European Journal of Cancer 169 (2022) 135–145

Available online at www.sciencedirect.com

ScienceDirect

journal homepage: www.ejcancer.com

Original Research

First-in-man, first-in-class phase I study with the monopolar spindle 1 kinase inhibitor S81694 administered intravenously in adult patients with advanced, metastatic solid tumours

Patrick Schöffski [a,*], Ahmad Awada [b], Anne-Marie de la Bigne [c], Zakia Felloussi [c], Mike Burbridge [c], Frederique Cantero [c], Riccardo Colombo [d], Sara Maruzzelli [d], Katia Ammattatelli [d], Maja de Jonge [e], Philippe Aftimos [b], Herlinde Dumez [a], Stefan Sleijfer [e]

[a] *Department of General Medical Oncology, University Hospitals Leuven, Leuven Cancer Institute, Leuven, Belgium*
[b] *Clinical Trials Conduct Unit, Institut Jules Bordet, Université Libre de Bruxelles, Brussels, Belgium*
[c] *TA Oncology, Institut de Recherches Internationales Servier, Suresnes, France*
[d] *Nerviano Medical Sciences S.r.l., Nerviano, Milan, Italy*
[e] *Medical Oncology, Erasmus MC Cancer Institute, Rotterdam, the Netherlands*

Received 11 February 2022; received in revised form 28 March 2022; accepted 1 April 2022
Available online 11 May 2022

KEYWORDS
Phase I;
S81694;
MPS1;
Spindle assembly checkpoint inhibitor;
Monopolar spindle 1 kinase

Abstract ***Background:*** S81694 is an inhibitor of monopolar spindle 1 kinase, a target expressed in proliferating cells. CL1-81694-001 was the first-in-human study aiming at identifying a safe dosing schedule in solid tumour patients.
Patients and methods: This trial was based on inter-individual dose-escalation of single agent S81694 in cohorts of ≥3 patients to assess the safety and tolerability and determine dose-limiting toxicities (DLTs), maximum tolerated dose (MTD) and recommended phase II dose (RP2D), with S81694 given on days 1,8,15 of a 28-day cycle as 1-h infusion.
Results: 38 patients were treated at doses ranging from 4 to 135 mg/m^2/week; 144 cycles were administered (median 2/patient; range 1–32 cycles). Patients discontinued treatment for disease progression (78.9%), adverse events (AE; 18.4%) or withdrawal of consent (2.6%). Treatment modifications occurred in 22 patients (57.9%; 49 cycles). Common treatment-emergent AEs were fatigue (22 patients;57.9%), anaemia (17;44.7%) and nausea (12;31.6%).

* *Corresponding author*: Department of General Medical Oncology, Leuven Cancer Institute, University Hospitals Leuven, and Faculty of Medicine, Department of Oncology, Laboratory of Experimental Oncology, KU Leuven, Herestraat 49, B-3000, Leuven, Belgium. Tel.: +32 16 346900; fax: +32 16 346901. .
E-mail address: patrick.schoffski@uzleuven.be (P. Schöffski).
@schoffski (P. Schöffski)

https://doi.org/10.1016/j.ejca.2022.04.001

Haematological toxicity was mild, with Grade 3 anaemia observed in three patients and neutropenia mainly seen at the 135 mg/m^2 dose level. Three first cycle DLTs included G3 anaemia (4 mg/m^2 dose), G4 hypertension (20 mg/m^2), G3 fatigue (135 mg/m^2). MTD was not reached due to premature discontinuation of enrolment based on a sponsor decision. Among 35 patients evaluable for response, one (renal cell carcinoma) had a complete response, one (hepatocellular carcinoma) had a transient decrease of target lesions and 13 had stable disease. Seven patients remained on study for ≥6 cycles, two at the 135 mg/m^2 dose.

Conclusions: S81694 can be administered safely as a single agent in adults with solid tumours on days 1,8,15 of a 28-day cycle up to a dose of 135 mg/m^2/week without reaching MTD. The RP2D was not defined due to the prioritization of the use of S81694 in combination with cytotoxic agents, based on emerging preclinical data.

Trial registration: EudraCT number: 2014-002023-10; ISRCTN registry ISRCTN35641359.

1. Introduction

Monopolar spindle 1 (MPS1) kinase is a dual tyrosine/serine–threonine kinase highly expressed in proliferating cells and a potential target for anti-cancer treatments [1]. Upon activation by phosphorylation, MPS1 plays a critical role in controlling mitosis by regulating the Spindle Assembly Checkpoint through kinetochore recruitment of essential proteins. This complex regulates a mechanism required for chromosome alignment, influencing the stability of the kinetochore-microtubule interaction and ensuring that cells do not divide until sister chromatids align in metaphase [2–7]. MPS1 is highly expressed in a number of tumours of different origins (melanoma, bladder, anaplastic thyroid, breast, lung, oesophageal, colon, pancreas, prostate cancer and glioblastoma).

MPS1 inhibitors are preferentially active in tumours with aneuploidy, a common feature of cancer cells: approximately 90% of solid tumours and 70% of haematological cancers are aneuploid, most having gains in chromosomes. High aneuploidy tumours overexpress MPS1 [3,8]. Triple-negative breast cancer is among tumour types dependent on MPS1 activity and potentially more sensitive to MPS1 inhibition than other tumour types [9–11]. When compared to widely-used anti-mitotic agents such as taxanes and vinca-alkaloids [12], MPS1 inhibitors do not induce but arrest mitosis.

S81694, a pyrazoloquinazoline, is a highly potent and selective small-molecule inhibitor of MPS1. S81694 causes mitotic checkpoint override, accelerates mitosis, induces chromosomal misalignment, reduces mitotic marker expression, induces apoptosis and cancer cell death. S81694 has a long residence time on the target and a brief treatment of 2 h is sufficient to commit cells to death. *In vivo*, the compound demonstrated significant anti-tumour activity in a number of cell lines, xenografts and transgenic tumour models (colon, mammary, melanoma, leukaemia, prostate). Significant tumour growth inhibition of up to 95% was seen with an intermittent schedule at well-tolerated doses.

Non-clinical safety of S81694 was characterized after intravenous (iv.) administration in rats, dogs and monkeys. In 4-week toxicity studies, the major target organs following repeated administration were the haemolymphopoietic system in rats and monkeys, the intestinal tract and male reproductive organs in rats. Injection site alterations were seen in both species. S81694 was not genotoxic in the Ames test but induced micronuclei in human peripheral lymphocytes *in vitro*. Haemolysis was observed *in vitro* at high concentrations. In rabbits, S81694 did not irritate the skin but induced ocular irritation.

A dose-related inhibition of the IKr potassium channel was observed in the hERG assay. A transient increase in arterial pressure was observed in monkeys but had no effect on heart rate, electrocardiogram or body temperature. According to preclinical toxicity, the safety profile was considered manageable, with target organ toxicity in line with its mechanism of action.

Here we present the results of trial CL1-81694-001 (EudraCT 2014-002023-10; ISRCTN registry reference ISRCTN35641359.), the first in human study of S81694, aimed at identifying a safe dosing schedule suitable for the development of the compound in patients with advanced/metastatic solid tumours.

2. Material and methods

2.1. Objectives

This study was a first-in-human, open-label, non-randomized, multi-centre, multinational, non-comparative dose-escalation trial in cohorts of adult patients with solid tumours who had exhausted standard treatment options. The study was based on inter-individual dose-escalation in cohorts of at least 3 patients each. The primary objective was to determine the maximum tolerated dose (MTD) and dose-limiting toxicities (DLTs) of S81694. Secondary objectives were to describe the safety and tolerability of S81694; define the recommended phase II dose (RP2D); determine the

pharmacokinetic (PK) profile of S81694 and its metabolite(s); explore the relationship between PK and selected adverse events (AEs); explore any potential exposure-response relationship for safety, efficacy and pharmacodynamics (PD); detect early signs of antitumour efficacy; and to identify potential predictive biomarkers of efficacy.

The study was approved by institutional review boards and ethics committees in participating institutions, by the competent authorities in participating countries and performed according to local and international guidelines.

2.2. Primary and secondary end points

The primary end point was to identify DLT occurring during the first treatment cycle, defined as 28 days. Secondary and exploratory end points included: safety and tolerability characterized by type, frequency, severity, intensity, timing and relationship AEs and laboratory abnormalities; PK parameters in plasma and urine; objective tumour response, as per Response Evaluation Criteria in Solid Tumors (RECIST version 1.1) [13]; biomarkers associated with treatment outcome.

2.3. Patient selection criteria

Male or female patients ≥18 years were included after informed consent, provided they had a confirmed diagnosis of an advanced or metastatic solid tumour for which no effective standard therapy was available or where standard therapy was considered unsuitable. At least 4 weeks/5 half-lives elapsed after completion of prior therapy and the first administration of S81694. Patients with controlled central nervous system involvement were included provided corticosteroids and/or anticonvulsants were not required. Resolution of toxicities due to prior treatment, an Eastern Cooperative Oncology Group performance status of 0–1, effective contraception and adequate organ function were required.

Baseline laboratory values fulfilled the following requirements: neutrophil count ≥1500/mm^3, platelets ≥100,000/mm^3, haemoglobin ≥10 g/dL, creatinine ≤1.5 times upper limit of normal (ULN) or creatinine clearance >60 ml/min, bilirubin ≤1.5 ULN, transaminases ≤3.0 ULN (≤5.0 ULN in presence of metastasis), alkaline phosphatase ≤2.5 ULN (≤5.0 ULN in presence of liver metastasis), direct Coombs test negative, Cold agglutinins negative (titer <64) and negative pregnancy test in females.

Patients were excluded if they had a blood transfusion ≤3 weeks before treatment, a history of haemolytic anaemia, glucose-6-phosphate-dehydrogenase-deficiency, thrombotic thrombocytopenic purpura, microangiopathic haemolytic anaemia or haemolytic uremic syndrome. Other exclusion criteria included concomitant treatment with inducers of flavin-containing monooxigenase 1 or 3, sensitive CYP3A4 substrates or CYP3A4 and BCRP substrates with narrow index. Other generic phase I exclusion criteria were applied.

2.4. Dose escalation strategy and cohort size

Patients were allocated to sequential cohorts of progressively increasing dose levels of S81694. Initially, dose escalation was based on 100% dose increments until treatment-related grade ≥2 toxicity was observed during the first cycle. Afterwards, increments ≤50% were adopted based on the evaluation of safety and PK.

Initially, three patients were treated at each dose level. The first patient was observed for DLT for at least 2 weeks before entering a new participant. In absence of DLT, the second and the third patient were enrolled together. If a patient discontinued treatment before completing Cycle 1 for reasons other than drug-related AEs, the patient was replaced. If 0/3 patients experienced DLT in the DLT window, the next cohort had to be treated at one dose level higher. If 1/3 patients experienced a DLT, up to three more were treated at the same level and were recruited in parallel. If <2/6 experienced DLT, the next cohort could start at one dose level higher. All patients at a given dose level had to complete the DLT window before a new patient could be treated with a higher dose. After completion of each cohort, safety and PK were reviewed and dose-escalation was decided jointly between investigators and sponsor. If ≥2/3 or >2/6 patients experienced DLTs in the DLT window MTD was reached. A new cohort (3–6 patients) was to be treated at a lower dose level to identify the RP2D.

The initial 8 dose levels were tested according to the design described above. After that, a Bayesian Logistic Regression Model (BLRM) was used to administer patients more accurate doses following a prediction MTD [14,15].

2.5. Treatment

S81694 was supplied as glass vials containing 30 mg/vial powder for solution for infusion formulated in mannitol 4%, polysorbate 80 0.5%, to be reconstituted in 10 ml of water for injection for a final solution of 3 mg/ml. The supplied solution had to be diluted to 250 ml or 375 ml (according to the dose of S81694) with saline; the final concentration was to range between 0.0272 mg/ml and 1.28 mg/ml.

S81694 was administered as single agent at increasing doses, starting from 4 mg/m^2 weekly, and given as a 1-h iv. infusion (if the absolute dose was ≤255 mg) or over 1 h 30 min (if dose was >255 mg and <480 mg) either by central venous catheter or peripherally, using an infusion pump. The duration of the infusion had the purpose of reducing haemolytic effects observed after

bolus administration in blood samples in animals during toxicology. The drug was administered on days 1, 8 and 15 in a 28-day cycle. A week of rest was introduced to allow recovery from expected myelotoxicity.

2.6. Starting dose

A starting dose of 4 mg/m^2/week (12 mg/m^2/cycle) was considered safe, corresponding to the lower dose between 1 and 6 of the highest non-severely toxic dose in a 4-week toxicity study in monkey (2 mg/kg = 24 mg/m^2, 1/6 = 4 mg/m^2), the most sensitive non-rodent species and to 1/10 of the severely toxic dose 10 in a 4-week toxicity study in rat (8 mg/kg = 48 mg/m^2, 1/10 = 4.8 mg/m^2).

2.7. Dose and schedule modifications

Patients were monitored for AEs. Dose modifications during treatment were guided by protocol. Dose modifications were based on the most severe, causally related AEs observed in the previous cycle. If multiple toxicities were observed, dose modifications were based on the most severe event.

Doses reduced for drug-related toxicity were not re-escalated. In case a patient experienced a DLT, it was recommended to reduce one level down. Patients experiencing DLT during the DLT window could be re-treated as described in the protocol. Patients who could not be re-treated because of toxicity within 36 days went off study.

2.8. Expansion cohorts

Expansion cohorts in selected tumour type were planned to further evaluate toxicity, PK, PD and to explore the efficacy of the compound. Due to early termination of the study no expansion phase occurred and planned exploratory work was not pursued.

2.9. Concomitant treatment

Concomitant treatments were administered according to local practice. Treatment with haematopoietic growth factors and transfusions was at investigator's discretion. Bisphosphonates or denosumab were allowed. In case of intolerance to polysorbate 80 anti-histaminics or corticosteroids were permitted. Strong inhibitors of Flavin-containing monooxigenase 1 and 3, breast cancer-resistant protein substrates with no narrow therapeutic index and substrates metabolized by cytochrome P3 A4 were used cautiously.

2.10. Treatment discontinuation

Treatment was discontinued in case of disease progression; any medical event requiring administration of an unauthorized concomitant treatment or jeopardizing patient's safety; occurrence of permanent or significant incapacity or disability; conditions making it impossible to comply with study protocol; and/or patient's refusal to continue.

2.11. Safety assessment and adverse events

AEs were graded according to NCI-CTCAE version 4.03. Patients were seen weekly during the DLT window, defined as the interval between the first administration in cycle 1 and the day of the first dose administration in cycle 2 which was expected to be 28 days or up to 35 days in case of delay.

2.12. Definition of dose-limiting toxicity

DLT was defined as any of the following events, for which a relationship to treatment with S81694 could not be excluded: Grade 4 neutropenia >7 days; febrile neutropenia Grade ≥3; Grade 4 thrombocytopenia; Grade 3 thrombocytopenia >7 days or associated with bleeding; any ≥3 grade non-haematological toxicity or inability to deliver cycle 2 on day 35 due to persisting toxicity. Considering preclinical toxicology, the following events were also defined as DLT: haemolysis Grade ≥3; Grade ≥3 anaemia with/without haemolysis. In case a patient did not receive at least 66% of the intended total dose during cycle 1 this was defined as technical DLT, but the patient was not replaced.

2.13. Response assessment

Tumour evaluation was based on computer tomography or magnetic resonance imaging performed at baseline and every 2 cycles starting with cycle 2, every 4 cycles from cycle 12 onwards and at the end of the last cycle. Efficacy was assessed according to RECIST 1.1 [13]. The objective tumour response rate was defined as the percentage of patients achieving a complete (CR) or partial response (PR). Patients discontinuing treatment for progression prior to the first on-treatment tumour assessment were classified as early PD.

2.14. Statistical analysis

The statistical analysis plan described a treated patients safety set, a PK set, a set evaluable for DLT and a set evaluable for efficacy. The analysis was descriptive and performed by CLIOSS S.r.l (Nerviano, Milan, Italy).

2.15. Pharmacokinetic and pharmacodynamics analysis

The PK analysis quantified S81694, its N-oxide metabolite (M1) and other metabolites. Assessments were performed on days 1–4, 8, 15–18 and 22 of cycle 1, and

on days 1 and 15 of cycle 2. Urine was collected on days 1 and 15 in cycle 1.

Plasma and urine concentration data points were used to calculate PK parameters using a non-compartmental approach, performed by Accelera (Nerviano, Milan, Italy). Plasma concentrations were analysed by population approach. Potential PK/PD relationships with activity, efficacy or safety were investigated through an exploratory analysis. A planned PD evaluation and molecular characterization of fresh tumour samples were not performed as the expansion phase of the trial was not activated.

2.16. Changes in the conduct of the study and planned analyses

The most important changes were introduced by Amendment 4, which revised the dose-escalation design, changing from a 3 + 3 design to BLRM, and Amendment 5, which updated the infusion procedure, extending the infusion duration to 1 h 30 min for any administered dose between ≤225 and 480 mg.

The sponsor discontinued the study before the completion of dose escalation and the expansion part was not implemented. This decision was related to the discontinuation of the development of S81694 as a single agent and the strategic decision to pursue further development in combination with other therapies.

3. Results

3.1. Patient characteristics

Thirty-nine patients were enrolled and assigned to increasing dose levels, including one who was not treated due to early deterioration of liver function. Deviations from selection criteria were observed in three patients (abnormal serum creatinine, positive cold agglutinins, recent cardiac disease). The median age at entry was 58.5 years (range 44–73), 10 patients (26.3%) were ≥65 years old. Gender was balanced and the majority of patients were Caucasian (94.7%). The performance status was 0 in 16 patients (42.1%) and 1 in 22 (57.9%). The most frequent cancer types were gastrointestinal tumours (23.7%), lung (13.2%), head and neck cancer (10.5%), sarcoma (7.9%), mesothelioma, breast cancer and endocrine malignancies (5.3% each). All but three patients had metastatic disease. Frequent sites of metastasis were lung (50%), liver (42.1%), lymph nodes (36.8%) and bone (23.7%). All patients had received prior systemic therapy, 9 (23.7%) had >5 lines of prior therapy and 20 (52.6%) had received 3–5 lines. Twenty-five (65.8%) had prior surgery, 14 (36.8%) radiotherapy and 24 (63.2%) had received hormonal, immune- and/or targeted therapies. Table 1 summarizes patient characteristics.

3.2. Patient disposition

Thirty-eight patients were treated on 9 dose levels, with 3–7 patients/level. S81694 was administered at 4 mg/m^2/week (6 patients), 6 mg (3), 9 mg (3), 13.5 mg (3), 20 mg (6), 30 mg (3), 45 mg (3), 67.5 mg (4) and 135 mg (7). A total of 144 treatment cycles were administered (median 2, range 1–32 cycles/patient).

A total of 38 patients received S81694 (safety set). The evaluable set for DLT consisted of 36 patients. All treated patients were included in the PK set. Thirty-six were evaluable for efficacy; one had no target lesion at baseline and one had no on-treatment assessment.

3.3. Reasons for treatment discontinuation

Patients discontinued treatment for disease progression in 30 cases (78.9%), AE or condition incompatible with further administration in 7 cases (18.4%) and withdrawal of consent in one case (2.6%). The conditions incompatible with further administration were anaemia G2, blood creatinine increased G2, pulmonary haemorrhage G3, emotional distress G3 and 2 cases of dyspnoea G3 combined with fatigue G3. There were no discontinuations due to DLTs.

3.4. Dose modifications and dose intensity

Treatment modifications (cycle/dosing delay, dose reduction, drug omission, infusion interruption, slowing of infusion) occurred in 22 patients (57.9%) in 49 cycles (34%). The median relative dose intensity for all patients

Table 1
Patient and tumour characteristics.

		Treated patients	
		n	%
Gender	Male	20	52.6
	Female	18[a]	47.4
Age (years)	<65	28	73.7
	65 - <85	10	26.3
ECOG PS	0	16	42.1
	1	22	57.9
Tumour Type	Mesothelioma	2	5.3
	Cancer of head and neck	4	10.5
	Cancer of the breast	2	5.3
	Cancers of lung	5	13.2
	Cancers of the endocrine system	2	5.3
	Cancers of the gastrointestinal tract	9	23.7
	Cancers of the genitourinary system	1	2.6
	Gynecologic cancers	1	2.6
	Melanoma	1	2.6
	Other	8	21.1
	Sarcomas of the soft tissue and bone	3	7.9

[a] One female patient was enrolled but never treated due to early deterioration of liver function.

was 98.2% (range 33.3–109.8%). At 135 mg/m^2/week the median relative dose intensity was 77.9% (range 47.1–94.4).

3.5. Clinical safety and toxicity

Thirty patients (78.9%) had treatment-emergent AE (TEAE) related to S81694, and the frequency ranged from 33.3 to 100% across dose levels, without clear relationship with dose. Most commonly (>5 patients) affected by TEAEs were blood/lymphatic system disorders (17, 44.7%), general disorders and administration site conditions (15, 39.5%), gastrointestinal (14, 36.8%), metabolism and nutrition (9, 23.7%) and nervous system disorders (6, 15.8%).

The most frequent TEAEs reported in at least 15% of patients were fatigue (22 patients, 57.9%), anaemia (17, 44.7%), nausea (12, 31.6%), decreased appetite (11, 28.9%), constipation, pain, vomiting (9, 23.7% each), cough (8, 21.1%), diarrhoea (7, 18.4%) and dyspnoea (6, 15.8%).

No significant ECG abnormalities were detected at baseline. During treatment, 25 patients had a maximum increase in QTc of ≤30 msec, 10 had > 30–60 msec and 2 had an increase of >60 msec, the latter also experiencing atrial fibrillation.

No Grade 3–4 drug-related TEAEs were reported up to dose level 13.5 mg, except for one case of Grade 3 anaemia (DLT) occurring in the first patient treated at 4 mg/m^2/week.

In subsequent dose levels, the following Grade 3–4 drug-related TEAEs were reported: nausea and hypertensive crisis (one case each) at 20 mg/m^2/week, hypophosphataemia (1) at 30 mg, gamma-glutamyltransferase increased (1) at 45 mg, platelet count decreased (1) at 67.5 mg, anaemia, keratitis, mucosal inflammation (1 case each), neutropenia (4) and fatigue (1 case, DLT) at 135 mg. These events were Grade 3, except for the Grade 4 hypertensive crisis. Table 2a shows the most frequently reported TEAEs in the safety population related to S81694.

3.6. Haematological toxicity

Abnormalities in haemoglobin were observed in 25 patients (all dose levels), Grade 3 alterations were reported in three patients only. Anaemia was seen in 17 patients (in 14 patients considered drug-related), with Grade 3 anaemia in three cases (2 patients only with Grade 3 drug-related anemia, including one DLT). Six patients had blood transfusions. Grade 1 haemolysis was reported in three patients.

Neutropenia was negligible at dose levels up to 67.5 mg/m^2/week. At 135 mg 4/7 patients experienced Grade 3 neutropenia. Median time to nadir was 22 days (range, 15–25) and median time to recovery was 27 days (range, 21–46). The median neutrophil value for four patients with Grade 3 neutropenia was 0.8 × 10^3/mm^3 (range, 0.5–1.0). No relevant effects were observed on platelets, except one thrombocytopenia Grade 3 at 67.5 mg/m^2/week. Table 2b presents haematological AEs.

3.7. Non-haematological blood chemistry abnormalities

Grade 3 increases in ALT/AST were reported in one patient each, 8 had Grade 3 gamma GT increases. The majority of patients with liver enzyme abnormalities had liver metastases. Phosphate was abnormal in 6 patients. A Grade 3 decrease in potassium was reported at the dose of 135 mg/m^2/week; a Grade 3 decrease in sodium was observed in another patient (67.5 mg). No Grade 4 blood chemistry alterations were reported. Non-haematological laboratory AEs are summarized in Table 2c.

3.8. Dose-limiting toxicity and maximum tolerated dose

Among 36 patients evaluable for DLT, three events were reported during the first cycle of treatment. Patient 0001 (4 mg/m^2/week) experienced Grade 3 anaemia; the event started with Grade 2 severity on cycle 1 day 1 and worsened to Grade 3 on cycle 1 day 15, was considered drug related and the patient came off due to PD. The cohort was expanded to 6 patients with no additional DLTs. Patient 0019 (20 mg/m^2/week) had a Grade 4 hypertensive crisis on day 2 cycle 1, considered drug related and leading to dose omission on days 8 and 15. In preclinical toxicology studies, a slight increase (10–15 mmHg) in systemic arterial pressure was observed after treatment in laboratory animals. For this reason, the clinical event was assessed as related, though the pathomechanism of blood pressure increase remains unknown. The patient went off treatment for PD; the cohort was expanded and no additional DLTs were seen. Patient 0036 (135 mg/m^2/week) had Grade 3 fatigue occurring on cycle 1 day 21, lasting one week and was considered drug-related, leading to dose reduction in cycle 2. When the patient withdrew consent, the cohort was expanded and no further DLTs were observed. The primary end point of defining the MTD was not reached.

Overall 18 patients experienced a SAE; three events were considered treatment-related, two occurring at the highest dose tested. SAEs included Grade 3 pain, Grade 2 acute kidney injury, Grade 3 pulmonary haemorrhage, Grade 1 pyrexia, Grade 2 tumour haemorrhage, Grade 3 tumour pain, Grade 3 diarrhoea, Grade 4 hypertensive crisis, Grade 3 radiculopathy, Grade 3 infection, Grade 3 cervical vertebral fracture, Grade 3 emotional distress, Grade 3 intestinal obstruction, Grade 5 malignant neoplasm progression, Grade 1 accidental overdose, Grade 5 fatal malignant neoplasm progression, Grade 3

Table 2a
Most frequently reported (>5%) treatment emergent adverse events (TEAE), related to S81694 by any CTC Grade 3-4.

	CTC Grade	Assigned Dose Level (mg/m^2/week)																	
		Any Dose Level (N = 38)		4 (N = 6)		6 (N = 3)		9 (N = 3)		13.5 (N = 3)		20 (N = 6)		30 (N = 3)		45 (N = 3)		67.5 (N = 4)		135 (N = 7)	
		n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%
Any Term	Any	30	78.9	6	100.0	3	100.0	1	33.3	1	33.3	4	66.7	3	100.0	3	100.0	3	75.0	6	85.7
	3–4	11	28.9	1	16.7							2	33.3	1	33.3	1	33.3	1	25.0	5	71.4
Abdominal pain	Any	2	5.3			1	33.3							1	33.3						
Anaemia	Any	14	36.8	3	50.0	1	33.3			1	33.3	2	33.3	2	66.7			1	25.0	4	57.1
	3–4	2	5.3	1	16.7															1	14.3
Decreased appetite	Any	6	15.8	1	16.7							1	16.7					1	25.0	3	42.9
Diarrhoea	Any	4	10.5											1	33.3					3	42.9
Dyspnoea	Any	2	5.3									1	16.7	1	33.3						
Fatigue	Any	11	28.9	1	16.7	2	66.7					3	50.0	1	33.3	1	33.3	1	25.0	2	28.6
	3–4	1	2.6																	1	14.3
Gamma- GT increased	Any	1	2.6													1	33.3				
	3–4	1	2.6													1	33.3				
Haemolysis	Any	3	7.9									1	16.7							2	28.6
Headache	Any	3	7.9	2	33.3							1	16.7								
Hypertensive crisis	Any	1	2.6									1	16.7								
	3–4	1	2.6									1	16.7								
Hypophosphatemia	Any	2	5.3									1	16.7	1	33.3						
	3–4	1	2.6											1	33.3						
Keratitis	Any	1	2.6																	1	14.3
	3–4	1	2.6																	1	14.3
Mucosal inflammation	Any	1	2.6																	1	14.3
	3–4	1	2.6																	1	14.3
Nausea	Any	8	21.1	1	16.7							2	33.3			1	33.3	2	50.0	2	28.6
	3–4	1	2.6									1	16.7								
Neutropenia	Any	5	13.2	1	16.7															4	57.1
	3–4	4	10.5																	4	57.1
Pain	Any	2	5.3									1	16.7							1	14.3
Paraesthesia	Any	2	5.3	1	16.7							1	16.7								
Platelet count decreased	Any	1	2.6															1	25.0		
	3–4	1	2.6															1	25.0		

N: Number of evaluable patients.
n: Number of observed values.

Table 2b
Haematological abnormalities: Worst CTC grade on treatment by dose level.

Parameter	CTC Grade[a]	Assigned Dose Level (mg/m^2/week) N = 38									Any Dose Level	
		4	6	9	13.5	20	30	45	67.5	135		
		N = 6	N = 3	N = 3	N = 3	N = 6	N = 3	N = 3	N = 4	N = 7		
		n	n	n	n	n	n	n	n	n	N = 38	100%
Haemoglobin	Any	3	2	1	2	4	3	1	3	6	25	65.8
	3	1			1					1	3	7.9
Lymphocytes	Any	2	2	2	1	2	2	2	2	2	17	44.7
	3			1			2				3	7.9
Neutrophils	Any	1	1			1	1			4	8	21.1
	3									4	4	10.5
Platelet Count	Any					1			1		2	5.3
	3								1		1	2.6
Leukocytes	Any	1	1			1	1			5	9	23.7
	3									3	3	7.9

N: Number of evaluable patients.
n: Number of observed values.
[a] No CTC Grade 4 and 5 were reported.

hypokalaemia and Grade 2 vomiting, Grade 3 keratitis, and Grade 2 diarrhoea.

3.9. Clinical activity

A total of 35 patients were evaluable for response. Patient No. 0006 with metastatic clear renal cell cancer (4 mg/m^2/week) achieved a PR from Cycle 4 to Cycle 18, later converting to CR from Cycle 20 up to Cycle 32, with a response duration of 112.7 weeks. The patient had received three lines of prior systemic therapy, including sunitinib, everolimus and sorafenib. Three patients were not evaluable for response (no on-treatment tumour assessment 2 patients, patient not treated and no tumour assessment on trial 1 patient).

RECIST stable disease (SD) as best response was seen in 13 patients. The duration of the stabilization ranged from 6.4 to 67.3 weeks with a median duration of

Table 2c
Blood chemistry abnormalities: Worst CTC grade emerged or worsened on treatment by dose level.

Parameter	CTC Grade[a]	Assigned Dose Level (mg/m^2/week) N = 38									Any Dose Level	
		4	6	9	13.5	20	30	45	67.5	135		
		N = 6	N = 3	N = 3	N = 3	N = 6	N = 3	N = 3	N = 4	N = 7		
		n	n	n	n	n	n	n	n	n	N = 38	100%
ALT	Any	1	2	1		1	1	1		2	8	21.1
	3									1	1	2.6
AST	Any	1	2	1		4	2	1	1		12	31.6
	3	1									1	2.6
Creatinine	Any	1				2	1			3	7	18.4
	3											
GammaGT	Any	5	1	2		3	1	2	2	4	20	52.6
	3	1		1		2		1	1	2	8	21.1
Phosphate	Any	2	2	2	2	3	2		1	4	18	47.4
	3	1		1	1	1	1			1	6	15.8
Hypokalaemia	Any		2	1	1		1			1	6	15.8
	3									1	1	2.6
Hyperkalaemia	Any	2			1	2	3	2	0	4	14	36.8
	3											
Hyponatremia	Any		2	1		2		1	3	3	12	31.6
	3								1		1	2.6
Hypernatremia	Any			1							1	2.6
	3											

N: Number of evaluable patients.
n: Number of observed values.
[a] No Grades 4 and 5 were reported.

Table 3
Objective response rate and best overall response.

	Patients evaluable for efficacy N = 36				Patients evaluable for efficacy treated at 135 mg/m^2/week N = 7	
	n	%	95% CI - LL	95% CI - UL	n	%
Objective response (CR + PR)	1	2.8	0.07	14.53	–	–
Complete response (CR)	1	2.8	–	–	–	–
Stable disease (SD)	13	36.1	–	–	4	57.1
Progressive disease (PD)	21	58.3	–	–	3	42.9
Not assessed[a]	1	2.8	–	–	–	–

95% CI: lower (LL) and upper (UL) 95% exact confidence limits for objective Response rate.
[a] Not assessed since the patients died before his first on-treatment assessment.

24.3 weeks (95%CI: 12.3–67.3). Patient 0038 with hepatocellular carcinoma had a decrease in target lesions, but progressed soon after that in a non-target site. The patient had received three lines of prior systemic therapy and was treated at 135 mg/m^2/week. Most patients had PD as best response. Seven patients remained on study for ≥6 cycles, including two on 135 mg/m^2/week. The median progression-free survival in the evaluable population was 8.1 weeks (95% CI 7.3–12.3). Table 3 summarizes the response assessment.

3.10. Pharmacokinetics

Plasma concentrations of S81694 showed a poly-exponential decline with overall average half-life of about 100 h. Plasma clearance was low and the volume of distribution was high, suggesting tissue distribution. Day 1 and 15 on systemic exposure to S81694 increased proportionally with dose and the accumulation ratios were in agreement with the administration schedule and half-life of the compound. The N-oxide NMS-03593478 metabolite was not quantifiable or present at very low levels in plasma. Renal clearance of S81694 and NMS-03593478 was low. Exposure seen at higher dose levels corresponded with drug concentrations in experimental animals that did respond to S81694 during preclinical testing.

3.11. Biomarker analysis

Archival biopsies from tumour material were obtained from 25 patients, but the biomarker analysis was not performed.

4. Discussion

MPS1 kinase is a dual tyrosine/serine–threonine kinase highly expressed in proliferating cells, playing a critical role in the control of mitosis, being involved in proper chromosome alignment, stabilizing the kinetochore-microtubule interaction and ensuring that cells do not divide until sister chromatids correctly align to the metaphase plate. MPS1 is highly expressed in a number of important human malignancies. Aneuploidy is a common feature of many of these cancers, and MPS1 inhibitors have been described to be more active in such tumours. Published data suggest that triple-negative breast cancer is among the tumour types more dependent on MPS1 activity and thus potentially more sensitive to specific inhibitors. Inhibition of MPS1 results in induction rather than arrest of mitosis, which differentiates such compounds from established spindle poisons such as vinca alkaloids and taxanes, which makes the development of MPS1 inhibitors a promising concept [16].

Based on preclinical testing, S81694 is a very potent and selective small-molecule inhibitor of MPS1 kinase activity, causing mitotic checkpoint override, acceleration of mitosis, induction of chromosomal misalignment and reduced mitotic marker expression. While some MPS1 inhibitors are undergoing preclinical development, S81694 was likely the first to enter the clinic in a typical phase I, first-in-human, dose-escalation study.

The patients treated here represented a broad range of malignancies and a very typical, non-selected phase I population. S81694 was given at weekly doses ranging from 4 to 135 mg/m^2/week, and the treatment was generally well tolerated as <20% of patients came off study due to AEs and the incidence of SAEs was low. DLTs were sporadic, occurred on different dose levels and expansion of cohorts did not result in further events. MTD was not reached, as the sponsor decided to terminate single-agent dose finding in this trial based on emerging translational evidence.

Dose escalation until 135 mg/m^2/week allowed to perform a detailed analysis of human PK. Plasma concentrations showed a poly-exponential decline with overall average half-life of about 100 h. Plasma clearance was low and the volume of distribution was high. The systemic exposure to S81694 increased proportionally with dose. The exposure at higher dose levels corresponded with active doses during preclinical testing.

Among patients evaluable for response, a durable CR of a renal cell carcinoma and a reduction of target lesions in hepatocellular carcinoma (RECIST 1.1-SD) were observed, 13 patients had SD and 7 remained on active treatment for 6 cycles, with up to 32 cycles given in individual patients. The 8.1-week median

progression-free survival in this trial was similar to the outcome of other traditional solid tumour dose finding trials.

The sponsor decided to focus on further development of S81694 in combination with paclitaxel in patients with breast cancer. The combination of S81694 with paclitaxel was found to be synergistic in TNBC cell lines (MDAMB231, MDAMB468 and HCC70 *in vitro*) and increased the efficacy of each single agent. This finding was validated *in vivo* in xenograft models established from cell lines and patient-derived biopsies (MDAMB231, MAXF1384 models; unpublished data). In mice xenografted with patient-derived TNBC, residual tumours after neo-adjuvant therapy showed positive results for the combination with docetaxel compared to their respective agents. The combination of S81694 with paclitaxel in MDA-MB-231-luciferase orthotopically implanted TNBC induced nearly complete inhibition of luciferase signal intensity assessed by *in vivo* imaging and was associated with significant survival improvement compared to paclitaxel alone ($p < 0.001$). In TNBC PDX models, the combination of paclitaxel with S81694 induced complete regression.

This strategic decision also had a negative impact on planned exploratory analyses, e.g. the tissue-based biomarker work and PK/PD analyses that were planned. Safety results and PK from the dose-finding trial did nevertheless provide guidance for a safe starting dose for the consecutive combination trial in breast cancer. As starting dose for S81694 in combination with 80 mg/m^2 paclitaxel, a dose of 13.5 mg/m^2 was chosen. A randomized phase I/II study (NCT03411161) with the combination was launched in the 1st line setting of metastatic TNBC but was then discontinued due to a lack of significant clinical activity. The phase II part was never initiated, as the treatment landscape for TNBC changed with the regulatory approval of anti-PD(L)1 immune checkpoint inhibitors in combination with chemotherapy in this setting. At present, S81694 still remains under clinical development.

4.1. Overall conclusions

To the best of our knowledge, we report one of the very first clinical trials with an MPS1 inhibitor in patients with solid tumours. S81694 was found to be safe and tolerable when given weekly iv. for 3 weeks every 4 weeks. The safety profile was consistent of haematological toxicity, general disorders and gastrointestinal symptoms, and effects on the haemolymphopoietic system as observed in animals were transient and clinically manageable. Plasma clearance was low and the volume of distribution was high. Early signs of clinical activity were seen, and the drug was tolerated well for a considerable period of time in individual patients. Dose escalation was safe up to 135 mg/m^2/week without reaching MTD. S81694 is a promising novel compound with a favourable safety and pharmacological profile and requires further clinical testing in selected malignancies, alone or in combination with other anti-cancer agents.

Ethics, consent and permissions

This study was conducted in accordance with Good Clinical Practice guidelines and the Declaration of Helsinki. Approval for the protocol was obtained from independent ethics committees at participating institutions. All patients provided written informed consent.

Author contributions

Patrick Schöffski was involved in the conceptualization, design and methodology of the study, proposed participating sites and wrote the manuscript. Patrick Schöffski and all other investigators were involved in the acquisition of data, all were involved in the analysis and interpretation of data, and contributed to the revisions of this manuscript.

Funding

This study was funded by Institut de Recherche Internationale Servier, Suresnes (France).

Conflict of interest statement

The following authors declare the following financial interests/personal relationships which may be considered as potential competing interests:Anne-Marie de La Bigne is an employee of Servier. Zakia Felloussi is an employee of Servier. Mike F. Burbridge was an employee of Servier. Frédérique Cantero was an employee of Servier. Riccardo Colombo is an employee of Nerviano Medical Sciences S.r.l. Sara Maruzzelli is an employee of Nerviano Medical Sciences S.r.l. Katia Ammattatelli was an employee of Nerviano Medical Sciences S.r.l. Philippe Aftimos: Consulting: Boehringer Ingelheim, Macrogenics, Roche, Novartis, Amcure, Servier, G1 Therapeutics, Radius, Deloitte. Honoraria: Synthon, Amgen, Novartis, Gilead. Travel grants: Amgen, MSD, Pfizer, Roche. Research funding to my institution: Roche.

Acknowledgements

We would like to thank the patients, families, nurses, and investigators who participated. We want to acknowledge Servier and Nerviano staff for their contribution: Bérengère de Moucheron, Patrizia Crivori,

Maria Teresa De Pietro, Claudia Di Giulio, Anna Amalia Bartosek, Sonia Colombini, Marina Ripamonti, Laura Torretti, Alessia Montagnoli and Paola Zugnoni.

References

[1] De Cárcer G, Pérez de Castro I, Malumbres M. Targeting cell cycle kinases for cancer therapy. Curr Med Chem 2007;14:969–85.
[2] Janssen A, Kops GJ, Medema RH. Elevating the frequency of chromosome mis-segregation as a strategy to kill tumor cells. ProcNatlAcadSci USA 2009;106(45):19108–13.
[3] Weiss E, Winey M. The Saccharomyces cerevisiae spindle pole body duplication gene MPS1 is part of a mitotic checkpoint. J Cell Biol 1996;132:111–23.
[4] Stucke VM, Sillje HH, Arnaud L, et al. Human Mps1 kinase is required for the spindle assembly checkpoint but not for centrosome duplication. EMBO J 2002;21:1723–32.
[5] Tighe A, Staples O, Taylor S. Mps1 kinase activity restrains anaphase during an unperturbed mitosis and targets Mad2 to kinetochores. J Cell Biol 2008;181:893–901.
[6] Hogg D, Guidos C, Bailey D, et al. Cell cycle dependent regulation of the protein kinase TTK. Oncogene 1994;9:89–96.
[7] Kang J, Chen Y, Zhao Y, et al. Autophosphorylation-dependent activation of human Mps1 is required for the spindle checkpoint. ProcNatlAcadSci USA 2007;104:20232–7.
[8] Vasudevan A, Schukken KM, Sausville EL, et al. Aneuploidy as a promoter and suppressor of malignant growth. Nat Rev Cancer 2021;21(2):89–103.
[9] Maire V, Baldeyron C, Richardson M, et al. TTK/hMPS1 is an attractive therapeutic target for triple-negative breast cancer. PLoS One 2013;8(5):E63712.
[10] Gyorffy B, Bottai G, Lehmann-Che J, et al. TP53 mutation-correlated genes predict the risk of tumor relapse and identify MPS1 as a potential therapeutic kinase in TP53-mutated breast cancers. Mol Oncol 2014;8(3):508–19.
[11] Balko JM, Giltnane JM, Wang K, et al. Molecular profiling of the residual disease of triple-negative breast cancers after neoadjuvant chemotherapy identifies actionable therapeutic targets. Cancer Discov 2014;4(2):232–45.
[12] Mukhtar E, Adhami VM, Mukhtar H. Targeting microtubules by natural agents for cancer therapy. Mol Cancer Therapeut 2014;13(2):275–84.
[13] Eisenhauer EA, et al. New response evaluation criteria in solid tumours: revised RECIST guideline (version 1.1). Eur J Cancer 2009;45:228–47.
[14] Babb J, Rogatko A, Zacks S. Cancer phase I clinical trials: efficient dose escalation with overdose control. Stat Med 1998;17:1103–20.
[15] Neuenschwander B, Branson M, Gsponer T. Critical aspects of the Bayesian approach to phase I cancer trials. Stat Med 2008;27:2420–39.
[16] Colombo R, Caldarelli M, Mennecozzi M, et al. Targeting the mitotic checkpoint for cancer therapy with NMS-P715, an inhibitor of MPS1 kinase. Cancer Res 2010;70(24):10255–64.