A Multicenter Phase II Study of Pazopanib in Patients with Unresectable Dermatofibrosarcoma Protuberans


To cite this version:


HAL Id: hal-03623658
https://amu.hal.science/hal-03623658

Submitted on 22 Mar 2023

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L’archive ouverte pluridisciplinaire HAL, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d’enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

Distributed under a Creative Commons Attribution - NonCommercial 4.0 International License
ORIGINAL ARTICLE

A multicenter phase II study of pazopanib in patients with unresectable dermatofibrosarcoma protuberans

Julie DELYON1 (0000-0002-4557-3377), Raphael PORCHER2 (0000-0002-5277-4679), Maxime BATTISTELLA3 (0000-0002-7053-7431), Nicolas MEYER4 (0000-0003-4519-8666), Henri ADAMSKI5 (0000-0001-9117-9306), François BERTUCCI6 (0000-0002-0157-0959), Bernard GUILLOT7 (0000-0001-6139-4136), Thomas JOUARY8 (0000-0002-9080-3661), Marie-Thérèse LECCIA9 (0000-0003-1222-0396), Sophie DALAC10 (0000-0002-9101-3119), Laurent MORTIER11 (0000-0002-2644-1790), Zineb GHRIEB12 (0000-0002-7471-4271), Laetitia DA MEDA13 (0000-0003-2988-6792), Eric VICAUT14 (0000-0001-6303-8557), Florence PEDEUTOUR15 (0000-0002-9072-5472), Samia MOURAH16 (0000-0002-9283-1068), Celeste LEBBE1 (0000-0002-5854-7290)

1 Université de Paris, INSERM U976, Team 1, HIPI, F-75010, Paris, France; AP-HP Hôpital Saint-Louis, Department of Dermatology, F-75010 Paris, France
2 Université de Paris, CRESS-UMR1153, INSERM, INRA, F-75004 Paris, France; Centre d’Épidémiologie Clinique, Hôpital Hôtel-Dieu, AP-HP, F-75004 Paris, France.
3 Université de Paris, INSERM U976, Paris, France; Pathology department, Hopital Saint-Louis, AP-HP, Paris, France
4 Dermatologie, Institut Universitaire du Cancer et CHU de Toulouse, France; INSERM UMR 1037-CRCT, Toulouse, France
5 Department of Dermatology, Rennes University Hospital, Rennes, France
6 Department of Medical Oncology, Institut Paoli-Calmettes, Marseille, France
7 Montpellier University Hospital F34095 Montpellier, France

© 2020 published by Elsevier. This manuscript is made available under the CC BY NC user license https://creativecommons.org/licenses/by-nc/4.0/
8 Department of Dermatology, Hôpital François Mitterrand, 4 Bd hauterive, 64000 Pau, France

9 Department of Dermatology, Grenoble University Hospital, Grenoble, France

10 Department of Dermatology, CHU Dijon Bourgogne, Dijon, France

11 University of Lille, INSERM U1189, Department of Dermatology, CHU Lille, 59000 Lille, France.

12 Centre d’Investigations Cliniques (CIC-1427), AP-HP Hôpital Saint Louis, F-75010 Paris, France

13 AP-HP, Hôpital Saint-Louis, Service de Dermatologie, Paris, France

14 Unité de Recherche Clinique, Hôpital Fernand Widal, Paris, France

15 Laboratory of Solid Tumors Genetics, Institute for Research on Cancer and Aging of Nice (IRCAN), CNRS UMR 7284/INSERM U1081 and Nice University Hospital affiliated to Nice University Côte d'Azur, Nice, France

16 Université de Paris; Assistance Publique-Hôpitaux de Paris, Hôpital Saint-Louis, Laboratoire de Pharmacogénomique, INSERM U976 Paris, France

**Corresponding author:** Dr Julie DELYON; Dermatology department, Hôpital Saint-Louis; 1 avenue C. Vellefaux, 75475 Paris cedex 10; Julie.delyon@aphp.fr

**Funding sources:** grant from Novartis to support the study overcost

**Promoter:** Assistance Publique-Hôpitaux de Paris (APHP)

**Keywords:** DFSP; dermatofibrosarcoma protuberans; pazopanib
ABSTRACT

Dermatofibrosarcoma protuberans (DFSP) is a soft-tissue sarcoma characterized by a high risk of local infiltration. The identification of the *COL1A1-PDGFB* t(17;22) translocation activating the PDGF pathway led to the use of imatinib in unresectable DFSP, with a response rate of 36-80%. Pazopanib is a multitarget tyrosine kinase inhibitor approved for soft tissue sarcomas. We conducted a phase II study of patients with unresectable DFSP to evaluate the efficacy and safety of pazopanib. Patients received 800 mg pazopanib daily. The primary endpoint was the objective response rate defined as the reduction of the largest diameter of the tumor ≥30% at 6 months or at surgery. Twenty-three patients, including one pre-treated with imatinib, were enrolled. With a median follow-up of 6.2 months (interquartile range 5.6-7.8), 5 patients (22%, 95%CI: 7-22%) had a partial response to pazopanib. The best objective response rate was 30% (95%CI 13-53%) using RECIST. One patient with metastatic DFSP previously treated with imatinib died after 2.4 months. Nine (39%) patients discontinued the treatment due to adverse events. Pharmacodynamics analyses of tumor samples were conducted: the enrichment of EGF and the EGFR-associated gene panel was associated with resistance, suggesting that EGFR-targeted therapies could be a therapeutic option to explore in DFSP.

**Trial registration:** ClinicalTrials.gov identifier: NCT01059656.
INTRODUCTION

Dermatofibrosarcoma protuberans (DFSP) is a rare tumor accounting for 6% of soft-tissue sarcomas. DFSP is characterized by a slow growth rate and a low metastatic potential but is at risk of local infiltration and recurrence. Wide surgical excision is the standard of care, sometimes with multiple procedures required to obtain complete resection (Fiore et al. 2005). However, the management of unresectable or metastatic DFSP remains challenging. Moreover, DFSP with fibrosarcomatous transformation, which accounts for 5-15% of DFSP, is associated with an increased risk of metastases and a worse prognosis (Liang et al. 2014; Rutkowski et al. 2017).

DFSP biology is characterized in most cases by rearrangement of chromosomes 17 and 22, involving the PDGFβ (platelet-derived growth factor β) gene on chromosome 22, which is fused with the COL1A1 (collagen 1 α1) gene on chromosome 17 (Dadone-Montaudié et al. 2018). The COL1A1-PDGFB fusion is transcriptionally upregulated and constitutively activates PDGF receptor β and its downstream signaling pathway (Giacchero et al. 2010; Greco et al. 1998; Simon et al. 1997). Thus, the first tyrosine kinase receptor (TKR) inhibitor approved in unresectable DFSP was imatinib, which targets PDGFR among other TKRs. This drug has shown efficacy in advanced DFSP with a response rate of 36-90%, and a progression-free survival (PFS) ranging from 11 months to 1.7 years (McArthur et al. 2005; Rutkowski et al. 2010; Stacchiotti et al. 2016b), including in the neoadjuvant setting (Kérob et al. 2010; Ugurel et al. 2014). However, imatinib does not provide sufficient tumor regression in some patients, and secondary resistance may develop (Rutkowski et al. 2010; Stacchiotti et al. 2016b), underlying the need for alternative therapeutic strategies (Fu et al. 2015; Kamar et al. 2013).

Angiogenesis plays a critical role in tumor growth, invasion and metastasis. In soft-tissue sarcoma, the interaction between VEGF and its main receptor VEGFR-2 promotes
tumor progression and is associated with more advanced tumor grades and worse prognosis (Chao et al. 2001; Iyoda et al. 2001). In DFSP, in addition to PDGFR, NRPI, a coreceptor of VEGFR-2, was significantly overexpressed (Baird et al. 2005), and we observed that VEGFR2 was overexpressed at the protein level in a series of 14 DFSP tumors (unpublished data). These results suggest that targeting VEGFR2 could be an alternative therapeutic approach in DFSP.

Pazopanib, a TKR inhibitor, exerts antiangiogenic effects by inhibiting VEGF receptors with high affinity (VEGFR-1/2/3). Several lines of data led us to hypothesize that pazopanib could be an effective therapeutic option in DFSP. First, in addition to VEGFR, pazopanib targets PDGFR, the main driver of DFSP biology, as well as FGFR and c-KIT (Sloan and Scheinfeld 2008). Second, pazopanib was shown to be efficacious in other advanced soft tissue sarcomas in a phase III trial, with a PFS of 4.6 months (vs 1.5 months in the placebo arm) (van der Graaf et al. 2012; Sleijfer et al. 2009) and is now approved as a second line treatment. More recently, pazopanib showed efficacy in advanced solitary fibrous tumors, a rare subset of soft-tissue sarcomas with sensitivity to antiangiogenic drugs (Martin-Broto et al. 2019). Finally, pazopanib was investigated in gastrointestinal stromal tumors (GIST), which, similar to DFSP, are treated with imatinib as first-line therapy. Pazopanib showed efficacy in patients with GIST with prior resistance to imatinib. The PFS with pazopanib was 3.4 months vs. 2.3 months for the best supportive care (Mir et al. 2016).

Recently, anecdotal cases suggested that pazopanib might be effective in DFSP (Miyagawa et al. 2017), although no prospective studies have been reported.

We conducted a phase II trial of pazopanib in the treatment of unresectable DFSP designed to assess the efficacy of pazopanib in DFSP and to identify biomarkers associated with response.
RESULTS

Patient characteristics

Between July 2010 and February 2014, 23 patients with unresectable DFSP were enrolled from nine centers in France. Table 1 shows the patient and tumor clinicopathological characteristics. Eighteen patients had primary DFSP, 4 had recurrent DFSP and 1 had metastatic DFSP. Four patients had previous surgery, and one patient had previously been treated with imatinib. A fibrosarcomatous transformation was detected in 6 patients (26%). The presence of the \textit{COL1A1-PDGFB} gene fusion was found in 18 patients (not evaluable in 5 patients). All cases of fibrosarcomatous DFSP harbored the \textit{COL1A1-PDGFB} fusion.

Efficacy

The median follow-up was 6.2 months (interquartile range, IQR, 5.6-7.8 months). At the final analysis, 5 patients (22%, 95% CI: 7-22%) had partial response (PR) following the primary outcome (reduction of the largest diameter of the tumor $\geq 30\%$ at 6 months or at surgery if performed before 6 months). Based on the standard RECIST criteria, 2 patients had PR (9%) and 12 had stable disease (SD, 55%) at 6 months or surgery, and the BORR until 6 months or surgery was 30% (7 patients, 95% CI: 13-53%) (figure 1A). Based on the WHO criteria, 4 patients (18%) had PR at 6 months or surgery; the WHO-criteria BORR until 6 months or surgery was 45% (10 patients) (figure 1B).

The only patient with metastatic DFSP, who was the only patient in the study previously treated with imatinib, did not respond to pazopanib and died after 2.4 months of treatment.

Finally, 18 patients (78%) had surgery, at a median time of 6.5 months from starting pazopanib (IQR, 5.4-7.8 months), with free pathological margins obtained for 12 patients (67%). The 6 patients with incomplete surgery underwent additional surgery. Overall, 14 out
of 18 patients with surgery finally had complete resection with free margins (61% of the total population).

Among patients with fibrosarcomatous DFSP (N=6), 2 patients had PR at 6 months or at surgery following the primary outcome, and 4 patients had PR as best response following standard RECIST criteria. Finally, 4 patients with fibrosarcomatous DFSP underwent surgery, 2 of whom had complete resection and 2 of whom requiring adjuvant radiotherapy.

**Safety and quality of life**

The median duration of pazopanib treatment was 3.8 months (IQR 2.1-6.0 months). Treatment was discontinued because of adverse events (N=9) or progression (N=7). All 23 patients (100%) experienced grade 2 or more clinical or biological adverse events, and 17 (74%) experienced grade 3-4 adverse events (table 2). Gastrointestinal disorders, hypertension, fatigue and transaminitis were the most frequently reported events. Grade 3-4 adverse events were transaminitis (6; 26%), cholestasis (3; 13%), hemolytic and uremic syndrome (one grade 4), and nephrotic syndrome (one grade 4). No drug-related death occurred. Nine (39%) patients discontinued pazopanib for toxicity because of transaminitis (N=4), renal toxicity (N=2), neutropenia (N=1), hypertension (N=1) and diarrhea (N=1). Adverse events led to reduced dosing or temporary drug withdrawal for 11 (48%) and 6 (26%) patients, respectively.

Significant decreases from baseline to 6 months of assessment were found for global quality of life, function and symptoms (P=0.012, after Holm’s correction for multiple testing). In particular, the global quality of life score decreased from a mean 66.7 (SD 29.8) at baseline to a median 57.4 (SD 31.7) at 6 months. Symptoms related to adverse events such as fatigue, nausea, diarrhea and appetite loss were more frequent upon treatment (figure S1).
Signaling pathways targeted by pazopanib

We focused on VEGFR-2 as a main target of pazopanib. First, we studied the plasma level of soluble VEGFR-2 (sVEGFR-2), which was previously shown to be a biomarker modified during treatment with antiangiogenic therapy (Llovet et al. 2012; Peña et al. 2010). Among the 23 patients tested, those with clinical benefit from pazopanib (PR) had significantly higher plasma levels of sVEGFR-2 at baseline than patients with SD or PD as best response ($P=0.04$) (figure S2a). However, the $VEGFR-2$ mRNA expression in tumor specimens did not significantly vary from baseline to 6 months or between the responders (PR) and the nonresponders (SD or PD as best response) in matched tumor samples ($N=5$, Figure S2b). This finding suggests that VEGFR-2 was correctly targeted by pazopanib in patients, as its soluble level was associated with the tumor response but without modifying its expression within the tumor. $PDGFRB$ mRNA expression was not modified during treatment between the responders and the non-responders ($N=5$, figure S3).

As the genotypes of VEGF receptors have been reported to be associated with sVEGFR-2 levels or with responses to tyrosine kinase inhibitors, including pazopanib (Beuselinck et al. 2016; Maitland et al. 2015), we studied the VEGFR-1 (rs9582036) and VEGFR-2 (rs34231037) genotypes. No association between the VEGFR-1 genotype and response to pazopanib was found; only one patient had a VEGFR-2 mutation (AG genotype).

Biomarkers of response to pazopanib and gene expression profiling

To gain insight into the biological activity of pazopanib in DFSP, we measured the mRNA expression of 302 genes involved in pathways related to VEGFR signaling, the cell cycle, and apoptosis in tumor samples using qPCR arrays. Sixteen patients had baseline and follow-up tumor samples available ($N=11$, baseline and month 1; $N=5$, baseline, month 1 and month 6). Patients were classified as responders or nonresponders (PR or SD+PD as best response,
respectively). No difference in gene expression was observed at baseline between the responders and the nonresponders that could not have been obtained by chance (figure 2A). Gene set enrichment analysis (GSEA) was then performed by testing the enrichment of 35 sub-pathways involving at least 3 genes. The nonresponder baseline samples had the highest score enrichment for 4 gene signatures: EGF and receptors, interferon/interleukin, angiogenic factors and positive regulators of apoptosis, while the TIMP and AKT gene signatures were higher in the responder samples than in the nonresponder samples. The enrichment of EGF and the EGFR-associated gene panel was significantly increased in the nonresponder samples ($P=0.035$) (figure 2B). We thus focused on the EGFR pathway components during treatment with pazopanib. After one month of pazopanib exposure, no difference was found in the variation of the mRNA levels of EGFR, ERBB2 and EGF between the responders ($N=10$) and the nonresponders ($N=5$) (figure 2C).

**Cell cycle analysis and response to pazopanib**

We studied the role of the p16/cyclin D-CDK4 pathway in tumors, as this pathway is known to be involved in DFSP progression (Eilers et al. 2015; Park et al. 2018; Siref et al. 2018; Stacchiotti et al. 2016a). We focused on the expression of cell cycle regulators in the tumor samples. At baseline, the mRNA level of $CDKN2A$ (coding for p16) ($N=16$) did not vary significantly between the responders and the non-responders, and was highly variable among the patients. Immunohistochemistry (IHC) showed that p16 expression was lost at baseline in 1 patient with SD and remained expressed in 3 patients with PR (figure 3A, table S1).

The variation in the expression of CDK4, a cyclin-dependent kinase promoting cell-cycle progression targeted by p16, was significantly reduced in the responders as compared to the nonresponders patients during pazopanib treatment ($P=0.02$, Wilcoxon test). The mRNA expression of CDK1 and cyclin D1 was reduced as well, but not significantly (figure 3B).
High cyclin D1 expression has been associated with worse outcomes in DFSP (Park et al. 2018). In our study, four patients had matched baseline and follow-up (month 1) tumor samples available for IHC (2 patients with SD and 2 patients with PR as best response). The expression of cyclin D1 was significantly decreased from baseline to month 1 in one patient with PR, while it remained stable in the other 3 patients (figure 3C, table S1). Altogether, these results suggested the involvement of the p16/cyclin D1-CDK4 pathway in the tumor response to pazopanib, and the decreased cyclin D1 and CDK4 expression during pazopanib exposure was associated with tumor response.
DISCUSSION

In our study, pazopanib induced tumor responses in 22% of the patients with unresectable DFSP. Most patients underwent first-line treatment for a non fibrosarcomatous DFSP.

Usually, advanced DFSP is treated with imatinib as the first-line therapy, which has proven efficacy in 36% to 90% of patients depending on the setting (neoadjuvant or advanced) (Kérob et al. 2010; McArthur et al. 2005; Rutkowski et al. 2010; Stacchiotti et al. 2016b; Ugurel et al. 2014). In a recent meta-analysis, the response rate was 60% (Navarrete-Dechent et al. 2019), highlighting the need for alternative treatments for patients with primary or acquired resistance to imatinib. In light of our study, the response rate to pazopanib first line in DFSP is deceptive. Notably, 48% of the patients had a dose reduction, and 39% had permanent dose interruption for adverse events. This rate was higher than in previous studies of pazopanib, in which 6% to 17% of patients had permanent discontinuation for toxicity (van der Graaf et al. 2012; Mir et al. 2016; Samuels et al. 2017; Sleijfer et al. 2009), probably because the acceptability of toxicity is higher in patients with metastatic disease. For several TKR inhibitors, the plasma concentration, which is related to the dose, has been associated with tumor response (Mir et al. 2016), suggesting that the efficacy of pazopanib might have been decreased by a low exposure at the effective dose in our study. However, the clinical benefit of pazopanib was significant in our study, as 18 patients (78%) finally underwent surgery, and 14 patients (61%) obtained complete resection with free pathological margins at the end of the study. A reduction in tumor size below the threshold of partial response was probably sufficient for these tumors to become resectable after treatment with pazopanib.

We performed a large analysis of mRNA expression to identify pathways involved in the response to pazopanib. While the mRNA expression of VEGF receptors, the main pazopanib target, was not modified during treatment with pazopanib between the responders and the nonresponders, the plasma protein level of sVEGFR2 was higher in the responders. In
a study conducted in soft tissue sarcoma, patients with clinical benefit from pazopanib had a lower baseline plasma VEGF than those who did not (Glade Bender et al. 2013), which is consistent with our results, as sVEGFR-2 binds its ligand the VEGF, thus preventing the activation of VEGFR-2 by VEGF on tumor cells. As the tumor response was correlated with sVEGFR2 expression, we concluded that the angiogenic pathway was more prevalent in the DFSP tumors of the responders than the nonresponders and was possibly involved in the tumor response induced by pazopanib.

EGFR signaling has been associated with the progression and transformation of DFSP (Osio et al. 2018). In the present study, among 6 patients with fibrosarcomatous DFSP, 4 had partial response as best response. Moreover, the overexpression of transcripts involved in the EGF/EGFR pathway at baseline was associated with resistance to pazopanib. Ugurel et al. observed a significant activation of EGFR in 100% of the DFSPs tested before treatment with imatinib (Ugurel et al. 2014). EGFR and pazopanib targets (VEGFR, PDGFR) share the same downstream signaling pathways, involving the mitogen-activated protein kinases and the phosphoinositide 3-kinase pathways. In our study, an increase in baseline EGFR signaling was associated with resistance to pazopanib. Altogether, these data suggest that EGFR-targeted therapies could be an interesting option to explore in DFSP.

Pharmacodynamics analyses also showed that the p16/cyclin D-CDK4 pathway was associated with tumor response. In DFSP, the deletion of CDKN2A and the resulting loss of P16 expression was identified in a significant proportion of DFSPs, including fibrosarcomatous DFSP, and was shown to contribute to the progression of DFSP (Eilers et al. 2015; Siref et al. 2018; Stacchiotti et al. 2016a). Although the number of samples was low, our results were consistent with the p16 loss found in one patient without clinical benefit from pazopanib and the remaining expression in 3 patients with response. Eilers et al. developed a rationale for targeting CD4/CDK6 in imatinib-resistant DFSP (Eilers et al. 2015), and we
observed a strong inhibition of CDK4 expression in the patients with clinical benefit from pazopanib, both suggesting that targeting the CDK4/CDK6 axis could be a therapeutic alternative that deserves further investigation.

Considering the rarity of this disease, we had to conduct our study in a large population that finally included locally advanced, metastatic and fibrosarcomatous DFSP. Resistance to imatinib was too rare to be retained as an inclusion criterion. Our results are not in favor of using pazopanib as a first-line therapy considering the response rate and the toxicity profile, but its place in the armamentarium against DFSP, particularly in resistance to imatinib, will remain to be defined.

In conclusion, our results suggested that pazopanib is a therapeutic option in DFSP, but with a relatively poor tolerability and low response rate compared to those in previous studies with imatinib. The involvement of the EGFR signaling pathway suggests an additional therapeutic strategy that deserves further investigation.
MATERIAL AND METHODS

Patients

We conducted an open-label phase II multicenter trial in 9 cancer centers or university hospitals in France (ClinicalTrials.gov: NCT01059656). The inclusion criteria were as follows: patients with histologically proven, unresectable DFSP, which was either primary, either locally recurrent or metastatic, and measurable according to the response evaluation criteria in solid tumors (RECIST) version 1.1; ECOG (Eastern Cooperative Oncology Group) performance status ≤1; adequate hematologic, hepatic and renal functions; and left ventricular ejection fraction within the local normal ranges (>45%). The unresectable stage of DFSP had to be assessed by a multidisciplinary team expert in sarcoma management prior to enrollment.

The presence of the COL1A1-PDGFB fusion was assessed at baseline using fluorescence in situ hybridization. Patients with fibrosarcomatous DFSP were included if molecularly confirmed by the detection of the t(17;22) translocation. Patients previously treated with surgery, radiotherapy or imatinib could be included. Exclusion criteria were patients with concomitant active cancers; other anticancer treatment within 4 weeks before enrollment; severe gastrointestinal disorders that might interfere with treatment absorption; any hemorrhagic risk or predispositions; uncontrolled hypertension; history of cardiovascular events in the last 6 months; and expected poor compliance to treatment. The study protocol was approved by French Ethics Committee. All patients provided written informed consent before enrollment.

Study design

Patients received 800 mg of pazopanib orally once daily. Dose reduction to 600 mg or 400 mg once daily was allowed in case of toxicity in accordance with good clinical practice and defined in the protocol (i.e., recurrent grade 2 or any grade 3 toxicity that resolved to grade
Study treatment was continued until disease progression or occurrence of unacceptable adverse events. The tumor response was assessed by investigators based on physical examination at months 1, 3 and 6, and the Physician Global Assessment tool (PGA). Patients were followed up for safety through monthly clinical and biological data. Adverse events were assessed and graded according to the Common Terminology Criteria Adverse Events (CTCAE) version 4. Quality of life was assessed using the EORT QLQ-C30.

Study endpoints
The primary endpoint was the objective response rate, obtained from a physical examination following the RECIST requirements of the tumor at 6 months or at surgery if performed before 6 months. PR was defined as a reduction of the largest diameter of the tumor ≥ 30% from baseline, as with standard RECIST criteria, but it did not require confirmation by a follow-up assessment 4 weeks later (to avoid an additional delay before surgery if some tumors became resectable).

Secondary endpoints were as follows:

- Other parameters of efficacy assessed by physical examination according to standard RECIST criteria version 1.1 (Eisenhauer et al. 2009) and WHO criteria (WHO 1979): objective response rate at 6 months or surgery, i.e. proportion of patients still considered as in complete response (CR) or partial response (PR) at 6 month or at time of surgery, and best overall response rate (BORR), defined as the best response recorded from the start of the study treatment until 6 months or surgery;
- Safety;
- Quality of life as evaluated by the European Organization for Research and Treatment of Cancer QLQ-C30 version 3.0 questionnaire at 1, 3 and 6 months;
- Correlation of tumor response with molecular analyses.
Pharmacodynamics analyses

Molecular analyses were performed on tumor biopsies and blood samples at baseline, months 1, 3 and 6 or at progression. In tumor samples, total mRNA was isolated using TRIzol reagent (Thermo Fisher Scientific, Waltham, USA) according to the manufacturer’s protocol. mRNA expression was measured using the SignArrays® 384 system (Anygenes, France), including 302 genes involved in angiogenesis, the cell cycle, apoptosis, migration and senescence, such as PDGF-A/B, PDGFRα/β, VEGF, and VEGFR-1, 2, 3 and their downstream signaling pathway components. The protein expression levels of cell-cycle components were assessed using IHC. Formalin-fixed paraffin-embedded (FFPE) tumor tissue samples were analyzed by central pathologic review. IHC staining was performed on a Benchmark Ultra automatic system (Roche Ventana, United States), with the anti-cyclin D1 (Clinisciences, France), anti-p16 (Roche Ventana, United States) and anti-RB1 (Abcam, United Kingdom) antibodies, and revealed with the OptiView kit (Roche Ventana, United States). In plasma, soluble VEGFR-2 (sVEGFR-2) was measured at baseline using a human sVEGFR-2/KDR enzyme-linked immunosorbent assay (ELISA) Quantikine kit (R&D Systems, France). Single SNP genotyping of VEGFR-1 (rs9582036) and VEGFR-2 (rs34231037) was performed using commercially available TaqMan® assays (Thermo Fisher Scientific, Illkirch, France).

Statistical methods

All patients enrolled in the trial were included in the primary analysis, and death was considered nonresponse. The study was designed according to an exact single stage phase II design (A’Hern 2001) and powered to detect an increase in the response rate from 20% to 50% at a one-sided 0.025 significance level with 90% power. Accordingly, the null hypothesis would be rejected if 10 or more responses would be observed out of 26 patients.
Due to difficulties in accrual, the trial was stopped after enrollment of 23 patients before any data analysis, which lowered the power to 80%.

Gene mRNA expression was compared to the best response (CR+PR, responders, vs. SD+PD, nonresponders) using Student’s t-tests; p-values were corrected to control the false discovery rate (FDR) over the multiple analyses were computed (Benjamini and Hochberg 1995). In addition, gene set enrichment analysis (GSEA) was used to interpret gene expression data (Subramanian et al. 2005).

All analyses used R statistical software version 3.3.3 (The R Foundation for Statistical Computing, Vienna, Austria).

**DATA AVAILABILITY**

The gene expression data discussed in this article have been deposited in NCBI's Gene Expression Omnibus and are accessible through GEO Series accession number GSE150787 (https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE150787).

The study protocol including the statistical analysis plan will be shared upon request. The initial protocol did not include the possibility of sharing data of participants with researchers not involved in the study. However, access to individual patient data that underline the results reported in this article, after deidentification, can be granted upon reasonable request. A protocol should then be submitted to the corresponding author and will be transmitted to the principal investigator of the trial to be examined by the trial scientific committee. After evaluation of the scientific and medical content of the protocol, if access is granted, ethical clearance will be sought from the ethical review board (CPP) by the principal investigator.
Disclosure of potential conflict of interest:

- MB received honoraria from BMS, Leo Pharma, Takeda, Innate Pharma, outside the submitted work.
- CL Avantis Medical Systems (board); received research grants or honoraria from Roche, BMS, MSD, GSK, Novartis, Amgen, Pierre-Fabre, Pfizer, Incyte, Roche, Merck Serono, Sanofi, and travel accommodations (Roche, BMS, MSD), outside the submitted work.
- JD: travel accommodations (Pierre Fabre, Roche)
- FP reports grants from Novartis Pharma, grants from Merck Serono, grants from Roche, personal fees from Astra Zeneca, outside the submitted work.
- SM received research grants or honoraria from Roche, Janssen, BMS, Biocartis, Novartis, outside the submitted work.
- NM received research grants and/or honoraria and/or travel accommodation from Roche, Novartis, GSK, BMS, MSD, Amgen, Merck GmBH, Incyte, Pierre Fabre, Sun pharma, Abbvie, Sanofi, outside the submitted work.
- LM reports travel for congress: BMS, Roche, Novartis, MSD
- EV: Abbott, Astra Zeneca, Bayer Health Care, Bristol Myers Squibb, Daiichi-Sankyo, Pfizer
- no COI to declare: RP, HA, LDM, MTL, SD, TJ, ZG.

Acknowledgments

The authors wish to thank Novartis to support the study over costs.

Author contribution – CrediT statement

Conceptualization: CL, RP, SM

Formal analysis: RP, SM, MB, FP

Investigation: NM, HA, FB, BG, TJ, MTL, SD, LM, CL
Methodology: RP

Project administration: ZG, LDM, EV

Supervision: CL

Writing – original draft preparation: JD

Writing – review and editing: JD, CL, SM, RP
REFERENCES


1998;17(10):1313–9


Peña C, Latham C, Shan M, Escudier B, Bukowski RM. Biomarkers predicting outcome in patients with advanced renal cell carcinoma: Results from sorafenib phase III Treatment


### Table 1: Patients and tumor characteristics at baseline

<table>
<thead>
<tr>
<th>Patients characteristics</th>
<th>No. (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Sex</strong></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>16 (70)</td>
</tr>
<tr>
<td>Female</td>
<td>7 (30)</td>
</tr>
<tr>
<td><strong>Age (median (min, max), years)</strong></td>
<td>48 (28; 77)</td>
</tr>
<tr>
<td><strong>ECOG</strong></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>21 (91)</td>
</tr>
<tr>
<td>1</td>
<td>2 (9)</td>
</tr>
<tr>
<td><strong>Disease stage</strong></td>
<td></td>
</tr>
<tr>
<td>Primary site</td>
<td>18 (78)</td>
</tr>
<tr>
<td>Local recurrence</td>
<td>4 (17)</td>
</tr>
<tr>
<td>Metastatic disease</td>
<td>1 (4)</td>
</tr>
<tr>
<td>Fibrosarcomatous DFSP</td>
<td>6 (26)</td>
</tr>
<tr>
<td>Unresectable DFSP</td>
<td>23 (100)</td>
</tr>
<tr>
<td><strong>Location of primary tumor</strong></td>
<td></td>
</tr>
<tr>
<td>Trunk</td>
<td>12 (52)</td>
</tr>
<tr>
<td>Head or neck</td>
<td>8 (35)</td>
</tr>
<tr>
<td>Upper limbs</td>
<td>2 (9)</td>
</tr>
<tr>
<td>Lower limbs</td>
<td>1 (4)</td>
</tr>
<tr>
<td><strong>Longest diameter (median (min; max), cm)</strong></td>
<td>6.5 (1.2; 16)</td>
</tr>
<tr>
<td><strong>Clinical presentation</strong></td>
<td></td>
</tr>
<tr>
<td>Plaque</td>
<td>3 (13)</td>
</tr>
<tr>
<td>Nodule</td>
<td>11 (48)</td>
</tr>
<tr>
<td>Plaque + nodule</td>
<td>4 (17)</td>
</tr>
<tr>
<td>Other</td>
<td>4 (17)</td>
</tr>
<tr>
<td>NA</td>
<td>1</td>
</tr>
<tr>
<td><strong>Prior treatment</strong></td>
<td></td>
</tr>
<tr>
<td>Surgery</td>
<td>4</td>
</tr>
<tr>
<td>Radiotherapy</td>
<td>0</td>
</tr>
<tr>
<td>Imatinib</td>
<td>1</td>
</tr>
</tbody>
</table>

NA, not available
<table>
<thead>
<tr>
<th>Adverse events</th>
<th>Total No. (%)</th>
<th>Grade 2 No. (%)</th>
<th>Grade 3-4 No. (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gastrointestinal</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>- Diarrhea</td>
<td>11 (48)</td>
<td>6 (26)</td>
<td>0</td>
</tr>
<tr>
<td>- Abdominal pain</td>
<td>8 (35)</td>
<td>7 (30)</td>
<td>0</td>
</tr>
<tr>
<td>- Dysguesia</td>
<td>3 (13)</td>
<td>2 (9)</td>
<td>0</td>
</tr>
<tr>
<td>- Vomiting</td>
<td>2 (9)</td>
<td>2 (9)</td>
<td>0</td>
</tr>
<tr>
<td>- Anorexia</td>
<td>2 (9)</td>
<td>1 (4)</td>
<td>0</td>
</tr>
<tr>
<td>- Constipation</td>
<td>1 (4)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Cutaneous</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>- Hair depigmentation</td>
<td>10 (43)</td>
<td>1 (4)</td>
<td>0</td>
</tr>
<tr>
<td>- Cutaneous rash</td>
<td>5 (22)</td>
<td>2 (9)</td>
<td>0</td>
</tr>
<tr>
<td>- Alopecia</td>
<td>4 (17)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>- Hand-foot syndrome</td>
<td>3 (13)</td>
<td>3 (13)</td>
<td>0</td>
</tr>
<tr>
<td>Renal</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>- Hemolytic and uremic syndrome</td>
<td>1 (4)</td>
<td>0</td>
<td>1 (4)</td>
</tr>
<tr>
<td>- Nephrotic syndrome</td>
<td>1 (4)</td>
<td>0</td>
<td>1 (4)</td>
</tr>
<tr>
<td>- Creatinine increased</td>
<td>4 (17)</td>
<td>0</td>
<td>1 (4)</td>
</tr>
<tr>
<td>Liver</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>- Transaminitis</td>
<td>19 (83)</td>
<td>3 (13)</td>
<td>6 (26)</td>
</tr>
<tr>
<td>- Cholestasis</td>
<td>12 (52)</td>
<td>2 (9)</td>
<td>3 (13)</td>
</tr>
<tr>
<td>Haematological</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>- Platelet count decrease</td>
<td>11 (48)</td>
<td>1 (4)</td>
<td>0</td>
</tr>
<tr>
<td>- Neutrophil count decrease</td>
<td>6 (26)</td>
<td>1 (4)</td>
<td>2 (8)</td>
</tr>
<tr>
<td>Other</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>- Fatigue</td>
<td>10 (43)</td>
<td>4 (17)</td>
<td>0</td>
</tr>
<tr>
<td>- Hypertension</td>
<td>20 (87)</td>
<td>7 (30)</td>
<td>0</td>
</tr>
<tr>
<td>- Headache</td>
<td>2 (9)</td>
<td>1 (4)</td>
<td>0</td>
</tr>
<tr>
<td>- Arthralgia</td>
<td>2 (9)</td>
<td>2 (9)</td>
<td>0</td>
</tr>
<tr>
<td>- Epistaxis</td>
<td>2 (9)</td>
<td>1 (4)</td>
<td>0</td>
</tr>
<tr>
<td>- Metrorragia</td>
<td>1 (4)</td>
<td>1 (4)</td>
<td>0</td>
</tr>
</tbody>
</table>

**Table 2: Treatment-related adverse events**

Data are No. (%) where n is the number of patients experiencing each adverse event.
FIGURE LEGENDS

Figure 1: Tumor response
A. Change from baseline of the largest diameter of the target lesion during study treatment (continuous lines) or after permanent discontinuation (dotted lines) in patients with response (yellow) or without response (blue) according the primary criterion.

B. Maximum percentage change in tumor size from baseline to 6 months, following the primary criterion (biggest diameter according to RECIST, blue) and the WHO criteria (yellow). One patient only had unidimensional tumor measure and was not assessed by WHO criteria.

Figure 2: Tumor response and gene expression profiling
A. P-values comparing the mRNA expression of 302 genes in the baseline tumor sample between responders and non-responders in tumors. Left sub-panel: cumulated distribution of p-values and FDR-adjusted q-values. The dashed line represents a p-value of 0.05. Right sub-panel: quantile-to-quantile plot of observed p-values giving vs. what would be expected by chance only.

B. GSEA analyses for the 6 pathways with the highest enrichment score (EGF and EGFR, interferons and interleukins, angiogenic factors, tissue inhibitor metalloproteinase (TIMP), AKT signaling pathway, and positive regulators of apoptosis) ; R, responders samples; S, non-responders samples.

C. Variation of relative mRNA expression of EGFR2, ERBB2 and EGF between baseline and month 1 in patients with response (PR) (blue) or not (SD or PD) (yellow) as best response (mean +- SD).

PR, partial response; SD, stable disease; PD, progressive disease
Figure 3: Cell cycle analysis and response to pazopanib

A. mRNA expression of *CDKN2A* at baseline in patients with response (PR, n=6; blue) or not (SD or PD, n=10; yellow) as best response (left) (mean ± SD) (n=16); representative images of p16 IHC staining in 2 tumor samples at baseline, one with PR to pazopanib (top) and one with SD (bottom) (right); x400 magnification.

B. Variation of mRNA expression of *CDK4, CDK1* and *CCND1* in patients with response (R for PR, n=6)) or not (NR for SD+PD, n=10)) as best response between baseline and 1 month of treatment. Mean ± SD; unpaired t-test; *, P<0.05.

C. Representative images of cyclin D1 IHC staining in 2 cases (one PR and one SD) at baseline and month 1; x400 magnification.

IHC, immunohistochemistry; PR, partial response; SD, stable disease; PD, progressive disease