Sorafenib and everolimus for patients with unresectable high-grade osteosarcoma progressing after standard treatment: a non-randomised phase 2 clinical trial

Sorafenib and everolimus combination in non-resectable high-grade osteosarcoma progressing after standard treatment: a non-randomized phase II clinical trial from the Italian Sarcoma Group.

Abstract

Background: Unresectable advanced/metastatic osteosarcoma represents an unmet medical need in which we demonstrated promising but short-lasting activity of sorafenib. We showed mTOR pathway is involved in sorafenib failure and represents a reasonable co-target to hit using everolimus.

Methods: Patients>17 years affected by relapsed/unresectable osteosarcoma, progressing after standard treatments, received sorafenib 800 mg plus everolimus 5 mg daily until progression or unacceptable toxicity in a Simon two-stage study (NCT01804374). Primary endpoint was 6-month progression-free survival rate 6mPFS. Setting α=5%, β=10%, at least 37 patients were needed to test if 6mPFS was ≤25%=P0 (9 patients) or ≥50%=P1 (19 patients). Secondary endpoints were PFS, overall survival, RECIST 1.1 objective response rate (ORR), safety and their correlations with biomarkers. Survival endpoints were estimated by Kaplan-Meier method with 95% confidence intervals (CI). Tests were two-sided when indicated.

Findings: Between June 2011 and June 2013, 38 patients were enrolled. 6mPFS was 45% (95%CI 28-61%, 17 patients). Median PFS and overall survival were 5 (95%CI 2-7) and 11 (95%CI 8-15) months, respectively. We observed two (5%) partial responses (PR), two minor responses (5%), 20 (53%) stable diseases (SD) for an ORR of 10%. PR/SD lasted more than six months in eight (21%) patients (6, 6, 7, 8, 8, 8, 10+, 11). One patient interrupted the study to undergo lung metastasectomy after ten months of disease control. Treatment was feasible, but toxicity led to dose reductions and/or short interruptions in 25 (66%) patients and permanent discontinuation in two (5%) patients. P-ERK1/2 and P-RPS6 immunohistochemical expression positively correlated with primary and secondary endpoints.

Interpretation: This combination showed activity as further-line treatment in advanced/unresectable osteosarcoma with some long-lasting responses, but failed to reach the pre-specified target of 6mPFS. Toxicity

was as expected. P-ERK1/2 and P-RPS6 expression may contribute to identify patients likely benefitting from this therapy.

Funding: Italian Sarcoma Group

Introduction.

High-grade osteosarcoma (HG-OS) is a rare sarcoma affecting approximately 1150 new patients per year in the European Union.[1] As of today, a multidisciplinary treatment encompassing chemotherapy and complete surgical removal of the tumor cures roughly 70% of the patients.[2] The single most important predictive factor for cure remains the possibility to completely resect the tumor with adequate margins both in the localized[3] and in the relapsed/metastatic setting.[4,5] Unfortunately, the most active chemotherapy with high-dose methotrexate, doxorubicin, cisplatin, +/- ifosfamide (MAP/I) and mifamurtide,[2] may eradicate micro-metastatic disease, but does not cure non-resectable disease. Several second- and further-line treatments have been tested6-12 showing a marginal activity at most. The observed response rates were in the order of 3.1%11 to 29%7-9 with a median progression-free survival (PFS) ranging from 1.411 to about 4 months.8,9

The increased knowledge of oncogenic pathways involved in HG-OS pathogenesis along with the advent of target therapies, prompted the exploration of drugs hitting identified key-proteins. Consequently, small inhibitors as imatinib and monoclonal antibodies as trastuzumab), bevacizumab13 and anti-Insulin-like Growth Factor-1 Receptor (IGF-1R)[13,14] have been tested without evidence of significant activity in advanced HG-OS. In this context, phospho-extracellular-regulated kinase 1/2 (P-ERK1/2) were shown to be involved into HG-OS growth, survival, neoangiogenesis and metastatic potential[15,16]. Interestingly, sorafenib, a multikinase inhibitor of proven efficacy in renal, hepatic and thyroid cancers[17], was demonstrated to abrogate growth and metastatization of a spectrum of osteosarcoma cell lines both in i*n vitro* and *in vivo* models[15]. These evidences led to a phase II trial to explore sorafenib activity in patients affected by HG-OS relapsed

or progressed after standard treatment and deemed unresectable[18]. In this scenario, sorafenib showed hints of antitumor activity in terms of response rate (14%), reduction of both metastases ^{18}FDG-uptake and tumor density and, finally, improvement in pain control. Unfortunately, these attractive results were relatively short lasting with a 4- and 6-month PFS rate (6mPFS) of 46% and 29%, respectively[18].

Later, it was shown that Akt-mTOR pathway is involved into the mechanisms of resistance to sorafenib in HG-OS[19]. Indeed, while sorafenib abrogates mTORC1 complex activity, mTORC2 complex, on the contrary, is activated leading to tumor progression. These results were consistent with several lines of evidences demonstrating that on one hand, inhibition of mTORC1 complex alone was ineffective[20] and, on the other hand, inhibition of key tyrosine kinase receptors such as IGF-1R induces an increased activity of mTOR pathway by means of mTORC2 complex[13,21,22]. Interestingly, in preclinical models this mechanism of resistance was shown to be effectively overcome by the combination of sorafenib with the mTOR inhibitor everolimus[19]. In phase I/II trials this combination has been extensively studied in renal and hepatic cancers at several different doses[23-25] showing that it is feasible. Given our former experience with sorafenib at full dose, we regarded sorafenib and everolimus at the daily dose of 800 and 5 mg, respectively, as the most appropriate dose to be explored in HG-OS. Finally, we hypothesized that in patients' specimens the phosphorylation of down-stream key signaling proteins as P-ERK1/2 and phospho-ribosomal protein S6 (P-RPS6) targeted by sorafenib and everolimus, respectively, could be used to identify patients most likely to benefit from this combination.

On these basis, we designed a phase II trial aiming to investigate the activity of the combination of sorafenib and everolimus in patients affected by HG-OS progressing after MAP/I treatment and deemed inoperable.

Methods

Patients

Patients with not surgically amenable, histologically documented, locally advanced/metastatic HG-OS having progressed after first- or second-line treatments for relapsing/metastatic disease were enrolled at 3 Italian Sarcoma Group centers. Eligibility criteria included progressive and measurable disease according to RECIST 1.1 (bone lesions allowed),[26] 18 year-old or greater, Eastern Cooperative Oncology Group (ECOG) performance status (PS) of 0/1, life expectancy ≥3 months, adequate organ and bone marrow function. Details on inclusion and exclusion criteria are available in appendix 1.

Study Design and Treatment

Patients were treated with sorafenib 400 mg twice daily and everolimus 5 mg daily given in an open-label fashion until progression, unacceptable toxicities or patient's refusal. Adverse events (AEs) were evaluated and graded according to Common Terminology Criteria for Adverse Event v4.03 (CTCAE). AEs management followed predefined rules (see supplementary material, online only). Each participating center Institutional Review Board and Independent Ethics Committee revised and approved all protocol documents. The study was conducted according to the Declaration of Helsinki and the International Conference on Harmonization of Good Clinical Practice guidelines.

The primary endpoint of the study was the 6mPFS. Secondary end points included: PFS; overall survival (OS); overall response rate (ORR), defined as complete responses (CRs) + partial responses (PRs) + Minor Responses (MRs) (shrinkage of less than 30% but more than 10% in sum of the widest diameter of the lesions); disease control rate [DCR = ORR + stable diseases (SDs)]; duration of response (DOR); pain improvement, and safety. On paraffin-embedded tumor specimens immunohistochemical expression of P-ERK1/2 and P-RPS6 was correlated with outcome and scored as follows: <10% positive cells: 0+, 10-50% positive cells:1+; >50% positive cells and high staining intensity: 2+.

PFS was calculated from study entry until progression, unacceptable toxicity or death whichever came first. OS was calculated from study entry until death. DOR was calculated from first non-progression assessment until either progression/death. In absence of the event or loss to follow-up, all survival endpoints were censored at the last date the patient was known to be event-free. Any sign of tumor-related pain improvement was evaluated by means of the Pain and Analgesic Score (PAS)[27] and the Brief Pain Inventory (BPI)[28] form filled in autonomously by the patients.

Chest and abdomen Computed Tomography (CT) and/or Magnetic Resonance Imaging (MRI) were performed at baseline, repeated every 2 months and anticipated if clinically indicated. CRs, PRs and MRs needed confirmation after at least 4 weeks. ^{18}FDG-PET was suggested but not mandatory and was performed at baseline, during the third week of treatment and then if clinically indicated. Its impact on tumor response assessment was purely exploratory.

Statistical analysis

This was a non-randomized, multicenter, open-label phase II trial (NCT01804374). We used Simon's optimum two-stage design[29] with 6mPFS as the primary end point. Patients alive and free from progression after 6 months were considered as successes.

Based on our previous study with sorafenib alone, the trial was designed to rule out a 6mPFS of 25% (null hypothesis) and target a 6mPFS of 50% (alternative hypothesis). Setting α-error at 0.05 and β-error at 0.·10, in presence of at least six successes observed within the 17 patients enrolled in the first stage, the trial was allowed to proceed to the second stage in which 20 more patients needed to be enrolled, for a total of at least 37 patients .

In presence of 14 or more successes the experimental treatment could be considered worth further studies. The intention-to-treat analysis included all patients who received at least one pill of each drug. The efficacy analyzable population included all patients for whom a disease evaluation (either clinical or radiological) was performed. Survival endpoints were estimated according to the Kaplan and Meier method with their respective 95% confidence intervals (CIs). RECIST ORR and DCR

were calculated and reported with their 95% CIs. The impact of P-ERK1/2 and P-RPS6K expression was evaluated by comparing survival outcomes using the two-sided Mantle-Cox log-rank test, Fisher's exact test, and Mantel-Haenszel Odds Ratio (OR) estimate. Baseline *vs.* on-treatment PAS and BPI scores were compared using paired student's *t* test. All statistics were computed using IBM SPSS Statistics v.20 and GraphPad Prism v.5.

Role of the funding source

The Italian Sarcoma Group sponsored the trial through an unrestricted grant by Bayer and Novartis. Pharmaceutical companies had no role in data collection and interpretation, or writing of the report. All authors had access to the data, vouch for the completeness and accuracy of the data and analyses and approved the final version of the manuscript. The corresponding author (GG) had final responsibility for the decision to submit for publication.

Results

Patient characteristics

From June 2011 and June 2013, 38 patients affected by relapsed and inoperable HG-OS were enrolled at three Italian Sarcoma Group centers. Table 1 describes patients' baseline characteristics. All patients had already received MAP/I chemotherapy and the median number of previous systemic regimens was two (range one to three). All patients were treated according to the protocol and included in the safety and efficacy analyses. All analyses were performed after the last patient had been followed for at least 6 months.

Safety

The median follow up for safety analyses was 6 months (95% CI: 2-9). At last follow-up no patient was still on therapy. We observed at least one AE in all patients (100%). Of these adverse events,

9% were grade 3-4. All AEs occurring in at least one patient are listed in Table 2. The most common grade 3-4 AEs were lymphopenia in six (16%), hypophosphatemia in six (16%), hand and foot syndrome in five (13%), thrombocytopenia in four (11%), fatigue, oral mucositis, diarrhea and anemia in two (5%) of the patients. All of these AEs were causally related to the study drugs. One patient (3%) experienced a pneumothorax which required a trans-thoracic drainage (G3 according to CTCAE) and recurred at the time of PD. In both cases it was regarded as a Serious Adverse Event (SAE) related to the study drugs. No other SAEs were reported during the whole study. No death was related to the experimental treatment and all deaths were attributed to disease progression.

Study drugs needed to be reduced or temporarily suspended in 25 (66%) patients because of toxicity (for details see table 3). In general, short drug interruptions were deemed useful in 22 (58%) patients to recover from toxicity and occurred more commonly during the first month of treatment than later on. The administered doses of sorafenib and everolimus were 77% (95% CI: 69-84%) and 82% (95% CI: 75-89%) of the expected ones, respectively. The mean durations of temporary interruptions were 7 days (95% CI 5-9) and 7 days (95% CI 5-8) for sorafenib and everolimus, respectively (Table 3).

Efficacy

Progression in 34 (89%), toxicity in two (5%), lost to follow-up during treatment in one (3%) and lung metastasectomy in one (3%) patients were reasons to stop the experimental treatment. Eight patients (21%) received sorafenib and everolimus for more than eight months (8, 8, 9, 10, 10+, 11, 11, 12 months, respectively). Within the 17 patients enrolled in the first stage we observed nine (53%, 95% CI: 26-79%) successes. According to the intention-to-treat analysis, of the 38 patients enrolled, 17 (45%, 95% CI: 28-61) were progression-free at 6 months (Figure 1). The median PFS and DOR were 5 (95% CI: 2-7) and 5 months (95% CI: 4-6), respectively. The median follow-up of

patients surviving was 10 months (range 6-14). The median OS was 11 months (95% CI: 8-15) with a 12- and 24-month survival rate of 37% (95% CI: 21-53) and 5% (95% CI: 0-13%), respectively.

We observed 2 (5%) PRs and 2 (5%) MRs for an ORR of 10% (Figure 2). Twenty (53%) patients achieved a SD and 14 (37%) had a progression. The DCR was 63% (95% CI: 47-79%). The median duration of treatment was 5 months (95% CI: 2-7). No CR was observed. On the contrary, we recorded 10 (33%) non-dimensional responses by means of ^{18}FDG-PET in the 30 patients who underwent a PET-scan (mean 52% reduction in SUV within responding patients). One patient underwent lung metastasectomy after 10 months of disease control with stable disease according to RECIST criteria.

We track and recorded self-perceived improvement. Pain improvement was not observed in terms of PAS score reduction (mean baseline: 1.9; mean best on treatment: 1·8, p=0·619), but a strong improvement was observed in 22 patients fully evaluable for BPI questionnaire (mean baseline score: 36 points; mean best on treatments score: 24, p=0·004).

Biomarkers

Immunohistochemical expression of P-ERK1/2 and P-RPS6 was fully evaluable in 33 (87%) and 35 (92%) patients, respectively. We could not perform high-quality immunohistochemistry in 5 (P-ERK1/2) and 3 (P-RPS6) HG-OS samples because of technical problem likely due to prolonged decalcification that impaired sample antigenicity. P-ERK1/2 staining was positive in 20 (61%) of the specimens and was significantly correlated with a better 6mPFS (OR 5, 95% CI: 1·04-24·03), p = 0·045). P-RPS6 staining (score 2+) was positive in 17 (49%) of the specimens and was associated with a better 6mPFS (61% vs 0%, p=0·008; OR not assessable), p<0·001). P-ERK1/2 and P-RPS6 expression were both positive in 17 (51%) patients and predicted a better median PFS (7 *vs.* 2 months, p=0·021) with a higher 6mPFS (71% *vs.* 19%, OR 10·4, 95% CI: 2·03-53·2, P=0·005). Figure 3.

Discussion

In HG-OS preclinical data had shown that the inhibition of mTOR pathway by means of everolimus[19] might increase the activity of sorafenib. We assessed this hypothesis studying the combination of sorafenib and everolimus in patients affected by inoperable HG-OS progressing after standard multidisciplinary treatment. Taking into consideration our previous study achieving a 29% 6mPFS with sorafenib alone[18], as primary endpoint, we deliberately set a high PFS rate of 50% at a reasonably prolong time-point of 6 months. Regrettably, the present trial has not met the pre-specified threshold of activity to consider sorafenib and everolimus combination worth further phase III study. Nevertheless, in the grim context of unresectable/relapsed HG-OS, our 6-month progression-free rate of 45% compares very favorably with former published studies addressing cohorts of patients with similar clinical characteristics (table ???). Moreover, this result stands clearly above the widely accepted 3- and 6mPFS of 40% and 20%, respectively[27], to consider active a drug.[31] In addition, Leary and Colleagues reported a median PFS of 1.8 months with a 6mPFS of less than 10% in pediatric patients affected by relapsed HG-OS who failed to achieve a second CR.[32] Finally, our patients belonged to a very unfavorable subset (inoperable, median age 30, heavily pretreated).[33]

To assess the activity of target therapies is always challenging and even more so in osteosarcoma in which either calcification or necrosis can occur in absence of tumor shrinkage.[18] It could easily be argued that the observed ORR of 10% was unsatisfactory. However, this is consistent with the ORR observed in other tumors with target therapies.[34] Furthermore, it is well known that the correct response evaluation of bone lesions is always demanding in any tumor.[26] Therefore, to improve our capability to interpreter imaging findings, we performed also ^{18}FDG-PET. As in the example reported in figure 4, in a dimensionally stable disease the ^{18}FDG uptake reduction was a further signal of study drugs anti-tumor activity. Moreover, since all enrolled patients had confirmed progression at study entry, long-lasting disease stability may hardly be referred to disease

"dormancy". To further detail this aspect, we show the progression-free survival of the whole cohort of patients during the therapy received before study enrollment (either surgery, chemotherapy or radiotherapy) and we found a median PFS of 2 months (CI95%: 2-3). (Figure 5). Indeed, in an unquestionably aggressive disease, 8 (21%) patients had an interesting tumor arrest lasting more than 8 months. Taken altogether these data support the concept of drugs affecting both tumor biology and progression.

The reported AEs are consistent with those described in phase I trials. Notwithstanding, our younger cohort tolerated this combination slightly better than what is reported in previous experiences[23-25]. In synthesis, toxicity was relevant, but manageable in most cases. Of course, different drug dosages and clinical settings explain some of the observed differences. In general, drug related AEs were very common and required close contact between clinicians and patients to improve their best management. In fact, we had foreseen some enhancement of toxicity due to sorafenib and everolimus overlap on skin toxicity, stomatitis, thrombocytopenia and fatigue. Consequently, short interruptions as well as drug dose modulations were very helpful to permit drug re-introduction and prolonged use in responsive patients.

There is a strong rational to combine a multikinase inhibitor (hitting tyrosine kinase receptors as PDGFR, VEGFR as well as ERK1/2)[35] with a selective inhibitor of mTOR[19,36] . This prompted us to explore the clinical validity of phosphorylated-ERK1/2 and -RPS6 as predictive biomarkers of sorafenib and everolimus combination activity. Our results support the concept to assess P-ERK1/2 and P-RPS6 because their expression was associated with a statistically significant improved activity of the combination. Acknowledging the absence of a control group, our data are consistent with the predictive role of both P-ERK1/2 and P-RPS6 in renal and hepatic cancers.[37] Moreover, the present data support the analytic validity of these biomarkers which had been previously studied by several groups on different series.[13-15,18] Therefore, these biomarkers might be helpful to further study this or similar combinations in HG-OS as well as in other tumors.

The major limitation of this study is the lack of a control group to compare the results. We conceived this trial in order to quickly gather enough information on whether preclinical results on sorafenib and everolimus could be translated into the clinical scenario. In a rare tumor a randomized design would have required longer time to complete the study. We tried to strengthen the results by choosing objective and easily assessable endpoints at a reasonably distant time-point so as to minimize on one hand "Hawthorne effect"[38] and, on the other hand, to generate clinical useful information on this combination activity. The challenge of studying the young population of relapsed and inoperable HG-OS in a randomized fashion is certified by the complete lack of randomized trial in the same setting.

In conclusion, despite the fact that sorafenib and everolimus showed some degree of activity in relapsed and inoperable HG-OS, this combination did not significantly affect its dismal prognosis (no CR and only 1 patient made eligible to surgery). Awaiting significant advancement in the knowledge on osteosarcoma biology or innovative chemotherapy, any further study targeting these pathways should select the experimental population on the basis of the proposed biomarkers. In a subset of patients affected by tumor expressing P-ERK1/2 and/or P-RPS6, this experimental combination might be worth further prospective controlled clinical trials.

Contributors

GG, SF, PP and MA designed and developed the study. GG, EP, VF, RB, AT, LD, RB, SDA, FF, PGC, SF, MA were responsible for patient inclusion. GG, YP, SD, LD, MM and MG collected data. YP and FC performed biomarker analysis. GG, YP, SD and LD analyzed and interpreted the data. GG and MA wrote the manuscript. All the authors reviewed the article for intellectual content, provided comments, and gave final approval.

Conflicts of interest

GG and MA gave expert testimony for Bayer. PGC has consultant advisory role for Novartis and Bayer, receive research founding from Novartis and Bayer, honoraria and other remuneration from Novartis. All other authors declare no relevant relationship to disclose.

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	N°	%
Patients	38	100%
Age (years) - median (range)	31 (18-64)	
Sex - male - female	23 15	61% 39%
Metastatic at diagnosis - yes - no	9 29	24% 76%
ECOG PS at start - 0 - 1 - 2*	16 20 2	42% 53% 5%
Lines of chemotherapy after MAP = 1 > 1	2 36	5% 95%
Previous surgery - median (range) - 0 - 1 - 2 - >2	2 (0-7) 1 6 16 15	 3% 16% 42% 39%
Sites of metastases - Lung only - Lung + bone or viscera - Bone only	12 22 4	32 58 10

Table 1. Demographics and baseline characteristics. MAP, Methotrexate Adriamycin Cisplatin; ECOG PS, Eastern Cooperative Oncology Group Performance Status. *Patients with EOCG PS 2 depending solely on orthopedic problems were eligible.

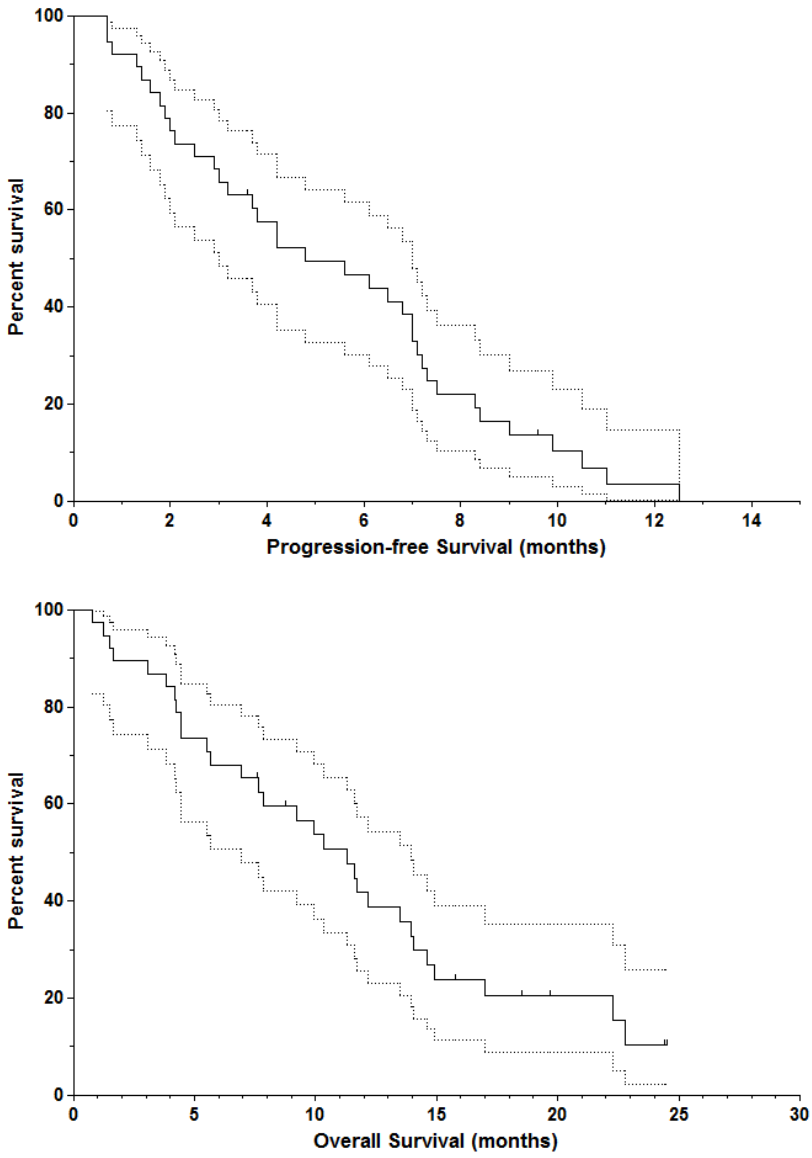


Figure 1. Kaplan-Meier plot for Progression-free Survival and Overall Survival

Adverse Event	All	All %	G1	G1%	G2	G2%	G3	G3%	G4	G4%
thrombocytopenia	22	58%	11	29%	7	18%	4	11%	0	0%
anemia	19	50%	14	37%	3	8%	2	5%	0	0%
lymphopenia	14	37%	7	18%	1	3%	3	8%	3	8%
leucopenia	12	32%	8	21%	3	8%	1	3%	0	0%
neutropenia	10	26%	6	16%	3	8%	1	3%	0	0%
febrile neutropenia	1	3%	0	0%	0	0%	1	3%	0	0%
mucositis	20	53%	11	29%	7	18%	2	5%	0	0%
diarrhea	18	47%	5	13%	11	29%	2	5%	0	0%
hypophosphatemia	18	47%	5	13%	7	18%	6	16%	0	0%
fatigue	16	42%	8	21%	6	16%	2	5%	0	0%
hypercholesterolemia	15	39%	14	37%	1	3%	0	0%	0	0%
nausea	14	37%	10	26%	3	8%	1	3%	0	0%
hypokalemia	14	37%	10	26%	3	8%	1	3%	0	0%
hypertriglyceridemia	14	37%	9	24%	5	13%	0	0%	0	0%
weight loss	13	34%	8	21%	4	11%	1	3%	0	0%
transaminase increase	12	32%	10	26%	2	5%	0	0%	0	0%
hyperglicemia	11	29%	9	24%	2	5%	0	0%	0	0%
abdominal cramps	11	29%	7	18%	4	11%	0	0%	0	0%
CK increase	10	26%	8	21%	1	3%	1	3%	0	0%
infection	9	24%	1	3%	7	18%	1	3%	0	0%
hypomagnesemia	9	24%	9	24%	0	0%	0	0%	0	0%
vomiting	8	21%	7	18%	1	3%	0	0%	0	0%
ggt increase	8	21%	4	11%	4	11%	0	0%	0	0%
costipation	8	21%	6	16%	2	5%	0	0%	0	0%
myalgia/arthralgia	6	16%	2	5%	3	8%	1	3%	0	0%
hypertension	6	16%	4	11%	1	3%	1	3%	0	0%
bilirubin increase	5	13%	4	11%	1	3%	0	0%	0	0%
amylase/lipase increase	4	11%	4	11%	0	0%	0	0%	0	0%
creatinine increase	3	8%	1	3%	2	5%	0	0%	0	0%
cough	3	8%	3	8%	0	0%	0	0%	0	0%
headache	2	5%	2	5%	0	0%	0	0%	0	0%
ejection fraction decrease	2	5%	2	5%	0	0%	0	0%	0	0%
bleeding	2	5%	2	5%	0	0%	0	0%	0	0%
dysphagia	1	3%	0	0%	0	0%	1	3%	0	0%
pneumothorax	1	5%	0	0%	0	0%	1	3%	0	0%
hfsr	27	71%	9	24%	13	34%	5	13%	0	0%
rash	24	63%	18	47%	5	13%	1	3%	0	0%

acneiform eruption	16	42%	14	37%	1	3%	1	3%	0	0%
xerosis	11	29%	11	29%	0	0%	0	0%	0	0%
pruritus	5	13%	5	13%	0	0%	0	0%	0	0%

Table 2. Adverse Events occurring in at least one patient.

	value	95% CI
Patients	38	100%
Sorafenib dose (administered/expected)	77%	69-84%
Everolimus dose (administered/expected)	82%	75-89%
Treatment permanent interruption because of toxicity - n of patients - % of patients	 2 5%	 0-5 0-13%
Treatment dose reduced/temporary interrupted - n of patients - % of patients	 25 66%	 19-31 50-82%
Treatment temporary interrupted - n of patients - % of patients	 22 58%	 16-28 42-74%
Dose levels sorafenib used 800 mg per day, n° (%) 600 mg per day (-1 dose level), n° (%) 400 mg per day (-2 dose level), n° (%)	 19 (50%) 6 (16%)* 19 (50%)	 33-67% 4-28% 33-67%
Days of interruption of sorafenib for single interruption - mean - median	 7 6	 5-9
Days of interruption of sorafenib during whole study - mean - median	 24 15	 12-35
Dose levels everolimus used 5 mg per day, n° (%) 2.5 mg per day (-1 dose level), n° (%) 2.5 mg every other day (-2 dose level), n° (%)	 25 (66%) 13 (34) 0 (0%)	 19-31 (50-82%) 7-19 (18-50%)
Days of interruption of everolimus for single interruption - mean - median	 7 5	 5-8
Days of interruption of everolimus during whole study - mean - median	 27 17	 15-39

Table 3. Dose Reductions. *Six (16%) of the 19 (50%) patients who reduced sorafenib to 400 mg were also temporary treated at an intermediate dose level of 600 mg per day (-1 dose level). Thrombocytopenia (9 patients, 24%), hand and foot syndrome or other skin toxicities (7 patients, 18%), hypertension and diarrhea (2 patients each, 5%) were the most common causes for sorafenib dose level reductions. Everolimus had to be reduced of one dose level (2·5 mg per day) mainly

because of thrombocytopenia. Causes for temporary interruptions were: skin toxicity in 13 cases (10 patients stopped both drugs, 2 sorafenib only, 1 everolimus only); thrombocytopenia in 10 cases (8 both drugs, 2 everolimus only); diarrhea in 2 cases (1 both drugs, 1 sorafenib only); hypertension in 2 cases (both drugs); pneumothorax in 2 cases in the same patient (both drugs); creatinine increase, febrile neutropenia and mucositis in 1 case each (both drugs stopped in all cases).

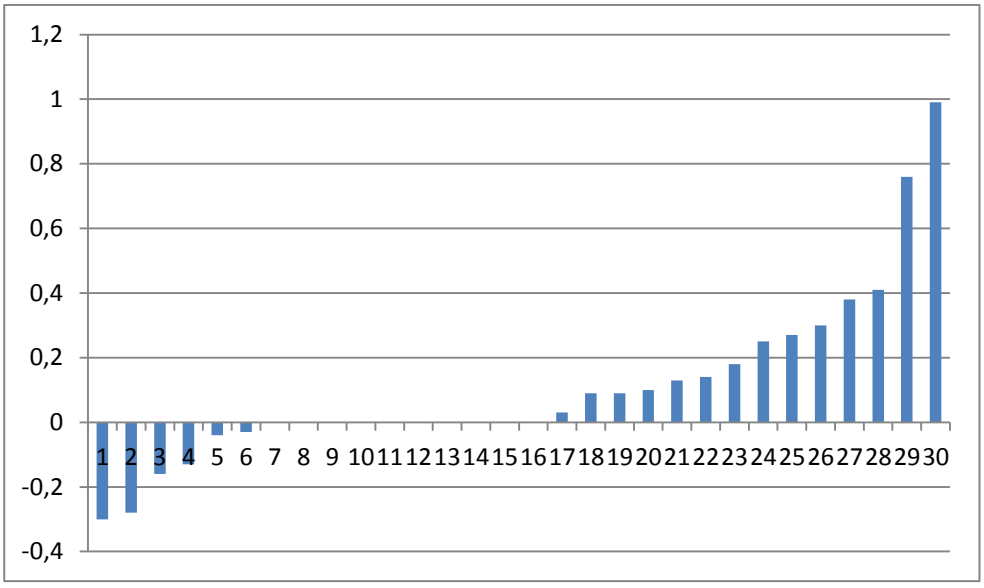
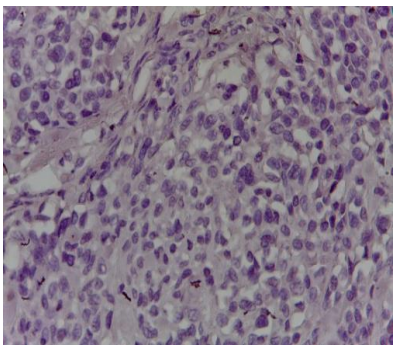
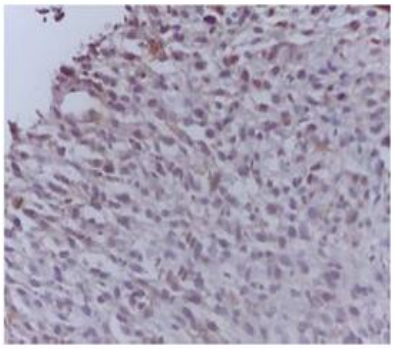


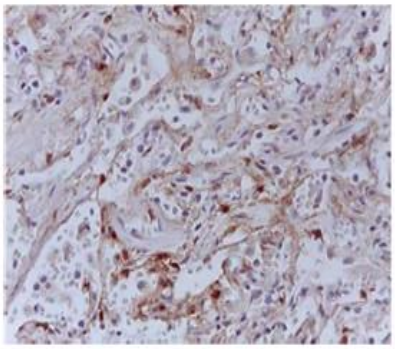
Figure 2. Waterfall plot



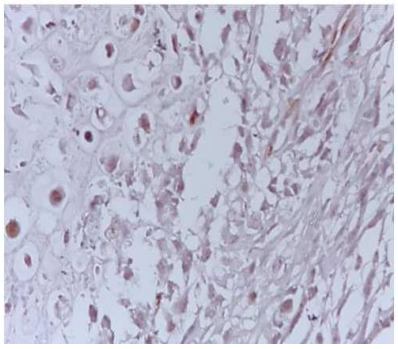
P-ERK1/2 0+



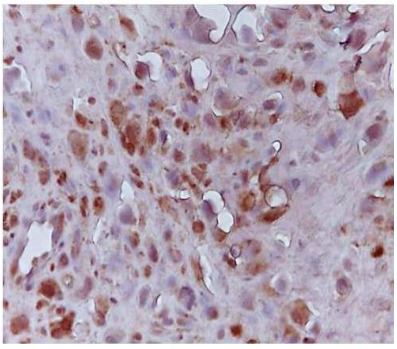
P-ERK1/2 1+



P-ERK1/2 2+



P-RPS6 1+



P-RPS6 2+

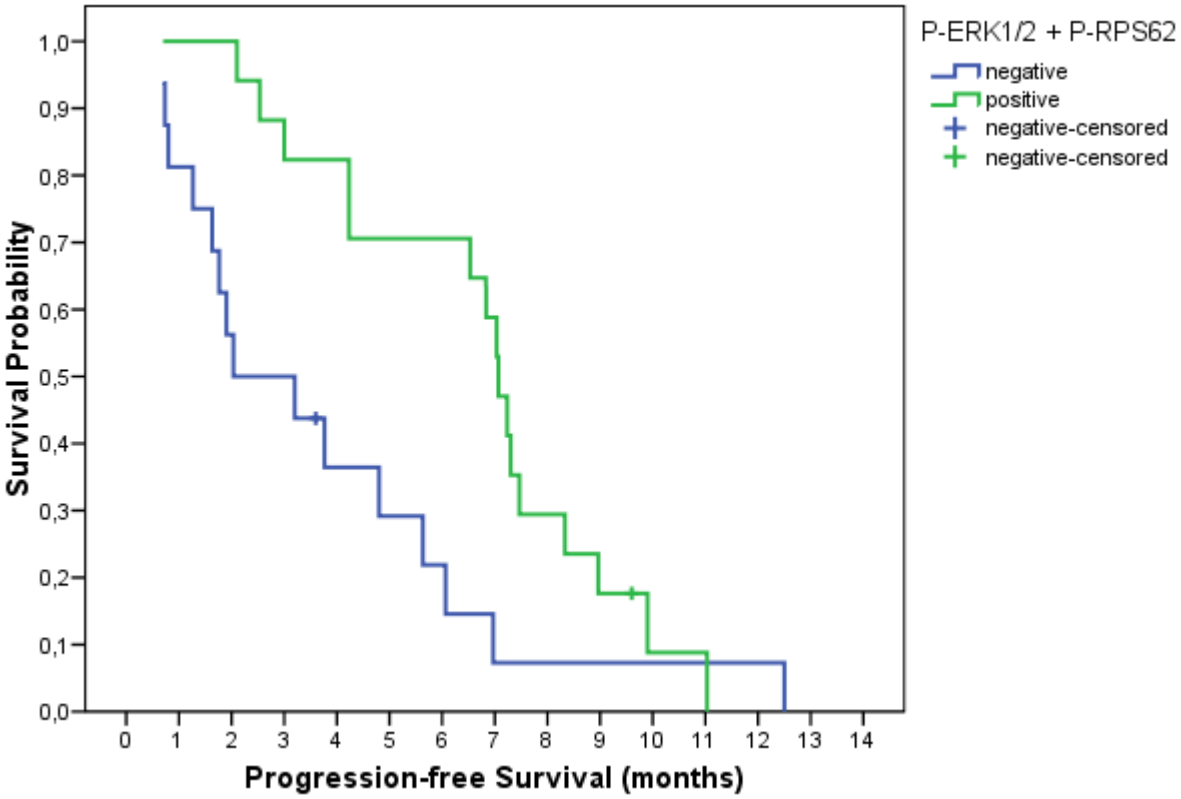


Figure 3. Immunohistochemical scores for P-ERK1/2 and P-RPS6. 0+ <10% positive cells; 1+ 10-50% positive cells; 2+ >50% positive cells and high staining intensity (Panel A).

Progression-free Survival curves for P-ERK1/2 positive and P-RPS6 2+ positive patients *vs.* negative ones (log-rank test p=0·021) (panel B).

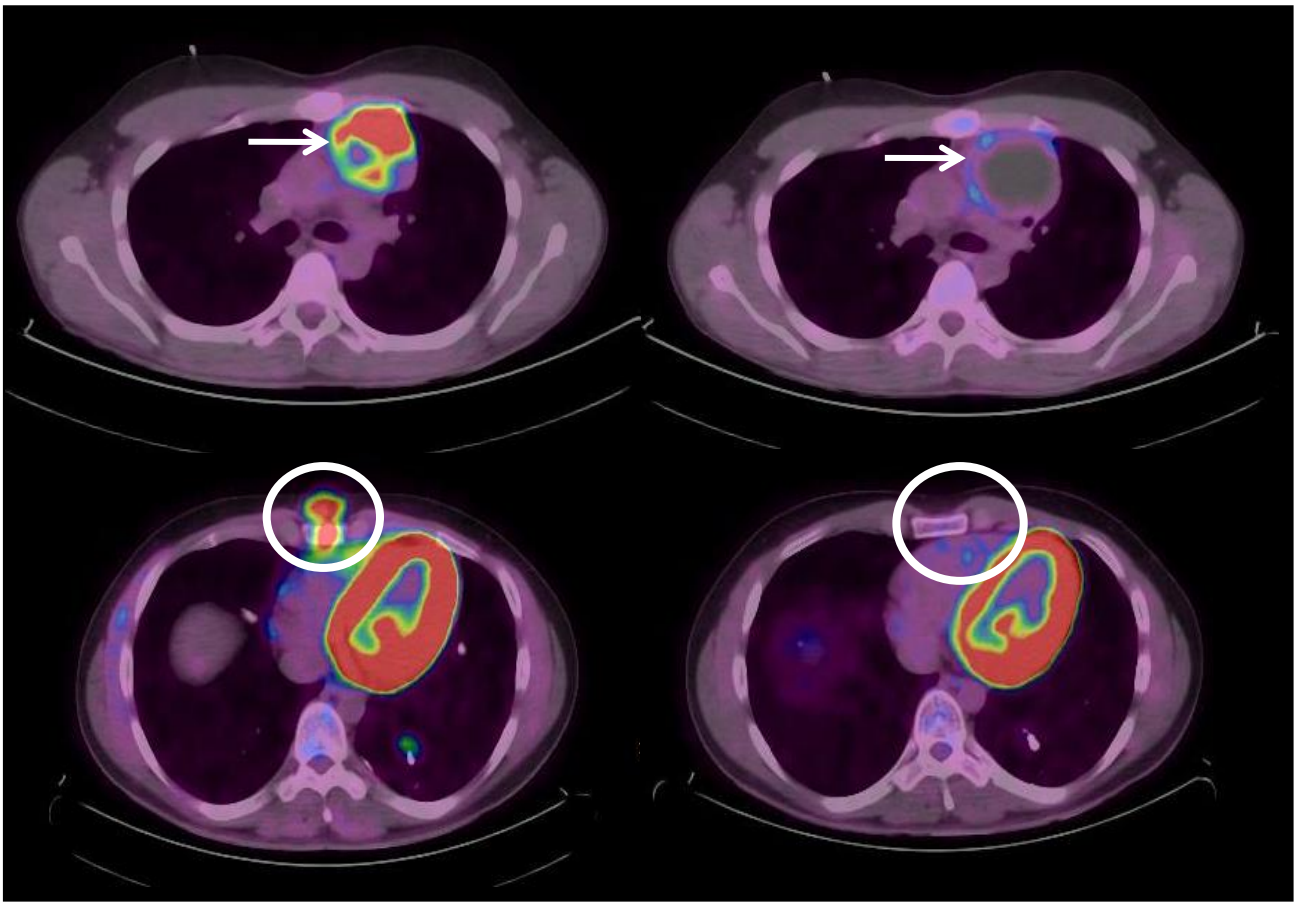


Figure 4. PET response after 2 months of therapy. On the left PET scan performed at baseline; on the right PET scan performed after 2 months of treatment in two patients.

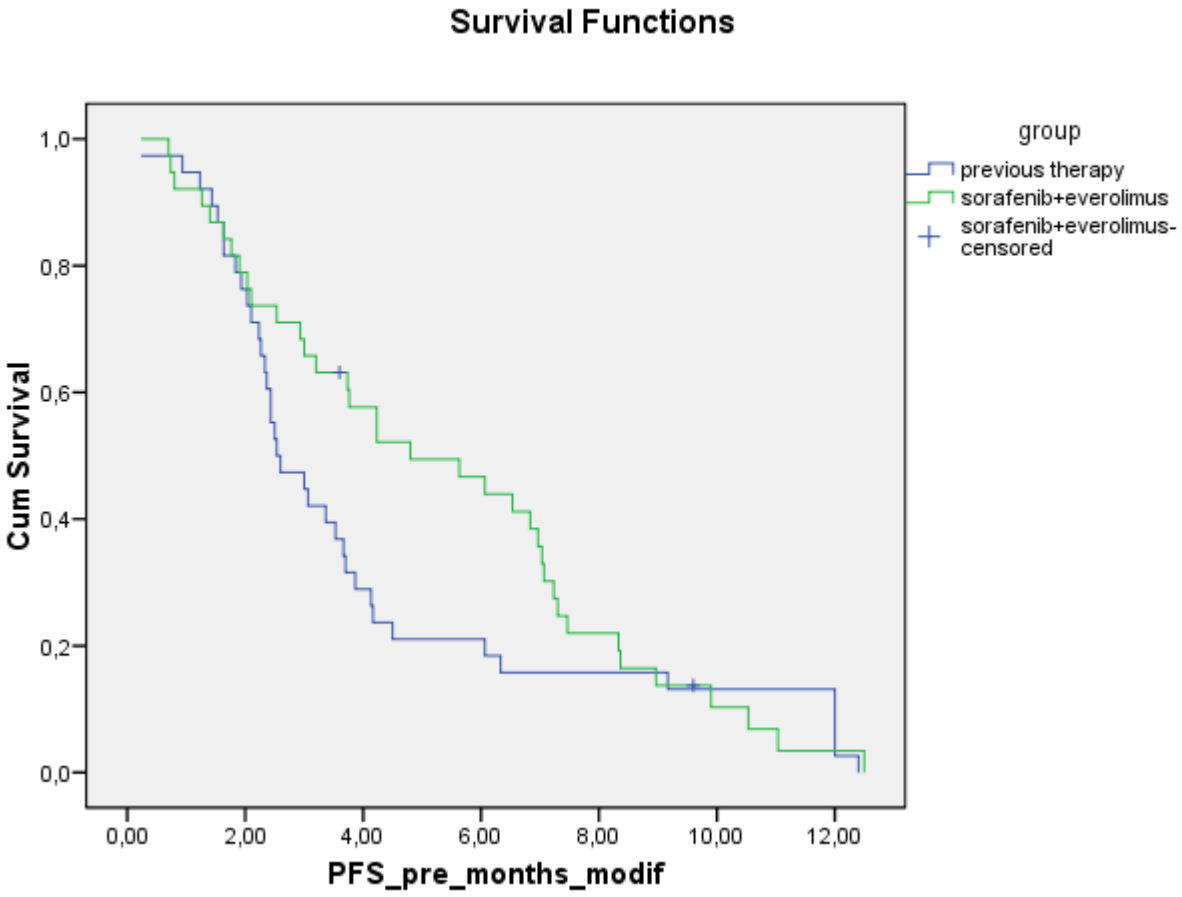


Figure 5. Progression-free Survival of last treatment (either chemotherapy, radiotherapy or surgery) performed before study enrollment vs. the one obtained with sorafenib + everolimus