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A phase I study of the combination of ro4929097 and cediranib in patients with advanced solid tumours (PJC-004/NCI 8503)

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Background: The Notch signalling pathway has been implicated in tumour initiation, progression, angiogenesis and development of resistance to vascular endothelial growth factor (VEGF) targeting, providing a rationale for the combination of RO4929097, a γ -secretase inhibitor, and cediranib, a VEGF receptor tyrosine kinase inhibitor.

Methods: Patients received escalating doses of RO4929097 (on a 3 days-on and 4 days-off schedule) in combination with cediranib (once daily). Cycle 1 was 42 days long with RO4929097 given alone for the first 3 weeks followed by the co-administration of both RO4929097 and cediranib starting from day 22. Cycle 2 and onwards were 21 days long. Soluble markers of angiogenesis were measured in plasma samples. Archival tumour specimens were assessed for expression of three different components of Notch signalling pathway and genotyping.

Results: In total, 20 patients were treated in three dose levels (DLs). The recommended phase II dose was defined as 20 mg for RO4929097 on 3 days-on and 4 days-off schedule and 30 mg daily for cediranib. The most frequent treatment-related adverse events (AEs) were diarrhoea, hypertension, fatigue and nausea. Eleven patients had a best response of stable disease and one patient achieved partial response. We did not detect any correlation between tested biomarkers of angiogenesis or the Notch pathway and treatment effect. There was no correlation between mutational status and time to treatment failure.

Conclusion: RO4929097 in combination with cediranib is generally well tolerated at the DLs tested. Preliminary evidence of antitumour efficacy with prolonged disease stabilisation in some patients with progressive malignancies warrants further clinical investigation of this treatment strategy.

The Notch signalling pathway is a key developmental signalling system that has been implicated in tumour initiation and progression (Tien *et al*, 2009). Several studies have highlighted the aberrant activation of this pathway in different tumour types. Dysregulation of this pathway promotes tumour growth by keeping the tumour cells/cancer stem cells in a pluripotent proliferative state and by enhancing tumour angiogenesis (Dufraigne *et al*, 2008; Rizzo *et al*, 2008). Mammalian cells possess four Notch receptors (Notch1–Notch4) and two families of ligands,

Jagged (Jagged1 and -2) and Delta-like (Dll-1, -3 and -4) (Dufraigne *et al*, 2008; Tien *et al*, 2009). Binding of Notch ligand to its receptor activates the pathway through a cascade of proteolytic cleavages, which are mediated by γ -secretase (Okochi *et al*, 2002).

The Notch pathway has been implicated in tumour angiogenesis. Components of the Notch pathway, especially Dll4, a Notch ligand with a key role in angiogenesis during embryonic development, are overexpressed in tumour vasculature (Patel *et al*, 2006; Jubb *et al*, 2009). Moreover, preclinical data have shown

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that Dll4–Notch signalling promotes tumour growth by improving the structure and function of tumour vasculature (Noguera-Troise *et al*, 2006; Ridgway *et al*, 2006; Li *et al*, 2007).

Targeting tumour angiogenesis via inhibition of vascular endothelial growth factor (VEGF) and its receptors (VEGFRs) has been a successful strategy in the treatment of several solid malignancies. Cross-talk between Notch and VEGF pathways is a potential mechanism of developing resistance to VEGF-targeted therapies (Patel *et al*, 2006; Li *et al*, 2011; Benedito *et al*, 2012). In preclinical studies, the Dll4–Notch signalling has been implicated in the development of resistance to VEGFR-targeted multi-kinase inhibitors and Notch pathway inhibition has been able to restore sensitivity to these agents (Li *et al*, 2011). In a glioblastoma xenograft mouse model, resistance to bevacizumab was abolished by a combination of γ -secretase inhibitor of the Notch pathway with bevacizumab (Li *et al*, 2011). Moreover, the combination of bevacizumab and a γ -secretase inhibitor synergistically inhibited tumour growth in bevacizumab-sensitive models, providing a rational base for a combination of γ -secretase inhibitor with VEGFR inhibitors in a clinical setting (Li *et al*, 2011). This phase I trial was initiated to evaluate the safety and tolerability, as well as antitumour activity of cediranib, a multi-kinase VEGFR inhibitor, with an inhibitor of the Notch signalling pathway, RO4929097.

Cediranib (AZD2171) is a highly potent inhibitor of VEGF signalling, with activity against all three VEGF receptors (VEGFR-1, VEGFR-2 and VEGFR-3), and c-Kit (Wedge *et al*, 2005). In a phase I trial, cediranib at 45 mg once daily dose was well tolerated and the most frequently reported AEs were diarrhoea, dysphonia and hypertension (Dreves *et al*, 2007). Antitumour efficacy of cediranib has been investigated in several phase II/III trials (Batchelor *et al*, 2010; Campbell *et al*, 2012; Hoff *et al*, 2012; Mulders *et al*, 2012; Schmoll *et al*, 2012).

RO4929097 is a potent and selective γ -secretase small-molecule inhibitor with antitumour activity in xenograft models, including colon cancer, pancreatic carcinoma and non-small cell lung cancer (Luistro *et al*, 2009). In a phase I study for patients with advanced malignancies, RO4929097 was well tolerated at 270 mg on a 3 days-on 4 days-off schedule, with skin, gastrointestinal events and fatigue being the most common toxicities (Tolcher *et al*, 2012).

This report summarises the final data of safety, efficacy and pharmacokinetics of RO4929097 in combination with cediranib in patients with advanced solid tumours.

MATERIALS AND METHODS

Study objectives. The primary objectives of this phase I clinical trial were to evaluate the safety and tolerability of escalating doses of RO4929097 in combination with cediranib, to characterise dose-limiting toxicities (DLTs) and to determine the recommended dose for phase 2 trials (RP2D). The secondary objectives were to assess pharmacokinetic properties, pharmacodynamic effects and to make a preliminary assessment of tumour response in patients with advanced solid tumours.

Patient eligibility. Patients were eligible if they had histologically or cytologically documented advanced solid malignancy, refractory to standard therapy or for which conventional therapy was not effective. Other key eligibility criteria included: age ≥ 18 years; Eastern Cooperative Oncology Group performance status of ≤ 1 ; life expectancy ≥ 12 weeks; adequate haematological (leukocyte count ≥ 3000 per mcl; absolute neutrophil count ≥ 1500 per mcl; haemoglobin ≥ 9 g dl⁻¹; platelet count $\geq 100\,000$ per mcl), hepatic (total bilirubin within normal institutional limits; aspartate aminotransferase (AST) and alanine aminotransferase (ALT) $\leq 2.5 \times$ institutional upper limit of normal), renal (creatinine within institutional normal limit or creatinine clearance

≥ 60 ml min⁻¹ per 1.73 m² for patients with creatinine more than institutional normal limits) and cardiac function (left ventricular ejection fraction $\geq 50\%$); negative serum pregnancy test in women of childbearing potential. Treatment could begin > 30 days after the patient's last therapy. Key exclusion criteria included previous treatment with a γ -secretase inhibitor and/or cediranib, meningeal or untreated brain metastases, major organ dysfunction, concurrent use of medications that are strong inducers/inhibitors or substrates of CYP3A4 enzyme, QTc interval greater than or equal to 450 ms in men and 470 ms in women by Bazett's correction and pregnancy or lactation. Previous therapy with other VEGFR tyrosine kinase inhibitors, such as sunitinib, was allowed.

Study design. This was an open-label, dose escalation phase I trial. It followed the standard 3 + 3 rule and no intra-patient dose escalation was allowed. Initial design of study consisted of two stages: (1) dose escalation and (2) expansion cohort, including patients with breast cancer, malignant melanoma, colorectal cancer, pancreatic cancer, renal cancer, high-grade glioma, non-small cell lung cancer, or ovarian cancer. However, accrual was terminated on 7 April 2012, after completion of three dose levels (DLs), due to discontinuation of RO4929097 development. Patients who were felt to be deriving clinical benefit were allowed to remain on cediranib monotherapy on study. The trial was approved by all relevant institutional ethical committees.

Patients in DL-1 received RO4929097 at 10 mg and cediranib at 20 mg. At DL-2, both RO4929097 and cediranib were administered at 20 mg. At DL-3, RO4929097 and cediranib were given at 20 mg and 30 mg, respectively. The rationale for starting dose of cediranib at 20 mg was based on results of other phase I trials where cediranib was administered in combination with chemotherapy or targeted agents (Goss *et al*, 2009; van Cruisen *et al*, 2010; Trarbach *et al*, 2012). In these studies, the maximum-tolerated dose of cediranib was always lower than 45 mg once daily. Furthermore, because an interim analysis of the BR24 phase II clinical trial demonstrated higher rates of serious AEs when cediranib at 30 mg per day was added to carboplatin and paclitaxel, the dose of 20 mg once daily was selected as starting dose of cediranib (Goss *et al*, 2010). At the time of study design, there were no available data on RO4929097 in combination with other agents. However, given the issues of CYP3A4 induction as well as autoinduction by RO4929097, the starting dose of 10 mg once daily was selected for RO4929097 in combination with cediranib (Tolcher *et al*, 2012). Cycles of treatment consisted of 21 days except for cycle 1, which was 42 days long consisting of a 3 weeks run-in period of RO4929097 alone. RO4929097 was administered on a 3 days-on and 4 days-off schedule (days 1–3, 8–10, 15–17, 22–24, 29–31 and 36–38) in combination with cediranib once daily starting from day 22 (days 22–42). Cycle 2 and onwards were 21 days in duration and consisted of once daily cediranib with RO4929097 on days 1–3, 8–10 and 15–17 (Supplementary Appendix Figure A1). Patients were treated until disease progression, occurrence of intolerable AEs, or study withdrawal.

The doses and schedule of RO4929097 and cediranib were chosen based on good tolerability in previous single-agent phase I trials of patients with advanced solid tumours (Dreves *et al*, 2007; Tolcher *et al*, 2012).

Safety. The safety and tolerability of combination regimen were assessed by collecting the incidence and severity of AEs, using the National Cancer Institute Common Terminology Criteria for Adverse Events, version 4.0.

The DLT was assessed during the first cycle (42 days) and defined as a treatment-related event meeting the following criteria: grade 4 neutropenia lasting ≥ 7 days, febrile neutropenia (absolute neutrophil count $< 1.0 \times 10^9$ per l and fever $\geq 38.5^\circ\text{C}$), grade 4 thrombocytopenia or thrombocytopenic bleeding (i.e., platelet count $< 50 \times 10^9$ per l and associated with clinically significant

bleeding), grade 4 diarrhoea, grade 3 diarrhoea lasting >72 h, despite medical management, any other non-haematologic grade 3 or 4 treatment-related toxicity lasting >72 h, despite appropriate medical therapy, failure to receive 80% of the planned dose of RO4929097 or cediranib, and inability to resume dosing for cycle 2 within 14 days due to treatment-related toxicity.

Pharmacokinetic assessment. To detect potential drug interaction, blood samples for pharmacokinetic evaluation of RO4929097 were serially collected on days 1, 10 and 22 of cycle 1 before dosing and at 0.5, 1, 2, 4, 6 and 24 h after dosing. Blood samples for pharmacokinetic analysis of cediranib were collected on day 22 of cycle 1 before dosing and at 0.5, 1, 2, 4, 6 and 24 h after dosing. The unbound RO4929097 samples were obtained by filtrating plasma samples using Amico Centrifree Micropartition devices (Millipore Corp., Bedford, MA, USA) (Zhang *et al*, 2008). Concentrations of RO4929097, total and unbound, and cediranib were determined using validated HPLC tandem mass spectrometry methods. Pharmacokinetic parameters were calculated by non-parametric methods using WinNonlin (Version 5.3, Pharsight Corp., Sunnyvale, CA, USA).

Pharmacodynamic assessment

Circulating biomarker evaluation. Soluble markers of angiogenesis were measured in blood samples collected before dosing on day 1 of cycle 1, day 22 of cycle 1 and day 1 of cycle 2. Plasma analysis was carried out for circulating VEGF-A, VEGF-B, VEGF-C, basic fibroblast growth factor, stromal cell-derived (SDF)-1-alpha factor, interleukin-6, and interleukin-8 levels using enzyme-linked immunosorbent assay kit from R&D System (Minneapolis, MN, USA). The assays were performed according to manufacturer's instructions. A triplicate was performed when the duplicate values differed by more than 15%. If at least two results were not within 15% of each other, results were discarded.

Archival tumour biomarker. Archival tumour specimens were assessed for expression of three different components of the Notch signalling pathway: Jagged-1, Notch-3 receptor and Notch-1 intracellular domain (NICD). Our initial plan was to test a panel of Notch pathway components. However, because of the discontinuation of treatment and limited number of patients, we selected to study the abovementioned three key biomarkers. Overexpression and prognostic significance of these markers have been reported in multiple studies (Reedijk *et al*, 2005; Dickson *et al*, 2007; Lin *et al*, 2010; Wu *et al*, 2011). Immunohistochemistry was performed using standard techniques. Anti-NICD (Cell Signaling #2421; Danvers, MA, USA) and anti-Notch-3 (Santa Cruz Biotechnologies #sc-5593; Santa Cruz, CA, USA) staining was performed using VECTASTAIN ABC rabbit IgG kit (Vector Labs #PK-4001; Burlingame, CA, USA). For anti-Jagged-1 (R&D Systems #AF1277; Abingdon, UK) staining, Cell and Tissue Goat IgG Staining kit (R&D Systems #CTS008; Abingdon, UK) was utilised. All immunohistochemistry was performed using Shandon Sequenza immunostaining cover plates (Fisher #7219950) and Peroxidase Substrate Kit, DAB (Vector Labs #SK-4100).

Expressions of these three markers were quantified by a pathologist (B.C.) who was blinded to the clinical outcome data and by using the Allred score (Allred *et al*, 1998; Dickson *et al*, 2007).

In patients with available formalin-fixed paraffin-embedded (FFPE) specimens with sufficient DNA, genotyping was performed with either Sequenom PMH v1.0 panel, a customised Sequenom MassArray for solid tumours that includes 280 mutations in 23 genes, or MiSeq TruSeq Amplicon Cancer Panel.

RESULTS

Patient characteristics. From 31 May 2010 through 6 November 2012, 20 patients were recruited at two centres (Princess Margaret Cancer Center and Juravinski Cancer Center), with baseline characteristics as shown in Table 1. Three DLs were evaluated (Table 2). As of data cut-off date on 6 November 2012, patients received a median number of three cycles (range 1–28+). At the time of this report, two patients are still receiving therapy.

Safety. All 20 treated patients were evaluable for non-haematologic and haematologic toxicities. Combination of RO4929097 with cediranib was generally well tolerated. The most frequent treatment-related AEs (occurring in greater than 30% of patients) of all grades and those of grades 3 or higher are described in Table 3. The most common treatment-related AEs during cycle 1 are presented in Supplementary Appendix Table A1. Briefly, the most frequently reported treatment-related AEs were diarrhoea, hypertension, fatigue, nausea, headache, hypothyroidism,

Table 1. Baseline demographics and patient characteristics (N=20)

Age, years	
Median	54
Range	18–87
Gender, n (%)	
Male	10 (50)
Female	10 (50)
ECOG score, n (%)	
0	4 (20)
1	16 (80)
Tumour type, n (%)	
Colorectal	6 (30)
Renal cell	3 (15)
Leiomyosarcoma	3 (15)
Other	8 (40)
Previous therapies	
Systemic therapy	19 (95)
Antiangiogenic agents	11 (55)
Radiotherapy	12 (60)
Abbreviation: ECOG = Eastern Cooperative Oncology Group.	

Table 2. Dose level evaluated and encountered DLTs

Dose level	Drugs	Number of patients	Number of observed DLTs	DLT description
1	RO4929097 10 mg Cediranib 20 mg	7	1	Grade 3 hypertension
2	RO4929097 20 mg Cediranib 20 mg	7	1	Grade 4 elevation of AST and grade 3 elevation of ALT
3	RO4929097 20 mg Cediranib 30 mg	6	0	
Abbreviations: ALT = alanine aminotransferase; AST = aspartate aminotransferase; DLT = dose-limiting toxicity.				

hypophosphatemia, increased ALT, and elevated AST. The majority of these were grade 1 or 2 events, but 4 (20%) patients experienced grade 3 AEs consisting of hypertension ($n=3$) and hypophosphatemia ($n=1$). Both conditions were reversible with

temporary discontinuation of study drugs. One patient experienced grade 4 elevation of AST and grade 3 elevation of ALT as discussed below.

Two patients experienced DLTs. In DL-1, one patient experienced grade 3 hypertension on day 38 of cycle 1, which was reversible after temporary discontinuation of treatment. The patient tolerated retreatment with RO4929097 at 10 mg per day dose and a reduced dose of cediranib (15 mg). At DL-2, one patient experienced grade 4 elevation of AST and grade 3 elevation of ALT on day 36 of cycle 1, which was resolved after permanent discontinuation of treatment.

Pharmacokinetics. In our study, pharmacokinetic parameters of cediranib were similar to the previously observed monotherapy data (Drevs *et al*, 2007). Pharmacokinetic analysis of RO4929097 showed similar Cmax and AUCt as phase I monotherapy study

Table 3. Common treatment related adverse events

		Dose level		
		1 (n=7)	2 (n=7)	3 (n=6)
Drug-related AE (%)	Grade	Cediranib 20 mg RO4929097 10 mg	Cediranib 20 mg RO4929097 20 mg	Cediranib 30 mg RO4929097 20 mg
Diarrhoea	All 3-4	6 (86%) 0 (0%)	3 (43%) 0 (0%)	4 (66%) 0 (0%)
Hypertension	All 3-4	6 (86%) 2 (28%)	2 (28%) 0 (0%)	4 (66%) 1 (17%)
Fatigue	All 3-4	3 (43%) 0 (0%)	3 (43%) 0 (0%)	4 (66%) 0 (0%)
Nausea	All 3-4	3 (43%) 0 (0%)	4 (57%) 0 (0%)	4 (66%) 0 (0%)
Hypothyroidism	All 3-4	3 (43%) 0 (0%)	2 (28%) 0 (0%)	3 (50%) 0 (0%)
Headache	All 3-4	4 (57%) 0 (0%)	1 (14%) 0 (0%)	3 (66%) 0 (0%)
Hypophosphatemia	All 3-4	3 (43%) 1 (14%)	2 (28%) 0 (0%)	2 (33%) 0 (0%)
Increased ALT	All 3-4	2 (28%) 0 (0%)	3 (43%) 1 (14%)	3 (50%) 0 (0%)
Increased AST	All 3-4	0 (33%) 0 (0%)	5 (71%) 1 (14%)	2 (33%) 0 (0%)

Abbreviations: ALT = alanine aminotransferase; AST = aspartate aminotransferase.

Table 5. Pharmacokinetic parameters of cediranib at different DLs

Cediranib	DL-1 (cediranib = 20 mg)	DL-2 (cediranib = 20 mg)	DL-3 (cediranib = 30 mg)
Tmax, h			
Median	4	2	2
Range	2-6	2-6	1-4
C max, nmol l⁻¹			
Mean	36.78	36.9	77.58
s.d.	23.94	25.06	33.99
AUC t ng*h ml⁻¹			
Mean	483.21	467.19	870.29
s.d.	176.05	287.89	378.79

Abbreviations: AUC = area under curve; DL = dose level.

Table 4. Pharmacokinetic parameters of RO4929097 at different DLs

	DL-1 (RO4929092 = 10 mg) (n: 5)			DL-2 (RO4929092 = 20 mg) (n: 7)			DL-3 (RO4929092 = 20 mg) (n: 5)		
	Day 1	Day 10	Day 22	Day 1	Day 10	Day 22	Day 1	Day 10	Day 22
Tmax, h									
Median	2	2	2	2	1	1	4	2	2
Range	1-2	1-4	1-24	0.5-24	0.5-24	1-24	0.5-6	0.5-4	1-2
C max, nmol l⁻¹									
Mean	296.4	296	288.6	465.14	778.85	482	437.8	788.2	408
s.d.	178.6	176.93	106.22	125.55	385.82	244.74	273.73	230.63	172.83
AUC t ng*h ml⁻¹									
Mean	2678.07	3441.80	3547.46	5367.68	11237.66	6360.44	7467.97	12164.95	6085.72
s.d.	1445.30	3025.29	1661.92	3374.58	7663.55	3919.95	5368.38	3688.61	2536.22
Unbound RO4929097									
C max, nmol l⁻¹									
Mean	18.06	21.44	16.48	42.57	63.34	37.42	32.48	53.52	25.68
s.d.	9.99	10.34	5.91	10.21	22.26	18.46	19.85	21.77	12.77

Abbreviation: DL = dose level.

(Tolcher *et al*, 2012). Furthermore, the AUCt of RO4929097 on day 10 was higher than day 1 implying no CYP3A4 autoinduction at the dose range and schedule administered in this study (10–20 mg per day). A summary of the pharmacokinetic profile of both drugs at different DLs are represented in Tables 4 and 5 and Supplementary Appendix Figure A2.

It is important to mention that these analyses are limited, as only three DLs of combination therapy were studied.

Antitumour activity. Nineteen of the 20 patients enrolled on the study had at least one follow-up tumour assessment and were evaluable for objective response assessment. One partial response was observed. Eleven of the 19 evaluable patients (58%) had a best response of stable disease, whereas 7 (37%) patients had disease progression.

As of the data cut-off date on 6 November 2012, prolonged stable disease of ≥ 6 cycles was observed in 7 patients; 1 with uterine leiomyosarcoma received 15 cycles of treatment before disease progression, 2 with colorectal cancer (7 and 11 cycles),

another 2 with renal cell carcinoma (11 and 13 cycles); 1 with cholangiocarcinoma who received 6 cycles of treatment and 1 patient with endometrial stromal sarcoma, a notably slow-growing low-grade malignancy, currently remains on study after 37 cycles of treatment and has achieved partial response after the data cut-off date (Figure 1).

Pharmacodynamics. We did not detect any correlation between the baseline values of circulating angiogenesis markers and time to treatment failure. Increases in VEGF-A, VEGF-C and SDF-1 were detected after dosing with cediranib, but there was no suggestion of relationship with dose or time to treatment failure (Figure 2).

No significant correlation was observed between expression of the three Notch pathway biomarkers evaluated (Jagged-1, Notch-3 receptor and NICD) and time to treatment failure (data not shown).

Mutational profiling was performed on DNA isolated from FFPE samples of 18 patients. Mutations were detected in samples of eight patients (Supplementary Appendix Table A2). None of

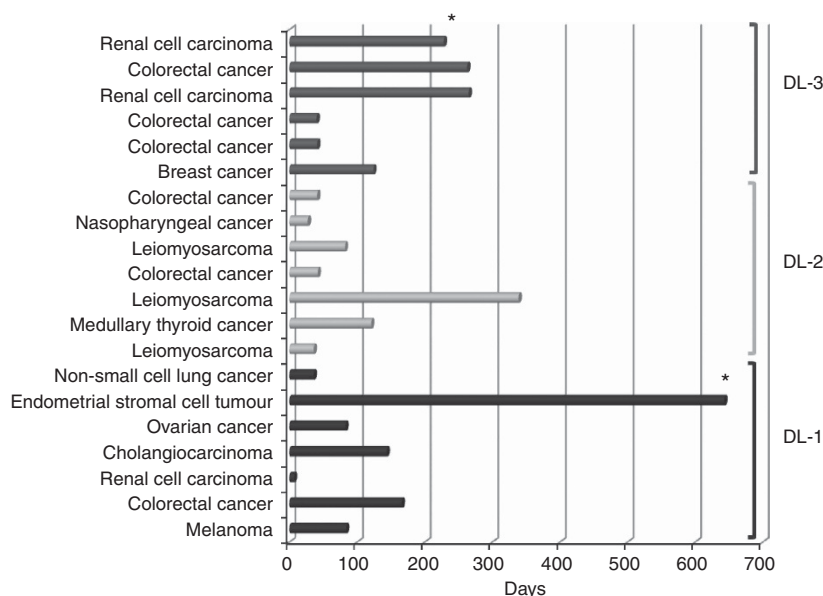


Figure 1. Duration of exposure as of data cutoff date on 6 November 2012. *Patient continues on treatment. Abbreviation: DL, dose level.

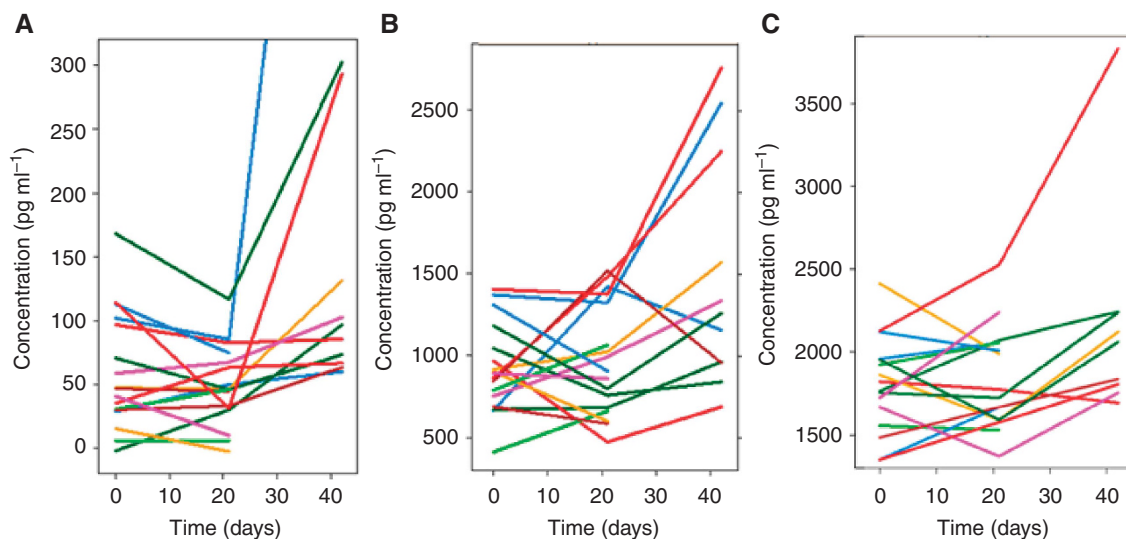


Figure 2. Concentration of circulating angiogenic factors measured in the sera of patients during cycle 1 of treatment. (A) VEGF-A, (B) VEGF-C and (C) SDF-1. Increases in these factors were detected after dosing with cediranib, but there was no suggestion of relationship with dose or time to treatment failure.

these mutations were Notch or angiogenesis pathway related and there was no correlation between mutational status and time to treatment failure.

DISCUSSION

This study demonstrated that a combination of RO4929097 with cediranib is tolerable in patients with advanced solid tumours. The RP2D was defined as 20 mg for RO4929097 on 3 days-on and 4 days-off schedule and 30 mg daily for cediranib. It is important to mention that RP2D was defined based on the available toxicity data from three doses that have been completed by the time this study was discontinued. Both agents could have potentially been escalated further if the study was carried out as per the initial protocol. Toxicities of this combination are similar to those observed with single agents in phase I clinical trials (Dreves *et al*, 2007; Tolcher *et al*, 2012). These non-life-threatening AEs were effectively addressed by dosing delay and dose reduction. They are also commonly observed in patients receiving cediranib and we saw no evidence of increased frequency or severity of these toxicities in the current study.

Preliminary evidence of antitumour activity were observed in some patients; 11 patients had stable disease and 1 patient with endometrial stromal sarcoma, a notably slow-growing low-grade malignancy, achieved partial response. Nine of them continued treatment for ≥ 4 cycles, most of whom had been heavily pretreated. There is a scientific rationale to target both Notch and VEGF signalling pathways and, hence, large number of prolonged disease stabilisation in this study may be a result of such effects.

The complexity of this combination approach underlines the importance of identifying suitable markers that can predict clinical benefit. In this study, we had planned testing of a large panel of the Notch and VEGF pathway components. However, after the decision of early study termination, we chose to test Jagged-1, Notch-3 receptor and NCID, which are overexpressed in multiple malignancies and associated with poor prognosis (Reedijk *et al*, 2005; Dickson *et al*, 2007; Lin *et al*, 2010; Wu *et al*, 2011). In future studies evaluating similar treatment strategies, it would be of great interest to assess the expression of DLL4, VEGFR1, VEGFR2 and VEGFR3, which are important components of cross-talk between VEGF and Notch pathways and tumour angiogenesis (Li *et al*, 2011). In our study, we did not detect association between the tested biomarkers of angiogenesis or the Notch pathway with treatment effect. However, these results are limited by the small number of treated patients and diversity of tumour types. Increases in VEGF and PlGF after dosing cediranib have been reported in monotherapy with cediranib and has been related to the antiangiogenic effect of the drug (Dreves *et al*, 2007). Similar patterns in levels of VEGF-A, VEGF-C and SDF-1 were detected in some of our patients. However, there was no correlation between these changes and time to treatment failure.

Overall, comparison of the RO4929097 and cediranib pharmacokinetic profile with historical monotherapy pharmacokinetic data at the same doses did not reveal any significant difference when they are administered in combination. As RO4929097 is highly bound in plasma to $\alpha 1$ -acid glycoprotein and albumin, monitoring of unbound RO4929097 has been recommended particularly when it is administered with other compounds that may impact protein binding (Wu *et al*, 2012). The effect of protein binding on cediranib pharmacokinetics has not been reported. In the present study, we did not detect any significant alteration in the pharmacokinetic profile of the total and unbound RO4929097 when combined with cediranib. It should be mentioned that because of the study schedule, pharmacokinetic sampling was

limited to 24 h after administration of RO4929097 and cediranib. Therefore, the elimination half-life and AUC_{0- ∞} cannot be reliably calculated. Although the effect of RO4929097 on CYP3A4 induction has been shown in a previous phase I study of RO4929097 (Tolcher *et al*, 2012), no evidence of autoinduction was observed in the pharmacokinetic analysis on this study. One possibility is that in the present study, maximal daily dose of RO4929097 was only 20 mg and autoinduction is unlikely to occur at this low dose. Indeed, in the single agent phase I study of RO4929097, reversible CYP3A4 autoinduction was detected at daily doses above 24 mg in the 3 consecutive days per week schedule (Tolcher *et al*, 2012).

In conclusion, combination of RO4929097 and cediranib is well tolerated with no evidence to suggest a significant change in the pharmacokinetics of these drugs. Preliminary evidence of anti-tumour efficacy with prolonged disease stabilisation in some patients with progressive malignancies warrants further clinical investigation of this treatment strategy. Unfortunately, because of the discontinuation of the RO4929097 and cediranib development programmes, further studies will need to occur with other agents that target Notch and VEGF pathways, respectively. A particularly interesting research strategy may be to inhibit Notch upon progression on an antiangiogenic agent in patients who had previously responded to the agent.

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