Phase I Study of Sequence-Selective Minor Groove DNA Binding Agent SJG-136 in Patients with Advanced Solid Tumors

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Abstract

Purpose: This phase I dose-escalation study was undertaken to establish the maximum tolerated dose of the sequence-selective minor groove DNA binding agent SJG-136 in patients with advanced solid tumors. The study also investigated antitumor activity and provided pharmacokinetic and pharmacodynamic data.

Experimental Design: Sixteen patients were assigned sequentially to escalating doses of SJG-136 (15-240 \( \mu \)g/mL) given as a 10-minute i.v. infusion every 21 days. The dose was subsequently reduced in incremental steps to 45 \( \mu \)g/mL due to unanticipated toxicity.

Results: The maximum tolerated dose of SJG-136 was 45 \( \mu \)g/mL. The main drug-related adverse event was vascular leak syndrome (VLS) characterized by hypoalbuminemia, pleural effusions, ascites, and peripheral edema. Other unexpected adverse events included elevated liver function tests and fatigue. The VLS and liver toxicity had delayed onset and increased in severity with subsequent cycles. Disease stabilization was achieved for >6 weeks in 10 patients; in 2 patients this was maintained for >12 weeks. There was no evidence of DNA interstrand cross-linking in human blood lymphocytes with the use of the comet assay. Evidence of DNA interaction in lymphocytes and tumor cells was shown through a sensitive \( \gamma\)-H2AX assay. SJG-136 had linear pharmacokinetics across the dose range tested.

Conclusions: SJG-136 was associated with dose-limiting VLS and hepatotoxicity when administered by short injection every 21 days. DNA damage was noted, at all dose levels studied, in circulating lymphocytes. The etiology of the observed toxicities is unclear and is the subject of further preclinical research. Alternative clinical dosing strategies are being evaluated.

SIG-136 (BN2629/NSC 694501/SG2000) is a rationally designed, sequence-selective pyrrolobenzodiazepine dimer that forms covalent bonds with guanine in the minor groove of DNA (1, 2). SIG-136 has potent activity in the National Cancer Institute anticancer drug screen, resulting in 50% net growth inhibition within the concentration range 0.14 to 320 nmol/L (mean, 7.4 nmol/L). Pattern recognition analysis (COMPARE) and molecular target analysis of SIG-136 have been compared with that of >60,000 compounds tested in the National Cancer Institute 60 cell line screen. Although SIG-136 has similarity to other DNA binding agents, the pattern of activity for this drug does not fit within the clusters of any known agents, suggesting it possesses a distinct mechanism of action.

The molecule spans six DNA base pairs with a preference for purine-GATC-pyrimidine sequences (3). The two imine moieties at the top of each seven-membered ring of SJG-136 bind covalently to the N2 positions of guanines on opposite strands of DNA to form a cytotoxic interstrand cross-link spanning four base pairs (4).

In mice bearing the LS174T human colon xenograft, DNA interstrand cross-links can be detected in tumor cells with the use of a modification of the single cell gel electrophoresis (comet) assay after administration of a therapeutic dose (5). Cross-links in the tumor increase with dose and are clearly detectable at 1 hour after i.v. administration. The level of cross-linking persists over a 24-hour period in this tumor, which is significantly longer than the effects produced by conventional cross-linking agents observed over the same time period.

Testing in the National Cancer Institute standard hollow fiber assay produced prominent growth inhibition in 20 of 24 i.p. and 7 of 24 s.c. test combinations, with 5 of 12 cell lines exhibiting cell kill (5). SIG-136 displayed potent activity against several human tumors in vivo (6). In addition, SIG-136 produced...
antitumor activity in mice bearing CH1 and CH1cisR xenografts, a cisplatin-resistant human ovarian tumor model (5).

In toxicology studies, the maximum tolerated dose (MTD) in mice was 300 μg/kg after a single dose. With the use of a 5-day dosing regimen, the MTD was 25 μg/kg/day in rats and 1 to 2 μg/kg/day in dogs. Dose-limiting myelotoxicity and gastrointestinal toxicity were seen in all species.

Based on the preclinical activity of SJG-136, a phase I dose-finding study was undertaken in patients with advanced solid tumors. It was considered appropriate to include patients with a variety of solid tumor types because no particular cancer cell lineage specificity was shown in preclinical studies. The objectives of the study were to establish the MTD of SJG-136 when administered as a 10-minute i.v. infusion once every 21 days and to determine the recommended dose for phase II evaluation. The study also sought to investigate possible antitumor activity and provide pharmacokinetic and pharmacodynamic data in patients.

**Materials and Methods**

This two-centered, open-label phase I dose-escalation study was the first clinical evaluation of i.v. SJG-136. The study protocol was reviewed by the Central Institutional Review Board of the Cancer Research UK Drug Development Office and the Royal Free Hospital research ethics committee.

Between April 2004 and July 2006, adult patients with histologically confirmed advanced and/or metastatic solid cancer, refractory to conventional treatment, or for whom no conventional therapy existed were recruited into the trial.

The patients had a life expectancy of at least 3 mo with a WHO performance status of 0 or 1. Before starting treatment, all patients had neutrophils ≥1.5 × 10⁹/L, platelets ≥100 × 10⁹/L, serum bilirubin ≤1.5 × upper limit of the normal range, and alanine aminotransferase and/or aspartate aminotransferase ≤2.5 × upper limit of the normal range unless due to tumor, in which case to 5 × upper limit of the normal range was permissible. In addition, all patients had serum albumin ≥30 g/dL and creatinine clearance ≥50 mL/min.

None of the patients had undergone major thoracic and/or abdominal surgery in the preceding 4 wk, and none were at medical risk because of nonmalignant systemic disease or active uncontrolled infection. Patients who had received a bone marrow transplant or who had undergone radiotherapy (except for palliative reasons), endocrine therapy, immunotherapy, or chemotherapy during the previous 4 wk (6 wk for mitomycin C) were excluded from entering the study as were those with a compromised bone marrow reserve. Additional reasons for exclusion included positive serology for hepatitis B or C, or HIV; coexisting heart failure or a history of New York Heart Class IV cardiac disease.

**Figure 1.** Summary of dose-escalation scheme.
Association class III/IV cardiac disease; and symptomatic pulmonary disease, dyspnea, pleural effusions, or ascites.

Female patients of childbearing potential had a negative serum pregnancy test before enrolment and agreed to use appropriate medically approved contraception from 4 wk before entering the study until 6 mo after completing the study. Male patients were using appropriate medically approved contraception during the study and for 6 mo afterwards. Pregnant or lactating women were excluded.

All patients provided signed written informed consent. The ability of the patients to cooperate with treatment and follow-up procedures was ensured and documented.

**Treatment.** SJG-136 was administered as a 10-min i.v. infusion once every 21 d. The 21-d schedule was selected in light of the predicted major myelosuppression following bolus administration with alkylating agents. The starting dose of 15 \( \mu g/m^2 \) was selected on the basis of preclinical toxicology studies. This dose was equivalent to approximately one sixteenth of the total dose of SJG-136 administered to dogs (daily for 5 d) that did not cause severe irreversible toxicity.

Treatment could continue for 6 cycles unless there was evidence of disease progression, unacceptable toxicity, or the patient requested withdrawal. If the patient was responding or had stable disease, further cycles were given at the discretion of the responsible physician. Patients who showed progressive disease were removed from the study.

Concomitant medication was given as medically indicated. The use of colony-stimulating factors was confined to the treatment of neutropenia, and antiemetic drugs were only given from cycle 2 onwards in established cases of nausea or vomiting. Radiotherapy was allowed for established cases of bone pain. Concurrent steroid treatment was limited to approximately one sixteenth of the total dose of SJG-136 administered to dogs (daily for 5 d) that did not cause severe irreversible toxicity.

Toxicity assessments were determined with the use of the National Cancer Institute Common Terminology Criteria for Adverse Events (version 3.0).

The primary end points were determination of dose-limiting toxicity (DLT) and the MTD. DLT was defined by the presence of the following drug-related events:

- Any drug-related death
- Hematologic events (grade 4 neutropenia lasting \( >7 \) d, grade 3 or 4 febrile neutropenia, infection with grade 3 or 4 neutropenia, grade 4 thrombocytopenia, or grade 3 thrombocytopenia with hemorrhage)
- Hepatic events (grade 3 or 4 increases in bilirubin, grade 3 or 4 increases in aspartate aminotransferase or alanine aminotransferase or alanine aminotransferase \( >5 \times \) the pretreatment level in patients with liver metastases)
- Other events (grade 3 or 4 vomiting despite optimal antiemetic therapy, grade 3 or 4 stomatitis or diarrhea, or any grade 3 or 4 nonhematologic event that increased in grade by at least two increments from baseline)
- Adverse event (during the first 2 cycles only) that delayed treatment and which did not resolve within 1 wk

The MTD was the dose of SJG-136 that resulted in DLT in at least two of three to six patients during the 1st cycle of treatment. The recommended dose for subsequent phase II clinical trials was defined as the dose level below the MTD.

**Criteria for dose escalation.** Initially, an accelerated dose-escalation strategy based on design 3B described by Simon et al. (7) was used. Doses were escalated in single-patient cohorts by doubling the dose from 15 to 30, 60, 120, and 240 \( \mu g/m^2 \) until drug-related adverse events were observed. Dose escalation continued until either one patient exhibited 1st cycle DLT or two patients experienced 1st cycle drug-related grade 2 or greater toxicities (excluding alopecia, and nausea and/or vomiting without appropriate treatment). The intention was to treat three patients per dose level in the case of drug-related grade 2 toxicities, and up to six patients per dose level were enrolled in the case of DLT (Fig 1). If DLT was not observed, the patients would receive escalating doses of SJG-136 that were 33% higher than the previous dose tested. If expansion of the single-dose cohort was indicated but the toxicities seen indicated that further patients at that dose level would be unsafe, the next lowest dose level was expanded to include an additional two patients.

A minimum interval of at least 3 wk was required between dose levels during the dose-doubling phase of the study. A 3-wk interval was also required between the first patient treated at an expanded dose level and the remainder of the patients treated at the same dose level. Intrapatient dose escalation was permitted after 2 cycles of treatment in the absence of significant toxicity in the subject and tolerance of treatment in an initial patient treated at the higher dose level.

**Tumor response.** Tumor response was assessed with the use of the Response Evaluation Criteria in Solid Tumors. Tumor assessments, based on magnetic resonance imaging, computed tomography, X-ray, or clinical measurement, were done within 1 wk of starting treatment with SJG-136 and then at the end of every 2 cycles.

**Pharmacokinetic assessments.** Pharmacokinetic sampling was done during cycle 1 and during the cycle at which the dose of SJG-136 was escalated for a given patient. Samples were taken pre-dose; 5, 10, and...
within 1 h of collection, the sample was centrifuged at 1,200 g at 4°C for 10 min. At least 3 aliquots of 1.5 mL of plasma were transferred immediately (with the use of a sterile pipette) to three polypropylene storage tubes. The samples were frozen immediately at -80°C and were kept upright until shipment. SJG-136 was extracted from plasma with the use of either zinc sulfate or acetonitrile to precipitate proteins before analysis by gradient reversed-phase high-performance liquid chromatography with the use of fluorescence detection.

Urine samples were collected post dose with timed collections 0 to 8 and 8 to 24 h on days 1 to 2 of cycle 1. The total urine volume was recorded, and 10 mL aliquots of urine were placed on ice and frozen at -80°C until shipment. SJG-136 was extracted from urine through solid phase extraction before reversed-phase high-performance liquid chromatography. The methodology was validated between 0.3 and 10 nmol/L.

Pharmacokinetic data were modeled with the use of a two-compartment infusion model in WinNonlin. Pharmacokinetic parameters were also calculated with the use of noncompartmental analysis to ensure a correlation with both the area under the concentration-time curve (AUC) to the last actual time point (AUC0-τ) and AUC to 8 h (AUC0-8 h) calculated with the use of the two-compartment model.

Pharmacodynamic methods. Whole blood samples (8 mL) for pharmacodynamic studies were collected during cycle 1 (pre-dose; 1, 4, and 24 h after the end of infusion) and cycle 2 (pre-dose; 1 and 4 h after the end of infusion). Samples were also taken on days 8 and 15 of cycles 1 and 2.

Tumor samples for pharmacodynamic assessment were collected before treatment, and between 2 and 4 h after the end of the first infusion of SJG-136.

DNA interstrand cross-linking was measured in peripheral blood lymphocytes and single-cell suspensions prepared from tumor biopsies with the use of a modification of the single cell gel electrophoresis (comet) assay (8). Cells were irradiated with 12.5 Gy to introduce a fixed level of random DNA single-strand breaks. This level of radiation produces an 50:50 distribution of DNA in the comet head and tail of control cells under the conditions used and is optimal for the detection of DNA interstrand cross-links. The cells were then embedded in agarose on a microscope slide and lysed. The resulting DNA was unwound under alkaline conditions, electrophoresed, and stained with propidium iodide. The extent of DNA damage was quantified by

Table 2. Actual dosage-escalation scheme

<table>
<thead>
<tr>
<th>Dose escalation</th>
<th>No. of patients (patient no.)</th>
<th>No. of cycles</th>
<th>Inpatient changes</th>
</tr>
</thead>
<tbody>
<tr>
<td>15 μg/m²</td>
<td>1 (1)</td>
<td>3</td>
<td>Dose escalation in cycle 4</td>
</tr>
<tr>
<td>30 μg/m²</td>
<td>2 (1, 2)</td>
<td>3</td>
<td>None</td>
</tr>
<tr>
<td>60 μg/m²</td>
<td>1 (3)</td>
<td>3</td>
<td>Dose escalation in cycle 4</td>
</tr>
<tr>
<td>120 μg/m²</td>
<td>2 (3, 4)</td>
<td>3</td>
<td>None</td>
</tr>
<tr>
<td>240 μg/m²</td>
<td>1 (6)</td>
<td>1</td>
<td>None</td>
</tr>
</tbody>
</table>

Dose de-escalation

| 120 μg/m²       | 1 (7)                         | 1            | None |
| 60 μg/m²        | 3 (8-10)                      | 9            | None |
| 45 μg/m²        | 7 (11-17)                     | 20           | Dose reduced* |
| 30 μg/m²        | 1 (14)                        | 4            | None |

* Dose was reduced in patient 14 who developed grade 2 VLS with grade 3 dyspnea in cycle 3.

Table 3. Summary of 10 cases of VLS

<table>
<thead>
<tr>
<th>Dosage (μg/m²)</th>
<th>Days to onset of VLS</th>
<th>Total with VLS (%)</th>
<th>Patient (no.)</th>
<th>Grade</th>
<th>Cycle</th>
<th>Symptoms</th>
</tr>
</thead>
<tbody>
<tr>
<td>30 (n = 1)</td>
<td>5</td>
<td>1 (100)</td>
<td>2</td>
<td>2</td>
<td>1</td>
<td>Ascites, hypoalbuminemia, limb edema, dyspnea</td>
</tr>
<tr>
<td>45 (n = 7)</td>
<td>14</td>
<td>4 (57)</td>
<td>11</td>
<td>1</td>
<td>3</td>
<td>Abdominal distension, hypoalbuminemia, limb edema, dyspnea, weight gain</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>Hypoalbuminemia, limb edema</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>14</td>
<td>1</td>
<td>2</td>
<td>Abdominal distension, ascites, hypoalbuminemia, limb edema, dyspnea, pleural effusion, weight gain</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>21</td>
<td>1</td>
<td>3</td>
<td>Abdominal distension, hypoalbuminemia, limb edema, dyspnea</td>
</tr>
<tr>
<td>60 (n = 4)</td>
<td>3</td>
<td>2 (50)</td>
<td>3</td>
<td>2</td>
<td>3</td>
<td>Abdominal distension, hypoalbuminemia, limb edema dyspnea, weight gain</td>
</tr>
<tr>
<td></td>
<td>8</td>
<td>10</td>
<td>2</td>
<td>1</td>
<td></td>
<td>Abdominal distension, hypoalbuminemia, limb edema, trunk edema, dyspnea</td>
</tr>
<tr>
<td>120 (n = 2)</td>
<td>7</td>
<td>2 (100)</td>
<td>4</td>
<td>3</td>
<td>2</td>
<td>Hypoalbuminemia, limb edema, pleural effusion, weight gain</td>
</tr>
<tr>
<td>240 (n = 1)</td>
<td>10</td>
<td>1 (100)</td>
<td>6</td>
<td>3</td>
<td>2</td>
<td>Hypoalbuminemia, limb edema, dyspnea, pleural effusion</td>
</tr>
</tbody>
</table>

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Komet analysis software to produce a tail moment, defined as the product of the percentage DNA in the comet tail and the distance between the means of the head and tail distributions based on the definition by Olive et al. (1990; ref. 9). The percentage decrease in comet tail moment compared with pre-dose irradiated controls was used to quantify the level of DNA interstrand cross-linking. Validation experiments in lymphocytes treated ex vivo with SJG-136 gave a linear increase in percentage decrease in tail moment over a dose range of 1 to 100 nmol/L.

Samples used for comet assay analysis were also assayed with the use of a research end point (γ-H2AX foci measurement), which will be reported in detail elsewhere (article in preparation). Briefly, the cells were fixed onto microscope slides, permeabilized, and blocked at 4°C. The blocked cells were then incubated overnight at 4°C with mouse monoclonal anti-γ-H2AX antibody, washed, and then incubated with Alexa Fluor 488 goat anti-mouse 2° antibody. After washing, the slides were counterstained with 2 mg/mL propidium iodide. Images were visualized with the use of a Zeiss LSM 510 Axiohot confocal microscope equipped with argon (488 nm) and helium-neon (543 nm) lasers for γ-H2AX and propidium iodide detection, respectively. Foci were counted in 50 cells per sample and the results expressed as the mean number of foci per cell. Validation experiments in lymphocytes treated ex vivo with SJG-136 gave a linear increase in foci per cell over a dose range of 0.05 to 5 nmol/L.

Results

**Patient demographics.** Sixteen patients with WHO performance status of 0 or 1 were selected from the two trial centers for treatment with SJG-136. The patient demographic details are shown in Table 1. All 16 patients had measurable target lesions, and all but 1 patient (who had primary stomach cancer) had >1 site of disease. Seven patients had liver metastases, 5 had lung metastases, 4 each had other metastatic or regional nodes, 2 had soft tissue metastases, and 1 had skin metastases. Each of the 16 patients had previously undergone one or more courses of chemotherapy. In addition, 15 of them had undergone prior surgery, 4 had previously received hormonal or biological therapy, and 3 had undergone radiotherapy.

**Treatment protocol.** The actual dose-escalation and de-escalation scheme used in the study is shown in Table 2.

The accelerated dose-escalation strategy planned at the start of the study (see Fig. 1) resulted in unexpected toxicity in the form of delayed vascular leak syndrome (VLS). This was seen in 4 of 5 patients (patients 2, 3, 4, and 6) treated at doses between 30 and 240 μg/m². The dose was de-escalated to 60 μg/m², and 3 additional patients were treated at this dose level. However, 2 of the 3 patients (patients 9 and 10) developed delayed reversible liver toxicity. Thereafter, new patients were recruited at the lower dose level of 45 μg/m². This was administered in combination with dexamethasone, 8 mg twice daily for 3 days starting 24 hours before treatment with SJG-136, to prevent any inflammatory effect of the drug on the bile duct and the appearance of VLS.

**Toxicity assessment.** The main drug-related adverse events observed in this study were VLS, elevated liver function tests, and fatigue. These adverse events generally had a delayed onset and increased in severity with subsequent cycles.

VLS was associated with one or more of the following events: hypoalbuminemia, peripheral edema, dyspnea, pleural effusion, abdominal distension, ascites, and weight gain. The emergence of this toxicity was unexpected. Grades 1 to 3 drug-related VLS was reported in 10 (62.5%) patients. An overview of all 10 cases of VLS is provided in Table 3. Symptoms were often debilitating and continued for prolonged periods (up to 7 months or beyond the scheduled follow-up period until the patient’s death). Two patients developed grade 3 VLS, one at 120 μg/m² and one at 240 μg/m², both during the 2nd cycle of treatment. Signs of VLS were detected from 1 to 21 days after treatment (median, 9 days).

Elevated liver function tests (grades 1 to 4) were reported in 12 (75%) patients across all dose levels. Three patients had dose-limiting, grades 3 to 4 elevations in hepatic transaminases (i.e., γ-glutamyl transpeptidase, aspartateaminotransferase, or alanine aminotransferase). There were six additional cases of grades 3 to 4 drug-related increases in liver transaminases, each in patients with liver metastases: one case at 45 μg/m², two at 60 μg/m², two at 120 μg/m², and one at 240 μg/m² every 21 days.

There were three cases of elevated bilirubin considered possibly or probably related to SJG-136 also reported in patients with liver metastases. One case occurred at 30 μg/m², one at 45 μg/m², and one at 120 μg/m² every 21 days.

Drug-related fatigue (grades 1 to 3) was reported in 14 (87.5%) patients. The time to onset of fatigue varied, but in 10 of the 14 patients, the first occurrence was in cycle 1. Three patients had grade 3 fatigue that interfered with activities of daily living. Although VLS is not usually associated with fatigue, the 10 patients with VLS also reported fatigue as an adverse event. VLS and fatigue commonly started in the same treatment cycle although fatigue was of shorter duration than VLS. Fatigue was sometimes associated with other adverse events, such as nausea and vomiting.

Drug-related hematologic adverse events consisted of grades 1 to 3 lymphopenia in 12 patients (75%), grades 1 to 2 anemia (i.e., low hemoglobin) in 8 patients (50%), grades 1 to 2 leukopenia in 2 patients (12.5%), and grade 3 neutropenia and grade 1 thrombocytopenia in 1 patient each (6.25%).

Abnormal metabolic and laboratory findings included grades 1 to 2 reductions in total protein (37.5%), and grades 1 to 2 proteinuria (25%), hyperuricemia, hyponatremia, and hyperphosphatemia (each in 12.5% of patients).
Drug-related gastrointestinal adverse events (apart from disease-related ascites and distension) included grades 1 to 3 nausea (44%), mucositis (25%), taste alteration, anorexia (both 19%), constipation, diarrhea (both 12.5%), and vomiting (6%).

Two patients developed cardiac adverse events that were possibly related to treatment with SJG-136. One patient was withdrawn from the study during cycle 4 (120 μg/m²) because of grade 2 palpitation and reversible grade 2 left ventricular dysfunction. The other patient developed grade 3 sinus tachycardia while receiving SJG-136 at 240 μg/m² every 21 days. This patient also had VLS.

One patient died 10 days after cycle 2 of treatment with SJG-136 at 30 μg/m². The patient had developed possible VLS with symptoms of ascites, ankle edema, and dyspnea. The causes of death were pulmonary edema, aspiration of gastric contents (aspiration pneumonitis), and progressive metastatic liver disease, unrelated to treatment with SJG-136. There were four other deaths, each due to underlying disease, occurring 30 days, and 6, 20, and 22 weeks after the final cycle of SJG-136.

**DLT and MTD.** A summary of DLTs seen in the study is shown in Table 4.

The protocol defined the MTD as the dose of SJG-136 that resulted in DLT in at least two of three to six patients in the 1st cycle of treatment. In this study, all DLTs had a delayed onset, starting in cycles 2 to 4; therefore, the protocol definition could not be followed. In light of this, a dose of 45 μg/m² every 21 days was considered to be the MTD, with two of seven patients receiving this dose level experiencing a DLT at some point during treatment. However, in view of the fact that VLS toxicity occurred even below this dose, alternative dosing schedules should be evaluated before the phase II evaluation of SJG-136.

**Clinical efficacy.** Twelve of the 16 patients were evaluable for efficacy at the end of cycle 2. The other 4 patients only received a single cycle of treatment. The best tumor response was stable disease in 10 patients. In 2 of the 10 patients, stable disease was maintained for 4 cycles. One of the 2 patients had colorectal cancer and received SJG-136 at 60 μg/m². The other patient had malignant melanoma and was treated with SJG-136 at 45 μg/m² every 21 days for 3 cycles, followed by 4 cycles at 30 μg/m² every 21 days. Two patients, one with melanoma and the other with cancer of the ampulla of Vater, developed progressive disease after 2 cycles.

**Pharmacokinetics.** Pharmacokinetic analysis was undertaken in all 16 patients treated with SJG-136. Systemic clearance varied from 1.41 to 12.8 L/hour/m² (mean, 5.0 L/hour/m²). There was a linear relationship between dose and AUC₀⁻⁸ although there was considerable variability at each dose level (Fig. 2). AUC values varied from 4.2 to 79 nmol/L/hour over the dose range 15 to 240 μg/m², and Cₘₐₓ varied from 5.3 to 91 nmol/L. The volume of distribution across the dose range 15 to 240 μg/m² ranged from 1.8 to 57 L/m². Modeling the data at 45 μg/m² (MTD) with the use of a two-compartment infusion model suggested elimination with a mean distribution phase half-life of 0.16 hours (range, 0.04-0.34 hours) and a mean terminal elimination half-life of 2.6 hours (range, 0.79-5.3 hours). The mean predicted Cₘₐₓ at this dose was 29 nmol/L (range, 13-54 nmol/L), and the mean AUC₀⁻₈ was 20 nmol/L/hour (range, 10-52 nmol/L/hour). The mean urinary recovery was 9.5% of the dose in 24 hours (range, 6.1%-14%).

**Pharmacodynamic measurements.** No evidence of DNA interstrand cross-linking was found in peripheral blood lymphocytes or in tumor cells when assessed with the use of a validated modification of the single cell gel electrophoresis (comet) assay.

In 16 patients, peripheral blood lymphocytes were analyzed for γ-H2AX foci after cycle 1 of treatment. Maximum induction of foci occurred 24 hours post dose, with mean foci counts between 16 and 76 per 50 cells. This represented a large increase from pre-dose, when the mean number of foci per 50 cells varied between 0.8 and 10.5. No evidence of a dose response was seen.

Tissue biopsies, taken pre-dose and 2 to 4 hours post infusion, were taken from 2 of the 16 patients. Increases in γ-H2AX foci induction were seen in both tissue biopsies after drug treatment (Table 5). The pre-dose level of foci was much higher in tumor samples than in lymphocytes from the same patient. The increase in foci formation resulting from drug treatment was much higher in the tumor samples than in the lymphocytes taken at a similar time point (4 hours) post infusion.
Discussion

Many effective agents used in the treatment of solid tumors, including alkylating agents, platinum drugs, and anthracyclines, interact with DNA. Despite the ability of these agents to cure cancers, such as testicular cancer and Hodgkin’s disease, the majority of patients with solid tumors develop resistance to chemotherapy. A critical factor in the development of resistance to this type of chemotherapeutic agent is the enhanced ability of cancer cells to repair drug-induced lesions in DNA (10, 11). Therefore, there remains an important need to optimize the use of current agents and to develop novel DNA-active agents for therapeutic use, which are less readily identified by DNA damage-repair mechanisms.

The pyrrolobenzodiazepine dimer SJG-136 is a novel agent with a unique mechanism of action. Unlike conventional alkylating agents, SJG-136 binds covalently in the minor groove of DNA, forming an interstrand cross-link. Evidence from in vitro experiments indicates that this family of agents inhibits transcription in a sequence-selective manner (12), and it is also possible that DNA replication may be inhibited through the cross-linking process (13). In addition, SJG-136 DNA adducts are resistant to repair compared with those of other DNA-active agents, and the compound retains full potency in cisplatin-resistant tumors (5). This is thought to be because SJG-136 adducts are nondistortive and so are not easily recognized by DNA repair enzymes.

Our results with the use of the γ-H2AX assay indicated that SJG-136 increased γ-H2AX foci in the peripheral blood lymphocytes of all 16 patients and in tumor tissue from the 2 patients in whom this was tested. Peak foci formation in lymphocytes was seen 24 hours post treatment, and higher levels of foci were observed in tumor than in lymphocytes taken at similar time points in the same patient. The tumor samples were assessed at 2 to 4 hours because preclinical studies in mice suggested that interstrand cross-links were detectable at this time point. Preclinical studies showed that γ-H2AX foci are detectable later than the DNA interstrand cross-links as measured with the use of comet assay. Although a previous study has used measurement of γ-H2AX phosphorylation by flow cytometry in leukemic cells (14), this is the first study to use the immunocytochemical measurement of γ-H2AX to detect DNA damage response to a cross-linking agent in clinical material, including solid tumors. In our preclinical validation studies, the γ-H2AX assay was found to be at least 10× more sensitive than comet assay at detecting DNA damage induced by SJG-136 in isolated human lymphocytes (article in preparation). The absence of any detectable DNA interstrand cross-links by comet assay in the current study would therefore indicate that the level produced was below the limit of detection for this assay. The findings with the γ-H2AX assay, however, provide conclusive evidence of the ability of SIG-136 to interact with DNA in vivo.

The major toxic effect of SJG-136 was the unexpected occurrence of vascular leak characterized by hypoalbuminemia accompanied by pleural effusions, ascites, and marked peripheral edema. The consequent reduction in the study dose to 45 µg/m² with the addition of steroid premedication did not significantly alter the incidence and severity of this complication. Although VLS was not a life-threatening toxicity in our study, it was severely debilitating and had major adverse consequences on quality of life. Additionally, the delayed occurrence of hepatotoxicity, although reversible, was difficult to monitor because of timing and made decisions on dose adjustments difficult.

The occurrence of VLS and liver toxicity could not have been predicted from the preclinical safety profile of the drug. VLS is usually associated with endothelial cell injury in response to chronic inflammation as seen in a variety of infections, autoimmune diseases, graft-versus-host disease, and during treatment of cancer patients with high doses of interleukin 2 (15–17). This adverse effect has been recorded previously with FK973, a DNA minor groove binding drug structurally similar to mitomycin (18). There were several features of the VLS secondary to SJG-136 with similarities to that found with FK973. In that study, cumulative dose-related toxicity with pleural and pericardial effusions, ascites, and peripheral edema occurred 1 to 2 weeks after treatment. The delay in VLS after administration of SJG-136 differs from that seen in immunotoxins, which occurs within 2 to 4 days of administration (19).

The low blood albumin concentrations that characterize the syndrome may have been caused by hepatocyte damage (16). Results of urinary protein measurement and small bowel biopsy excluded renal and enteric loss, respectively. Additionally, despite the finding of VLS in three patients <5 days after treatment, the occurrence of VLS in seven patients >7 days after infusion is similar to the time of onset after FK973 treatment. Although no morphologic evidence of endothelial damage was found in two liver biopsies done in patients with VLS, we did not specifically investigate γ-H2AX foci in endothelial cells from these specimens.

In patients with colorectal liver metastases, hypoalbuminemia has been shown to be associated with increased levels of C-reactive protein, indicating that it may be caused by an inflammatory response rather than being related to tumor volume per se (20). Dexamethasone, 8 mg twice daily for 3 days starting 24 hours, was given before treatment with SJG-136 to prevent any inflammatory effect of the study drug. However, this intervention was unable to prevent the occurrence of VLS in our study. The etiology of VLS with SJG-136 is unclear and is currently the subject of preclinical studies.

Table 5. γ-H2AX foci induction in tumor tissue and lymphocytes from two patients after treatment with SJG-136

<table>
<thead>
<tr>
<th>Patient</th>
<th>Primary tumor</th>
<th>Dose (µg/m²)</th>
<th>Tumor</th>
<th>Lymphocytes</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Pre-dose 2-4 h post infusion</td>
<td>Pre-dose 4 h post infusion</td>
</tr>
<tr>
<td>12</td>
<td>Melanoma</td>
<td>45</td>
<td>11.44 ± 1.70</td>
<td>44.88 ± 4.54</td>
</tr>
<tr>
<td>13</td>
<td>Melanoma</td>
<td>45</td>
<td>10.10 ± 0.85</td>
<td>31.53 ± 4.88</td>
</tr>
</tbody>
</table>

5.49 18.34

2.35 4.88

4.54 1.04

4.88 4.20

27.34 2.35
Efficacy was not a primary end point in this phase I study of patients with advanced solid tumors. No objective responses were obtained. However, stable disease for between 77 and 184 days was documented in 10 of the 12 patients with evaluable lesions. Dose escalation was limited by the delayed and unexpected occurrence of VLS, and the patient with the maximal duration of stable disease developed significant toxicities necessitating treatment discontinuation.

A linear relationship was found between AUC0-8 h or Cmax and dose over the dose range studied (15-240 μg/m²) although there was considerable variability in these parameters within each dose level.

We have confirmed that SJG-136 increases γ-H2AX foci in peripheral blood lymphocytes and post-treatment tumor biopsies, suggesting that, at clinically attainable concentrations, the drug causes DNA damage. However, the toxicity is such that we do not recommend further development of this agent with the use of this schedule unless the mechanism underlying VLS can be identified and prevented. Other phase I studies underway will clarify if protracted regimens also result in a similar toxicity profile. Results from a study investigating infusion on 3 consecutive days every 21 days suggest that the toxicity of VLS after treatment with SJG-136 may be significantly reduced by concomitant administration of steroids and diuretics (21).

Disclosure of Potential Conflicts of Interest

J.A. Hartley, employment, ownership interest, Spirogen. P. Loadman, employment, Spirogen.

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References

Phase I Study of Sequence-Selective Minor Groove DNA Binding Agent SJG-136 in Patients with Advanced Solid Tumors


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