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Phase I evaluation of seliciclib (R-roscovitine), a novel oral cyclin-dependent kinase inhibitor, in patients with advanced malignancies

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ABSTRACT

Aim: Phase I study of seliciclib (CYC202, R-roscovitine), an inhibitor of cyclin-dependent kinases 2, 7 and 9, causing cell cycle changes and apoptosis in cancer cells.

Patients and methods: This phase I trial aimed at defining the toxicity profile, the maximum tolerated dose (MTD), the recommended phase II dose (RD) and the main pharmacokinetic and pharmacodynamic parameters of oral seliciclib. Three schedules were evaluated: seliciclib given twice daily for 5 consecutive days every 3 weeks (schedule A), for 10 consecutive days followed by 2 weeks off (schedule B) and for 3 d every 2 weeks (schedule C).

Results: Fifty-six patients received a total of 218 cycles of seliciclib. Dose-Limiting Toxicities (DLT) consisting of nausea, vomiting, asthenia and hypokalaemia occurred at 1600 mg bid for schedule A and in schedule C, DLT of hypokalaemia and asthenia occurred at 1800 mg bid. The evaluation of longer treatment duration in schedule B was discontinued because of unacceptable toxicity at lower doses. Other adverse events included transient serum creatinine increases and liver dysfunctions. Pharmacokinetic data showed that exposure to seliciclib and its carboxylate metabolite increased with increasing dose. Soluble cytokeratin 18 fragments allowed monitoring of seliciclib-induced cell death in the blood of patients treated with seliciclib at doses above 800 mg/d. One partial response in a patient with hepatocellular carcinoma and sustained tumour stabilisations were observed.

Conclusions: The MTD and RD for seliciclib are 1250 mg bid for 5 d every 3 weeks and 1600 mg bid for 3 d every 2 weeks, respectively.

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1. Introduction

CDK/cyclin complexes are regulated by CDK inhibitors such as the INK4 family and the CIP/KIP family.[1] Mutations and/or deletions of CDK inhibitors are observed in several human cancers and can promote tumour development.[2–4] Screening for small molecules that inhibit CDK function led to the discovery of seliciclib (CYC202, *R*-roscovitine), a tri-substituted purine analogue that competes with ATP for its binding site on CDKs.[1] Seliciclib preferentially inhibits the activity of CDK2/cyclin E complex with an $IC_{50} \leqslant 0.1\,\mu M$, as well as CDK7/cyclin H, CDK2/cyclin A and CDK9/cyclin T1 complexes.[5]

Seliciclib has been shown to block RNA synthesis in human colon carcinoma cell lines by inhibiting the CDK7- and CDK9-dependent phosphorylation of the carboxyl-terminal domain of RNA polymerase II, leading to the inhibition of transcription and to the accumulation of p53.[6] Seliciclib can also exert antitumour activity by inducing the expression of the pro-apoptotic gene Bcl-x.[7] Antiproliferative effects of seliciclib *in vitro* were reported in a panel of human cancer cell lines at concentrations ranging from 4.4 to 31 μM and *in vivo* after oral dosing in animal with xenografted tumours, as a single agent,[8] and in combination with other anticancer agents.[9] Pharmacokinetic studies in rodents showed that a single oral daily dose of 500 mg/kg (2750 mg/m^2) achieved plasma levels of seliciclib > 10 μM (3 μg/mL) for 24 h.[10] Seliciclib is rapidly metabolised by CYP3A4 and CYP2B6 with the main metabolites being the carboxylate PMF30-128 and two glucuronides termed M1 and M2.

Based on preclinical results, a phase I trial was initiated in patients with advanced malignancies with the aim of characterising the toxicity profile, the dose-limiting toxicities (DLTs), the maximum tolerated dose (MTD) and the recommended phase II dose (RD) and the main pharmacokinetic (PK) and pharmacodynamic (PD) parameters of seliciclib given by oral administration.

2. Patients and methods

2.1. Patient selection

Eligible patients had to meet the following criteria: histologically confirmed metastatic or locally advanced solid tumour or lymphoma, for which no standard treatment was indicated or exists, age ⩾ 18 years, World Health Organization performance status 0–2, life expectancy ⩾ 3 months, adequate biological values (absolute neutrophil count $\geqslant 1.5 \times 10^9$/l, haemoglobin ⩾90 g/l, creatinine clearance ⩾ 1 ml/s by Cockroft formula, bilirubin ⩽ 1.25 × the upper limit of normal (ULN), alkaline phosphatase (AP) and transaminases ⩽ 2.5 × ULN, prothrombin time within the normal range), negative pregnancy test and signed informed consent prior to start of any study procedures. Patients were not eligible if they had received previous chemotherapy, immunotherapy, radiotherapy or any investigational therapy <4 weeks (<6 weeks for mitomycin C and nitrosourea) prior to study entry, or extensive radiotherapy on 30% of bone marrow reserves, or bone marrow transplantation. Exclusion criteria also included inability to swallow, chronic gastrointestinal disease or conditions that could hamper compliance and/or absorption of seliciclib, prior malignancies with the exception of *in situ* cervical cancer and basal cell skin cancer, known HIV, HBC or HCV infection, major thoracic and/or abdominal surgery <3 weeks preceding study entry.

This trial and the consent documents were approved by the French Ethic Committee and was conducted in accordance with Good Clinical Practices and in compliance with the French national regulations.

2.2. Drug administration

Based on animal toxicology, the starting dose of seliciclib was 100 mg twice-daily (bid). Seliciclib was supplied by Cyclacel Ltd. (Dundee, UK) as gelatin capsules of 50 mg or 200 mg. Seliciclib capsules were stored at room temperature in a closed container. Seliciclib was given according to three schedules: schedule A consisted of 5 consecutive days every 3 weeks, schedule B of 10 consecutive days followed by 2 weeks off and schedule C of 3 consecutive days every 2 weeks. The choice of these intermittent schedules was based not only on preclinical data that first reported a short half-life for seliciclib favouring a daily schedule but also on the fact that the inhibition of Rb phosphorylation (that has not been eventually validated as an appropriate pharmacodynamic biomarker in the clinic) was maintained up to several days after the last administration of seliciclib in animal models, suggesting that a continuous, potentially more toxic, schedule might not be necessary.[8] Treatment was continued until tumour progression, unacceptable toxicity, patient refusal, unforeseen events, major violation of the protocol, dose interruption > 2 weeks or discontinuation by the investigator for clinical reasons not related to the study drug. Patients did not receive prophylactic antiemetic therapy.

2.3. Dose escalation and determination of the MTD and RD

Planned dose increments were 100% until >grade 1 drug-related toxicity was observed. All patients receiving at least one dose of the study treatment were evaluable for DLT assessment. The MTD was defined as the dose below that at which more than 2 out of 6 patients develop a DLT during the first cycle. A DLT was defined as grades 3 and 4 haematological or non-haematological toxicity (excluding grade 3 reversible asymptomatic increase in transaminases), and any treatment interruption due to safety/tolerance lasting >1 week despite appropriate treatment. If a DLT occurred, treatment was delayed until recovery to baseline levels. Treatment was resumed at the next lower level or 75% of the initial dose. If a DLT recurred, treatment was discontinued. The RD would be MTD or the lowest most active dose according to the pharmacokinetic and pharmacodynamic studies.

2.4. Pre-treatment and follow-up studies

Pre-treatment evaluation was performed within 4 weeks prior to the initiation of seliciclib dosing and included

documentation of medical history, electrocardiogram, chest X-ray, assessment of disease and concomitant medications. Physical examination, vital signs, WHO performance status, haematology, biochemistry and urinalysis were performed within 7 d prior to treatment. Tumour assessment was performed within 3 weeks prior to the start of treatment, and subsequently every 2 months. The RECIST criteria (version 1.0) were used to evaluate the response to treatment.[11] Adverse events were reported according to the National Cancer Institute Common Toxicity Criteria (version 2.0).

2.5. *Pharmacokinetic analysis*

Blood samples for PK analyses of seliciclib and its main metabolites (PMF30-128, M1 and M2) in plasma were taken from all patients at the following time points: before the morning dose, and at 0.5, 1, 1.5, 2, 4, 8, 12 (before the evening dose), 13, 14, 16 and 24 h after the morning dose on the first and last days of dosing during cycle 1. When the MTD was established on schedule C, 6 additional patients were to be included at the total daily MTD dose divided in three daily intakes (at 6-h intervals) for 3 consecutive days, every 2 weeks in order to perform additional clinical and PK evaluation. At each time point, 7 ml aliquots of blood were withdrawn from patients into heparinised tubes. The tubes were centrifuged at 1200*g* at 4 °C for 10 min. The plasma was transferred to a sterile 2 ml cryovial and stored at –70 °C until analysed. The plasma levels of seliciclib and its metabolites were determined by a validated assay using LC-MS/MS after solid phase extraction. The lower limit of detection was 1 ng/ml.

PK analysis of the plasma samples was performed using the trapezoidal method and log-linear regression. PK parameters were determined for the parent compound seliciclib and its metabolites using the software Kinetica™ (Innaphase Limited, Amersham, UK). The following parameters were determined: maximum plasma concentration after administration (C_{max}), sampling time of C_{max} (T_{max}), area under the concentration–time curve (time 0 to last sample with a quantifiable concentration: AUC_{last}) and terminal half-life ($T_{1/2}$).

2.6. *Pharmacodynamic analysis*

Various soluble cytokeratin 18 (CK18) fragments are released into the extracellular environment upon the loss of membrane integrity that occurs during either necrotic or apoptotic cell death.[12,13] The Tissue-Polypeptide-Specific antigen (TPS) ELISA detects fragments of CK18 released following either apoptotic or necrotic cell death. The neoepitope recognised by the M30 antibody is exposed only following cleavage of CK18 by either caspase-3 or -7 and therefore can be used for monitoring apoptotic-specific cleavage of CK18. The TPS ELISA (IDL Biotech Bromma, Sweden) and the M30 ELISA (Peviva AB. Bromma, Sweden) were performed using plasma samples collected throughout the first day and during the last day of treatment of cycle 1. The changes in M30 or TPS signals between the first and last days of treatment were compared using a non-paired unequal-variance Student *t*-test.

3. Results

3.1. *General*

Among 57 patients enrolled on the study, 56 patients received treatment and were evaluable for safety. One patient was not treated due to rapid deterioration in general condition. Patient characteristics are listed in Table 1. Most patients were heavily pretreated with a median of 4 previous lines of chemotherapy (range: 0–10). A total of 218 cycles of seliciclib were administered (median: 3 cycles, range: 1–24).

In schedule A, the starting dose of 100 mg bid was escalated up to 1000 mg bid without any DLTs (Table 2). At the dose of 1000 mg bid, 1/6 patients experienced grade 3 asthenia. At the dose of 1600 mg bid, grades 3 and 4 asthenia, nausea and vomiting occurred in 2 patients and grades 3 and 4 hypokalaemia in two other patients. The dose of 1600 mg bid was thus considered intolerable. The MTD and RD were defined as 1250 mg bid for 5 consecutive days every 3 weeks.

In schedule B, one patient experienced grade 3 asymptomatic hypokalaemia, and another patient a grade 3 skin rash at the starting dose of 1000 mg bid. Three additional patients were therefore treated at this dose. One of these patients experienced grade 4 asymptomatic hypokalaemia leading to treatment discontinuation. The MTD was exceeded at this dose and the study was amended to enroll a new cohort of

Table 1 – Patient characteristics.

Number of evaluable patients	56
Male/female	23/33
Age (years), median range	53 (30–75)
WHO performance status	
0	33
1	20
2	3
Site of primary disease	
Colorectal	19
Breast	10
Lung	4
Sarcoma	4
Prostate	3
Adrenal gland (corticosurrenaloma)	3
Pancreatic	2
Gastric	2
Thymic	2
ACUP	2
Uterine cervix	2
Liver	1
Ovarian	1
Parotid (cylindroma)	1
No. of prior lines of chemotherapy	
0	1
1	3
2	6
⩾3	46
Prior radiation therapy	35

WHO: World Health Organization; ACUP: Adenocarcinoma of Unknown Primary.

3–6 patients at a reduced dose of 800 mg bid. Amongst the first three patients assessed at this reduced dose, one patient experienced a DLT consisting of a grade 3 asymptomatic hypokalaemia during the first cycle. Two of the three additional patients treated at this dose experienced grades 3 and 4 asymptomatic hypokalaemia as DLTs, respectively. This dose was therefore considered not tolerable and the evaluation of this schedule was terminated.

Table 2 – Number of cycles and DLTs by dose escalation schedules.

Dose (mg bid)	No. of patients evaluable for DLT/entered	No. of cycles	DLT at cycle 1	Type of DLT
Schedule A: 5 d every 3 weeks				
100	3/3	10	0	–
200	2/2	5	0	–
400	1/1	8	0	–
800	4/4	13	0	–
1000	6/6	20	1	Asthenia
1250	3/3	12	0	–
1600	6/6	34	4	Vomiting, nausea and asthenia Vomiting, nausea and asthenia Hypokalaemia Hypokalaemia
Schedule B: 10 d followed by 2 weeks off				
1000	6/6	20	3	Hypokalaemia Hypokalaemia Skin rash
800	6/6	18	3	Hypokalaemia Hypokalaemia Hypokalaemia
Schedule C: 3 d every 2 weeks				
1200	3/3	10	0	–
1600	3/3	12	0	–
1800	6/6	32	3	Hypokalaemia Hypokalaemia and asthenia Asthenia
1000/1000/1200[a]	7/7	24	4	Asthenia Asthenia Hypokalaemia Hypokalaemia

DLT: Dose-limiting toxicity; bid: bi-daily.
[a] Tri-daily administration.

Table 3 – Relevant adverse events at least possibly related to study drug.

	Patients, *n* (%)					
	Schedule A (total = 25 patients)		Schedule B (total = 12 patients)		Schedule C (total = 19 patients)	
	G 1–2	G 3–4	G 1–2	G 3–4	G 1–2	G 3–4
Clinical toxicities						
Nausea	14 (56%)	4 (16%)	11 (92%)	–	17 (89%)	–
Vomiting	13 (52%)	4 (16%)	4 (33%)	–	14 (74%)	–
Asthenia	10 (40%)	3 (12%)	10 (83%)	1 (9%)	9 (47%)	10 (53%)
Anorexia	9 (36%)	-	2 (17%)	–	9 (47%)	2 (11%)
Hepatic dysfunctions						
Bilirubin increase	11 (44%)	1 (4%)	7 (58%)	–	8 (42%)	3 (16%)
GGT increase	9 (36%)	13 (52%)	5 (42%)	4 (33%)	8 (42%)	11 (58%)
AP increase	14 (56%)	3 (12%)	6 (50%)	1 (9%)	15 (79%)	1 (5%)
ALT increase	15 (60%)	3 (12%)	8 (67%)	–	13 (69%)	5 (26%)
AST increase	16 (64%)	4 (16%)	9 (75%)	–	15 (79%)	2 (11%)
Metabolic dysfunctions						
Hypokalaemia	10 (40%)	6 (24%)	4 (33%)	5 (42%)	9 (47%)	6 (32%)
Creatinine increase	14 (56%)	1 (4%)	9 (75%)	–	13 (69%)	–

G: grade of toxicity according to NCI-CTC version 2.0; GGT: γ-glutamyl transferase; AP: alkaline phosphatase; ALT: alanine amino transferase; AST: aspartate amino transferase.

In schedule C, no DLT was observed at doses up to 1800 mg bid. At this latter dose, grade 3 hypokalaemia and/or asthenia occurred in 3/6 patients. The MTD and RD were therefore established at 1600 mg bid. Given the short half-life observed in the preliminary results of the PK analysis, the RD of 1600 mg bid (corresponding to 3200 mg daily) was studied further by a tri-daily administration (1000/1000/1200). This led to DLT of asthenia or hypokalaemia in 4/6 patients, suggesting that the tri-daily administration was less tolerable than twice daily administration.

Dose-intensity achieved at the RD in schedule C was 15% higher than at the RD in schedule A. This might be explained by the poor tolerance of longer exposures, as illustrated by the toxic events reported in schedule B.

3.2. Toxicity

The most frequent toxicities (any grade) among all schedules were asthenia, anorexia, nausea, vomiting, liver function abnormalities, as well as hypokalaemia and serum creatinine increase (Table 3). No significant haematological toxicity was observed.

3.2.1. Nausea and vomiting

Mild to moderate nausea and/or vomiting were frequently observed on all schedules (Table 3). At the RD in schedule A, 2 of 3 patients had grades 1 and 2 nausea and vomiting. At the RD in schedule C, 2 of 3 patients had grades 1 and 2 nausea and all 3 patients had grades 1 and 2 vomiting. These side-effects could subsequently be prevented by the use of ondansetron and steroids.

3.2.2. Hepatic disorders

Hepatic enzyme abnormalities were reported following oral seliciclib administration and were without abnormalities of coagulation tests. An increase in AST/ALT levels usually occurred during the first 2 cycles of treatment. Increased bilirubin levels were also observed, but appeared to be more prevalent at higher doses in schedules A and C. These toxicities were reversible after treatment discontinuation. At the RD in schedule A, all three patients experienced grades 1 and 2 increase in AP, bilirubin and ALT/AST and transient grade 3 increase in GGT. At the RD in schedule C, all three patients experienced transient grades 3 and 4 GGT increase, and grades 1 and 2 AP increase. Two patients experienced grade 2 and one patient had grade 3 ALT increases, while two patients had grades 2 and 3 bilirubin increase.

3.2.3. Metabolic disorders

Most patients experienced hypokalaemia (any grade) during the study (any cycle). Fourteen had grade 3 hypokalaemia and three had grade 4. The occurrence of hypokalaemia appeared to be dose dependent; occurring only at higher doses, and did not seem to be related to the duration of drug administration. Hypokalaemia did not appear to be related to vomiting or diarrhoea and was not associated with cardiac disorders. Serum creatinine increase (all grades) occurred in 37 patients (66%). However, only one patient in schedule A experienced a grade 3 serum creatinine increase. At the RDs, one patient in schedule A and two patients in schedule C experienced grades 1 and 2 increase in creatinine levels.

3.3. Pharmacokinetic analysis

Overall, the exposure of seliciclib and its carboxylate metabolite (PMF30-128) increased with increasing doses. Within-group comparisons showed that the seliciclib exposure was generally higher on day 1 than on subsequent days in some patients. The median terminal half-life of seliciclib was similar in all dose groups (Table 4).

Seliciclib and metabolites' plasma concentrations at days 1 and 3 of cycle 1 at the RD in schedule C are shown in Fig. 1A. Concentrations of seliciclib often exceeded concentrations associated with *in vitro* activity.[10] Seliciclib and PMF30-128 concentrations on days 1 and 3 were roughly similar. In contrast, the M2 metabolite was produced at a high concentration with a peak plasma level reaching 40 μg/ml on day 3. The M1 metabolite PK profile suggested its accumulation over time.

Table 4 – Summary of seliciclib pharmacokinetic parameters on day 1 of cycle 1.

Dose mg/d	No. of patients	Median			
		C_{max} (μg/ml)	T_{max} (h)	$T_{1/2}$ (h)	AUC_{last} (μg*h/ml)
200 (A)	2	0.05	1.52	5.95	0.65
400 (A)	2	0.37	2.25	2.98	1.58
800 (A)	1	1.63	1.50	2.87	16.18
1600 (A)	4	3.76	2.00	3.11	24.73
1600 (B)	6	3.51	2.00	3.62	42.82
2000 (A)	6	2.87	4.00	3.63	33.43
2000 (B)	6	1.75	4.00	6.38	32.90
2400 (C)	3	4.90	1.52	3.95	39.40
2500 (A)	5	3.07	2.11	4.05	37.33
3200 (A)	3	3.75	4.00	3.25	46.18
3200 (C)	4	7.60	4.00	4.13	120.98
3200 (C[a])	6	6.43	8.03	3.30	84.75
3600 (C)	6	4.91	1.75	3.06	79.53

NA: not applicable; SD: Standard deviation; AUC: area under concentration–time curve; (A): schedule A; (B): schedule B; and (C): schedule C.
[a] Tri-daily administration.

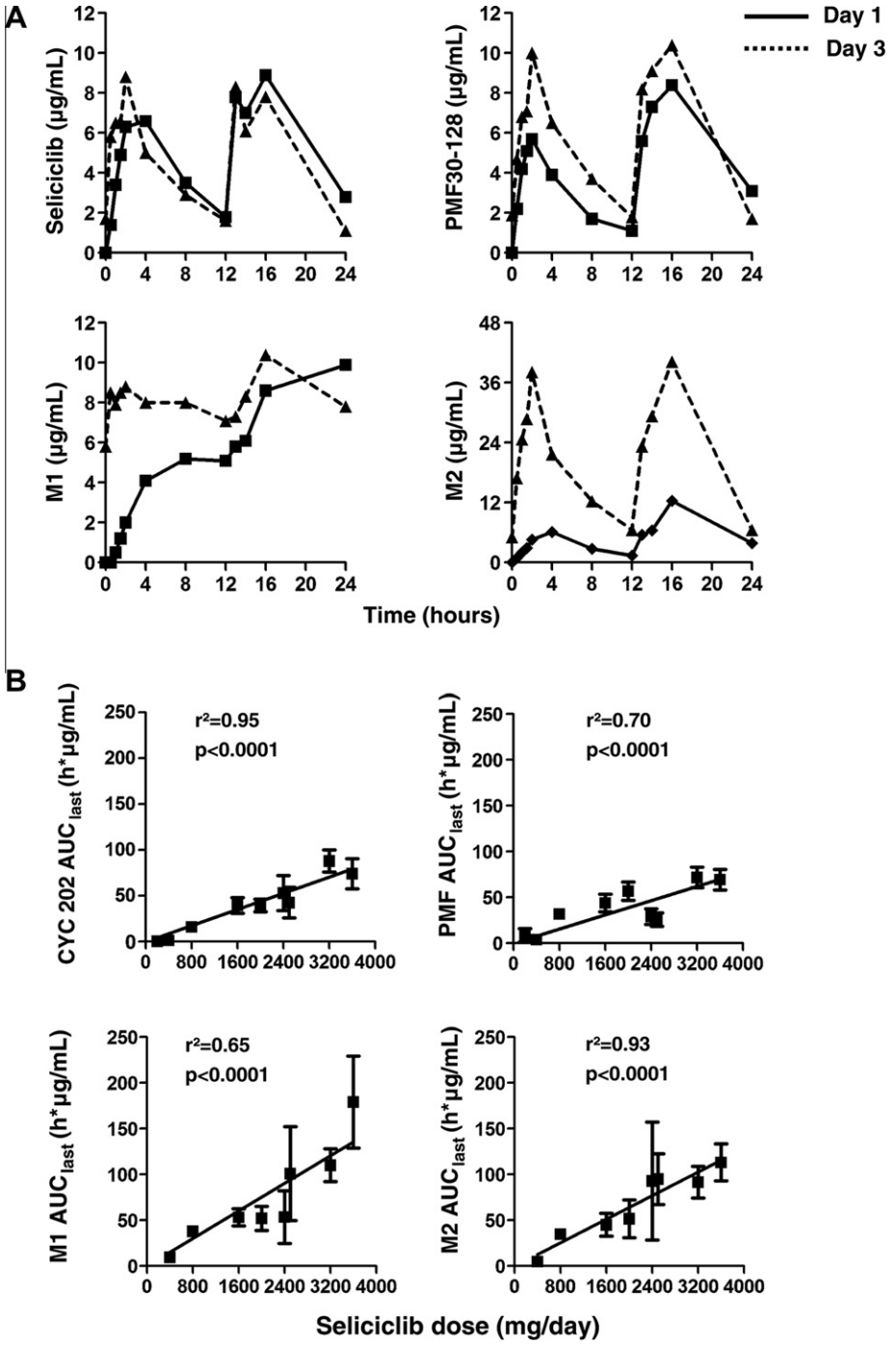

Fig. 1 – (A) Mean plasma concentrations of seliciclib, PMF30-128, M1 and M2 at days 1 and 3 when seliciclib was given at the recommended dose in schedule C (1600 mg bid for 3 d every 2 weeks). (B) Mean AUC_{last} of seliciclib, PMF30-128, M1 and M2 according to seliciclib dose. NB. Seliciclib standard curves were used for the determination of M1 and M2 concentrations in plasma as M1 and M2 standards were not available to quantify these metabolites in their own right.

Similar results were obtained at the RD in schedule A (data not shown).

Median AUC_{last} *versus* dose curves suggested that exposure to seliciclib and its metabolites increased incrementally with dose (Fig. 1B).

Overall, C_{max} and AUC_{last} obtained at the RD in schedule C were higher than at the RD in schedule A.

3.4. *Pharmacodynamic analysis*

Among 48 patients evaluable for pharmacodynamic analysis, 22 and 15 patients had a statistically significant increase in the levels of TPS and M30, respectively, from day 1 to the last day of treatment of cycle 1, respectively (Supplementary Fig. A1). Because of the small number of patients treated at

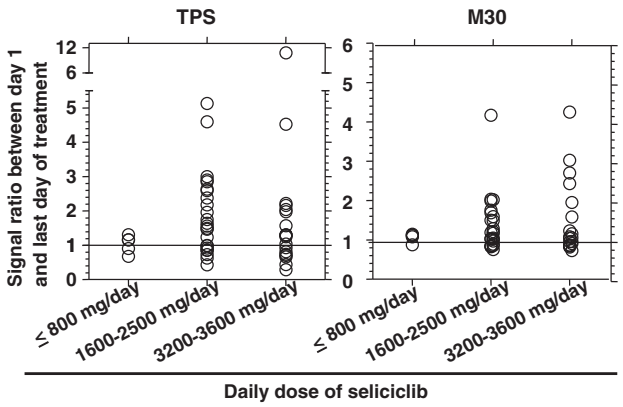

Fig. 2 – Ratio in the signals of tumour cell death TPS and M30 plasma markers between the first day and the last day of treatment. Patients were grouped into three categories depending on the administered daily dose of seliciclib (⩽800 mg/d, 1600–2500 mg/d and 3200–3600 mg/d).

each dose level, patients were grouped into three categories for the dose-dependency evaluation of these pharmacodynamic markers, depending on the administered daily dose of seliciclib: low (⩽800 mg/d), medium (1600–2500 mg/d) and high (3200–3600 mg/d). Both TPS and M30 levels increased significantly following seliciclib treatment for the medium and high-dose categories, but not for the low-dose category (Fig. 2).

3.5. *Antitumour activity*

One partial response was observed in a patient with hepatocellular carcinoma (800 mg bid, schedule B). Six patients achieved tumour stabilisation lasting ⩾4 months [range: 4–>18 months], including two patients with NSCLC (1600 mg bid, schedule A, and 1800 mg bid, schedule C) and one patient with each of the following tumour types: parotid cylindroma (100 mg bid, schedule A), corticosurrenaloma (1000 mg bid, schedule A), thymic carcinoma (1000 mg bid, schedule B) and adenocarcinoma of unknown primary (400 mg bid, schedule A).

4. Discussion

CDKs expression is often perturbed in cancer and their inhibition can induce apoptosis. CDKs represent therefore rational targets for cancer therapy. Several CDK inhibitors have entered clinical evaluation.

Our study shows that seliciclib can be safely administered orally at the dose of 1250 mg bid for 5 d every 3 weeks (schedule A) and at the dose of 1600 mg bid for 3 d every 2 weeks (schedule C). Uninterrupted dosing for 10 d (schedule B) was poorly tolerated and not further evaluated. Pharmacokinetic data and dose–intensity analyses suggest that schedule C would be more appropriate to take forward in phase II trials.

In contrast to other CDK inhibitors such as flavopiridol and indisulam, no haematological toxicity was observed in this study.[14–16] This difference might be related to the different CDK targets of these compounds. DLTs were asthenia, hypokalaemia, nausea, vomiting and skin rash, all of them being reversible upon treatment discontinuation. Hypokalaemia was more frequently reported at higher doses. The mechanisms by which seliciclib induces hypokalaemia were investigated in a previous phase I trial of seliciclib,[17] but still remain unclear. This metabolic disorder was not observed with other CDK inhibitors. Increased hepatic enzymes and serum creatinine were frequent, and this was already reported in a previous phase I trial evaluating seliciclib bid for 7 d every 3 weeks.[17] Transient serum creatinine increase had been reported with other CDK inhibitors.[18,19]

The PK data obtained in this study were in accordance with those obtained in a previous phase I trial.[17] In this latter study, patients received doses of seliciclib ranging from 100 mg bid to 800 mg bid for 7 consecutive days every 21 d. Mean AUC_{last} of seliciclib observed in the current study was similar to that observed in the previous study at the same dose. The high plasma levels of seliciclib metabolites, in particular M2, at the RD for schedules A and C suggested that seliciclib was subject to extensive metabolism. The accumulation of the M1 metabolite and the high levels of the M2 metabolite might explain why prolonged administrations of lower doses of seliciclib were poorly tolerated in schedule B of this study and the previous phase I trial.[17] The PK analysis found no clear correlation between any toxicity and peak plasma level and/or exposure to seliciclib and its metabolites.

Several PD biomarkers directly reflecting cell death have been investigated in trials of CDK inhibitors. Pharmacokinetic–pharmacodynamic relationships established in HCT116 human colon cancer xenografts treated with seliciclib have shown reduced phosphorylation of the retinoblastoma protein and decreased expression of cyclin D1 in tumour tissue.[20] However, these markers did not change in peripheral blood mononuclear cells of patients treated with seliciclib in a previous phase I trial with seliciclib.[17] This was not entirely unexpected, considering that these fully differentiated cells are not cycling. Reduced tumoural phosphorylation of retinoblastoma protein inconsistently decreased in patients treated with CDK inhibitors such as indisulam and AZD5438,[19,21] but these effects were transient. Seliciclib was shown to induce cell death at doses ⩾800 mg/d as indirectly evidenced by the release of CK18 fragments in plasma. Two different assays were used, M30 monitoring the release of CK18 following the induction of apoptosis and TPS monitoring the release of CK18 by either necrosis or apoptosis. Both assays indicated that seliciclib was biologically active in patients since the increase in the levels of cell-death markers in patient's plasma following exposure to seliciclib was significant. In this study, a good correlation between the two assays was found, although the TPS assay had a greater sensitivity making it more reliable for monitoring samples from patients that had low plasma levels of CK18. Some data have been published suggesting that release of CK18 can be associated with hepatic and renal dysfunction.[22–24] In this study, the changes in the levels of either TPS or M30 did not statistically correlate with any changes in individual biochemical measurements taken from the patients experiencing liver and renal toxicities (data not shown).

Based on the results of this trial, the CDK inhibitor seliciclib is currently being investigated in phase II studies as a

single agent in patients with nasopharyngeal carcinoma and NSCLC. Some evidence of tumour reduction has been reported with seliciclib single agent in patients with treatment-naïve, locally advanced nasopharyngeal carcinomas.[25]

Conflict of interest statement

Sian Armour, Sheelagh Frame, Simon Green and Athos Gianella-Borradori are employed by Cyclacel. Christophe Le Tourneau, Sandrine Faivre, Valerie Laurence, Catherine Delbaldo, Karina Vera, Veronique Girre, Véronique Diéras and Eric Raymond do not have any conflict of interest to disclose.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.ejca.2010.08.001.

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