death without prior documented recurrence (event), or last contact, whichever occurred first. Patients alive and without recurrence at the date of their last contact were censored. Kaplan–Meier curves were used for estimating time-to-event distributions, while log-rank tests were used for assessing statistically predefined comparisons. Univariate Cox regression was further performed to estimate hazard ratios for certain factors in association to PFS and OS.

In the multivariate analysis, model choice was performed using backward selection criteria of $p < 0.10$, including the following parameters in the initial step: sex (women vs. men), age ($< 60$ vs. $\geq 60$), primary tumor site (right colon vs. left colon), performance status (1–2 vs. 0), and *TP53* status (wild type vs. mutated).

All tests were two-sided and significance level was set at 5%. The statistical analysis was performed using the SAS software (SAS for Windows, version 9.3, SAS Institute Inc., Cary, NC).

## Results

In total, 78 patients were enrolled in the study (CONSORT diagram described in Fig. 1). One patient was characterized as ineligible after re-evaluation due to mutated KRAS status (KRAS p.Gly13Asp), but continued treatment after principal investigator's decision. This decision was made after the target of 77 patients was reached. As a result, the final number of patients became 78. Basic patient and tumor characteristics are presented in Table 1. Seven patients (9.0%) were subjected to metastasectomy and forty-one patients (52.6%) received second-line treatment.

### Treatment

In total, 45 patients (57.7%) completed 6 cycles of treatment. The main reasons of not completing the minimum of 6 cycles are described in Fig. 1. Out of the 4 patients who died during the first 6 cycles of treatment, 2 died from their disease and 2 from toxicity. Also, 3 patients discontinued treatment before completing 6 cycles, because their doctor decided to refer them for metastasectomy.

Patients received 664 cycles of panitumumab (median 7; range 1–37) in total, 445 cycles of oxaliplatin (median 6; range 1–13), and 483 cycles of capecitabine (median 6; range 1–25). Median relative dose intensities in the first six cycles were 0.99 (range 0.42–1.13) for panitumumab, 0.99 (range 0.15–1.03) for oxaliplatin, and 0.88 (range 0.04–1.03) for capecitabine. Forty-two patients (53.8%) received capecitabine at full dose, while full dose of panitumumab and oxaliplatin was administered in sixty-seven patients (85.9%), respectively.

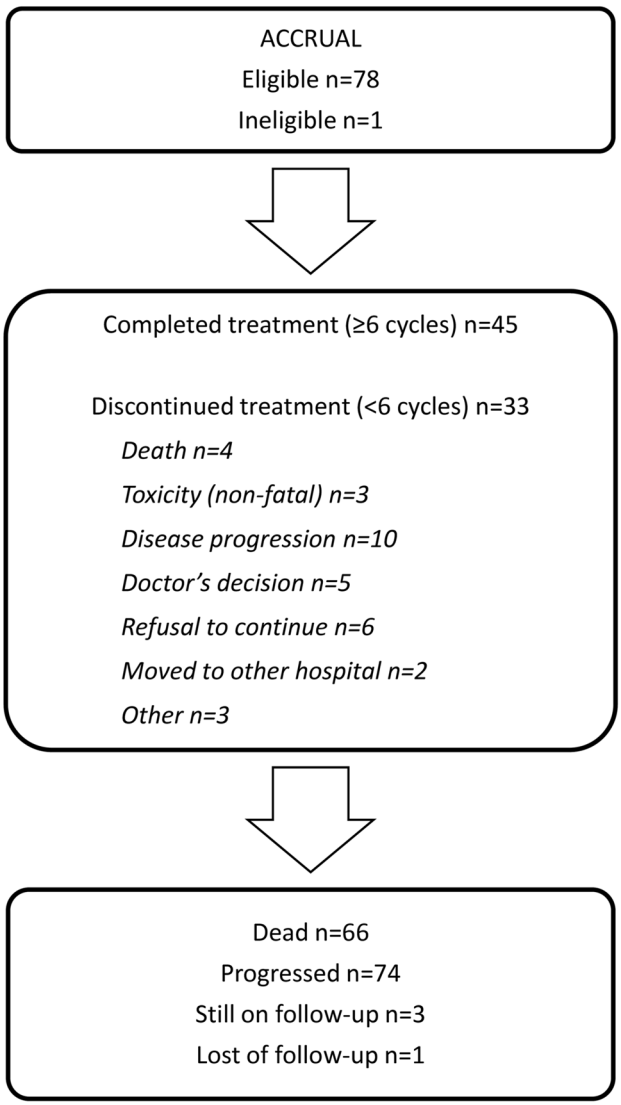


**Fig. 1** CONSORT diagram

### Toxicity

The incidence of toxicities by grade is presented in Table 2. The most commonly recorded toxicity was skin rash (acne) in 88.5% of the study population. Diarrhea was the second most common toxicity occurring in 40 patients (51.3%), while sensory neuropathy was recorded in 46.2%. Five episodes (6.4%) of deep vein thrombosis were recorded (one fatal). In total, 5 deaths were potentially related to the study

**Table 1** Basic patient and tumor characteristics

| Characteristic | *N* | % |
|---|---|---|
| Age | | |
| Median (range) | 65.8 (30.0–80.8) | |
| < 60 | 32 | 41.0 |
| ≥ 60 | 46 | 59.0 |
| Sex | | |
| Women | 33 | 42.3 |
| Men | 45 | 57.7 |
| PS (ECOG) | | |
| 0 | 62 | 79.5 |
| 1 | 15 | 19.2 |
| 2 | 1 | 1.3 |
| Primary location | | |
| Left colon | 66 | 84.6 |
| Right colon | 12 | 15.4 |
| Primary surgery | | |
| Yes | 59 | 75.6 |
| No | 19 | 24.4 |
| Adjuvant chemotherapy | 12 | 15.4 |
| Organs involved | | |
| Liver | 35 | 44.9 |
| Lung | 24 | 30.8 |
| Nodes | 11 | 14.1 |
| Peritoneum | 13 | 16.6 |
| Bones | 4 | 5.1 |
| Colon/rectum | 5 | 6.4 |
| Other | 6 | 7.7 |
| N of organs involved | | |
| 1 | 31 | 39.7 |
| 2 | 19 | 24.4 |
| ≥ 3 | 8 | 10.3 |
| Total | 78 | 100 |

*PS(ECOG)* performance status by Eastern Cooperative Oncology Group

treatment. Two of them (2.6%) were unanimously attributed to treatment toxicity, but the history cardiovascular disease might have been a contributory factor. The other 3 deaths were considered by the investigators as "cancer-related but the contributory role of the study treatment could not be excluded." These cases are described in Supplementary paragraph S1 (Online Resource 1).

## Response to treatment

Forty-three patients (55.1%) achieved an objective tumor response (complete response 6.4%; partial response 48.7%), 14 patients (17.9%) had stable disease, and 6 patients (7.7%) progressive disease. Fifteen patients (19.2%) were not evaluated for response due to: treatment discontinuation prior to evaluation ($N = 12$; 15.4%) or non-evaluable disease ($N = 3$; 3.8%). Reasons of treatment discontinuation prior to evaluation were early death from complications of the tumor ($N = 2$; 2.6%), early toxic death ($N = 1$; 1.3%), pulmonary embolism ($N = 1$; 1.3%), doctor's decision ($N = 2$; 2.6%), patient's decision ($N = 5$; 6.4%), and protocol deviation ($N = 1$; 1.3%). Therefore, the clinical trial met its primary endpoint of a minimum of 42 responses.

ORR did not differ significantly between patients with left vs. right colon tumors as well as between patients with skin rash (acne) grade 0–1 versus 2–4 in the first cycle of treatment (Table 3). Central radiology review by RECIST1.1 criteria could be performed in 50 cases. Among them, 3 achieved a complete response (6%), 32 a partial response (64%), while 6 patients (12%) had stable disease and 9 patients (18%) progressive disease. A waterfall plot of objective responses by RECIST1.1 criteria is depicted in Fig. 2.

## Survival and prognostic factors

After a median follow-up of 51 months (range 0.3–60.2), 74 patients (96.1%) progressed and 66 (84.6%) died. Two patients (2.6%) were lost to follow-up. Three out of the four patients that have not progressed yet are still on follow-up, while one patient was lost to follow-up due to refusal to continue. Median OS was 20.2 months (95% CI 15.4–26.4) and median PFS was 8.1 months (95% CI 6.8–9.8). Patients with tumors in the left colon had significantly longer PFS compared to those with tumors in the right colon, while no statistically significant difference was observed between the two subgroups in terms of OS (Table 4). Of note, among 7 patients who underwent metastasectomy, 6 had tumors in the left colon and 1 in the right colon. PFS and OS did not differ significantly between patients with grade 0–1 and grade 2–4 skin rash (acne) in the first cycle (Table 4). Kaplan–Meier curves for PFS and OS based on tumor's location and grade of skin rash are presented in Fig. 3a and S2 (Online Resource 1) and Fig. S3 (Online Resource 1), respectively.

## Retrospective tumor genotyping and TIL assessment

NGS revealed 108 mutations in 21 out of 51 interrogated genes (Supplementary Table S2, Online Resource 2) that were distributed in 53 out of 62 informative tumors (85.5%) (Supplementary Table S3, Online Resource 2). Four of these mutations were present in the germline of the corresponding patients (*MSH3* p.Gly896Arg; *BRCA2* p.Glu2856Ala; *IGF1R* p.Arg437His; *POLE* p.Ala430Thr); none of them is currently registered as pathogenic in ClinVar and all four were presented in the matched tumors at germline frequencies. All other mutations were tumor-private and were considered as somatic. The examined tumors had 1–4 mutations

**Table 2** Toxicities by grade according to NCI CTC 3.0

| Type of toxicity | Grade | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 1 | | 2 | | 3 | | 4 | | 5 | | Unknown | | Total | |
| | *N* | % | *N* | % | *N* | % | *N* | % | *N* | % | *N* | % | *N* | % |
| Hematological | | | | | | | | | | | | | | |
| Anemia | 15 | 19.2 | 11 | 14.1 | 3 | 3.9 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 29 | 37.2 |
| Leukopenia | 17 | 21.8 | 7 | 9.0 | 2 | 2.6 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 26 | 33.3 |
| Neutropenia | 13 | 16.7 | 9 | 11.5 | 2 | 2.6 | 1 | 1.3 | 0 | 0.0 | 0 | 0.0 | 25 | 32.1 |
| Thrombocytopenia | 16 | 20.5 | 7 | 9.0 | 4 | 5.1 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 27 | 34.6 |
| Non-hematological | | | | | | | | | | | | | | |
| Skin/nail | | | | | | | | | | | | | | |
| Acne | 19 | 24.4 | 34 | 43.6 | 15 | 19.2 | 0 | 0.0 | 0 | 0.0 | 1 | 1.3 | 69 | 88.5 |
| Hand-foot syndrome | 9 | 11.5 | 5 | 6.4 | 6 | 7.7 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 20 | 25.6 |
| Other abnormalities | 10 | 12.8 | 6 | 7.7 | 1 | 1.3 | 0 | 0.0 | 0 | 0.0 | 1 | 1.3 | 18 | 23.1 |
| Gastrointestinal | | | | | | | | | | | | | | |
| Nausea | 7 | 9.0 | 8 | 10.3 | 2 | 2.6 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 17 | 21.8 |
| Vomiting | 8 | 10.3 | 7 | 9.0 | 3 | 3.9 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 18 | 23.1 |
| Mucositis | 7 | 9.0 | 1 | 1.3 | 2 | 2.6 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 10 | 12.8 |
| Diarrhea | 14 | 18.0 | 12 | 15.4 | 14 | 18.0 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 40 | 51.3 |
| Constipation | 8 | 10.3 | 3 | 3.9 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 11 | 14.1 |
| Neuropathy | | | | | | | | | | | | | | |
| Sensory | 21 | 26.9 | 10 | 12.8 | 5 | 6.4 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 36 | 46.2 |
| Motor | 4 | 5.1 | 2 | 2.6 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 6 | 7.7 |
| Electrolytes of special interest | | | | | | | | | | | | | | |
| Hypocalcemia | 6 | 7.7 | 6 | 7.7 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 12 | 15.4 |
| Hypokalemia | 19 | 24.4 | 1 | 1.3 | 2 | 2.6 | 1 | 1.3 | 0 | 0.0 | 0 | 0.0 | 23 | 29.5 |
| Hypomagnesemia | 18 | 23.1 | 5 | 6.4 | 1 | 1.3 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 24 | 30.8 |
| Constitutional symptoms | | | | | | | | | | | | | | |
| Fatigue | 12 | 15.4 | 10 | 12.8 | 4 | 5.1 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 26 | 33.3 |
| Anorexia/taste alteration | 11 | 14.1 | 11 | 14.1 | 2 | 2.6 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 24 | 30.8 |
| Weight loss | 3 | 3.9 | 5 | 6.4 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 8 | 10.3 |
| Liver toxicity | 24 | 30.8 | 9 | 11.5 | 2 | 2.6 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 35 | 44.9 |
| Infection with normal ANC | 4 | 5.1 | 11 | 14.1 | 3 | 3.9 | 0 | 0.0 | 0 | 0.0 | 1 | 1.3 | 19 | 24.4 |
| Allergic Reactions | 2 | 2.6 | 2 | 2.6 | 3 | 3.9 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 7 | 9.0 |
| Ocular surface | 2 | 2.6 | 3 | 3.9 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 5 | 6.4 |
| Vascular | | | | | | | | | | | | | | |
| Deep vein thrombosis/embolism | 0 | 0.0 | 0 | 0.0 | 1 | 1.3 | 3 | 3.9 | 1 | 1.3 | 0 | 0.0 | 5 | 6.4 |
| Central venous catheter thrombosis | 0 | 0.0 | 0 | 0.0 | 1 | 1.3 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 1 | 1.3 |
| Superficial thrombosis | 0 | 0.0 | 2 | 2.6 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 2 | 2.6 |
| Stroke | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 1 | 1.3 | 0 | 0.0 | 0 | 0.0 | 1 | 1.3 |

*CNS* central nervous system, *ANC* absolute neutrophil count

and 1–3 mutated genes per tumor, while mutated allele frequencies (VAFs) were generally clonal [mean (±SD) 35.5% (±17.7); median 32%] (Supplementary Table S2, Online Resource 2). Top mutated genes were *TP53* in 34 tumors (64% of mutated, 55% of all tumors) and *APC* in 27 tumors (51 and 44%, respectively). Mutations in PI3K-pathway genes concerned *AKT1* in 2 tumors, *BRAF* in 5, *ERBB2* in 3, *NRAS* in 1, *KRAS* in 6, *PIK3CA* in 4, and *PTEN* in 1 tumor, the latter co-mutated in *PIK3CA* and *KRAS*. In 4 tumors, *KRAS* mutations were located in exon 3 (codon 61) and exon 4 (codon 146); these were not targeted upon prospective testing as per recommendations for panitumumab during the time of patient enrollment. One tumor carried a *KRAS* p.Gly12Val at 16% VAF that was missed upon prospective testing probably due to low frequency, while another carried a *KRAS* p.Gly13Asp that was already identified upon

**Table 3** Objective response rates (RECIST1.1) in the entire population and by location of primary tumor, development of early skin rash, and mutation status

| Group | Total | CR + PR | | *p* value |
|---|---|---|---|---|
| | *N* | *N* | % | |
| Total study group | 78 | 43 | 55.1 | |
| Evaluated for response | 63 | 43 | 68.3 | |
| Central radiology review | 50 | 35 | 70.0 | |
| Primary tumor location | | | | 0.75 |
| Left colon | 66 | 36 | 54.5 | |
| Right colon | 12 | 7 | 58.3 | |
| Early skin rash (at first cycle) | | | | 0.93 |
| Grade 0–1 | 58 | 32 | 55.2 | |
| Grade 2–4 | 19 | 10 | 52.6 | |
| Mutation status (*KRAS*/*NRAS*/*BRAF*) | | | | 0.72 |
| All wild type | 50 | 27 | 54.0 | |
| Any mutated | 12 | 6 | 50.0 | |
| Mutation status (*KRAS*/*NRAS*/*BRAF*/*PIK3CA*/*AKT1*/*ERBB2*) | | | | 0.60 |
| All wild type | 43 | 22 | 51.2 | |
| Any mutated | 19 | 11 | 57.9 | |
| Mutation status (*TP53*) | | | | 0.023 |
| Wild type | 28 | 10 | 35.7 | |
| Mutated | 34 | 23 | 67.6 | |

*CR* complete response, *PR* partial response

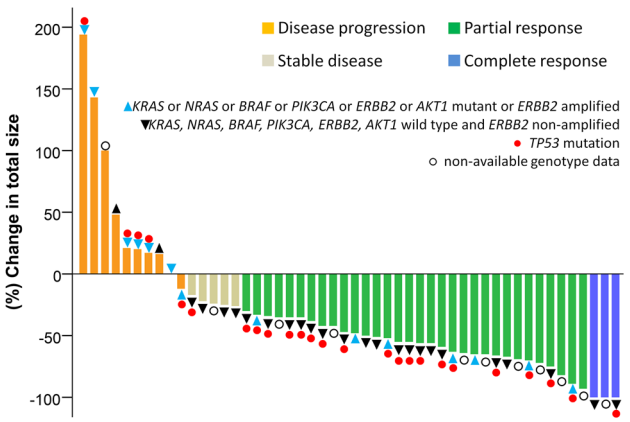


**Fig. 2** Waterfall plot of objective responses after central radiology reassessment. The genetic tumor profile is also shown for each case

prospective testing but the patient was enrolled based on the suggested indolent nature of this mutation for the administered drug [17]. As shown in Supplementary Table S3 (Online Resource 2), mutations in *AKT1, BRAF, KRAS, NRAS* were mutually exclusive, while *TP53* mutations coexisted with mutations in any gene including APC but these were mutually exclusive with *PIK3CA* mutations.

We only found one tumor with *HER2* amplification that also carried a mutation in the same gene and a *TP53* mutation as well. Only two tumors were classified as *EGFR* amplified; one probably had chromosomal instability based on the observed increased number of chromosome 7 and 17. There were additional 10 tumors with higher copies than our normal reference that were considered as potentially unstable at the chromosomal level, as previously suggested [13].

TIL density was not associated with either the status of *TP53* or any other genomic alteration or clinicopathological parameters under investigation. The only significant difference was observed between patients who underwent surgery and those who did not, with non-operated patients presenting with higher TIL density ($p = 0.041$).

## Treatment efficacy and prognostic factors according to tumor mutational status

Of 62 patients with NGS informative tumors, 50 (80.6%) were wild type for *KRAS, NRAS*, and *BRAF* genes and 43 (69.4%) were wild type for *KRAS, NRAS, BRAF, PIK3CA, AKT1*, and *ERBB2* genes. ORR did not differ significantly between groups with different mutation status (Table 3). The waterfall plot of responses by RECIST1.1 criteria (Fig. 2) demonstrates also the molecular alterations in the above genes for each case. The correlation of tumor sidedness and early skin rash with treatment outcome was confirmed in the above subgroup of 43 patients (Supplementary Tables S4 and S5, Online Resource 1). No significant difference in terms of PFS or OS was observed between patients with tumors *KRAS, NRAS,* and *BRAF* wild type or with tumors *KRAS, NRAS, BRAF, PIK3CA, PTEN*, and *ERBB2* wild type as compared to patients with mutations in at least one of these genes (Table 4). The only mutation-related impact with respect to ORR and to PFS derived from *TP53* mutations that were positively associated with CR/PR and with favorable PFS, as shown in Tables 3 and 4, respectively. Kaplan–Meier curves according to the status of *TP53* are depicted in Fig. 3b and Supplementary Fig. S4 (Online Resource 1).

Multivariate analysis (Fig. 4) confirmed the unfavorable prognostic significance for PFS of wild-type *TP53* and right-sided primaries. Additionally, women, older patients (≥60 years), and those with excellent PS (ECOG) 0 were in lower risk of disease progression, compared to men, younger patients (<60 years), and those with poorer PS (ECOG) 1–2, respectively.

TILs did not show any prognostic significance in the univariate analysis with respect to PFS or OS (Wald's $p = 0.95$ and $p = 0.61$, respectively, Supplementary Fig. S5, Online Resource 1).

**Table 4** Univariate Cox regression analysis for progression-free and overall survival

| Covariates | Progression-free survival | | | | |
|---|---|---|---|---|---|
| | N patients | N events | HR | 95% CI | Wald's *p* |
| Primary site | | | | | |
| Right colon versus left colon | 12 versus 66 | 12 versus 62 | 2.63 | 1.37–5.02 | 0.003 |
| Early skin rash (at first cycle) | | | | | |
| Grade 2–4 versus 0–1 | 19 versus 58 | 18 versus 56 | 0.88 | 0.52–1.50 | 0.64 |
| Mutation status (*KRAS*/*NRAS*/*BRAF*) | | | | | |
| All wild type versus any mutated | 50 versus 12 | 48 versus 12 | 0.60 | 0.31–1.16 | 0.13 |
| Mutation status (*KRAS*/*NRAS*/*BRAF*/*PIK3CA*/*AKT1*/*ERBB2*) | | | | | |
| All wild type versus any mutated | 43 versus 19 | 41 versus 19 | 0.57 | 0.32–1.01 | 0.054 |
| Mutation status (*TP53*) | | | | | |
| Wild type versus mutated | 28 versus 34 | 28 versus 32 | 2.71 | 1.54–4.77 | 0.001 |
| Covariates | Overall survival | | | | |
| | N patients | N events | HR | 95% CI | *Wald's p* |
| Primary site | | | | | |
| Right colon versus left colon | 12 versus 66 | 10 versus 56 | 1.26 | 0.64–2.48 | 0.50 |
| Early skin rash (at first cycle) | | | | | |
| Grade 2–4 versus 0–1 | 19 versus 58 | 16 versus 50 | 0.82 | 0.47–1.45 | 0.50 |
| Mutation status (*KRAS*/*NRAS*/*BRAF*) | | | | | |
| All wild type versus any mutated | 50 versus 12 | 42 versus 12 | 0.65 | 0.34–1.24 | 0.19 |
| Mutation status (*KRAS*/*NRAS*/*BRAF*/*PIK3CA*/*AKT1*/*ERBB2*) | | | | | |
| All wild type versus any mutated | 43 versus 19 | 36 versus 18 | 0.67 | 0.38–1.19 | 0.17 |
| Mutation status (*TP53*) | | | | | |
| Wild type versus mutated | 28 versus 34 | 25 versus 29 | 1.21 | 0.71–2.08 | 0.48 |

*CI* confidence intervals, *HR* hazard ratio

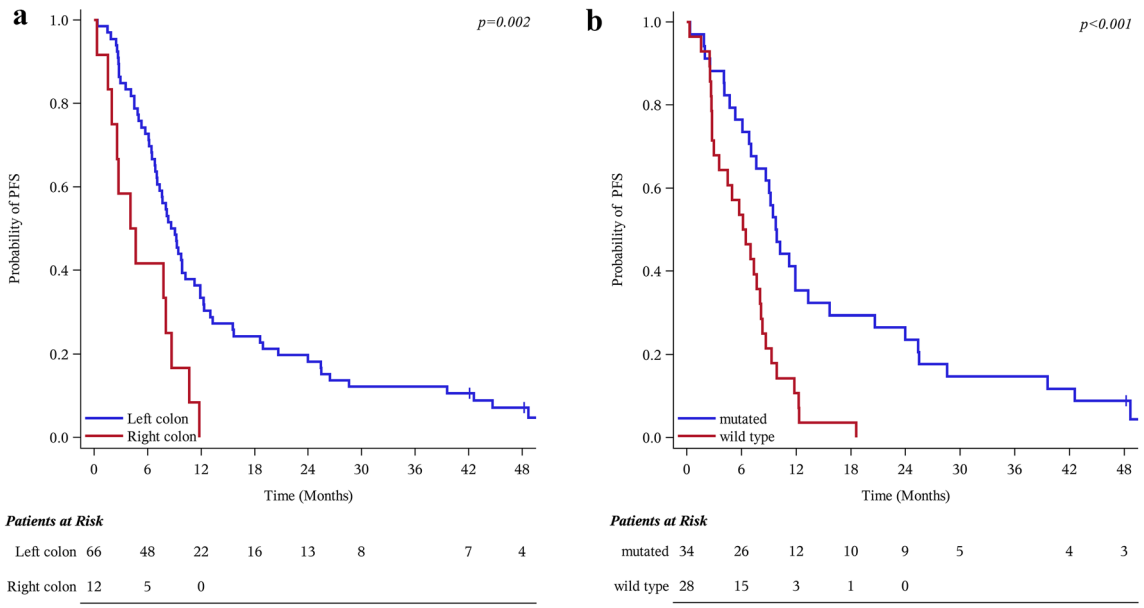


**Fig. 3** Progression-free survival by primary site (**a**) and *TP53* status (**b**)

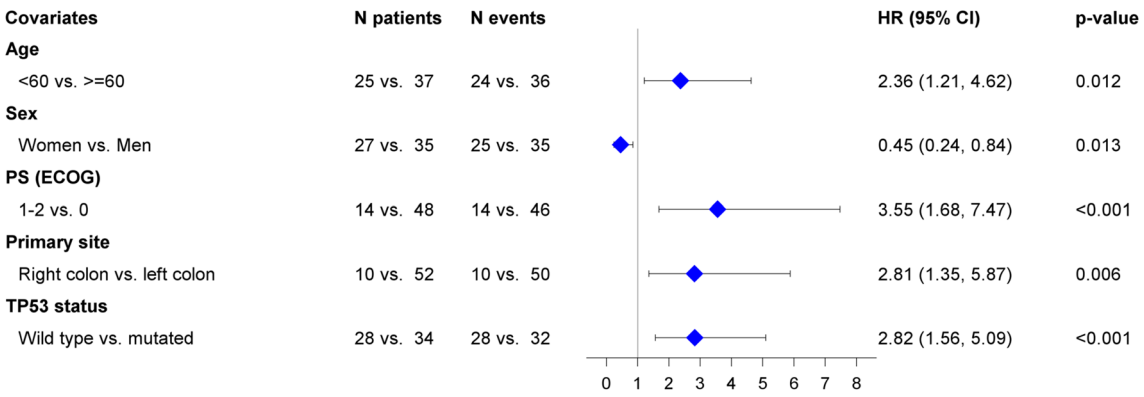


**Fig. 4** Forest plot describing multivariate Cox regression analysis of independent predictors for progression-free survival

## Discussion

The present trial met its primary endpoint with regard to the ORR achieved with panitumumab combined with CAPOX as first-line treatment in patients with RAS wild-type aCRC. However, the presented ORR of 55% and median PFS of 8 months were slightly inferior to what would be currently expected from the results of large clinical trials examining combinations of cetuximab or panitumumab with chemotherapy [18–22]. The best activity of anti-EGFR mAb combinations was shown with chemotherapy regimens combining infusional 5-FU (FOLFOX, FOLFIRI). In comparison, regimens including capecitabine tended to show inferior activity [23–26]. For example, although the COIN MRC clinical trial did not demonstrate benefit in survival from the combination of FOLFOX/CAPOX with cetuximab compared to FOLFOX/CAPOX alone, a subgroup analysis showed that only patients receiving FOLFOX-cetuximab had improved outcome. Since then, the combinations of EGFR--targeting mAbs with infusional 5FU-based chemotherapy have been established as the preferred regimens over the combinations with capecitabine-based chemotherapy. The present clinical trial was developed at a time when this information was not yet available. Nevertheless, our results on ORR and PFS with CAPOX-panitumumab are slightly better compared to those already published for this type of regimens (ORR 41–50%, PFS 6.2–7.2 months) [24–26].

Why capecitabine adversely interacts with anti-EGFR mAbs remains unanswered, especially since, for example, cetuximab does not adversely influence capecitabine blood levels and metabolism [27]. Moreover, it is important to note that 15 patients in the present trial discontinued treatment because of regimen-related toxicity, which may explain the limited activity of capecitabine-antiEGFRmAb regimens.

EGFR-targeting mAbs might exacerbate capecitabine-related diarrhea. In MRC COIN clinical trial, the incidence of grade 3–4 diarrhea was 30%, which led to the decision to reduce the dose of capecitabine from 1000 to 850 mg/m$^2$ [23]. This dose reduction might have negatively impacted the efficacy of the chemotherapy. In contrast, smaller studies showed a lower but still significant frequency of grade 3–4 diarrhea of 16–22% [24–26]. In the present clinical trial, diarrhea was the second more common grade 3–4 toxicity (18%) after skin rash, but still manageable with supportive treatment, dose reductions, and delays. Thus, only 53% of patients managed to receive capecitabine at full dose without dose deferrals. Moreover, the number of SAEs ($N=35$) and the number of deaths possibly related to the study treatment ($N=5$) are relatively high for a sample size of 78 patients. Therefore, the present study confirms that the combination of panitumumab with CAPOX has relatively limited activity probably because of excessive toxicity.

The present trial also confirmed the superior outcome of left-sided primary tumors compared to the right-sided cancers. Multiple lines of evidence have shown that the location of the primary tumor is prognostic and possibly predictive of benefit from mAbs. A recent large meta-analysis of 13 first-line randomized trials confirmed that right-sided tumors are associated with poorer OS and probably lack of benefit from anti-EGFR agents [3], although this statement needs prospective confirmation before justifying a change in clinical practice. With regard to earlier cancer stages, tumor sidedness seemed to affect only post-relapse OS, but not relapse-free survival, implying that it is prognostic only when the tumor becomes metastatic [28]. Multiple studies have consistently demonstrated the marked molecular heterogeneity between right colon and left colorectal tumors, which might explain the location-specific survival differences [29]. Interestingly, tumor sidedness was still prognostic even in multivariate analysis including MSI, chromosomal instability (CIN), *KRAS* and *BRAF* status [28–31]. Our extensive genomic analysis revealed a subgroup of 43 patients who did not harbor any *RAS-BRAF* and PI3K-AKT pathway mutations. Even in this *KRAS/NRAS/BRAF/PIK3CA/AKT1/ERB*

*B2* wild-type subgroup, the location of the primary remained a strong independent prognostic factor for PFS. To the best of our knowledge, this is a novel finding.

Other than anticipated, we show that response to the CAPOX-panitumumab regimen was not related to the presence of the retrospectively identified mutations in *KRAS, NRAS, BRAF*, and the rest of PI3K pathway genes that concerned 27% of the studied cohort, since 10 out of 17 patients with corresponding alterations responded to the combination. Responses were achieved in the presence of *KRAS* p.Gly13Asp, in line with a previous notion for this substitution [17], of *KRAS* p.Ala146Thr, p.Gln61His, and of *NRAS* p.Gly12Asp. On a case per case review, the subcloncal presence of these mutations in the tumors might, at least partially, explain these responses [32], as well as those observed for *BRAF* or *AKT1* mutations. However, partial responses were also observed in cases with clonal PI3K-pathway gene mutations, indicating that the apparent intracellular driver did not function independently of the assumed blockade of EGFR signaling on the cell surface. Nevertheless, although not statistically significant, patients with wild-type tumors for the above genes had an apparent trend for better PFS, but not OS. The small sample size and further lines of treatment might have influenced these results.

We also show that *TP53* mutations were significantly favorably related to ORR and PFS. Gain-of-function *TP53* mutations are established early during colon carcinogenesis and loss-of-function mutations develop late and contribute to metastasis [33], while metastatic lesions seem to be richer in *TP53* mutations compared to matched primary tumors [34]. Here, only few metastatic samples were available for analysis and all but one were mutated, while the rate of TP53 mutations was higher than usually reported [8, 35] probably due to the very high proportion of left-sided tumors in the cohort [8]. *TP53* mutations have generally been considered as unfavorable prognosticators in left-sided early CRC [36]. In the metastatic setting, however, the role of *TP53* mutations is still not clear [37]. Our data appear in line with those by Oden-Gangloff et al. [38], who demonstrated that *TP53* mutations were associated with higher sensitivity to cetuximab in irinotecan-refractory patients with *KRAS* wild-type CRC, although the tumors we examined were derived from patients treated in the first-line setting. Notably, Ciardiello et al. [35] could not confirm the favorable impact of *TP53* mutations in *KRAS* wild-type CRC patients treated with FOLFIRI + cetuximab, but again the chemo-regimen was different from that in our study. In contrast, Huemer et al. [39] observed a trend for adverse outcome of *TP53* mutations in a cohort study including only 30 patients treated with EGFR-targeting agents.

Suggesting a mechanism for this phenomenon can only be speculative in the absence of solid preclinical data for the specific disease stage and treatment setting. As described recently, albeit without treatment specifications, *APC*/*TP53* mutations seem to be associated with favorable outcome [40]. Indeed, the two genes were frequently co-mutated in our series, although *APC* mutations were unrelated to outcome. It should be noted that the small number of patients in the present study limited the combined analysis of mutations and clinicopathological characteristics. Thus, the present data, although interesting, should be considered as hypothesis generating, need validation in larger patient cohorts, and prompt for functional studies to unravel a possible role of *TP53* mutations upon chemotherapy/anti-EGFR mAb combinations.

## Conclusions

The current clinical trial confirmed the compromised tolerability and limited efficacy of CAPOX combined with panitumumab as a first-line treatment in CRC. Extended tumor genotyping showed the favorable prognostic significance of *TP53* mutations independently of primary tumor location and other prognostic factors in patients treated with CAPOX-panitumumab.

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**Author contributions** GPap conceived the study, participated in its design, contributed to the acquisition, analysis, and interpretation of data, and drafted the manuscript. VKot conceived the study, participated in its design, contributed to the acquisition, analysis, and interpretation of data and drafted the manuscript. EG contributed to the analysis and interpretation of data and revised critically the manuscript. GAK contributed to the analysis and interpretation of data and drafted the manuscript. VKar contributed to the acquisition of data and revised critically the manuscript. SL contributed to the acquisition, analysis, and interpretation of data and revised critically the manuscript. AKou contributed to the acquisition of data and revised critically the manuscript. MB contributed to the acquisition, analysis, and interpretation of data and revised critically the manuscript. EC contributed to the acquisition, analysis, and interpretation of data and revised critically the manuscript. ED contributed to the acquisition, analysis, and interpretation of data and revised critically the manuscript. KC contributed to the acquisition of data and revised critically the manuscript. GT contributed to the acquisition of data and revised critically the manuscript. EP contributed to the acquisition of data and revised critically the manuscript. SC contributed to the acquisition, analysis, and interpretation of data and revised critically the manuscript. ES contributed to the acquisition of data and revised critically the manuscript. IGK contributed to the acquisition of data and revised critically the manuscript. IV contributed to the acquisition of data and revised critically the manuscript. AKon contributed to the acquisition of data and revised critically the manuscript. KNS contributed to the acquisition of data and revised critically the manuscript. GPen conceived the study, participated in its design, contributed to the acquisition, analysis, and interpretation of data, and drafted the manuscript. DP conceived the

study, participated in its design, contributed to the acquisition of data, and drafted the manuscript. GF conceived the study, participated in its design, contributed to the acquisition, analysis, and interpretation of data, and drafted the manuscript. All authors approved the final version of the manuscript to be published and agreed to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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## Compliance with ethical standards

**Conflict of interest** VKar: Advisory Board of Amgen, Pfizer, Novartis, BI, Lilly, Astellas, Genesis-Pharma, and Janssen. SL: Employee of NEO New Oncology GmbH and Consultant BioTech AG. ES: Advisory Board of Merck, MSD, Astra-Zeneca, Roche, Amgen, and Genesis. GF: Advisory Board of Pfizer, Sanofi, and Roche. Honoraria from Astra-Zeneca. The rest of the authors declare that they have no conflict of interest.

**Ethical approval** All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. The clinical protocol was approved by the Institutional Review Boards of Papageorgiou General Hospital, Ioannina University Hospital, Agii Anargiri Cancer Hospital, Sotiria General Hospital, Alexandra Hospital, Patra University Hospital, Metropolitan Hospital, Hygeia Hospital, Chania General Hospital, Hippokration Hospital, and by the National Organization for Medicines. The trial was registered with the ClinicalTrials.gov identifier: NCT01215539. The translational research protocol was approved by the Institutional Review Board of the Papageorgiou General Hospital (08/04/2009 #233).

**Informed consent** Informed consent was obtained from all individual participants included in the study. In addition, patients who were willing to provide biological material for future translational research studies signed a separate informed consent.

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## Affiliations


**George Papaxoinis[1] · Vassiliki Kotoula[2,3] · Eleni Giannoulatou[4,5] · Georgia-Angeliki Koliou[6] · Vasilios Karavasilis[7] · Sotirios Lakis[3] · Andreas Koureas[8] · Mattheos Bobos[3] · Elpida Chalaralambous[3] · Emily Daskalaki[2] · Kyriakos Chatzopoulos[3] · George Tsironis[9] · Elisavet Pazarli[2] · Sofia Chrisafi[3] · Epaminontas Samantas[10] · Ioannis G. Kaklamanos[11] · Ioannis Varthalitis[12] · Athina Konstantara[13] · Konstantinos N. Syrigos[14] · George Pentheroudakis[15] · Dimitrios Pectasides[1] · George Fountzilas[3,16]**

1 Oncology Section, Second Department of Internal Medicine, Hippokration Hospital, 11527 Athens, Greece

2 Department of Pathology, Faculty of Medicine, School of Health Sciences, Aristotle University of Thessaloniki, Thessaloniki, Greece

3 Laboratory of Molecular Oncology, Hellenic Foundation for Cancer Research/Aristotle University of Thessaloniki, Thessaloniki, Greece

4 Victor Chang Cardiac Research Institute, Darlinghurst, NSW, Australia

5 The University of New South Wales, Kensington, NSW, Australia

6 Section of Biostatistics, Hellenic Cooperative Oncology Group, Data Office, Athens, Greece

7 Department of Medical Oncology, Faculty of Medicine, School of Health Sciences, Papageorgiou Hospital, Aristotle University of Thessaloniki, Thessaloniki, Greece

8 Department of Radiology, Aretaieio Hospital, National and Kapodistrian University of Athens, Athens, Greece

9 Department of Clinical Therapeutics, Alexandra Hospital, National and Kapodistrian University of Athens School of Medicine, Athens, Greece

10 Third Department of Medical Oncology, Agii Anargiri Cancer Hospital, Athens, Greece

11 Department of Surgery, School of Health Sciences, General Oncologic Hospital of Kifisia, National and Kapodistrian University of Athens, Athens, Greece

12 Oncology Department, General Hospital of Chania, Crete, Greece

13 Department of Surgery, St. Luke's Hospital, Thessaloniki, Greece

14 Oncology Unit GPP, Sotiria General Hospital, National and Kapodistrian University of Athens School of Medicine, Athens, Greece

[15] Department of Medical Oncology, Ioannina University Hospital, Ioannina, Greece

[16] Aristotle University of Thessaloniki, Thessaloniki, Greece