LETTER

Phase 0 trial investigating the intratumoural concentration and activity of sorafenib in neurofibromatosis type 2

INTRODUCTION

Schwannomas, meningiomas and ependymomas are tumours of the nervous system which occur sporadically or as part of the hereditary disease neurofibromatosis type 2 (NF2). Mutations in neurofibromin 2/NF2 gene cause all NF2-related schwannomas, meningiomas and ependymomas, 77% sporadic schwannomas, 60% sporadic meningiomas and 30% sporadic ependymomas.[1] Schwannomas are the hallmark of NF2 and develop in all patients with NF2. Current treatments for NF2-related tumours are surgery or radiosurgery. Radiation may induce additional mutations and formation of secondary tumours in NF2 whereas surgery has limited use in patients with high tumour load or tumours located at the sites where resection would cause neurological complications.[2] Avastin is effective but only in a fraction of patients.[2] Published consensus recommendations suggest that the development of effective therapies for NF2 is urgent, with great potential for clinical progress.[2]

Platelet-derived growth factor receptor β (PDGFRβ) is overexpressed and activated in human primary schwannoma cells leading to increased proliferation.[3] Using orally available, Food and Drug Administration (FDA)-approved cRAF/VEGFR-2/PDGFRβ inhibitor, sorafenib, this was successfully inhibited in vitro.[3]

Here we report an open-label, phase 0, single-agent trial (EudraCT: 2011-001789-16, REC: 11/LO/0771) testing sorafenib in patients with NF2 with the aim to determine whether molecular target inhibition occurs with oral sorafenib in patients with NF2 and whether target inhibition in plasma with oral sorafenib in patients with NF2 can act as a biomarker.

METHODS

Per protocol minimal recruitment target depending on the pharmacodynamic (PD) response rate was three patients (two out of three or three out of five with 60% PD response).[4] Here, five adult patients with NF2 with peripheral schwannomas (PS), diagnosed according to National Institute of Health (NIH) Diagnostic Criteria for NF, were treated with sorafenib administered orally at maximum tolerated dose (MTD) of 400 mg, two times a day, for 10 days, and with a single 400 mg dose on day 11 and two (seperate PS except one patient) PS biopsied at day 0 and 11 as part of the trial protocol. The steady-state plasma concentrations and intratumoural concentrations of sorafenib (primary outcome) in PS were measured after the treatment (NorthEast Bioanalytical Laboratories, Hamden, Connecticut, USA). The coprimary outcome was efficacy of sorafenib on target inhibition. This was assessed by determining the levels of active/phosphorylated PDGFRβ, ERK1/2, AKT and S6 ribosomal protein (ps6), expression of proliferation marker cyclin D1, apoptosis marker cleaved caspase-3 in PS and the levels of active/phosphorylated PDGFRβ, ERK1/2, AKT in peripheral blood mononuclear cells (PBMCs) before and after treatment in each patient. Methods used were western blotting and immunohistochemistry (IHC).[5] Evidence for a reduction in each molecular target of sorafenib was sought from one-sided t-tests performed, using R V.3.3.3, on the log-transformed ratios of the before-and-after measurements, which assumed a common variance for each marker pooled from across the patients. According to the phase 0 design of Murgo *et al*,[4] the mean logged ratios of at least three out of five patients had to be less than 0 to conclude a significant effect of sorafenib on a particular target.

RESULTS

Most patients experienced mild-moderate adverse events using CTCAE, (online supplementary table 1). Sorafenib was detected at 3316.9–20 792.2 ng/mL in plasma and 1425.9–6242.1 ng/g in PS samples from all patients with NF2 similar to other trials (online supplementary table 2).

Western blot analysis of PS samples from five patients demonstrated non-significant changes of $pPDGFR\beta^{Y857}$ in all patients (figure 1A); non-significant changes of pERK1/2 (figure 1B) in four patients and significant reduction in one patient; non-significant changes of $pAKT^{S473}$ in all five patients (figure 1C); non-significant changes of ps6 in three patients and significant reduction in two (figure 1D); non-significant changes of cyclin D1 (figure 1E), cleaved caspase-3 (figure 1F) and total ERK1/2 and AKT online supplementary figure 1A,C) in all five patients. Results from PBMC: $pPDGFR\beta^{Y857}$ (figure 1G), pERK1/2 (figure 1H), $pAKT^{S473}$ (figure 1I), total ERK1/2 and total AKT (online supplementary figure 1B,D) showed no significant changes.

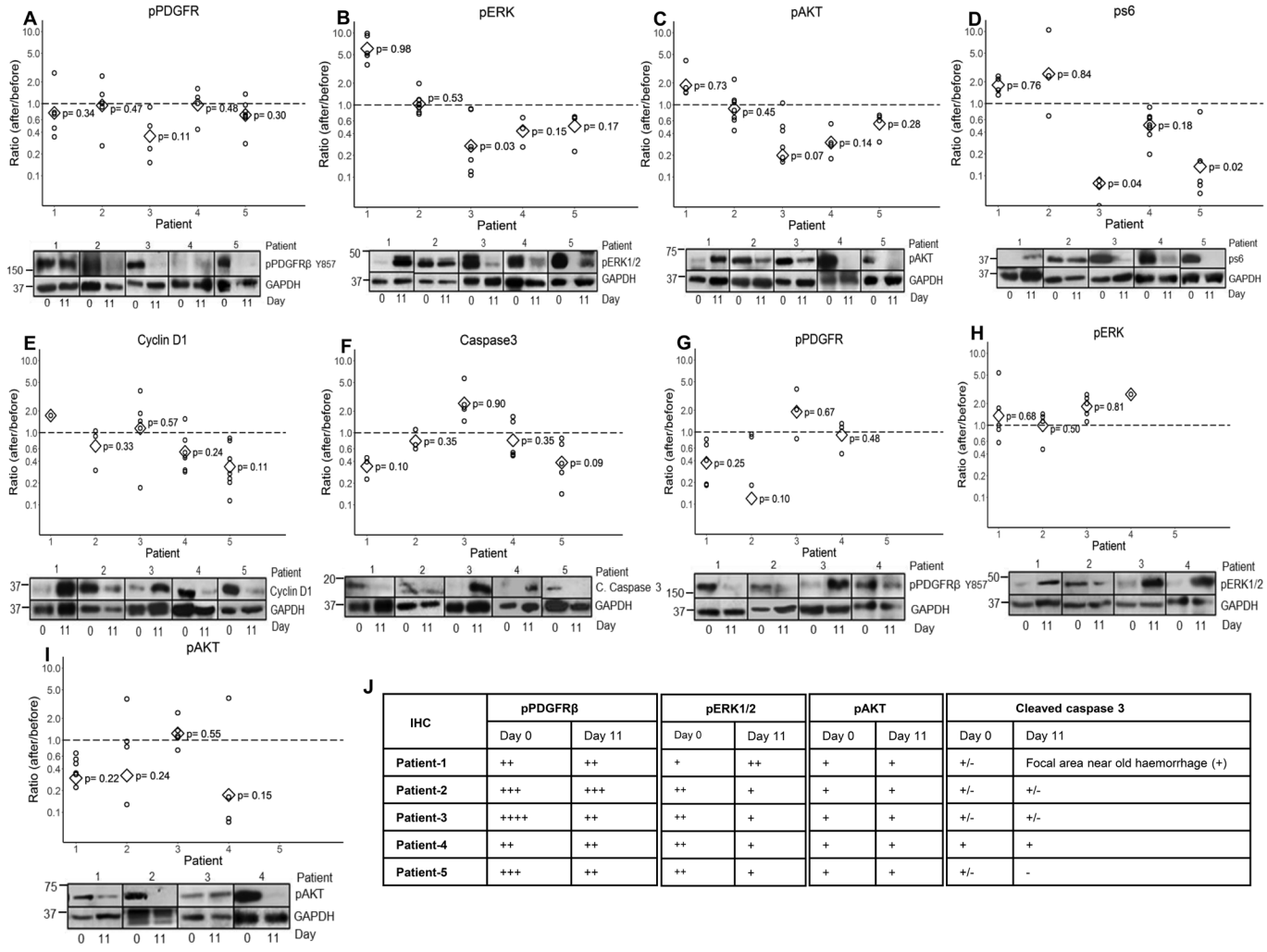
Additional semiquantitative IHC demonstrated decreased $pPDGFR\beta^{Y857}$ staining in PS samples from two patients, pERK1/2 in four and cleaved caspase-3 in one (figure 1J). No changes in $pAKT^{S473}$ (figure 1J) were observed. Sorafenib increased number of proliferating cells (MIB1) in PS tissues from three patients and had no effect in two (online supplementary figure 1E). It also increased number of intratumoural macrophages (CD68) in PS tissue from one patient and decreased in PS tissues from two patients (online supplementary figure 1E). The number of tumour-infiltrating CD3 +T cells increased in tissue samples from four patients (online supplementary figure 1E). Double staining for MIB1 and macrophage marker CD63, and MIB1 and T-cell marker CD3 revealed that the majority of proliferating cells were tumour cells although, in two cases, up to 24% were macrophages. No proliferating lymphocytes were observed (online supplementary figure 1E).

No correlation between target inhibition and drug concentrations in PS and in plasma were observed.

DISCUSSION

To select the right drug to take forward into larger clinical trials in patients with NF2, we report the first phase 0 clinical trial[4] allowing rapid assessment of the usefulness of sorafenib. Our within-patient measurements allowed a direct estimate of patient-specific variability.

We show that at standard MTD (400 mg, two times a day), sorafenib was detected in plasma 3316.9–20 792.2 ng/mL) and in tumour tissue 1425.9–6242.1 ng/g) in all patients. At MTD, sorafenib caused adverse events in all patients including fatigue, diarrhoea/constipation and rash which were mostly mild to moderate but in one patient rash was severe. PDs effects, even at MTD with reasonable bioavailability, were lower than expected from our preclinical in vitro studies in human primary schwannoma cells where sorafenib was highly effective.[3] The primary PD endpoint was negative since samples from only two out of five patients displayed significant results. IHC staining for MIB1/Ki67 revealed no changes in proliferation on sorafenib treatment in two patients and a slight increase in three patients. This increase is potentially due to accumulation of MIB1/Ki67-positive macrophages and active tumour-infiltrating CD8 +T



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IHC	pPDGFRβ		pERK1/2		pAKT		Cleaved caspase 3	
	Day 0	Day 11	Day 0	Day 11	Day 0	Day 11	Day 0	Day 11
Patient-1	++	++	+	++	+	+	+/-	Focal area near old haemorrhage (+)
Patient-2	+++	+++	++	+	+	+	+/-	+/-
Patient-3	++++	++	++	+	+	+	+/-	+/-
Patient-4	++	++	++	+	+	+	+	+
Patient-5	+++	++	++	+	+	+	+/-	-

Figure 1 Analysis of the efficacy of sorafenib in target inhibition in peripheral schwannoma samples (PS; western blotting and immunohistochemistry) and peripheral blood mononuclear cells (PBMCs; western blotting) from patients with neurofibromatosis type 2 (NF2). (A) Sorafenib had no significant effect on pPDGFRβ in PS samples of any patients (five in five). (B)No significant changes of pERK1/2 were observed in PS samples from four patients (four in five) and significant reduction of pERK1/2 was detected in PS sample from one patient (one in five). (C) pAKT^{S473} did not significantly change in PS samples from any patients (five in five). (D) No significant changes of ps6 were observed in PS samples from three patients (three in five) and significant reduction was detected in PS samples from two patients (two in five). (E and F) Cyclin D1 and cleaved caspase-3 were not significantly changed in PS samples from any of patients (five in five). (G–I) All results from PBMC: pPDGFRβ (G), pERK1/2 (H) and pAKT^{S473} (I) showed no significant change in any of patients. (J) Immunohistochemistry demonstrated decreased pPDGFRβ staining in PS samples from two patients (two in five), pERK1/2 in four (four in five) and cleaved caspase-3 in one (one in five). No significant changes in pAKT^{S473} were observed in any of patients. Technical repeats: Patient 1, n=5 (A), n=5 (B), n=3 (C), n=4 (D), n=1 (E), n=3 (F), n=5 (G), n=5 (H), n=5 (J); Patients 2, n=5 (A), n=6 (B), n=7 (C), n=3 (D), n=3 (E), n=3 (F), n=3 (G), n=4 (I); Patient 3, n=4 (A), n=6 (B), n=6 (C), n=3 (D), n=5 (E), n=4 (F), n=3 (G), n=5 (H), n=5 (I); Patient 4, n=4 (A), n=3 (B), n=4 (C), n=8 (D), n=6 (E), n=5 (F), n=3 (G), n=1 (H), n=4 (I); Patient 5, n=6 (A), n=3 (B), n=4 (C), n=5 (D), n=7 (E), n=5 (F). PDGFRβ, platelet-derived growth factor receptor β.

cells.[6] Post-treatment PS samples demonstrated increased macrophages and T cells which agree with previous studies.[6] Double staining for MIB1/Ki67, macrophage marker CD63 and, MIB1/Ki67 and T-cell marker CD3 revealed that most of proliferating cells were tumour cells. However, a percentage of CD63 positive macrophages double stained with MIB1. No CD3 positive cells costained with MIB1. Thus, lack of reduction or slightly increased proliferation after the treatment could partly be due to increased number of proliferating macrophages.

This study demonstrates that sorafenib can be detected in plasma and in PS and its effect on target inhibition can be successfully determined confirming the usefulness of phase 0 trials in patients with NF2. However, despite some PD effects, the trial was negative for the primary outcome and patients experienced relevant side effects.

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