Clinical phase I and pharmacokinetic study of S 16020, a new olivacine derivative: report on three infusion schedules


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S 16020, a new 9-OH olivacine derivative, is a novel topoisomerase II inhibitor with activity in cell lines presenting the classical multidrug resistance phenotype. This report summarizes, in addition to pharmacokinetic data, the whole phase I clinical experience of S 16020 using three different infusion schedules. Asthenia and skin toxicity were the main side effects. In an attempt to understand the skin toxicity mechanism, experiments in animals were performed, the results of which are reported. S 16020 showed rapid tumor necrotizing activity in some patients, with soft tissue metastases of epidermoid tumors and pain at the tumor site. To document the side effects of S 16020 and tumor site reactions (pain, edema, inflammatory signs), inflammatory parameters and some cytokines were measured. In our patients there was no hemolysis and no detection of anti-S 16020 antibodies, confirming the absence of immunogenicity of the compound. Based on the overall data of the three infusion schedules of S 16020, the dose of 100 mg/m² over 3 h every 3 weeks was selected for phase II studies.

Key words: clinical phase I, olivacine derivative, S 16020, solid tumors

Introduction

S 16020 is a cytotoxic agent derived from 9-hydroxy olivacine, structurally related to the ellipticine family [1], which acts by intercalation within the DNA and by topoisomerase II inhibition. S 16020 was also shown to induce apoptosis in vitro, independent of the cell p53 status [2].

In vitro, S 16020 is a potent cytotoxic compound with antineoplastic activity comparable to that of doxorubicin [3]. According to the National Cancer Institute (NCI) panel it demonstrates significant cytotoxic activity in human leukemic, ovarian, lung and renal cancer cell lines.

In vivo, the antitumor activity of S 16020 was as important as that of doxorubicin in murine leukemia and metastatic lung tumor models [4]. S 16020 administered intravenously using different schedules showed significant antitumor activity against bronchial, ovarian and breast carcinoma human xenografts in nude mice [5, 6]. Multidrug-resistant cell lines were sensitive to S 16020, suggesting weak cross-resistance with anthracyclines.

Single and repeated-dose toxicity studies carried out to meet regulatory requirements have shown a dose-dependent hemato-logical effect and an effect on the genital organs [7]. Nephro-toxic effects with tubular and glomerular lesions were observed by electronic microscopy in the rat at doses of ≥20 mg/kg after three injections at 3-week intervals. This toxicity was not found in the monkey with the same treatment schedule [7].

The spectrum of activity, mechanism of action and toxicological profile of S 16020 justified its clinical development. Phase I studies were conducted in patients with incurable advanced solid cancer in order to evaluate the tolerance and determine the maximum tolerated dose (MTD) according to three different schedules. Secondary objectives were the characterization of the human pharmacokinetics and the recording of any sign of antitumor activity.

As recommended by the Committee for Proprietary Medicinal Products (CPMP) guidelines [8] for a first phase I trial, a 60-min intravenous infusion of S 16020 every 3 weeks was tested first (schedule A). Subsequently, two other schedules (B and C) were explored in an attempt to improve the therapeutic index. In fact, the observation of an unexpected cutaneous toxicity at the fourth dose level with schedule A did not allow further dose escalations, and led us to amend this schedule based on the available pharmacokinetic data using a prolonged time of infusion (schedule B) and to explore a third schedule using a fractionated dose over 3 days every 3 weeks (schedule C).
Considering the preclinical results and the fact that S 16020 is chemically related to the ellipticine family, which is known for its ability to induce anti-drug antibodies in humans, particular attention was also given to the surveillance of the immunological and renal parameters. Indeed, the clinical use of the ellipticine derivative ellipitnium acetate or Celiptium® was limited by renal toxicity and an immune-mediated hemolytic reaction due to the induction of anti-elliptinium IgM antibodies [9, 10].

Patients and methods

Patients

The eligibility criteria were: age >18 years; refractory and progressive tumor; written informed consent; Eastern Cooperative Oncology Group (ECOG) performance status <2; no chemotherapy or radiotherapy within the last 4 weeks; no hormono- or immunotherapy within the last 2 weeks; polynuclear cell count ≥2 × 10⁹/l; hemoglobin ≥100 g/l; platelet count ≥100 × 10⁹/l; aspartate aminotransferase (AST); alanine aminotransferase (ALT); alkaline phosphatases <2.5 × upper normal limit (UNL); bilirubin <1.5 × UNL; creatinine clearance ≥60 ml/min; proteinuria <0.3 g/l (≤+ on test tape); normal left ventricular ejection fraction (LVEF) in patients who received doxorubicin (Adriamycin) ≥450 mg/m² or equivalent, i.e. epirubicin 700 mg/m² and mitoxantrone 120 mg/m²; and at least one evaluable and/or measurable tumor target.

The study was conducted in two centers, and each institutional ethics committee approved the protocol and its amendments. The study initiation date was December 1996. Schedule A was completed in May 1998, and schedule B started in April 1998 and was completed in September 1999. Schedule C also started in April 1998 and was completed in August 1998.

Treatment plan

Schedule A. S 16020 was administered as a 60-min intravenous infusion every 3 weeks.

The starting dose of 30 mg/m² was determined from toxicological studies in animals [7], and corresponded to 1/17 of the mouse LD₅₀ (10% lethal dose), 1/10 of the rat LD₅₀ and the NOAEL (no observed adverse effect level) in the beagle dog. The dose escalation was planned according to the modified Fibonacci scale for six dose levels, i.e. 30, 60, 100, 150, 210, 270 and 330 mg/m²/administration. A minimum of three patients had to be included at each dose level and if the occurrence of a dose-limiting toxicity (DLT) at a given dose level, three additional patients were to be included at this dose level.

Schedule B. S 16020 was administered by intravenous infusion over 3 h every 3 weeks.

The starting dose of 100 mg/m² was based on a pharmacokinetic simulation showing that following an infusion of this dose over 3 h, the peak plasma concentration would be equivalent to that obtained after an infusion of 60 mg/m² over 1 h, which was well tolerated during the first part of the study (schedule A).

Schedule C. S 16020 was administered as a 60-min intravenous infusion on 3 consecutive days (corresponding to one cycle), which was repeated every 3 weeks.

The starting dose of 35 mg/m²/day was defined from the preclinical toxicological studies as for schedule A. The dose escalation was planned in increments of 10 mg/m²/day by groups of three patients until the observation of a DLT. In that case, three additional patients were to be included.

For all three schedules, toxicity was assessed weekly according to NCI criteria, and when not available according to UICC (Union Internationale Contre le Cancer) criteria. A DLT was defined as follows: grade 4 leucopenia and/or neutropenia lasting for >7 days, or febrile neutropenia (neutropenia grade ≥3 with temperature ≥38.5°C); grade 4 thrombocytopenia; signs of hemolysis; alteration of renal function with a reduction of at least 50% of creatinine clearance and/or an increase of at least 50% of creatininemia (in such a case, hyperhydration was planned), and/or proteinuria with tape test ≥++ or ≥3 g/l, and/or nephrotic syndrome; and all other toxicities grade ≥3, except nausea/vomiting and alopecia. If, at a given dose level, a DLT was observed in half of the six patients included, the dose escalation was stopped and this dose level was considered to be the MTD.

If the hematological or biological parameters did not return to normal before the next infusion, the treatment was delayed by 1 or 2 weeks. After a 2-week treatment postponement, the patient was withdrawn from the study and not replaced. No dose reduction was planned.

Antitumor measurements using the World Health Organization (WHO) criteria were scheduled after three drug administrations, or after the second course for patients who could not receive the third course, and then every 6–8 weeks.

Drug supply and administration

The study drug was supplied by Les Laboratoires Servier Industrie (Gidy, France) in vials of 100 mg of freeze-dried powder, which were dissolved in 10 ml of solvent (a mixture of sodium hydroxide and sodium chloride in water for injection).

Taking into account the patient’s body surface area and the proposed dose level, the corresponding volume of reconstituted solution was dissolved in 250 ml of isotonic sodium chloride 0.9% solution for infusion. This solution was stable for 6 h at room temperature.

S 16020 was administered by regular intravenous infusion via a medium size peripheral vein or an implantable device.

Concomitant treatments

Antiemetics were not required a priori in schedule A, but due to vomiting in the majority of patients they were given systematically in schedules B and C using the standard regimen in each institution (metoclopramide or 5HT3 antagonists).

Investigations at selection and during follow-up

Investigations into the following were performed at selection: demographic; medical history; complete physical examination; assessment of the extension of the disease and of measurable lesions according to the tumor location, but including at least a chest X-ray and a liver echography; concomitant medication report; and hematological, biological and immunological tests (complete blood cell count, reticulocytes, platelets, haptoglobin, direct Coomb’s test, detection of anti-S 16020 antibodies as negative control, tonogram, calcium, magnesium, total protein, AST, ALT, alkaline phosphatase, γ-glutamyl transferase, total and indirect bilirubin, cholesterol, creatinine, creatinine clearance and urine examination by tape test).
Before each S 16020 infusion and at 0.5, 1, 2, 6 and 24 h after infusion, body temperature, blood pressure and heart rate were all monitored. Physical examination including recording all adverse events, and biological tests were repeated before each administration and weekly up to 3 weeks after the last drug administration.

**Pharmacokinetic analyses**

**Blood sampling: schedule A.** Blood and plasma were collected in heparinized tubes on day 1 only: pre-dose, 30 min (during infusion), 1 h (end of infusion), then at 10 min, 20 min, 30 min, 1 h, 2 h, 4 h, 8 h and 24 h after the end of the infusion.

**Blood sampling: schedule B.** Blood only was collected on day 1 at the following time points: pre-dose, 3 h (end of infusion), then at 5, 7, 11, 19 and 24 h after the start of the infusion.

**Blood sampling: schedule C.** Blood only was collected: pre-dose and at 1 h (end of infusion) on days 1 and 3, then at 2, 4 and 8 h after the S 16020 infusion was started on day 3 only.

All samples were kept frozen at −18°C until analysis.

**Assay procedure.** S 16020 in plasma and blood (limit of quantitation 2 and 10 ng/ml, respectively) and S 16018 (N-demethylated metabolite) in blood only (limit of quantitation 10 ng/ml) were quantified by high performance liquid chromatography with ultra-violet (UV) detection after solid/liquid extraction. Both methods had previously been validated and used S 16016 (9-methoxy-derivative of S 16020) as internal standard.

**Pharmacokinetic interpretation.** A non-compartmental pharmacokinetic analysis was performed on the individual blood and plasma concentrations of S 16020 and blood concentrations of S 16018, and the following parameters were determined: C₀,₀ observed concentration at the end of infusion; Cₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙₙ¢...
groups of patients treated at these dose levels. At the dose level of 150 mg/m², 120 mg of methylprednisolone was administered by i.v. infusion 1 h before the start of S 16020 infusion, followed by methylprednisolone 32 mg orally twice daily starting 24 h after S 16020 administration for 3 consecutive days, then 32 mg once daily for 3 more days. This steroid premedication was also initiated for patients treated at the 100 mg/m² dose level, with a reduction of the oral dose of methylprednisolone (32 mg once daily) over 4 days.

In schedules B and C, no steroid premedication was given.

After the observation of severe acne in two patients treated at the 100 mg/m² dose level, with a reduction of the oral dose of methylprednisolone (32 mg once daily) over 4 days.

In schedules B and C, no steroid premedication was given.

Toxicity: dose-limiting toxicities and adverse events

Schedule A (1-h infusion every 3 weeks). The DLT was cutaneous. Two patterns of cutaneous toxicity occurred: (i) a subacute erythematous maculo-papular skin rash of the face that could extend to the trunk and extremities; and (ii) delayed acne on the face and the back. The rash started within days after the first drug infusion. The erythema usually lasted for 1–2 weeks and was accompanied by pruritus in half of the cases. Although highly variable, the rash intensity was dose related and was observed in two out of six patients at dose levels 1 and 2, and in 23 out of 28 (82%) patients (i.e. in 39 out of 57 courses) at dose levels 3 and 4. In six out of 25 patients, desquamation and hyperpigmentation followed the rash. In patients with previously irradiated areas, redness was also observed within the limits of the radiation field. In six patients, biopsies were performed at the site of the erythematous rash. The skin lesions were specific to a neutrophilic eccrine hidradenitis corresponding to the necrosis of the excretory coils of the sudoral glands, with neutrophilic infiltrates in the surrounding edematous interstitial tissue. The epithelial cells of some sudoral glands displayed squamous syringometaplasia. Prophylactic medication with steroids was attempted in a further group of patients treated at the same dose levels (over 7 days at a dose level of 150 mg/m² and over 4 days at a 100 mg/m² dose level). This premedication succeeded in

<table>
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<tr>
<th>Table 1. Patient characteristics</th>
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<tr>
<td>Schedule A (1-h infusion on day 1 every 3 weeks)</td>
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<tr>
<td>No. of patients</td>
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<td>Median age, years (range)</td>
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<tr>
<td>1</td>
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<tr>
<td>Colon-rectum</td>
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<td>Kidney</td>
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<td>Soft tissue</td>
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<td>&gt;3</td>
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<tr>
<td>Prior hormonotherapy</td>
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reducing the intensity of the rash and the facial edema at the 150 mg/m² dose level, but not its frequency.

At dose levels 1 and 2, no cases of acne were reported. At dose level 3 (100 mg/m²), three out of 15 patients developed moderate acne. At dose level 4 (150 mg/m²), five out of 13 patients developed mild to moderate acne after one to three drug infusions (of which three patients received steroids, and one received steroids and isotretinoin) and two out of 13 patients had severe acne (of which one received steroids from the first S 16020 administration and the other one from the second because of an extended rash with fever and general symptoms after the first infusion; both patients received a total of three infusions). Out of the nine other patients with acne, five presented a moderate rash. The acne lesion onset was delayed by several weeks after the start of S 16020 infusion. In one patient, the biopsy corresponded to the keratinization of the excretory channels of the sebaceous glands. Acne was slowly reversible during the follow-up period of 2 months. The severity of acne in the two patients treated at 150 mg/m² did not allow continuation of the dose escalation or the inclusion of additional patients. Acne was therefore considered the DLT, and the MTD was set at 150 mg/m².

Facial or palpebral edema was observed in nine patients at dose levels 3 and 4.

Asthenia was also a significant toxicity, but only in the two groups of patients receiving prophylactic steroids: it was severe in eight out of 12 patients treated at 100 mg/m², and in five out of seven treated at 150 mg/m², while it was not reported in the nine patients treated at these same dose levels without prophylactic steroids. The onset of severe asthenia occurred within a few days of S 16020 administration and lasted for a median of 8 days.

Apart from the above-described toxicities, the most frequent adverse events related to the treatment with S 16020 were headache, vomiting, pain at the tumor site, myalgia and anorexia. These events were observed from the first dose level.

Headache and vomiting were observed in 79% and 74% of the patients experiencing mainly grade 1 or 2 episodes, and in only four patients having grade 3 episodes. The onset of headache was observed ~12 h after S 16020 infusion and was easily relieved with paracetamol. Its high frequency justified the preventive use of paracetamol at the third and fourth dose level. Vomiting, mainly grade 2, also appeared ~12 h after S 16020 infusion and the patients were treated with antiemetic drugs.

Pain at the tumor site occurred the day after the infusion in 25 out of 85 courses. In patients with soft tissue metastases, inflammatory signs were observed with redness and surrounding edema. In 13 patients with internal abdominal metastases, pain at the tumor site and transient intestinal occlusion or biliary tract dilatation occurred, and required hospitalization in four cases. In five out of 15 patients with intra-thoracic lesions, acute dyspnea developed during the infusion and/or a few hours later in two cases. The chest X-rays were suggestive of peri-tumoral edema. Coughing and dyspnea developed 2–3 days after drug infusion in the other three cases. In two of these, a spastic component was also observed. In two patients with head and neck cancer, laryngeal dyspnea developed within a few days due to peri-tumoral edema.

Myalgias, mainly leg pain, occurred in 11 out of 34 patients within 24–48 h after drug infusion. They were severe in two patients, who required major analgesics. In five patients, a flu-like syndrome with fever was observed and was controlled well with anti-inflammatory drugs.

Diarrhea occurred in eight patients and reached grade 3 in only one patient treated at 60 mg/m² after the third administration.

Mild to moderate anorexia was reported in 10 patients. In one patient it was severe and led to withdrawal of treatment after two courses.

No cases of alopecia or asialia were reported and there were no allergic reactions.

S 16020 induced a moderate dose-dependent myelosuppression. Grade 1–2 hematological toxicity was observed up to the dose level of 100 mg/m². Grade 3–4 leukopenia and neutropenia occurred at the dose level of 150 mg/m² in six out of 13 (46%) and three out of 13 (23%) patients, respectively. No neutropenic fever was observed. Grade 3 anemia occurred in five out of 15 patients (33%) treated at 100 mg/m², and in two out of 13 patients (15%) treated at 150 mg/m². Grade 3 thrombocytopenia occurred in one out of 15 patients (7%) treated at 100 mg/m², and in two out of 13 patients (15%) treated at 150 mg/m². All these patients had been heavily pretreated with four or more chemotherapy or high-dose chemotherapy regimens. There were no infectious complications or bleeding requiring platelet transfusion.

S 16020 did not mediate immune hemolytic reaction.

At dose levels 3 and 4, five out of 28 patients had a grade 3–4 increase in bilirubin value and a grade 3 increase in alkaline phosphatase. Out of those five patients, four had progressive liver metastases. A grade 3 increase in AST/ALT values was observed in three out of 28 patients.

A drop in creatinine clearance to <60 ml/min was measured in seven out of 34 patients based on the urine collection, and represented a >50% reduction in creatinine clearance in five patients. These low values were not accompanied by any increase in serum creatinine or proteinuria.

Table 2 shows the frequency (by patients and by courses) of the main side effects graded according to the NCI scale, except for asthenia which was graded according to the UICC scale.

Schedule B. At 125 mg/m², the DLT was edema occurring in the two enrolled patients. The first patient had a gastric carcinoma with hepatic metastases and sus-clavicular lymph nodes. After the first course he developed erythema with severe concomitant edema of the face, which decreased rapidly after steroid administration. He also had liver pain and grade 2 fever for several days. His general status deteriorated and he
died 1 month later from progressive disease. The second patient developed a pulmonary edema on day 4 after the first drug infusion. The patient had metastatic melanoma with large cutaneous nodules and pulmonary lymphangitis. The day of the infusion she developed a sepsis with *Staphylococcus aureus*. On day 4 she developed signs of respiratory distress syndrome. The chest X-ray showed interstitial edema. The clinical status improved within 2 days with antibiotics and high-dose steroids. Infection was considered to be a possible cause, but a drug-induced pneumonitis with peri-tumoral edema cannot be ruled out in view of the peritumoral inflammatory signs observed in other patients. The MTD was therefore established at 125 mg/m². The following patients were enrolled at lower dose levels (four patients at 80 mg/m² and eight additional patients at 100 mg/m² to document further the recommended dose).

In this schedule, acne was not dose limiting, although seven out of 14 patients developed grade 1 lesions with a rapid onset within days of drug infusion. All cases were rapidly reversible. An erythematous rash was observed in all 14 patients, but was neither severe nor prolonged. Grade 1 or 2 fever was the second most frequent side effect. Headache, vomiting, pain at the tumor site, myalgia and diarrhea were observed as in schedule A (Table 2). Except for pain at the tumor site, all these events were grade 1 or 2, and were manageable. Asthenia was severe in two patients treated at the 100 mg/m² dose level. These two patients had head and neck neoplasms and presented with a weight loss of 5–8 kg. At 100 mg/m², only one out of eight patients had grade 3 thrombocytopenia. At 125 mg/m², one out of the two enrolled patients had grade 3 neutropenia and grade 4 thrombocytopenia. Hepatic toxicity was observed in the two patients treated at 125 mg/m² who developed hyperbilirubinemia grade 4; this was concomitant to progressive liver metastases in one patient, and to a respiratory distress syndrome with right cardiac failure in the other.

Schedule C. A total of four patients were included. Overall, the four patients experienced multiple drug-related adverse events. All four patients experienced headache, erythematous rash with pruritus and vomiting related to S 16020 administration. Three out of the four patients experienced nausea and asthenia. Serious adverse events occurred in two patients. One patient with metastatic hepatocarcinoma had a septic-shock-like syndrome with major agitation and confusion the day after the first cycle, which required hospitalization in the intensive care unit. Although the white blood cell (WBC) count was 17,000, no infection could be documented, but the patient recovered rapidly after anti-biotherapy. Another patient with metastatic colon cancer of the lung, liver and bone developed hyperalgesia at the level of a vertebral lesion with epiduritis starting on day 2 of the first cycle. This painful episode was concomitant to prolonged severe agitation and required high doses of opioid analgesics.

Hematological toxicity with grade 3 neutropenia and thrombocytopenia was observed in one patient with breast carcinoma pretreated with five chemotherapeutic regimens.

### Tumor response

Schedule A. Among the 31 evaluable patients, two patients had a partial response; they were treated at 30 mg/m² (eight courses) and 100 mg/m² (four courses), respectively. Both had head and neck neoplasms that had been irradiated previously, and were treated with a platinum-based therapy.

The first patient, a 50-year-old woman, had a relapsing epidermoid tumor of the maxillary sinus with cervical lymph nodes and adrenal gland metastases. She had been treated previously with surgery, radiotherapy and two regimens of chemotherapy, including cisplatin and fluorouracil and one experimental cytotoxic drug. After eight courses of S 16020

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**Table 2. Main side effects (grade 3) by patient and by course**

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<th>Schedule</th>
<th>A</th>
<th>B</th>
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<tr>
<td>Dose level (mg/m²)</td>
<td>30</td>
<td>60</td>
</tr>
<tr>
<td>Headache</td>
<td>0/0</td>
<td>0/0</td>
</tr>
<tr>
<td>Vomiting</td>
<td>0/0</td>
<td>1/1</td>
</tr>
<tr>
<td>Asthenia</td>
<td>0/0</td>
<td>0/0</td>
</tr>
<tr>
<td>Pain at tumor site</td>
<td>0/0</td>
<td>0/0</td>
</tr>
<tr>
<td>White blood cell count</td>
<td>Grade 3</td>
<td>0/0</td>
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<tr>
<td></td>
<td>Grade 4</td>
<td>0/0</td>
</tr>
<tr>
<td>Platelets</td>
<td>Grade 3</td>
<td>0/0</td>
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<td></td>
<td>Grade 4</td>
<td>0/0</td>
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she had a complete response at the primary tumor site and underwent surgery to remove the remaining cervical lymph nodes. Upon microscopic examination, no cancer cells were found in three out of four lymph nodes. She did not relapse at the primary tumor site and had a stabilization of the adrenal metastases for 8 months. She then progressed at the abdominal site and died a few months later.

The second patient, a 57-year-old woman, had a nasopharynx carcinoma with one large pulmonary metastasis and bone metastases. She had been treated previously with radiotherapy and three lines of chemotherapy. After four courses of S 16020 she responded at the pulmonary site and no new bone metastases were documented. The main toxicity was prolonged asthenia and she decided to stop the treatment.

Stable disease was observed at 2 months in 10 patients, but was confirmed 1 month later in only five of them.

Schedule B. Among the 14 enrolled patients, antitumor activity was documented at the 100 mg/m² dose level in one patient, with a large loco-regional relapsing epidermoid tumor of the maxillary sinus that had been treated previously with radiotherapy and four chemotherapy regimens. Five days after the first drug infusion, tumor shrinkage was already apparent with continuing necrosis of the lesion. After 2 months of local control the patient developed new regional skin nodules.

In another patient with a nasopharynx carcinoma pre-treated with radiotherapy and chemotherapy, stabilization was obtained for 2 months, but due to asthenia the patient refused to continue the treatment. These observations confirmed the antitumor activity obtained in the same tumor types as with schedule A.

Schedule C. No response was observed in the three evaluable patients.

Immunology

No anti-S 16020 antibody was detected in any patient during the study for all three schedules.

Pharmacokinetics

Schedule A (1-h infusion). Pharmacokinetic evaluation was performed in 26 out of the 34 patients at all dose levels during the first administration of the compound, as shown in Figure 1. Peak blood concentrations of S 16020 at the end of the infusion (C_{inf}) ranged from 301 ± 32 ng/ml to 1669 ± 479 ng/ml between the 30 and 150 mg/m² dose levels, with plasma concentrations slightly lower at 30 and 60 mg/m², but with a blood/plasma ratio close to 1 at the two highest doses. Blood and plasma concentrations declined biphasically with a mean terminal half-life of ~4 h. S 16018, a metabolite of S 16020, was not detected at 30 mg/m², while low levels were quantifiable at 60 (11–20 ng/ml), 100 and 150 mg/m² (10–79 ng/ml). Exposure increased slightly more than dose, with a 13- and 9-fold increase in AUC, respectively, in plasma and blood for a 5-fold increase in dose, but these results could be influenced by the limited number of patients treated at 30 and 60 mg/m² compared with those treated at 100 and 150 mg/m². The V_{ss} was large (~2501) and clearance (~900 ml/min) appeared to be constant over the dose range.

Schedule B (3-h infusion). Eight out of 14 patients were sampled on day 1 (two at 80 mg/m², five at 100 mg/m² and one

Figure 1. Blood concentrations of S 16020 versus time on day 1.
at 125 mg/m²) during the first administration, and three were also sampled during the second administration.

The mean peak blood concentration of S 16020 at the end of the infusion (3 h) was 697 ± 75 ng/ml at the 100 mg/m² dose level, i.e. approximately half that obtained at the same dose at the end of the 1-h infusion, as predicted by a computerized simulation based upon the data obtained from the pharmacokinetics of schedule A. The other parameters (t½, Vss and AUC) remained unchanged and the clearance was therefore independent of the infusion rate.

For the three patients from whom blood samples were taken at the second administration, the parameters were identical to those recorded on day 1 of the first administration.

**Schedule C.** Blood was collected on day 3 in the three patients treated at 35 mg/m² (pre-dose, at the end of the infusion, and 2, 4, 8 and 12 h after the start of the infusion). On day 1, blood collection was limited to pre-dose and end of infusion in the four enrolled patients. On day 1, Cinf was 300 ng/ml (30 mg/m²) and 362 ± 76 ng/ml (35 mg/m²) for S 16020. On day 3 (35 mg/m²), S 16020 was still quantifiable in the pre-dose samples of the three patients (19–56 ng/ml), but the Cinf was slightly lower (at 279 ± 28 ng/ml) than on day 1. Two hours after the end of the infusion, S 16020 was hardly quantifiable, with very few concentrations above the limit of quantitation, indicating an increased clearance and non-linear pharmacokinetics with time. For S 16018, levels were below or close to the limit of quantitation (10 ng/ml) on day 1, and the Cinf was higher on day 3 (53 ± 19 ng/ml) compared with day 1.

The mean S 16020 AUCₜ (215 ± 109 ng.h/ml), recorded here on day 3 at the 35 mg/m² dose level (three patients), was 2.8-fold less than that obtained on day 1 at the 30 mg/m² dose level in the three patients of schedule A, where concentrations were still quantifiable up to 8 h after the end of infusion. These data show a nearly 3-fold increase in clearance on day 3 when administrations were performed on a daily basis.

Table 3 compares the main blood pharmacokinetic parameters in the three schedules for two dose levels. Values in schedule C refer to day 3 samplings.

**Discussion**

The expected toxicities of S 16020, based on preclinical studies, were hematomatological, immunological, renal and hepatic. However, the MTD defined in schedule A was based on an unexpected pattern of cutaneous toxicity described as ‘toxidermie annexeelle’, consisting of neutrophilic eccrin hidradenitis and acne-like lesions [12]. Attempts to decrease the occurrence of the hidradenitis and possibly the onset of acne by steroid pre-medication failed, although the intensity of both types of lesions seemed less severe at 150 mg/m², with none out of seven patients in the treated group presenting a grade 3 erythematous rash, compared with two out of six in the non-pre-treated group. Based on our data it is probable that the addition of isotretinoin did not modify the incidence of acne, but due to the small number of patients in our study, no firm conclusions can be drawn. Another attempt was made by prolonging the infusion duration over 3 h (schedule B). This prolongation also failed to decrease the occurrence of the rash and acne, but their reduced intensity allowed us to recommend S 16020 at 100 mg/m² over 3 h without systematic steroid premedication for phase II studies. In this schedule, seven out of 14 patients presented minor acne lesions with a more rapid onset but with a rapidly favorable outcome. It is possible that this decreased severity is due to the drop in the plasma concentration peak associated with the 3-h schedule. The last attempt through the fractionation of low doses of S 16020 over 3 days (schedule C) did not decrease the frequency of the skin toxicity.

Biopsies at the site of the erythematous skin rash and acne showed a necrosis of the sudoral glands (hidradenitis), as described after the use of chemotherapeutic agents such as cytarabin or bleomycin, and the keratinization of the excretory channels of the sebaceous glands as described with overdoses of amineptine and dioxin [13, 14]. An experiment aiming to reproduce the necrosis of the sudoral glands was performed using micro-pigs, whose skin structurally resembles that of humans most closely. The animals were twice given a hematotoxic dose close to the lethal dose of S 16020. No treatment-related histomorphological changes were noted in the multiple skin samples.

<table>
<thead>
<tr>
<th>Table 3. Comparison of the main pharmacokinetic parameters</th>
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<tr>
<td><strong>Dose (mg/m²)</strong></td>
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<td>-------------------</td>
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<tr>
<td></td>
</tr>
<tr>
<td>No. of patients</td>
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<td></td>
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<tr>
<td>C_{inf} (ng/ml)</td>
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<td></td>
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<td>t½ (h)</td>
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<tr>
<td>AUC (ng.h/ml)</td>
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<td>Clearance (ml/min)</td>
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<td>Vss (l)</td>
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The main site of erythema in humans was the face, which led us to suspect a photosensitivity reaction after UVA or UVB irradiation. However, experiments using guinea pigs receiving the MTD of S 16020 did not induce photo-irritation.

Similar lesions of both the eccrine glands and the sebaceous follicles have been described previously with overdoses of the antidepressant drug amineptine, whose metabolite concentration damaged the cutaneous glands [12, 13]. In one case, the persistence of acne could be related to the persistence of drug metabolites in the sebum and urine 90 days after drug intake [14]. No experiment aimed at dosing S 16020 in the sweat of the patients was undertaken, since it would have required painful techniques. The assay of S 16020 metabolites in the sebum was not explored in the two patients with severe acne. As a result of this, the mechanism leading to these types of lesions in our patients remains unclear, although a direct toxic effect due to the concentration of S 16020 in the cutaneous glands is strongly suspected.

Another rather unexpected limiting toxicity was asthenia. Its severity was related to the S 16020 dose, and all patients with grade 3 asthenia experienced this side effect while they were treated with steroids. Such a relationship may be coincidental in our small groups of patients. No such effect has been reported in the literature. On the contrary, small doses of steroids (smaller than those used here) are often given to patients in order to diminish the asthenia, probably through an anti-inflammatory and euphorizing effect [15]. In cancer patients, fatigue remains difficult to explain and its etiology is multifactorial. The role of the chemotherapeutic agents in the onset or aggravation of asthenia may involve the production of by-products of cell death and cytokines [16–18]. S 16020 showed extensive and rapid tumor necrotizing activity in well documented patients with soft tissue metastases. The release of cytokines following exposure to S 16020 could explain both effects: the tumor necrosis and the severe asthenia. To document this hypothesis, C-reactive protein (CRP), tumor necrosis factor-alpha (TNFα) and vascular endothelial growth factor (VEGF) levels were measured in the blood of all patients treated in schedules B and C. The CRP values (as measured by nephelemetry) were increased in all patients by between 2- and 10-fold, the maximum value being observed after 24 or 48 h and lasting for 1 week. The TNFα blood levels as measured by the ELISA test increased in only three patients. The VEGF level as measured by the ELISA test increased in seven patients at 24–48 h post-treatment. In vitro, S 16020 has been shown to induce an important and rapid mast cell degranulation, leading to the secretion of histamine and arachidonic acid. In another in vitro experiment, interleukin (IL)-6 and IL-8 concentrations as measured by the ELISA test were clearly increased in an S 16020 dose-dependent manner in the supernatant of one tumor (SKOV) cell line and two primary cultures of dermal fibroblasts (C. Dosquet, personal communication). These preclinical and clinical observations favor a significant inflammatory process induced by S 16020.

Other specific adverse effects of S 16020, such as the onset of moderate to severe pain (at the primary tumor or at the metastatic sites) and the onset of acute dyspnea (usually starting the day after the infusion), also favor the induction of an important inflammatory process by S 16020. Moreover, since recall reactions have been observed in some patients, the same type of inflammatory lesions can be suspected at the sites of previously irradiated visceral tumors, in particular at the oropharyngeal and pulmonary sites [19], providing an explanation for (sub)acute dyspnea in three patients with head and neck cancer, and in two breast cancer patients with lung metastases.

Acute respiratory distress syndrome has already been reported after treatment with some therapeutic agents such as gemcitabine and, in a series of 113 patients treated with cytarabine, 13 cases of acute respiratory failure occurred (of which nine were fatal) [20]. The respiratory syndrome is comparable to the clinical findings usually associated with cytokine release, which are particularly severe in patients presenting with tumor infiltrates at the pulmonary site. Indeed, all four patients in schedule A and the patient in schedule B who developed acute dyspnea had pulmonary metastases.

Transient myalgias occurred in 18 patients. This side effect was dose limiting with some ellipticines. In our study it occurred in the patients pretreated with a platinum derivative. It is therefore possible that it reactivated some previously induced neurological damage.

Hepatic and renal tolerance was good in all three schedules. Out of six patients with an increase in their bilirubin level, four had progressive hepatic metastases and one had cirrhosis. Hematological toxicity was mild and confined to heavily pretreated patients. There was no hemolysis and no detection of anti-S 16020 antibodies, confirming the absence of immunogenicity of the compound. It can therefore be postulated that the chemical changes introduced in the olivacine derivative S 16020 (i.e. the switch from the 11 position to the 1 position of the alkyl-side-chain associated with the methylation of the indolic ammonium) succeeded in preventing the formation of human anti-S 16020 antibodies.

Conclusion

Due to the particular toxicity pattern of S 16020 with the 1-h infusion schedule every 3 weeks, no dose could be recommended for phase II trials.

The fractionated schedule over 3 days was considered unsuitable for further development in view of the occurrence of serious adverse events in two patients, and the very poor tolerance in four patients.

Based on the good overall tolerance and the absence of severe cutaneous toxicity without steroid premedication at the dose of 100 mg/m² over 3 h every 3 weeks, this schedule was finally chosen for phase II studies, and another phase I study using a weekly schedule was undertaken.
Acknowledgements

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References


