A Phase I Clinical and Pharmacokinetic Study of Ro 31-7453 Given as a 7- or 14-Day Oral Twice Daily Schedule Every 4 Weeks in Patients with Solid Tumors

Ramon Salazar,[1] Donald Bissett,[2] Chris Twelves,[1,3] Lars Breimer,[4] Mark DeMario,[4] Sophia Campbell,[1] Jay Zhi,[4] Steve Ritland,[4] and Jim Cassidy[2]

[1]Cancer Research UK, Department of Medical Oncology, Beatson Laboratories, University of Glasgow, United Kingdom; [2]Department of Medicine and Therapeutics, Aberdeen University, Institute of Medical Sciences, Foresterhill, United Kingdom; [3]University of Leeds and Tom Connors Cancer Research Centre, Bradford, United Kingdom; and [4]Roche Laboratories, Hoffmann-La Roche, Inc., Nutley, New Jersey

ABSTRACT

Purpose: **This is a dose-finding Phase I study of oral Ro 31-7453, a new class of antimitotic drug with promising preclinical activity in several chemoresistant models.**

Experimental Design: **Two schedules of oral Ro 31-7453 (every 12 h) given for either 7 or 14 consecutive days repeated every 4 weeks were explored consecutively.**

Results: **Thirty-seven patients with refractory cancer entered the study (14 on the 7-day schedule and 23 on the 14-day schedule). Median age was 63 years (range, 40–77 years), and median Karnofsky performance status was 80 (range, 60–100); the most frequent diagnosis was colorectal carcinoma (16 patients). Dose levels of 100, 200, 240, and 280 mg/m^2 twice daily (bid) for 7 days and 70, 100, 125, and 150 mg/m^2 bid for 14 days were explored. A total of 110 cycles were administered, the median number of cycles received was 3 (range, 1–7); six patients completed 6 or more cycles. Myelosuppression and mucositis were dose-limiting with both schedules. Fatigue and gastrointestinal toxicities other than mucositis were frequent but generally mild. The maximum tolerated doses were 200 mg/m^2 bid and 125 mg/m^2 bid for the 7- and 14-day schedules, respectively. Pharmacokinetic analysis showed rapid absorption and metabolism. The area under the concentration-time curve and trough concentrations of Ro 31-7453 and two active metabolites appeared dose proportional with a $t_{1/2}$ of ~9 h and a t_{max} of ~4 h. One patient with pretreated lung cancer had a partial response.**

Conclusions: **Both Ro 31-7453 regimens were feasible, but the 14-day schedule at the recommended dose of 125 mg/m^2 bid was selected for further monotherapy Phase II evaluation because of its higher preclinical activity. This regimen is convenient, well tolerated, and has a favorable pharmacokinetic profile.**

INTRODUCTION

Ro 31-7453 belongs to a novel class of antimitotic and apoptosis-inducing agents with *in vitro* efficacy against a wide range of human tumor cell lines, including all five multidrug-resistant cell lines tested (1). Although the precise mechanism of action has not yet been identified, Ro 31-7453 inhibits cyclin-dependent kinases 1, 2, and 4. Ro 31-7453 also inhibits tubulin polymerization in cell-free systems (2), thus preventing normal progression through M phase by inhibiting formation of the mitotic spindle. In addition, Ro 31-7453 is synergistic with paclitaxel and vinorelbine, drugs that also alter tubulin kinetics, in breast carcinoma cell lines (1, 3). Ro 31-7453 has antitumor activity *in vivo* in 15 of 16 animal models at s.c., orthotopic, and pulmonary sites when administered by the oral, i.v., and i.p. routes. Antitumor activity in these preclinical models ranged from statistically significant growth inhibition in 11 models to regression in 4 models, depending on the cell line and schedule of administration. The sensitive models included xenografts derived from breast (MDA-MB-435), colorectal (RKO, HT-29, and HCT116), lung (A549), prostate (DU-145), paclitaxel- and multidrug-resistant colorectal (SW480 and LS1034), and uterine human tumor cell lines (4). Ro 31-7453 also induced regression and growth inhibition against a syngeneic rat mammary adenocarcinoma (MTLn3) and in the multiple intestinal neoplasia (Min) mouse model, but it was ineffective against the B16F10 murine melanoma (4).

Dose-limiting bone marrow and small intestine toxicity was observed in a series of rodent studies. These toxicities were reversible in rats after a 7-day recovery period (1, 5). In preclinical pharmacokinetic studies, elimination of Ro 31-7453 and its metabolites occurred primarily through biliary clearance, with renal clearance a minor component.[1] Several metabolites have been isolated and characterized preclinically; of these, Ro 27-4006 (M1) and 27-0431 (M2) have antitumor activity equivalent to that of the parent compound (6). Schedules of administration that achieved prolonged exposure to Ro 31-7453 had a

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Note: Ramon Salazar is currently at Medical Oncology Service, Vall d'Hebron University Hospital, Barcelona, Spain. Jim Cassidy is currently at Cancer Research Campaign UK, Department of Medical Oncology, Beatson Laboratories, University of Glasgow, Glasgow, United Kingdom.

Requests for reprints: Chris Twelves, Tom Connors Cancer Research Centre, University of Bradford, Richmond Road, Bradford, BD7 1DP, United Kingdom. Phone: 44-1274-233226; Fax: 44-1274-233234; E-mail: C.Twelves@bradford.ac.uk.

[1] Roche Laboratories, unpublished data.

better therapeutic index in preclinical models (5, 7). This led to the development of a novel oral sustained release microprecipitate formulation of Ro 31-7453, which was active in multiple animal tumor models, including MDA-MB-435 (breast cancer), HCT116 and SW480 (colon cancer), A549 (non-small cell lung cancer), and UACC-62 and LOX [melanoma (8)]. This oral formulation of Ro 31-7453 was developed for clinical Phase I evaluation.

The efficacy of RO31-7453 in a broad range of tumor models, including paclitaxel-resistant cell lines, the possibility of a unique target and mechanism of action, and synergy with other tubulin-interacting agents all made this compound worthy of clinical development. We report here the results of a Phase I and pharmacological study of Ro 31-7453 administered orally to adult patients with solid malignant tumors. This study aimed to define the maximum tolerated dose (MTD), feasibility of administration, and pharmacokinetic parameters of Ro 31-7453 when given orally twice daily (bid) using two different schedules of 7 and 14 days, repeated every 4 weeks.

PATIENTS AND METHODS

Patient Selection. Eligible patients had histologically or cytologically confirmed locally advanced and/or metastatic solid tumors for which there was no standard therapy. They were required to have a Karnofsky performance status of ≥60% and to be at least 18 years of age, with no recent chemotherapy and/or radiotherapy and adequate hematopoietic function [absolute neutrophil count $\geq 1.5 \times 10^9$/liter; platelet count $\geq 100 \times 10^9$/liter], renal function (serum creatinine $> 1.5 \times$ the upper limit of normal), and liver biochemistry [total serum bilirubin level $\leq 1.5 \times$ the upper limit of normal; alkaline phosphatase, serum aspartate aminotransferase, and alanine aminotransferase levels $\leq 2.5 \times$ the upper limit of normal; in the presence of liver metastasis, serum aspartate aminotransferase and alanine aminotransferase were allowed to be $\leq 4\times$ the upper limit of normal]. Patients with brain metastases or >grade 2 neuropathy at baseline were not eligible. The local ethics committees of the participating institutions approved the study protocol, and all patients gave written informed consent before entering the study.

Dosage and Dose Escalation. Patients were to receive Ro 31-7453 orally bid (with a 12-h interval) for 7 days in the first part of the study and for 14 days in the second part of the study. Treatment was repeated every 28 days for 24 weeks (6 cycles), as long as patients did not experience unacceptable toxicity or disease progression. Treatment could be continued beyond this point at the discretion of investigator.

Dose-limiting toxicity (DLT) was defined as grade 4 (National Cancer Institute Common Toxicity Criteria Version 2.0) neutropenia lasting more than 5 days or complicated by fever, grade 3 or 4 thrombocytopenia, and/or ≥grade 3 nonhematological toxicity, excluding fever, chills, and flu-like symptoms. If a patient experienced DLT, and continued treatment was deemed appropriate, the dose of Ro 31-7453 was reduced by one dose level. Treatment was given every 28 days, provided all toxicities had recovered to ≤grade 1, with the exception of neutropenia, for which retreatment was permitted with a grade 2 neutrophil count.

The dose escalation scheme was based on the National Cancer Institute-Food and Drug Administration accelerated design (9). Briefly, this involved single patient cohorts with dose doubling until one patient experienced ≥grade 2 toxicity during any course of treatment. At that point, the accelerated dose escalation stage terminated, and three patient cohorts were implemented with 20–60% dose increments, depending on toxicity at the prior dose level. If only one patient had DLT, three additional patients were accrued at the same dose level. If two or more patients of a three- or six-patient cohort experienced DLT, escalation ceased. Accrual then continued at the previous dose level forming a six-patient cohort, which was declared the MTD, provided no more than one patient experienced DLT.

The starting dose was 100 mg/m^2 bid for the 7-day schedule, which represented one third of the highest tolerable dose in the rat, the most sensitive species. Once the feasibility and MTD of the 7-day schedule had been established, the 14-day schedule was explored in the same fashion. The starting dose for the second part of the study was to be less than half the daily dose at the formerly established MTD and would depend on the pharmacokinetic and global safety profile observed with the 7-day schedule.

Drug Administration. Ro 31-7453 was supplied as 50-, 100-, and 200-mg capsules, packaged in bottles. For the first cycle only, on pharmacokinetic sampling days subjects fasted for at least 8 h and were then offered a light breakfast before dosing. On other treatment days, fasting was not required. Patients were asked to return all unused capsules and bottles to monitor compliance. No standard antiemetic treatment or prophylaxis was specified.

Treatment Assessment. Within 28 days of the planned start of study treatment, patients had an electrocardiogram, chest X-ray, and computed tomography tumor measurements. Within 7 days of planned treatment start, blood was drawn for a complete blood cell count including WBC differential and blood chemistry (including urea, bilirubin, aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase, total protein, serum albumin, serum creatinine, uric acid, glucose, sodium, potassium, chloride, calcium, and inorganic phosphate); urinalysis was also undertaken. Weekly evaluations included medical history, physical examination, toxicity assessment, and complete blood cell count. Blood chemistry was performed before starting each treatment cycle and on weeks 2 and 3 of the cycle, with urinalysis every month. Tumor evaluation was performed at baseline and after every two treatment cycles according to the WHO response criteria.

Sample Collection for Pharmacokinetic Analysis. Full pharmacokinetic sampling was performed on the first and last days of dosing during cycle 1. Blood samples (7 ml) were collected into Vacutainer tubes containing EDTA at 0 (predose), 1.5, 4, 8, 12, and 24 h after the first dose and then after the last dose of the first cycle (day 8 in the 7-day schedule or day 15 in the 14-day schedule). In addition, on day 8 of the 14-day dosing schedule, a single blood sample was taken before the morning dose to determine trough concentrations. Limited samples were also collected after the morning dose on the first and last day of dosing during cycle 2. For the purposes of pharmacokinetic sampling, only the morning dose was given on the first day of cycle 1, and treatment was extended to give a final morning dose so that the total dose administered was the same as on subse-

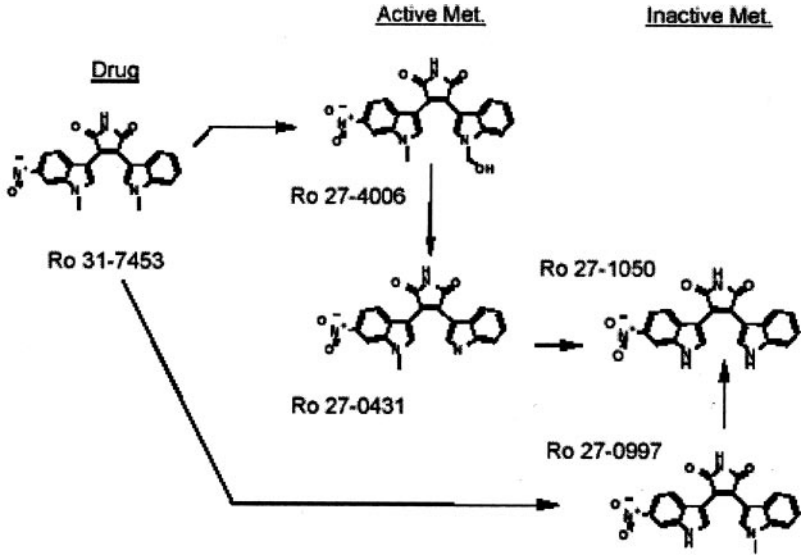

Fig. 1 Metabolic pathways for Ro 31-7453.

quent cycles. Pharmacokinetic blood sampling was discontinued in patients who vomited within 2 h of their morning dose of Ro 31-7453.

Immediately after sampling, tubes were placed on ice and then centrifuged at 2500 rpm for 15 min at 4°C as soon as possible (within 30 min of collection). After centrifugation, the plasma was transferred immediately into polypropylene tubes and frozen at −70°C. Blood samples were collected in an identical manner during the second cycle at 0 (predose) and 4 h after the morning dose on the first and last days of dosing.

A predose urine sample was taken on day 1 only. Additional urine samples were collected into polyethylene containers and then refrigerated 0–4, 4–12, and 12–24 h after drug administration on the first and last days of cycle 1. The total urine volume, pH, and temperature were measured for each time interval, and a 15-ml aliquot was transferred into a polypropylene tube and stored immediately at −70°C.

Pharmacokinetic Analyses. The liquid chromatography/mass spectrometry/mass spectrometry (LC/MS/MS) method for determination of Ro 31-7453 and its four metabolites, Ro 27-0431, Ro 27-0997, Ro 27-1050, and Ro 274006, in a single sample was validated over the concentration range 0.2 (the limit of quantitation)–200 ng/ml for all analytes. After addition of an internal standard (isotopically labeled Ro 31-7453-$^{13}C_6$), the analytes were isolated from EDTA human plasma by liquid-liquid extraction into an ethyl acetate/isopropyl alcohol mixture, concentrated, separated by high-performance liquid chromatography, and measured by positive ion TurboIon Spay MS/MS. The precision (average within-day and between-day percentage coefficient of variation for quality assurance samples) for Ro 31-7453, Ro 27-0431, Ro 27-0997, Ro 27-1050, and Ro 27-4006 was 3.00%, 3.35%, 3.75%, 4.70%, and 8.12%, respectively. The accuracy (percentage of bias) ranged from −1.46% to 0.95%, −2.10% to 2.42%, −2.32% to 2.09%, −2.93% to 2.40%, and −1.64% to 2.77% for Ro 31-7453, Ro 27-0431, Ro 27-0997, Ro 27-1050, and Ro 27-4006, respectively. Structures for the four metabolites are presented in Fig. 1.

Pharmacokinetic Data Analysis. A noncompartmental pharmacokinetic analysis of the plasma samples was performed using the WinNonLin pharmacokinetic program (Version 2.1; Pharsight; 2001). The following pharmacokinetic parameters of

Table 1 Patient characteristics

Characteristic	No. of patients	
	7-day schedule	14-day schedule
Patients entered	14	23
Patients assessable for toxicity	14	23
Age (yrs)		
Median	63.5	63.0
Range	40–72	41–77
Sex		
Female	4	4
Male	10	19
Karnofsky performance status		
Median	80	80
Range	60–100	60–100
Tumor type		
Colorectal CA[a]	6	10
Lung CA	2	1
Breast CA	0	1
Head and neck CA	0	2
Prostate CA	2	0
Renal cell CA	0	2
Melanoma	1	0
Mesothelioma	0	1
Carcinoma unknown to site	2	1
Other	1	5
Previous therapy		
Chemotherapy	14	23
No. of chemotherapy regimens		
0	0	0
1	6	11
2	3	8
3	3	2
>3	2	2
Radiotherapy and chemotherapy	6	6

[a] CA, cancer.

Ro 31-7453 and the four metabolites were estimated: empirical time of peak plasma level t_{max}; empirical peak plasma level (C_{max}); and apparent elimination rate constant (λ_z) calculated by linear regression of the terminal phase of the semilogarithmic plasma level curve, when this was clearly defined. The elimination half-life ($t_{1/2}$) was defined as $\ln 2/\lambda_z$. The area under the concentration-time curve (AUC) was estimated by the linear-log trapezoidal rule. Pharmacokinetic parameters obtained on the first day of cycle 1 at different dose levels were compared to assess dose proportionality. Similarly, pharmacokinetic parameters on the first and last day of the first cycle of treatment were compared to assess the potential of time-dependent effects on kinetics, such as drug metabolism, accumulation, or other changes in drug disposition. The amount of unchanged drug excreted in urine was expressed as a percentage of the administered dose. Pharmacokinetic data were reported as mean ± SD.

Statistical Methods. Clinical and pharmacokinetic data were summarized using descriptive statistics. For dose-AUC relationship plots and pharmacodynamic studies, each of the three active components (Ro 31-7453 and its two major metabolites) was analyzed. The sum of the pharmacokinetic parameters of the three components was also analyzed, given their equal antiproliferative activity in preclinical models.

RESULTS

Patients, Dose Escalation, and Safety. Thirty-seven patients entered the study (14 on the 7-day schedule and 23 on the 14-day schedule); their characteristics are listed in Table 1. All were eligible and assessable for both toxicity and antitumor activity. Median age was 63 years (range, 40–77 years), and median Karnofsky performance status was 80 (range, 60–100); 29 patients were male, and 8 were female. All had received prior chemotherapy (median number of regimens = 2); six patients had also received prior radiotherapy. Colorectal cancer was the most common tumor type.

Dose levels of 100, 200, 240, and 280 mg/m^2 bid and 70, 100, 125, and 150 mg/m^2 bid were explored with the 7- and 14-day schedules, respectively. A total of 110 cycles were administered. The median number of cycles received was 3 (range, 1–7 cycles), with six patients completing ≥6 treatment cycles. Four patients (29%) and three patients (13%) were withdrawn for treatment-related adverse events with the 7- and 14-day schedules, respectively. The MTD, defined as the highest dose at which not more than one of a six-patient cohort experienced DLT, was 200 mg/m^2 bid for the 7-day schedule and 125 mg/m^2 bid for the 14-day schedule, repeated every 4 weeks.

The 7-day treatment schedule used the initial accelerated dose escalation phase with a single patient for the 100 and 200 mg/m^2 dose levels. Grade 2 nausea and vomiting were observed in the second patient during cycle 2, so the next patient received Ro 31-7453 at a dose of 280 mg/m^2 bid. This patient experienced a DLT (prolonged grade 4 neutropenia) and was dose-reduced in subsequent cycles to 200 mg/m^2 bid, at which an additional five cycles were administered. Three patients were then treated with Ro 31-7453 (200 mg/m^2 bid) without DLT. An intermediate dose level of 240 mg/m^2 bid was next evaluated, at which two of six patients experienced DLT, rendering this also above the MTD. Of these two patients, one had grade 3 vomiting, skin rash on a lymphedematous region, and grade 4 mucositis; the other had grade 4 neutropenia and mucositis. The MTD was, therefore, 200 mg/m^2 bid for 7 days; this dose level was extended to six patients with only a single DLT (grade 4 diarrhea).

With the 14-day treatment schedule, four patients experienced DLTs. There were no DLTs with Ro 31-7453 (70 mg/m^2

Table 2 Description of most frequently reported drug-related adverse events

Adverse events with a reported rate of >10%. Multiple occurrences of the same adverse event in one individual counted only once, but some patients suffered a number of diverse adverse events. Data represent *N* (%).

	Ro 31-7453 (q[a] 12 h × 7 days; *N* = 14)		Ro 31-7453 (q 12 h × 14 days; *N* = 23)	
Disorder	AE	SAE	AE	SAE
Blood disorders and infectious complications				
Neutropenia	3 (21)	3 (21)	3 (13)	3 (13)
Anemia			3 (13)	
Febrile neutropenia	1 (7)	1 (7)	1 (4)	1 (4)
Thrombocytopenia			2 (9)	1 (4)
Pancytopenia			1 (4)	1 (4)
Neutropenic sepsis			2 (9)	2 (9)
Gastrointestinal disorders				
Nausea/vomiting	8 (57)	2 (14)	10 (43)	1 (4)
Diarrhea	4 (29)		7 (30)	1 (4)
Stomatitis	2 (14)		5 (22)	
Mucosal inflammation	3 (21)	1 (7)	1 (4)	
Abdominal pain	2 (14)		4 (27)	
Other disorders				
Fatigue/flu-like symptoms	8 (57)		10 (43)	
Anorexia	2 (14)		5 (22)	
Alopecia	3 (21)		2 (9)	
Dermatitis	3 (21)	1 (7)		

[a] q, every; AE, adverse event; SAE, serious adverse event.

Table 3 National Cancer Institute Common Toxicity Criteria grade worst hematological toxicity and dose-limiting toxicities of oral Ro 31-7453 (all cycles)

Dose (mg/day)	No. of patients/ no. of cycles	Neutropenia		Thrombocytopenia		Anemia	
		Grade 3	Grade 4	Grade 3	Grade 4	Grade 1–2	Grade 3–4
7-Day schedule							
100	1/2						
200	7[a]/19						
240	6/13		3				
280	1/1		1[b]				
14-Day schedule							
70	3/13						
100	8/25		1				1
125	6/18	1[b]					
150	6/14		4	1	1	1	1

[a] Including dose level after dose reduction.
[b] Febrile neutropenia.

bid) given for 14 days, but one of six patients at the 100 mg/m^2 bid dose level had prolonged grade 4 neutropenia. This cohort was expanded beyond the first six patients due to thrombocytopenia, subsequently judged spurious, in another patient. Two more patients were added with no DLTs. Therefore, the next cohort was opened at 125 mg/m^2 bid, at which one of the six patients experienced febrile neutropenia, thrombocytopenia, nausea, emesis, and diarrhea, all of which were grade 3 and dose-limiting. This patient, who had breast cancer and liver metastases, subsequently suffered a fatal intracranial hemorrhage. A previously undetected abnormality, probably a brain metastasis into which there had been hemorrhage, was identified on computed tomography. Finally, at the 150 mg/m^2 dose level, two of six patients had febrile neutropenia, so this was declared above the MTD of 125 mg/m^2 bid for 14 days.

All these DLTs occurred during the first cycle of therapy so they were the basis for decisions on dose escalation. A safety profile showing frequent (≥10%) treatment-related adverse events over all administered cycles is summarized in Table 2. The incidence and severity of toxic events were evenly distributed between the two schedules. Eight patients receiving each schedule experienced treatment-related grade 3/4 adverse events. Serious adverse events occurred in four (29%) and six (26%) of the patients in the 7- and 14-day schedule, respectively. Worst all-cycle hematological and nonhematological toxicities by dose level are summarized in Tables 3 and 4, respectively. The majority of serious adverse events were hematological. Eleven and three patients, respectively, required packed RBC and platelet transfusions. Alopecia was observed in only five patients (grade 1, three patients; grade 2, two patients). Other sporadic adverse events with a possible relation to study medication included skin rash, paresthesia, cramps, pain, weakness and stiffness of muscles in limbs, taste disturbance, and dizziness. Importantly, toxicity at the recommended dose level for the two schedules was easily manageable.

Antitumor Activity. One patient with non-small cell lung cancer who had received two previous lines of treatment (one of which was cisplatin-based therapy for 3 months as first-line chemotherapy) treated with 240 mg/m^2 bid Ro31-7453 for 7 days achieved a partial response after the first cycle of treatment; this response was maintained for was 6 months. Seven other patients had disease stabilization that lasted >16 weeks, including one who remained on study for seven treatment cycles.

Pharmacokinetics and Pharmacodynamics. Ro 31-7453 was well absorbed, with peak plasma concentrations reached approximately 4 h after oral dosing and a $t_{1/2}$ of approximately 9 h. Parent drug was extensively metabolized to two major metabolites (Fig. 1), Ro 27-4006 and 27-043, the concentrations of which peaked around 5 h after oral dosing (Fig. 2). Both of

Table 4 Worst National Cancer Institute Common Toxicity Criteria grade nonhematological toxicity (all cycles)

Dose (mg/day)	No. of patients/ no. of cycles	Vomiting/nausea		Mucositis/stomatitis		Diarrhea			Fatigue	
		Grade 2	Grade 3	Grade 3	Grade 4	Grade 2	Grade 3	Grade 4	Grade 2	Grade 3
7-Day schedule										
100	1/2									
200	7[a]/19	1					1	1	2	
240	6/13	1	3	1	1				1	
280	1/6	1								1
14-Day schedule										
70	3/13								1	
100	8/25		1	1		1			1	1
125	6/18	1	1			1			2	
150	6/14	2	1	2		2			1	

[a] Seven patients after dose reduction.

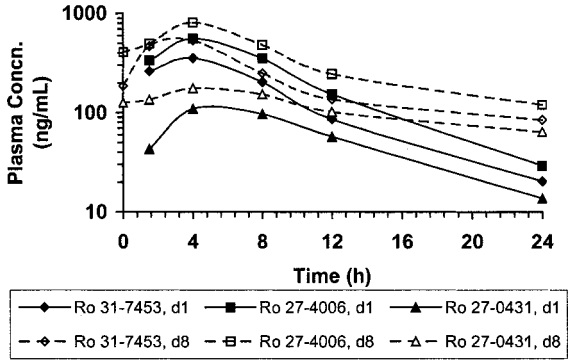

Fig. 2 Semilogarithmic plots of mean plasma concentrations *versus* time for Ro 31-7453 (*diamonds*), Ro 27-4006 (*squares*), and Ro 27-0431 (*triangles*) after Ro 31-7453 dosing on days 1 and 7 in six patients treated with 200 mg/m^2 twice daily in the 7-day schedule. *Solid lines with filled symbols* are profiles of the first-day treatment, and *dashed lines with open symbols* are those of the last-day treatment.

these metabolites have equal activity, as determined by antiproliferative assays (6), and comparable systemic exposure to the parent compound. Three additional inactive metabolites, Ro 27-0997, Ro 27-1050, and Ro 28-0351 were also identified. Ro 28-0351 was not measured due to lack of an available validated assay. Exposure to the other two inactive metabolites was minimal, with AUCs for each <5% of that for the active metabolites across all dose levels (data not shown). Less than 3% of the administered dose was recovered in urine among a limited patient sample. Individual pharmacokinetic parameters of the parent compound and major metabolites are summarized in Tables 5 and 6 for the 7- and 14-day schedules, respectively.

Although data were limited, when the three active species were combined to form an index of systemic exposure (total AUC), a relationship between dose and total exposure was suggested (Fig. 3). In addition, C_{max} appeared proportional to dose over the dose range studied in the two schedules, with clearance, t_{max}, and $t_{1/2}$ being independent of dose. There was minimal change, except for the accumulation expected with twice daily dosing of a drug with a $t_{1/2}$ of 9 h after 7 or 14 days of daily treatment (Fig. 2), suggesting no induction of metabolizing enzymes. Comparison of peak and trough data for cycles 1 and 2 showed no apparent cycle dependency (Table 7). A pharmacokinetic-pharmacodynamic relationship was observed between cycle 1 exposure (daily total AUC of three active species, mg × h/ml) and percentage of inhibition of neutrophils and platelets. However, interpatient variability was large, as observed in the related Fig. 4, *A* and *B*.

DISCUSSION

This study has confirmed the tolerability, bioavailability, and predictable pharmacokinetics of two oral schedules of Ro 31-7453 given twice daily for 7 or 14 days, repeated every 4 weeks. In common with other phase-specific cytotoxic agents, Ro 31-7453 showed more pronounced antitumor activity with protracted exposure in experimental models (5, 7). Prolonged exposure, either by continuous infusion or oral administration (10), also avoids the possibility of excessive toxicity from high peak drug concentrations and may increase the therapeutic index. Oral treatment is clearly preferred by patients (11), and

Table 5 Summary (mean ± SD) of pharmacokinetic parameters of Ro 31-7453 and two major metabolites, Ro 27-4006 and Ro 27-0431 (7-day regimen)

	100 mg/m^2 (N = 1)		200 mg/m^2 (N = 6)		240 mg/m^2 (N = 6)		280 mg/m^2 (N = 1)	
	Day 1	Day 8	Day 1 (N = 5)[a]	Day 8 (N = 5)[b]	Day 1	Day 8 (N = 4)[c]	Day 1	Day 8
C_{max} (ng/ml)								
Ro 31-7453	86.7	98.6	416 ± 176	608 ± 265	715 ± 423	841 ± 498	658	1650
Ro 27-4006	186	172	575 ± 107	800 ± 205	724 ± 352	994 ± 201	1230	1340
Ro 27-0431	39.4	48.9	293 ± 335	200 ± 61	115 ± 48	290 ± 180	180	529
t_{max} (h)								
Ro 31-7453	4	4	3.5 ± 1.1	3 ± 1	5 ± 4	4 ± 3	4	1.5
Ro 27-4006	4	4	4.3 ± 2.3	3 ± 1	6 ± 4	5 ± 2	4	1.5
Ro 27-0431	4	0	8.8 ± 8.7	8 ± 9	10 ± 8	5 ± 4	4	4
$t_{1/2}$ (h)								
Ro 31-7453	4.59	16.9	7.7 ± 5.7	7.1 ± 2.7	10.0 ± 7.8	6.1 ± 1.4	7.39	7.83
Ro 27-4006	4.00	21.7	6.7 ± 4.5	9.5 ± 10.6	13.9 ± 18.6	6.8 ± 2.2	6.86	8.42
Ro 27-0431	4.97	25.8	5.6 ± 1.0	10.8 ± 10.9	9.5 ± 5.7	7.5 ± 1.6	10.00	14.64
AUC[d] (ng × h/ml)								
Ro 31-7453	1021	703.5	6082 ± 6071	4194 ± 1520	6456 ± 2769	8830 ± 6035	5606	
Ro 27-4006	1934	1310	7093 ± 3579	6474 ± 2254	10504 ± 4407	12123 ± 5002	10668	
Ro 27-0431	509.8	398	1488 ± 564	2019 ± 625	1857 ± 1077	4208 ± 2903	2434	
C_{trough}[e] (ng/ml)								
Ro 31-7453		54.2		145 ± 72		361 ± 282		786
Ro 27-4006		96.5		325 ± 236		549 ± 294		721
Ro 27-0431		37.4		296 ± 283		209 ± 152		433

[a] N = 5, due to lack of pharmacokinetics data on day 1 data in patient 12.
[b] N = 5, due to lack of pharmacokinetics data on day 8 in patient 11.
[c] N = 4, due to abnormality of pharmacokinetics data in patient 10, who did not take the morning dose on day 8.
[d] AUC, area under the concentration-time curve. $AUC_{0-\infty}$ for day 1 and AUC_{0-12} for day 8.
[e] Average of two measures.

Table 6 Summary of pharmacokinetic parameters of Ro 31-7453 and two major metabolites, Ro 27-4006 and Ro 27-0431 (14-day regimen)

	70 mg/m^2 (N = 3)		100 mg/m^2 (N = 8)		125 mg/m^2 (N = 6)		150 mg/m^2 (N = 6)	
	Day 1	Day 15 (N = 2)[a]	Day 1 (N = 5)[b]	Day 15	Day 1[c]	Day 15	Day 1	Day 15
C_{max} (ng/ml)								
Ro 31-7453	181 ± 59	376 ± 115	359 ± 345	476 ± 104	366 ± 115	593 ± 113	316 ± 283	364 ± 214
Ro 27-4006	272 ± 108	486 ± 63	485 ± 333	748 ± 128	684 ± 304	931 ± 330	588 ± 329	763 ± 392
Ro 27-0431	81 ± 44	116 ± 46	73 ± 51.6	209 ± 42	109 ± 31	219 ± 64	74 ± 17	122 ± 22
t_{max} (h)								
Ro 31-7453	3.2 ± 1.4	4 ± 0	3.8 ± 2.6	3.58 ± 1.02	8 ± 8.9	4.8 ± 1.79	4 ± 0	4 ± 0
Ro 27-4006	4 ± 0	4 ± 0	3.8 ± 2.6	4 ± 0	8.8 ± 8.7	4 ± 0	4 ± 0	4 ± 0
Ro 27-0431	4 ± 0	4 ± 0	5.1 ± 2.8	4 ± 0	8 ± 8.9	4.8 ± 1.79	4 ± 0	6 ± 2.8
$t_{1/2}$ (h)								
Ro 31-7453	5.8 ± 2.0	6.2 ± 2.8	6.62 ± 1.37	5.78 ± 1.46	8.2 ± 2.8	7.4 ± 2.9	4.19 ± 0.44	4.9 ± 0.6
Ro 27-4006	5.0 ± 1.7	5.7 ± 2.4	6.04 ± 1.23	6.2 ± 1.35	6.4 ± 2.2	6.4 ± 2.8	3.38 ± 0.03	4.89 ± 0.01
Ro 27-0431	5.8 ± 1.6	6.0 ± 1.0	8.48 ± 3.35	8.58 ± 1.39	8.4 ± 4.2	8.7 ± 5.1	4.5 ± 0.01	6.9 ± 1.02
AUC[d] (ng × h/ml)								
Ro 31-7453	1411 ± 675	2432 ± 630	2064 ± 1220	3583 ± 968	2858 ± 1162	4068 ± 1298	1864 ± 916	1990 ± 282
Ro 27-4006	2381 ± 747	3815 ± 1294	3662 ± 1389	6200 ± 1160	7351 ± 4134	6605 ± 1993	4274 ± 1372	5634 ± 2136
Ro 27-0431	789 ± 395	1094 ± 139	883 ± 315	2076 ± 512	1377 ± 266	1937 ± 815	792 ± 158	1194 ± 291
C_{trough}[e] (ng/ml)								
Ro 31-7453		108 ± 68		162 ± 45		176 ± 127		51 ± 45
Ro 27-4006		122 ± 8		292 ± 55		301 ± 137		298.5 ± 64.3
Ro 27-0431		55 ± 18		137 ± 50		121 ± 76		88 ± 25

[a] N = 2 due to noncompliance of patient 10 on day 15 (no drug was taken on the morning of day 15).
[b] N = 5 due to the lack of pharmacokinetic data on day 8 for patient 11.
[c] Day 1 $t_{1/2}$ and AUC for patient 17 cannot be assessed.
[d] AUC, area under the concentration-time curve. $AUC_{0-\infty}$ for day 1 and AUC_{0-12} for day 15.
[e] Average of two measures.

animal data using a novel oral sustained release microprecipitate formulation of Ro 31-7453 (8) supported exploration of this route of administration in humans.

Oral administration of Ro 31-7453 is feasible in adults with solid tumors; bone marrow depression and mucositis are dose-limiting, as would be expected for an antiproliferative agent. Other side effects, which were quite frequent but generally mild, included fatigue and gastrointestinal disorders such as nausea, vomiting, and diarrhea; alopecia was uncommon and seen in only 5 of 37 patients. The number of patients requiring packed RBC and platelet transfusions [11 (30%) and 3 (8%), respectively] reflects the hematological toxicity of Ro 31-7453 as predicted from preclinical toxicology (5). The recommended doses for further testing the 7- and 14-day schedules are 200 mg/m^2 bid and 125 mg/m^2 bid, respectively. The patient population was heavily pretreated, but one patient with pretreated lung cancer had a durable partial response, and seven more patients achieved disease stabilization for more than four treatment cycles.

In the absence of major differences in the pattern of toxicity, the 125 mg/m^2 bid for 14 days schedule was selected for further development as monotherapy due to the more prolonged exposure to Ro 31-7453 that showed optimal antitumor activity in preclinical models (5, 7, 8). A parallel Phase I study with a 4-day every 3 weeks schedule was also shown to be feasible with a comparable safety profile; again, myelosuppression and stomatitis were the DLTs. The MTD with this regimen was defined as 560 mg/m^2 when the total daily dose was taken orally as a single dose or 340 mg/m^2 bid when the daily dose was split every 12 h (12). Thus, the 4-day schedule yields a significantly lower dose intensity than our more prolonged administration, but its administration interval renders it appropriate for combination therapy.

Limited data suggest that Ro 31-7453 and its two major metabolites (Ro 27-4006 and Ro 31-7453) have dose-proportional pharmacokinetics in relation to C_{trough} and AUC. This is further supported by analysis of pooled pharmacokinetic data from 62 patients included in a concurrent Phase I study using daily × 4 and bid × 4 day schedules (12, 13). However, interpatient pharmacokinetic variability is high. Exposure to the three inactive metabolites was minimal. Thus, AUC and trough concentrations of the parent drug and its active metabolites were

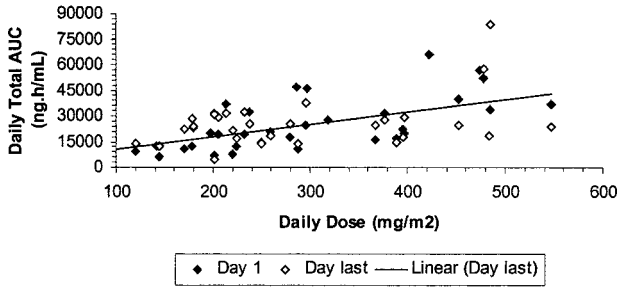

Fig. 3 Scatter plots depicting total area under the concentration-time curve (AUC) values of an index of systemic exposure (total AUC) formed by the combined AUCs of the three active species Ro 31-7453, Ro 27-4006, and Ro 27-0431 as a function of dose. *Filled diamonds* are day 1 AUC (0–∞), and *open diamonds* are day 8 AUC. *Lines* represent linear trends.

Table 7 Mean peak and trough plasma concentrations of cycles 1 and 2

	100 mg/m^2 q12 h (n = 3)[a]			125 mg/m^2 q12 h (n = 5)[b]		
	Cycle 1	Cycle 2	Ratio[c]	Cycle 1	Cycle 2	Ratio[c]
C_{peak}						
Day 1						
Ro 31-7453	242 ± 18	256 ± 11	1.03	341 ± 116	384 ± 163	1.33 ± 0.69
Ro 27-4006	542 ± 79	520 ± 141	1.04	788 ± 286	697 ± 292	0.99 ± 0.25
Ro 27-0431	106 ± 34	75 ± 11	0.64	115 ± 32	104 ± 45	0.95 ± 0.42
Total	890 ± 131	850 ± 162	0.98	1243 ± 403	1185 ± 466	1.07 ± 0.32
Day 14						
Ro 31-7453	443 ± 100	673 ± 101	1.39 ± 0.40	586 ± 115	501 ± 121	0.85 ± 0.19
Ro 27-4006	811 ± 159	1155 ± 78	1.29 ± 0.02	931 ± 330	909 ± 254	0.93 ± 0.13
Ro 27-0431	232 ± 45	353 ± 30	1.52 ± 0.28	209 ± 53	182 ± 37	0.93 ± 0.21
Total	1486 ± 287	2181 ± 6.4	1.35 ± 0.16	1725 ± 456	1591 ± 367	0.90 ± 0.15
C_{trough}						
Day 14						
Ro 31-7453	172 ± 87	220 ± 20	1.00 ± 0.20	209 ± 151	170 ± 61	1.19 ± 0.12
Ro 27-4006	348 ± 137	503 ± 10	1.20 ± 0.15	358 ± 159	402 ± 223	1.27 ± 0.38
Ro 27-0431	175 ± 68	223 ± 15.6	1.08 ± 0.15	136 ± 83	114 ± 33	1.81 ± 0.30
Total	696 ± 276	946 ± 14	1.11 ± 0.03	703 ± 359	686 ± 289	1.24 ± 0.28

[a] Cycle 1 day 1 peak levels are not available for patient 15, cycle 2 day 1 peaks are not available for patient 110. Cycle 2 day 4 peak levels are not available for patient 109. Cycle 2 trough levels are not available for patient 109.

[b] Cycle 1 day 1 peaks are not available for patient 15. Cycle 2 day 14 peaks are not available for patient 112. Cycle 2 trough levels are not available for patient 112.

[c] Ratio = cycle 2 level/cycle 1 level.

summed to represent the total effective exposure. Systemic exposure, in terms of total amount of the parent drug and two active metabolites, was within the range that demonstrated efficacy in preclinical models. Urinary excretion was low, indicating that elimination is mainly through metabolism, which involves formation of at least two active species as demonstrated in this study. With regard to pharmacokinetic-pharmacodynamic relationships, there appeared to be a correlation between exposure (daily total AUC of three active species, mg × h/ml) and the percentage of inhibition from baseline of neutrophils and platelets. However, due to large interpatient variabilities, evidenced by large coefficients of variation, this needs to be interpreted with caution. Finally, compared with other antimitotic agents in the preclinical setting, Ro 31-7453 has a wide range of *in vivo* antitumor activity (4) and is active *in vitro* against multidrug-resistant models (1). This study has demonstrated the feasibility of two oral schedules of Ro 31-7453 with an acceptable safety profile. In addition, the current available pharmacokinetic data compare favorably with the nonlinear kinetics of *Vinca* alkaloids (14, 15) and paclitaxel (16). The 14-day schedule of oral Ro 31-7453 at the recommended dose of 125 mg/m^2 bid is being evaluated in Phase II trials in patients with breast, lung, and colorectal cancer. Phase I trials of Ro 31-7453 given on a 4-day bid schedule (12) in combination with gemcitabine (17) and paclitaxel (18) are also under way.

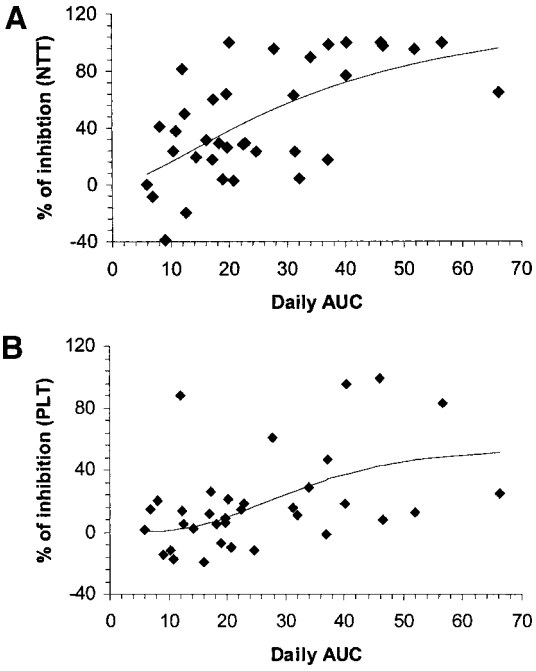

Fig. 4 Pharmacodynamic relationship between mean daily total AUC of three active species, Ro 31-7453, Ro 27-4006, and Ro 27-0431 (mg × h/ml), after first oral administration at the four different dose levels and percentage of inhibition of neutrophils (*A*) and platelets (*B*).

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