



Published in final edited form as:

Future Oncol. 2014 December ; 10(15): 2435–2448. doi:10.2217/fon.14.99.

Impact of adding the multikinase inhibitor sorafenib to endocrine therapy in metastatic estrogen receptor-positive breast cancer

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Abstract

Background—Targeting growth factor and survival pathways may delay endocrine-resistance in estrogen receptor-positive breast cancer.

Materials & methods—A pilot Phase II study adding sorafenib to endocrine therapy in 11 patients with metastatic estrogen receptor-positive breast cancer was conducted. Primary end point was response by RECIST after 3 months of sorafenib. Secondary end points included safety, time to progression and biomarker modulation. The study closed early owing to slow accrual.

Results—Eight out of 11 patients had progressive disease on study entry and three had stable disease. Of the ten evaluable patients, seven experienced stable disease (70%) and three experienced progressive diseases (30%), with a median time to progression of 6.1 months (8.4 months in the seven patients on tamoxifen). The serum samples demonstrated a significant reduction in VEGF receptor 2 and PDGF receptor- α . Microarray analysis identified 32 suppressed genes, no induced genes and 29 enriched Kyoto Encyclopedia of Genes and Genomes pathways.

Conclusion—The strategy of adding a targeted agent to endocrine therapy upon resistance may be worthwhile testing in larger studies.

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Ethical conduct of research

The authors state that they have obtained appropriate institutional review board approval or have followed the principles outlined in the Declaration of Helsinki for all human or animal experimental investigations. In addition, for investigations involving human subjects, informed consent has been obtained from the participants involved.

Financial & competing interests disclosure

This work was funded by a grant from Onyx-Bayer Pharmaceuticals to S Massarweh. Additional funding provided by grant number UL1RR033173 (TL1 RR033172, KL2 RR033171) from the National Center for Research Resources from the Office of the Director, NIH, and supported by the NIH Roadmap for Medical Research (the content does not necessarily represent the official views of National Center for Research Resources and NIH). Immunohistochemistry work was supported by the Markey Cancer Center Biospecimen and Tissue Procurement Shared Resource Facility. We are grateful to Brent Shelton from the Markey Cancer Center Biostatistics Core for his help with the original study design. This work was presented in part at the 33rd Annual San Antonio Breast Cancer Symposium in 2010 [52] and the 34th annual San Antonio Breast Cancer Symposium 2011 [53]. Trial registration: registered under ClinicalTrials.gov identifier NCT00525161.

No writing assistance was utilized in the production of this manuscript.

Keywords

angiogenesis; breast cancer; endocrine resistance; PDGF receptor- α ; Ras/Raf/MAPK; VEGF receptor 2

Estrogen receptor (ER) expression occurs in approximately 70% of all breast cancers and is a critical factor in cancer survival and disease progression [1]. Disruption of ER signaling using a variety of targeted endocrine strategies is a mainstay of treating ER-positive breast cancer and is routinely used for all stages of the disease. For metastatic breast cancer in particular, where the primary treatment objective is palliation and prevention of disease progression, endocrine therapy offers the advantages of ease of administration, low cost and minimal toxicity with preservation of a patient's quality of life. Despite the established benefit of endocrine therapy in ER-positive metastatic breast cancer; however, therapeutic resistance is routinely encountered. Chemotherapy is commonly used to control disease progression after endocrine therapy failure [2], particularly in women who are premenopausal, where endocrine therapy is limited to the use of tamoxifen with or without ovarian suppression [3]. Despite the potential benefits of chemotherapy in metastatic ER-positive breast cancer, it is associated with significant toxicity, expense and many patients do not gain benefit. Novel therapeutic strategies to improve endocrine therapy benefit while preserving quality of life are, therefore, of great interest in order to avoid the early use of chemotherapy.

As the understanding of ER signaling biology has increased in the last decade [2], it is now evident that cell signaling pathways that may coexist with ER can influence tumor response to endocrine therapy, and this interaction may promote endocrine resistance and treatment failure in patients [4,5]. More importantly, targeting this molecular 'crosstalk' can be exploited therapeutically in order to overcome endocrine resistance and prolong benefit of endocrine therapy [5].

One of the critical molecular pathways responsible for endocrine resistance in breast cancer is the Ras/Raf/MAPK signaling cascade in which MAPK can directly interact with and activate ER [6–8], promote endocrine resistance [9,10] and has been associated with risk of relapse after adjuvant endocrine therapy [11,12]. Interestingly, tumor hypoxia, a common feature of advanced solid tumors, leads to sustained MAPK activation [13,14], which in turn can activate hypoxia inducible factor- α (HIF1- α)-dependent transcription of VEGF, PDGF receptor- α (PDGFR- α) and other angiogenesis factors [15,16]. Increased HIF1- α is associated with ER degradation [17,18] and poor response to endocrine therapy [19], while inhibition of MAPK abrogates HIF1- α phosphorylation and can restore tamoxifen sensitivity by interfering with hypoxia-induced downregulation of ER [20]. In addition, multiple lines of evidence suggest that estrogen directly modulates angiogenesis [21], and that both estrogen and tamoxifen can promote transcription of potent angiogenesis factors such as VEGF [22,23], which promotes sustained angiogenesis – one of the hallmarks of cancer [24].

In this pilot study, we examined whether adding sorafenib, a multikinase inhibitor of VEGF receptor (VEGFR)/Ras/Raf/MAPK [25], to endocrine therapy can enhance endocrine

response and overcome resistance in patients with advanced ER-positive breast cancer. Patients with progression on endocrine therapy alone, or maximal response with significant residual disease, were given sorafenib – in addition to continuing the same endocrine agent – and followed for response and toxicity assessment. Serum biomarkers and tumor biopsies, when feasible, were obtained and correlated with response.

Materials & methods

Patients

Premenopausal or postmenopausal patients were required to have biopsy-proven metastatic ER-positive breast cancer and already receiving an endocrine agent. Patients were required to have either progressive disease (PD) or maximal response on the existing endocrine agent with measurable residual disease. Patients were required to have an Eastern Cooperative Oncology Group performance status of 0–2 and adequate baseline renal and hepatic function, defined as serum creatinine ≤ 1.5 -times the upper limit of normal (ULN), serum bilirubin ≤ 1.5 -times the ULN and aspartate aminotransferase–alanine aminotransferase ratio ≤ 2.5 -times the ULN. Key exclusion criteria included an Eastern Cooperative Oncology Group performance status of 3 or 4, rapidly PD requiring chemotherapy, known brain metastases and any prior use of antiangiogenic agents. The study was approved by the University Of Kentucky (USA) institutional review board and all patients signed informed consent.

Study design

This was originally a single-institution, pilot, Phase II study of adding sorafenib to pre-existing endocrine therapy. On study entry, eligible patients underwent serum sample collection and core biopsy of accessible disease (if applicable) on endocrine therapy and prior to starting sorafenib. Serum and a second biopsy were then collected on day 28. Sorafenib dose was 400 mg orally twice daily along with continuing the same endocrine agent. Patients were followed monthly for clinical and toxicity evaluation. Disease response by RECIST criteria [26] was assessed after 3 months by appropriate scans and these were obtained every 2 months thereafter until progression. Sorafenib and the endocrine agent were continued until disease progression or unacceptable toxicity (Figure 1). Inpatient dose reduction to 400 mg once daily and then 400 mg every other day was allowed depending on the type and severity of toxicity encountered, provided that criteria for patient withdrawal from study treatment have not been met.

Biopsies

Tumor tissue was obtained by core biopsy using a Bard® Max-Core® Biopsy Instrument (#MC1410; Bard Biopsy Systems, AZ, USA). The outside needle was 14 gauge (2.1 mm), with a smaller inside cutting needle to obtain the biopsy. The length of the cutting needle penetration was 22 mm and the total length of the needle was 10 cm. Multiple core biopsy samples of approximately 2 × 10–20 mm were divided in 10% formalin or immediately frozen in liquid nitrogen.

ELISA

Blood samples were collected from each patient on study entry (day 1) and after 28 days of adding sorafenib (day 28). Serum was separated from each sample and several 0.5 ml aliquots were made and stored in -80° Fahrenheit. Serum ELISA was measured in picograms per milliliter and compared between day 1 and 28 using kits for human VEGF-A, human soluble VEGFR-1 (sVEGFR-1; Flt-1), and VEGFR-2 obtained from R&D Systems (MN, USA). PDGFR- α was detected using PathScan[®] Sandwich ELISA (Cell Signaling Technology, MA, USA). All analyses were conducted in duplicates along with appropriate controls as per manufacturers' instructions in the respective kits and as described on the respective websites [27,28].

Immunohistochemistry

Tumor material obtained from four-paired core biopsies was fixed in 10% neutral-buffered for-malin overnight before processing and paraffin embedding. A 3- μ m-thick section was examined by hematoxylin and eosin staining to verify adequacy of tumor tissue. Immunohistochemistry was performed on 4- μ m-thick sections from these paraffin blocks. Deparaffinization, hydration and heat-induced epitope retrieval was performed in a PT Link pretreatment module with EnVision[™] FLEX Target Retrieval Solution, High pH (Dako, CA, USA) according to the manufacturer's instructions. The primary antibodies in this study included: ER (clone SP1) at 1:20 dilution (Dako); progesterone receptor (PgR; clone PgR 636) at 1:50 dilution (Dako); cyclin D1 (clone SP4) at 1:30 dilution (Dako); and Ki-67 (clone MIB-1) at 1:50 dilution (Dako). Secondary antibodies used were EnVision FLEX Target Retrieval Solution, High pH-labeled polymer horseradish peroxidase anti-mouse or antirabbit as appropriate. Staining was visualized using 3,3'-diaminobenzidine chromogen (Dako). Slides were lightly counterstained with hematoxylin. Breast specimens positive for cyclin D1, ER, PgR and tonsil specimens positive for Ki-67 were run simultaneously as positive controls. Each specimen was run simultaneously with mouse or rabbit IgG as appropriate for negative controls. Slides for ER, PgR and cyclin D1 were evaluated using the Allred scoring system [29]. For the Ki-67 proliferation index, we counted at least 500 cells and recorded the percentage of cells with positive staining. The pathologist reading the results was blinded to the treatment group.

Statistical considerations

This was originally planned as a two-stage, Phase II Simon design [30] with a primary end point of clinical response (complete response and partial response) after 3 months of adding sorafenib to endocrine therapy. Based on the known historical response rate to sorafenib of no better than 5–10% [31,32], the study was designed to test the null hypothesis that the clinical response rate is no better than 10% versus the alternative hypothesis that it is at least 25% when sorafenib is added to endocrine therapy. In the first stage, 18 patients were planned for enrollment, and if two or fewer responses were observed, the trial would be terminated. Sample size calculations were conducted using Number Cruncher Statistical Systems software [33]. Ultimately, the study was stopped after 11 patients secondary to slow accrual and withdrawal of funding by the sponsor.

Secondary study end points were time to progression (TTP), clinical benefit rate (CBR) defined as complete response/partial response/stable disease (SD) \geq or equal to 24 weeks, toxicity assessment, and serum and tissue biomarker analysis. TTP was analyzed using Kaplan–Meier methods. All adverse events (AEs) were recorded using the National Cancer Institute Common Terminology Criteria for Adverse Events v3.0 and were summarized overall and by grade (grade 1 and grades 2–3). Serum levels of VEGF, sVEGFR-1, VEGFR-2 and PDGFR- α in the ten paired samples (before and after sorafenib) were compared for statistical significance using paired student's *t*-test or the ranked sum test on the differences as appropriate after normality assessment. All analyses were completed using Statistical Analysis System 9.3.

Expression microarrays

Each of four-paired frozen samples were homogenized in TRI[®] Reagent (Sigma-Aldrich, MO, USA) and incubated at room temperature for 5 min. Chloroform was added to each sample. Samples were vortexed and then incubated at room temperature for 5 min. Samples were centrifuged at high speed for 10 min and the aqueous layer was transferred to a new tube. RNA was purified using RNeasy[®] minicolumns (Qiagen, CA, USA) following the manufacturer's protocol after ethanol addition. The resulting RNA was quantified, and the quality was assessed using the Agilent Bioanalyzer (Agilent Technologies, CA, USA). RNA was submitted to the University of Kentucky Microarray Core Facility for analysis on GeneChip[®] Human Genome U133 Plus 2.0 high-density DNA microarrays (Affymetrix, CA, USA). Briefly, 250 ng of RNA was processed to cDNA using the 3' IVT Express Kit (Affymetrix). cDNA was hybridized to the arrays and scanned using the GeneChip[®] 7G Scanner (Affymetrix). The resulting data were normalized using the Guanine Cytosine Robust Multi-array Analysis method. To identify differentially expressed genes comparing pre- and post-sorafenib treatment, we used the significant analysis of micro-arrays (SAM) method for paired data and calculated the false discovery rate (FDR) [34]. The cutoff value was chosen to be a FDR <0.20 and at least a twofold change. We also used the gene set enrichment analysis (GSEA) method [35] to identify pathways associated with the treatment. In GSEA, the genes were preranked based on the absolute values of their SAM test statistics. We investigated all the pathways in the Kyoto Encyclopedia of Genes and Genomes database [36]. Enriched pathways were identified based on the cutoff value of a FDR <0.20 . GSEA was performed based on the GSEA Java desk-top software application [37]. All other analyses were performed based on R/Bioconductor [38]. The microarray data was deposited on the Gene Expression Omnibus, accession number GSE40837 [39].

Results

Patient & disease characteristics

The study was closed after 11 patients were enrolled owing to slow accrual. Median age was 45 years, with a range of 39–72 years (Table 1). Five patients had locally advanced disease (45%) and all (100%) had metastatic disease. Most common sites of metastasis were bone and lung (Table 1) and in seven patients (64%) metastasis was present *de novo*. ER was positive in all patients and PgR was positive in nine. In nine out of 11 patients (82%) HER2 was negative; HER2 status was unknown in two patients (18%). Overall, nine out of 11

patients (82%) had ER⁺/PgR⁺/HER2⁻ disease. At the time of study entry, seven patients (64%) were on tamoxifen, three (27%) were on an aromatase inhibitor (AI) and one (9%) was on fulvestrant, with four patients (36%) having received prior endocrine therapy in the adjuvant setting. Eight patients (73%) had PD at study entry and three (27%) had confirmed SD on endocrine therapy alone. Three patients received prior chemotherapy, two as adjuvant, and eight patients were treated with endocrine therapy only (Table 1). Six patients were premenopausal and four were also receiving goserelin. Individual patient characteristics are described in Table 2.

Efficacy

Of the 11 patients enrolled, one discontinued treatment after 2 weeks owing to a grade 3 rash and was not evaluable for response. Of the ten remaining patients, seven (70%) experienced SD and three (30%) had progression after sorafenib. The clinical benefit rate was 50% (95% CI: 19–81%). One of the patients with SD had a minor response, but decided to discontinue sorafenib after 156 days owing to recurrent rash (patient 2, Table 2). The median TTP after adding sorafenib was 6.1 months (Figure 2A). Of the eight patients who entered the study with PD, five (62%) converted to SD after adding sorafenib with a median TTP of 6.4 months. Notably, in the seven patients receiving tamoxifen, median TTP was 8.4 months (Figure 2B) with one patient, who entered the study with PD on tamoxifen (patient 11, Table 2), remaining on treatment with SD for 36 months before experiencing disease progression.

Safety & tolerability

All patients developed some toxicity. Hypophosphatemia was the most common AE, followed by hypokalemia (Table 3). Weight loss and rash, including one patient with a Stevens–Johnson-like reaction, were the most common clinical AEs encountered, followed by hypertension (Table 3). One patient with SD at study entry discontinued sorafenib after 2 weeks due to a grade 3 rash. Overall, the majority of toxicities were grade 1 or 2, with seven grade 3 toxicities seen: two hypophosphatemia episodes and one each of rash, anorexia, weight loss, colitis and hypokalemia. There were no grade 4 toxicities and no patients experienced hand–foot syndrome. Treatment was reasonably tolerated and toxicities were reversible upon sorafenib discontinuation. Altogether, four patients had to interrupt sorafenib or reduce the dose in the first month of treatment owing to the development of rash, which was the main dose-limiting toxicity.

Serum ELISA

To examine the effect of adding sorafenib on serum markers of angiogenesis, we compared levels of VEGF, sVEGFR-1, VEGFR-2 and PDGFR- α in paired serum samples before and after sorafenib (Table 4), and levels were graphically represented in individual Figure 3). There was a slight increase in mean serum VEGF on day 28 (289.56 pg/ml) compared with day 1 (261.38 pg/ml), but the difference was not statistically significant ($p = 0.3223$). Similarly, sVEGFR-1, which acts as a decoy for VEGF, was slightly increased on day 28 (150.95 pg/ml) compared with day 1 (142.29 pg/ml), but the difference was not statistically significant ($p = 0.084$) (Table 4). VEGFR-2, on the other hand, was significantly reduced in all patients (6971.47 vs 8803.32 pg/ml) with a mean decrease of 1831.85 pg/ml, $p = 0.0035$ (Table 4). PDGFR- α , another marker of tumor angiogenesis associated with disease

progression in breast cancer [40] and a target of sorafenib, was also significantly reduced (0.7576 pg/ml on day 28 vs 0.8639 pg/ml on day 1), with an average reduction of 1064 pg/ml, $p = 0.017$. There were no significant differences in serum biomarkers tested when comparing patients with SD versus PD, probably owing to the smaller sample size in these subgroups (data not shown).

Immunohistochemistry

Four out of five patients with primary tumors in place agreed to have paired tumor biopsies carried out. We examined the effect of sorafenib on ER and PgR expression as well as its impact on tumor proliferation by measuring Ki-67 and the cell cycle regulator cyclin D1 (Figure 4). Overall, there were no significant changes in ER, PgR or Ki-67 on day 28 versus day 1 in this small patient sample (data not shown). ER decreased slightly in two out of the four patients (Figure 4), and PgR, which was negative in three patients on day 1, increased in one patient who had SD in response to sorafenib (patient 1, Table 2). Ki-67 decreased in response to sorafenib in two out of four patients, both whom had SD, and increased in the other two patients; one of them developed PD on sorafenib. The second patient only had a slight increase in Ki-67. Cyclin D1 decreased in three out of four patients, with no change in the fourth patient who had an Allred score of 8, with 100% nuclear staining (Figure 4).

Expression microarray analysis

To further analyze and identify differentially expressed genes after adding sorafenib, we compared microarray analysis between day 1 and 28 in the four-paired frozen biopsy samples obtained. Based on SAM with an FDR <0.20 and at least a twofold change, we identified a total of 32 suppressed genes represented by 33 probe sets with no induced genes (Figure 5 & **Supplementary Table 1**, see online at www.futuremedicine.com/doi/suppl/10.2217/fon.14.99). Based on GSEA, we identified 29 enriched Kyoto Encyclopedia of Genes and Genomes pathways comparing day 28 versus day 1 with an FDR <0.20 (Table 5). The table shows the name of each pathway, total number of genes in that pathway, the number of genes in the leading edge subset and the corresponding FDR q-value.

Discussion

In this pilot trial of advanced metastatic ER-positive breast cancer, we attempted to improve endocrine response and overcome resistance by adding sorafenib, a multikinase inhibitor of VEGFR/Ras/Raf/MAPK. Our planned strategy was focused on targeting endocrine resistance in residual disease, either after maximal response to endocrine therapy alone or after resistance had already started to manifest as PD. Despite the small number of patients studied, we saw an encouraging effect from adding sorafenib to endocrine therapy, particularly in patients experiencing disease progression, who subsequently converted to SD after sorafenib was added. The observed TTP of over 6 months and clinical benefit rate of 50% is of interest, considering that sorafenib as a single agent has limited activity on its own in unselected patients with metastatic breast cancer [31,32]. One patient in our trial has had SD for approximately 3 years after adding sorafenib to tamoxifen, after her cancer had already progressed on tamoxifen therapy alone. This observation suggests that, when added to endocrine therapy, sorafenib may have a collaborative effect on enhancing ER blockade

and restoring tumor growth inhibition. A recent report of adding sorafenib to an AI upon progression of ER-positive breast cancer also suggested that the observed clinical benefit rate of 23% reflects restoration of endocrine sensitivity since the AI was continued along with sorafenib [41].

This small pilot study suggests that the strategy of adding a targeted agent to endocrine therapy after progression is feasible, rather than upfront combination to delay resistance. This approach may have potential advantages over upfront combinations of endocrine and targeted therapies for treatment of metastatic disease. Using a targeted agent upon progression, once resistance develops, spares the patient likely toxicities during a period when cancer may still be quite sensitive to endocrine therapy alone, particularly in the first-line setting, and may reduce the expense of treatment as well. More importantly, it is possible that targeting a signaling pathway that is driving endocrine resistance and cancer growth may actually be a more effective strategy once this pathway is activated and cancer is most dependent on it for survival [2]. Recent studies in AI-resistant metastatic breast cancer are supportive of this hypothesis [42–44], while front-line combination approaches with endocrine therapy may be less rewarding [45]. The success or failure of a particular clinical trial strategy, of course, may also depend on the specific resistance pathway of interest and whether it can be activated early in the course of treatment with endocrine therapy or is it acquired once resistance develops [46,47]. It is, therefore, important whenever possible to collect tumor biopsy specimens upon disease progression and, while on therapy, to identify specific molecular aberrations in order to target therapy more specifically. In individual patients who experience dramatic benefit from a particular drug [48,49], identifying ‘targetable’ aberrations may allow better patient selection for future trials, which can potentially be carried out with significantly smaller sample size if enriched for a particular target.

In addition to identifying the dominant pathways responsible for cancer cell survival and the dynamics of molecular change with progression, it is also important to examine target modulation and correlate that with novel therapeutic interventions. Of particular note, serum angiogenesis markers can be modulated by endocrine therapy and their differential modulation by various agents may be a surrogate marker for treatment benefit [50]. This is especially true when examination of the tumor itself is not feasible to assess variables such as proliferation markers, which may provide prognostic information for outcome [51]. In our study, serum VEGFR-2 and PDGFR- α were both effectively reduced by sorafenib suggesting a pharmacodynamic correlation with reduced tumor angiogenesis – a hallmark of disease progression. Interestingly, serum sVEGFR-1, which acts as a sink for circulating VEGF and limits VEGF-induced angiogenesis [50], was increased in response to sorafenib, albeit not significantly, which may reflect the small sample size. Nonetheless, our observations suggest that sorafenib has a favorable impact on serum angiogenesis profile when added to endocrine therapy and examining serum angiogenesis biomarkers in a larger number of patients may prove useful as a surrogate to clinical end points when evaluating agents that target tumor angiogenesis.

Of note, microarray analysis of tumor samples before and after sorafenib in this trial showed a significant reduction in gene expression profile with no induced genes observed. This may

reflect the nature of the trial where baseline biopsies were obtained from tumors progressing on endocrine therapy and subsequent biopsies on sorafenib may be indicative of a pharmacodynamic drug effect, but should be interpreted with caution in view of the small number of samples examined.

Conclusion

This pilot study suggests that adding the multi-kinase inhibitor sorafenib to endocrine therapy upon progression, especially tamoxifen, produces meaningful clinical benefit with manageable toxicity and delays resistance and the otherwise inevitable use of chemotherapy.

Future perspective

The strategy of later addition of a targeted agent to overcome resistance may offer a rational alternative to upfront combination with endocrine therapy to delay resistance, with the advantage of reduced toxicity and cost. Future clinical trials may be appropriate to compare these alternative strategies and more effort is needed to emphasize feasibility of such designs. Regardless, the possibility of extending the efficacy of a hormonal agent for treatment of metastatic breast cancer, perhaps for years, is a worthwhile goal.

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EXECUTIVE SUMMARY

Background

- Endocrine therapy is simple, effective and relatively nontoxic, but resistance develops in essentially all patients with metastatic disease and progression eventually develops necessitating a change of therapy.
- Increasing evidence suggests that endocrine therapy failure and disease progression is driven by coexpressed growth factor and survival signaling pathways in estrogen receptor-positive disease that drive tumor growth, despite continued estrogen receptor blockade.
- Rational use of targeted agents may extend endocrine therapy benefit in patients with metastatic disease and avoid the otherwise early use of chemotherapy for endocrine therapy failure.

Results

- This small pilot trial suggests that sorafenib can halt disease progression when added to endocrine therapy, particularly tamoxifen, even though sorafenib has little clinical activity as a single agent.
- Median time to progression was 6.1 months after adding sorafenib, with one patient experiencing benefit for 3 years before disease progression occurred.
- Toxicity was manageable, but not insignificant, in this population of patients with advanced disease.
- Biomarker evaluation shows a significant impact of adding sorafenib on angiogenesis factors, although correlation with response was limited by the small sample size.

Conclusion

- As a future clinical trial development strategy, adding a targeted agent to endocrine therapy at the time of progression, perhaps guided by dominant molecular aberrations in individual tumors, may be an alternative strategy to upfront lengthy treatment combinations designed to prevent resistance.
- This proposed strategy could contribute to decreased cost and toxicity in individual patients, especially if their disease is initially sensitive to the endocrine agent alone.
- It is critical to further examine biomarkers that can predict benefit from targeted therapy in order to better select patients in future combination clinical trials.

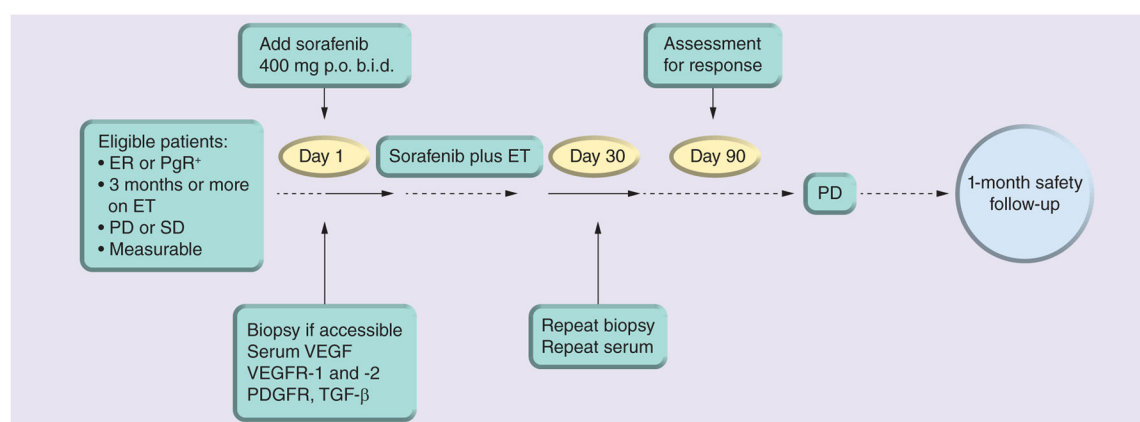


Figure 1. Clinical trial schema

Patients on ET for at least 3 months with PD or SD and measurable disease received sorafenib 400 mg b.i.d. daily and response was assessed after 3 months. Baseline serum and tumor biopsies, where applicable, were collected on study entry and after 28 days of sorafenib. Patients were followed until disease progression or unacceptable toxicity. b.i.d.: Twice daily; ER: Estrogen receptor; ET: Endocrine therapy; PD: Progressive disease; PDGFR: PDGF receptor; PgR⁺: Progesterone receptor positive; p.o.: Per os; SD: Stable disease; VEGFR: VEGF receptor.

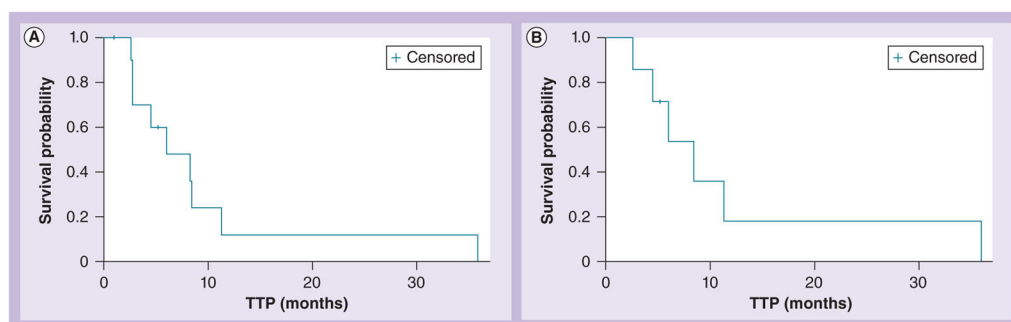


Figure 2. Kaplan–Meier survival graphs of median time to progression: product-limit survival estimates

(A) All patients in study ($n = 11$). Median TTP = 6.1 months (95% CI: 2.6–11.3). **(B)** Patients receiving tamoxifen on entry ($n = 7$). Median TTP = 8.4 months (95% CI: 2.6–36). TTP: Time to progression.

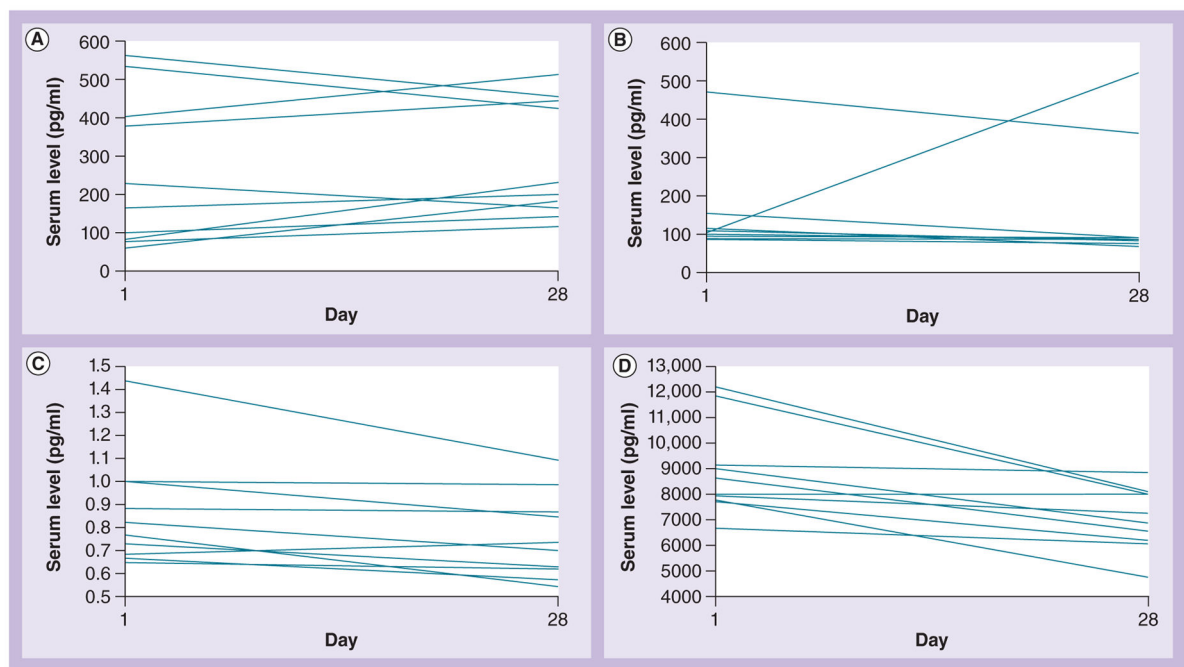


Figure 3. Comparison of serum biomarkers by ELISA in individual patient paired samples after adding sorafenib to endocrine therapy (day 1 vs 28)

Biomarkers shown are (A) VEGF, (B) human soluble VEGF receptor 1, (C) PDGF receptor- α and (D) VEGF receptor 2.

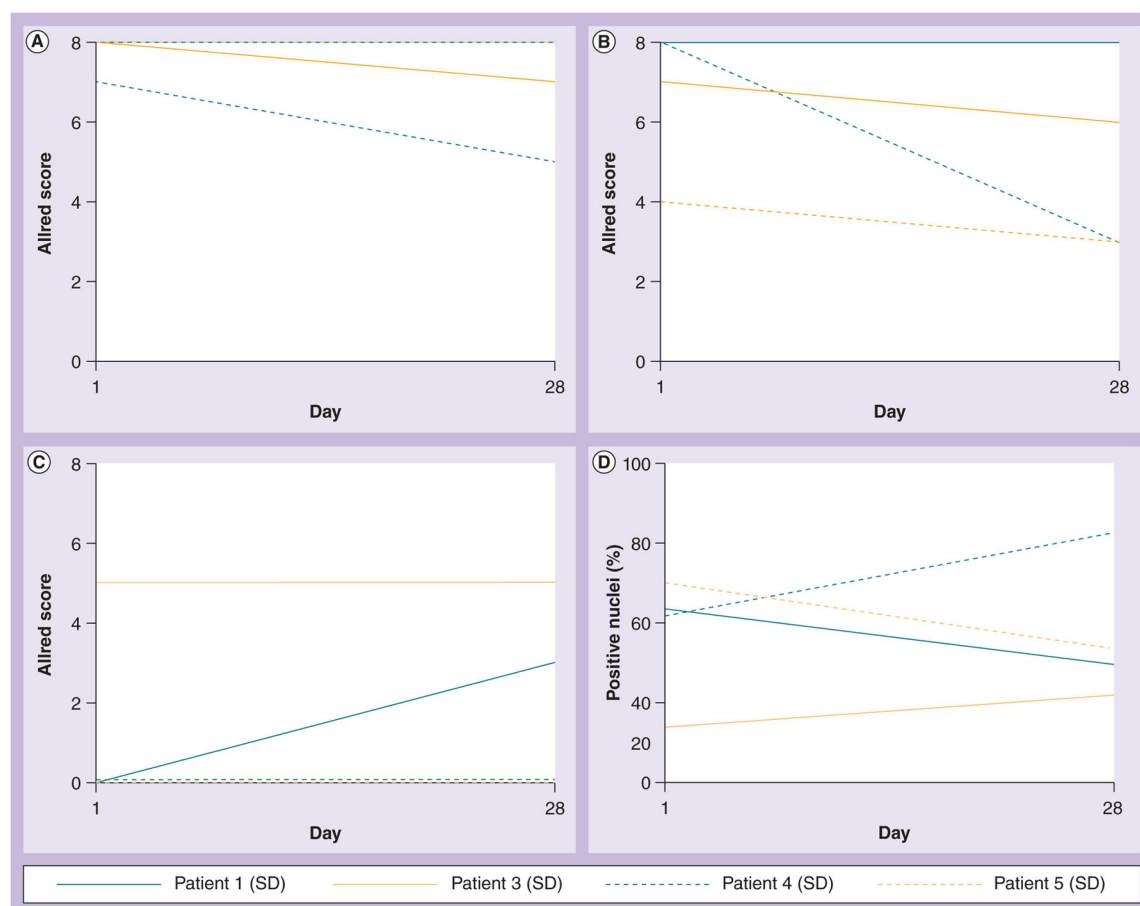


Figure 4. Comparison of immunohistochemistry markers from individual patient paired samples after adding sorafenib to endocrine therapy (day 1 vs 28)

Biomarkers shown are (A) estrogen receptor, (B) cyclin D1, (C) progesterone receptor and (D) Ki-67. Ki-67 is represented by the percentage of nuclear staining, while estrogen receptor, progesterone receptor and cyclin D1 were scored using the Allred method. Sample numbers correspond to patient numbers in Table 2.

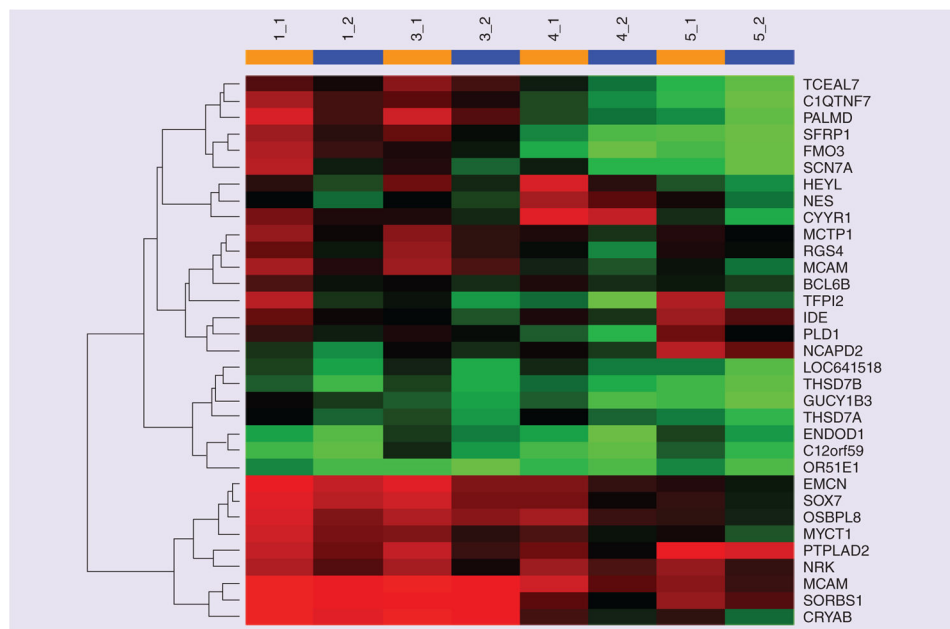


Figure 5. Gene expression heat map representing 33 probe sets for the top 32 genes with an FDR <0.2 and at least a twofold reduction from baseline

A list of the 32 genes is provided in **Supplementary Table 1**, see online at www.futuremedicine.com/doi/suppl/10.2217/fon.14.99. The captions at the top of the figure represent the sequence number of the patient from whom the sample was obtained and whether it was performed on day 1 or 28 (e.g., '1_1' is patient sequence 1 on day 1, '1_2' is patient sequence 1 on day 28, and so on). Patient sequence numbers correspond to the numbers from Table 2.

Table 1

Patient and disease characteristics (n = 11).

Characteristics	n (%)
Age	
Median, range	45, 39–72
<40	3 (27)
40–49	3 (27)
50–59	1 (9)
60–69	2 (18)
70	2 (18)
Sites of disease	
Locally advanced	5 (45)
Bone metastases	9 (82)
Lung metastases	5 (45)
Relapsed versus de novo metastasis	
<i>De novo</i>	7 (64)
Relapsed	4 (36)
Estrogen receptor status	
Positive	11 (100)
Negative	0
Progesterone receptor status	
Positive	9 (82)
Negative	2 (18)
HER2 status	
Negative	9 (82)
Unknown	2 (18)
Endocrine therapy at study entry	
Tamoxifen	7 (64)
Anastrozole	1 (9)
Letrozole	1 (9)
Exemestane	1 (9)
Fulvestrant	1 (9)
Line of current endocrine therapy	
First	9 (82)
Second	2 (18)
Disease status at entry	
Progressive disease	8 (73)
Stable disease with maximal response	3 (27)
Prior chemotherapy	
No	8 (73)

Characteristics	n (%)
Yes	3 (27)

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Table 2

Individual patient characteristics (n = 11).

Patient	Age (years)	Endocrine agent	Menopause	ER	PgR	HER2	Disease sites	Disease status	Best response	Biopsy	Reason off study	Days on therapy
1	44	TAM goserelin	Pre	+	+	-	Local, bone	PD	SD	Yes	PD: bone	339
2	72	TAM	Post	+	+	-	Local, bone	SD	SD	No	Pt choice	156
3	41	TAM	Pre	+	+	-	Local, bone	SD	SD	Yes	PD: peritoneum	182
4	45	TAM goserelin	Pre	+	+	-	Local, bone, lung	PD	PD	Yes	PD: local	79
5	63	Letrozole	Post	+	-	-	Local, bone, retro-orbital	PD	SD	Yes	PD: local	248
6	70	TAM	Post	+	+	-	Bone, lung	PD	SD	No	PD: bone	136
7	39	Anastrozole goserelin	Pre	+	+	-	Bone, lung	PD	PD	No	PD: lung, liver lesions	83
8	39	TAM goserelin	Pre	+	+	NA	Local, bone	PD	SD	No	PD: bone	252
9	66	Fulvestrant	Post	+	+	NA	Lung, pleura	PD	PD	No	PD: peritoneal	83
10	55	Exemestane	Post	+	-	-	Bone, LN	SD	NE	No	Toxicity grade 3 rash	NE, [‡]
11	39	TAM	Pre	+	+	-	Lung, pleural	PD	SD	No	PD: pleural disease, bone	1077

[‡]Stopped sorafenib after 2 weeks, day 28 serum not collected.

ER: Estrogen receptor; LN: Lymph node; NA: Not applicable; NE: Not evaluable; PD: Progressive disease; PgR: Progesterone receptor; Pt: Patient; SD: Stable disease; TAM: Tamoxifen.

Table 3

Adverse events (regardless of relation to study drug; n = 11).

Adverse event	Grade 1	Grade 2	Grade 3	Total (%)
Hypophosphatemia	8	1	2	11 (100)
Hypokalemia	9	0	1	10 (91)
Rash	3	5	1	9 (82)
Weight loss	6	2	1	9 (82)
Hypertension	1	5	0	6 (55)
Nausea/vomiting	6	0	0	6 (55)
Elevated ALT/AST	5	0	0	5 (45)
Anorexia	3	1	1	5 (45)
Diarrhea	1	3	0	4 (36)
Hypocalcemia	4	0	0	4 (36)
Fatigue	2	1	0	3 (27)
Mucositis	2	1	0	3 (27)
Leukopenia	1	2	0	3 (27)
Anemia	3	0	0	3 (27)
Thrombocytopenia	2	0	0	2 (18)
Colitis	0	0	1	1 (9)
Stevens–Johnson syndrome	0	1	0	1 (9)
Joint pain	1	0	0	1 (9)
Infection/Herpes zoster	0	1	0	1 (9)

ALT/AST: Aspartate aminotransferase–alanine aminotransferase ratio.

Table 4

Serum biomarkers by ELISA (in pg/ml).

Serum biomarker	Day 1 mean (median)	Day 28 mean (median)	Difference (day 28 - day 1) mean (median)	p-value
VEGF	261.38 (198.18)	289.56 (217.66)	28.13 (40.56)	0.3223 [†]
sVEGFR-1	142.29 (102.67)	150.94 (82.79)	8.65 (−30.48)	0.0840 [†]
VEGFR-2	8803.32 (8215.70)	6971.47 (6980.00)	−1831.85 (−1795.05)	0.0035
PDGFR-α	0.8639 (0.7948)	0.7576 (0.7156)	−0.1064 (−0.961)	0.0170

All p-values are based on the paired differences.

[†]Due to non-normality of the data, p-values are presented from the rank sum nonparametric test.

PDGFR-α: PDGF receptor-α; sVEGFR-1: Human soluble VEGF receptor 1; VEGFR-2: VEGF receptor 2.

Table 5

Enriched Kyoto Encyclopedia of Genes and Genomes pathways.

Name	Size	FDR q-value	Genes in leading edge (n) [†]
HSA05212_PANCREATIC_CANCER	73	0	45
HSA04350_TGF_BETA_SIGNALING_PATHWAY	87	0.019	80
HSA04110_CELL_CYCLE	104	0.021	92
HSA05210_COLORECTAL_CANCER	84	0.029	48
HSA04512_ECM_RECEPTOR_INTERACTION	87	0.033	53
HSA04510_FOCAL_ADHESION	191	0.038	168
HSA04320_DORSO_VENTRAL_AXIS_FORMATION	27	0.039	13
HSA00903_LIMONENE_AND_PINENE_DEGRADATION	26	0.035	20
HSA05222_SMALL_CELL_LUNG_CANCER	85	0.044	51
HSA04115_P53_SIGNALING_PATHWAY	65	0.048	51
HSA05220_CHRONIC_MYELOID_LEUKEMIA	74	0.066	70
HSA05211_RENAL_CELL_CARCINOMA	69	0.083	66
HSA04810_REGULATION_OF_ACTIN_CYTOSKELETON	200	0.112	104
HSA03030_DNA_POLYMERASE	24	0.108	15
HSA00190_OXIDATIVE_PHOSPHORYLATION	118	0.103	104
HSA05215_PROSTATE_CANCER	86	0.098	35
HSA05214_GLIOMA	61	0.13	27
HSA05219_BLADDER_CANCER	41	0.14	19
HSA00240_PYRIMIDINE_METABOLISM	84	0.136	42
HSA04360_AXON_GUIDANCE	127	0.142	110
HSA00051_FRUCTOSE_AND_MANNOSSE_METABOLISM	40	0.143	20
HSA04120_UBIQUITIN_MEDIATED_PROTEOLYSIS	39	0.146	33
HSA04150_MTOR_SIGNALING_PATHWAY	44	0.148	24
HSA05050_DENTATORUBROPALLIDOLUYSIAN_ATROPHY	15	0.155	12
HSA00632_BENZOATE_DEGRADATION_VIA_COA_LIGATION	24	0.152	17
HSA05221_ACUTE_MYELOID_LEUKEMIA	52	0.167	51
HSA00280_VALINE_LEUCINE_AND_ISOLEUCINE_DEGRADATION	44	0.164	28
HSA04520_ADHERENS_JUNCTION	75	0.173	71
HSA05218_MELANOMA	71	0.182	29

[†]The leading edge subset is the core of a gene set that accounts for the enrichment signal.