Featured Article

A Phase I Study of SR-4554 via Intravenous Administration for Noninvasive Investigation of Tumor Hypoxia by Magnetic Resonance Spectroscopy in Patients with Malignancy

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Abstract

Purpose: **To perform a Phase I study of SR-4554, a fluorinated 2-nitroimidazole noninvasive probe of tumor hypoxia detected by ^{19}F magnetic resonance spectroscopy (MRS).**

Experimental Design: **SR-4554 administration, on days 1 and 8, was followed by plasma sampling for pharmacokinetic studies and by three MRS studies performed over 24 h on days 8 and 9. Unlocalized MR spectra were acquired from tumor (10- or 16-cm dual resonant ^{1}H/^{19}F surface coil; 1.5 T Siemens Vision MR system; 2048 transients acquired over 34 min; 1.28-ms adiabatic pulse; repetition time, 1 s). Plasma drug concentrations were measured with a validated high-performance liquid chromatography method. Noncompartmental pharmacokinetic analysis was performed.**

Results: **Eight patients underwent pharmacokinetic studies, receiving doses of SR-4554 of 400-1600 mg/m^2. Peak plasma concentrations increased linearly with the SR-4554 dose ($r^2 = 0.80$; $P = 0.0002$). The plasma elimination half-life was relatively short (mean ± SD, 3.28 ± 0.59 h), and plasma clearance was quite rapid (mean ± SD, 12.8 ± 3.3 liters/h). Urinary recovery was generally high. SR-4554 was well tolerated. A single patient experienced dose-limiting toxicity (nausea and vomiting) at 1600 mg/m^2. The maximum tolerated dose was 1400 mg/m^2. SR-4554 was detected spectroscopically in tumors immediately after infusion at doses of 400-1600 mg/m^2. At the highest dose (1600 mg/m^2), SR-4554 was detectable in tumor at 8 h, but not at 27 h.**

Conclusions: **SR-4554 has plasma pharmacokinetic and toxicity profiles suitable for use as a hypoxia probe. It can be detected in tumors by unlocalized MRS. Additional clinical studies are warranted.**

Introduction

SR-4554 is a new 2-nitroimidazole agent that has been designed specifically as a noninvasive probe of tumor hypoxia detected by ^{19}F MRS[3] (Fig. 1). We describe a Phase I dose-escalation study of SR-4554 administered by i.v. infusion, assessing the plasma pharmacokinetic and toxicity profiles of SR-4554 and performing preliminary MRS studies to detect SR-4554 in tumors.

Nitroimidazoles have been used extensively as radiosensitizers, usually given daily before each fraction of a course of radiotherapy (1, 2). More recently, a new role for nitroimidazoles has emerged, as markers of tumor hypoxia (3). This function is by virtue of the selective bioreduction of nitroimidazoles under hypoxic conditions by intracellular nitroreductases to reactive metabolites, which are then covalently bound within the hypoxic cells. The detection of these metabolites thus indicates the presence of cellular hypoxia. The use of nitroimidazoles as hypoxia markers requires only a single administration of the agent. Cumulative doses are, therefore, considerably lower than those required for radiosensitization over a course of radiotherapy, reducing the risks of side effects encountered with multiple dose schedules. Pimonidazole (4–7) and EF5 (8, 9) are currently undergoing clinical evaluation as hypoxia markers detected by immunohistochemistry in biopsy specimens.

SR-4554 is a new nitroimidazole that has been designed specifically to be used as a noninvasive probe of tumor hypoxia (10–16). Extensive preclinical validation studies have provided strong evidence of oxygen-dependent intracellular binding of SR-4554 (10, 13) and of oxygen-dependent retention in animal tumor models (12, 14, 15). As a diagnostic agent, a key feature is that SR-4554 should have minimal or manageable toxicity. However, because it is detected by MRS, sensitivity is also an

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[3] The abbreviations used are: MRS, magnetic resonance spectroscopy; MTD, maximum tolerated dose; MRI, magnetic resonance imaging; DLT, dose-limiting toxicity; HPLC-UV, high-performance liquid chromatography-UV; TR, time to repetition; AUC, area under the time-concentration curve; CV, coefficient of variation.

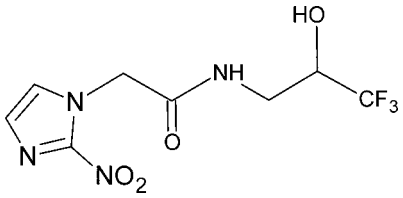

Fig. 1 Structure of SR-4554.

important consideration in terms of achieving maximum signal to noise ratio. This is directly dependent on the administered dose of the ^{19}F-containing agent. Thus, the use of SR-4554 in this context requires a balance between the need to give as high a dose as possible to optimize the ^{19}F signal acquired from tumor, with the need to avoid unacceptable toxicity. To our knowledge, there have been no previous studies in humans of ^{19}F MRS detection of fluorinated nitroimidazoles used specifically as hypoxia markers. However, experience with the cytotoxic agent 5-fluorouracil (which has one ^{19}F atom) indicates that it can be detected in tumors in humans by ^{19}F MRS after i.v. doses of 300-1000 mg/m^2 (equivalent to total doses of ~1200–2000 mg for an average-sized adult; 17–19) and oral doses of 50–100 mg (20). Thus, based on the previous clinical experience of related nitroimidazoles (21–26) and ^{19}F MRS studies of 5-fluorouracil (17–20), it was hypothesized that the use of SR-4554 (which has three ^{19}F atoms per molecule) administered as a single dose of a few grams could reasonably be expected to be detected in tumors by ^{19}F MRS and should also have an acceptable toxicity profile. On the basis of preclinical validation studies (12, 14–16) that supported the clinical development of SR-4554, a Phase I dose escalation study was planned and conducted. The overall aims of the study were: (*a*) to define the pharmacokinetic profile of SR-4554 in humans; (*b*) to document toxicity and define the MTD of SR-4554; (*c*) to perform preliminary MRS studies to determine whether SR-4554 can be detected in tumors; and (*d*) to develop a MRS scanning protocol that will allow calculation of a parameter of SR-4554 retention that would correlate with tumor hypoxia.

Patients and Methods

Study Design. SR-4554 was approved for clinical development by the Cancer Research UK New Agents Committee. The study was designed as a single-center Phase I dose escalation study. The protocol was reviewed and approved by the Cancer Research UK Internal Review Board and by the Royal Marsden Hospital Committees for Clinical Research and for Local Research Ethics. The primary end points of the study were: determination of the dose of SR-4554 required to produce ^{19}F signal of sufficient intensity to be reliably detected by localized MRS without causing toxicity unacceptable for a diagnostic procedure; and establishment of a MRS scanning protocol (with particular attention to timing of scans after i.v. administration of SR-4554) to produce a parameter of SR-4554 retention by tumor that can be used as a surrogate measure of tumor hypoxia.

Patients were treated in two cohorts. The first cohort underwent both pharmacokinetic and MRS studies, whereas the second cohort underwent pharmacokinetic studies alone. The study schedule for patients undergoing pharmacokinetic and MRS studies is shown in Fig. 2. A dynamic contrast-enhanced MRI scan was performed on day 1 before the SR-4554 infusion to provide current information on tumor site and size. SR-4554 was administered at the same dose on two separate occasions 1 week apart, because it was not practically possible simultaneously to perform MRS scanning and obtain all plasma samples required for pharmacokinetic studies. Plasma samples were taken after the first infusion on day 1 and analyzed before the second infusion on day 8. Up to three MRS scans were performed after the day 8 SR-4554 infusion, to gain time course information on the kinetics of the ^{19}F signal acquired from SR-4554 in the tumors of individual patients. The first scan was performed immediately after completion of the SR-4554 infusion. The plasma pharmacokinetic parameters were used to aid decisions on timing of the second and third scans. Urine was collected for 24 h after the day 1 SR-4554 infusion.

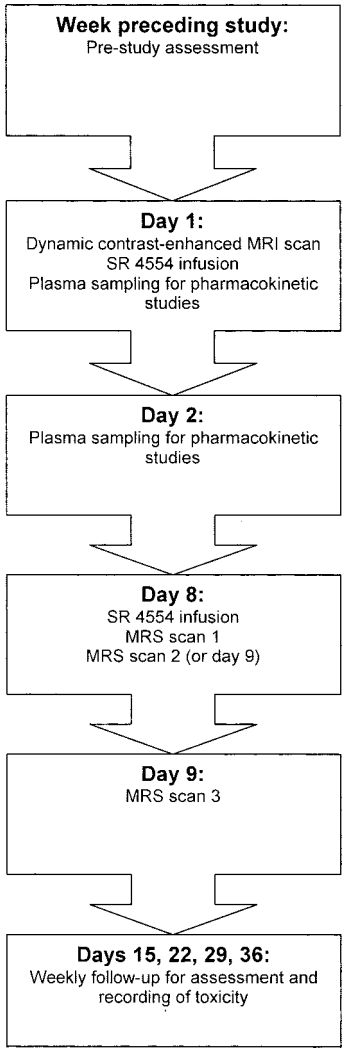

Fig. 2 Schedule of events for the Phase I clinical study of SR-4554.

Patient Eligibility. Inclusion criteria were: histologically proven solid malignancy that was stable or refractory, not cur-

rently on treatment (except for endocrine or cytokine therapies); age ≥18 years; life expectancy of at least 3 months; WHO performance status 0–2; patients receiving corticosteroids for symptom control, provided there has been no dose change during the 2 weeks before the study; hematological and biochemical indices within defined ranges (hemoglobin, ≥9 g/dl; white cell count, $>3.5 \times 10^9$/liter; neutrophils, $>1.5 \times 10^9$/liter; platelets, $\geq 100 \times 10^9$/liter; plasma creatinine, <130 μmol/liter; bilirubin, <30 μmol/liter; aspartate aminotransferase and alanine aminotransferase, ≤2.5 times the upper limit of normal); and ability to provide informed consent and to cooperate with treatment and follow-up. For patients undergoing MRS studies, the criteria were tumors of at least 3 cm in diameter and at a depth no greater than 4 cm.

Exclusion criteria were: metronidazole treatment; radiotherapy during the previous 4 weeks; chemotherapy during the previous 3 weeks; and pregnant or lactating women. For patients undergoing MRS studies, the criteria were implanted or prosthetic magnetic materials and cardiac pacemakers; and known allergy to gadolinium contrast agents.

SR-4554 Dosage and Dose Escalation. Preclinical toxicology studies of SR-4554 were conducted under the auspices of the Cancer Research UK New Agents Committee. The maximum dose administered in mice was 270 mg/kg, equivalent to 810 mg/m^2, limited by the solubility of SR-4554. Mild myelosuppression was the only adverse effect observed at this dose. A starting dose of ~50% of the maximum dose administered, 400 mg/m^2, was selected. For the present study, SR-4554 was administered as a single 30- to 60-min i.v. infusion. Patients also undergoing MRS studies were treated on a 2-week dosing schedule consisting of identical infusions on days 1 and 8. Patients were enrolled at a rate of one patient per dose level. The dose was doubled until dose-limiting grade 1 or 2 toxicity occurred. If one instance of DLT occurred, then three patients would be entered at the dose level below that at which DLT was encountered, which would be defined as MTD. However, when MTD was reached, the protocol was modified to explore an intermediate dose level between those of MTD and DLT, to maximize potential for ^{19}F signal acquisition from SR-4554 in tumor.

Toxicities were graded using the National Cancer Institute Common Toxicity Criteria, version 2.0. DLT was defined as any of the following: greater than grade 1 sensory neuropathy, motor neuropathy, neutropenia, thrombocytopenia, nonhematological toxicity; greater than grade 2 anemia; neutropenic fever; and drug-related death.

SR-4554 was supplied by SRI International. It was formulated by the Cancer Research UK Formulation Unit at the University of Strathclyde in 0.5-ml amber glass ampoules at a concentration of 200 mg/ml in 99% dimethyl sulfoxy methoxide and 1% Tween 80. These were stored out of light at 4°C. In preparation for i.v. administration, SR-4554 was diluted with 0.9% normal saline.

Pretreatment and Follow-Up Studies. Baseline evaluations were performed before SR-4554 administration. A chest X-ray and electrocardiogram were performed within 4 weeks of study commencement. A clinical examination was performed, and hematological and biochemical indices were evaluated within 1 week of study and also on study day 1. After SR-4554 administration, patients were reviewed weekly over 4 weeks for assessment of toxicity, clinical examination, and blood sampling for hematological and biochemical indices.

Table 1 Characteristics of patients entered into the Phase I study of SR-4554

Characteristics		No. of patients
Age (yr)		
Median	70	
Range	34–77	
Sex		
Male		7
Female		1
WHO performance status		
0		7
1		0
2		1
Tumor type		
Fibromatosis		1
Liposarcoma		1
Leiomyosarcoma		1
Adenocarcinoma of the prostate		4
Non-Hodgkin's lymphoma		1

Plasma Pharmacokinetics. Plasma samples were taken before SR-4554 infusion, at the end of infusion, and at 5, 10, 15, and 30 min and 1, 2, 4, 8, 12, and 24 h after SR-4554 infusion. In patients also undergoing MRS studies, plasma samples were taken according to the same schedule, but omitting samples scheduled to be taken when the patient was in the MR scanner. At each time point, a 5-ml aliquot of blood was collected into a heparinized tube and was centrifuged at $1200 \times g$ (or 3000 rpm) at 4°C for 10 min. The plasma was transferred into 2-ml Eppendorf tubes and stored at −20°C. Plasma samples were extracted and analyzed for the presence of parent SR-4554 by HPLC-UV by a method reported previously, adapted, and validated for human samples (15). Pharmacokinetic data were analyzed by noncompartmental analysis using Pharsight WinNonLin (version 3.0; Pharsight Corporation, Mountain View CA). Day 1 and day 8 pharmacokinetic parameters were compared using a paired Student's *t* test.

Urinary Excretion of SR-4554. Urine was collected for 24 h after the SR-4554 infusion. The total urine volume was measured, and a 5-ml aliquot was stored at −20°C. SR-4554 was extracted from urine samples and measured by HPLC-UV analysis. Duplicate standard curves were prepared by spiking with external SR-4554 at levels of 0.5, 1, 10, 50, 100, 150, and 200 μg/ml. Duplicate quality control samples were included at levels of 1.5, 80, and 180 μg/ml. Samples were prepared by spiking 250 μl of urine with 30 μl of internal standard (50 μg/ml) and adding 250 ml of 1% HCl and 1 ml of ethyl acetate. After centrifugation for 5 min at 5000 rpm, the organic phase was removed and dried and the residue was dissolved in 150 μl of methanol. Samples were then analyzed as for plasma.

MR Studies. Diagnostic dynamic contrast-enhanced MRI (proton density, longitudinal relaxation time- and total transverse relaxation time-weighted images) was performed on day 1, before the SR 4554 infusion.

MRS studies were performed using a 10- or 16-cm dual resonant ^{1}H/^{19}F surface coil and a 1.5 T MR system (Siemens

Table 2 Dose-escalation schedule and toxicity profile for the Phase I clinical study of SR-4554

Dose level (mg/m^2)	No. of patients	Total dose (mg)	No. of patients manifesting toxicity		
			Hematological toxicity	Neurological toxicity	Gastrointestinal toxicity
400	1	960	0	0	0
800	1	1440	0	0	0
1200	1	2520	0	0	0
1400	3	2520	0	0	0
		2660	0	0	0
		3080	0	0	0
1600	2	3200	0	0	0
		3200	0	0	1[a]

[a] Grade 2.

Vision, Erlangen, Germany). Unlocalized data were acquired from the sensitive region of the coil. To achieve uniform excitation over this sensitive region, a 1.28-ms adiabatic radiofrequency pulse was used. A reference bulb containing tetrafluorosuccinic acid, located within each surface coil, was used to check coil tuning. The 2048 transients at the ^{19}F frequency were acquired over 34 min using a pulse-acquire sequence with a TR of 1 s. Reference ^{1}H spectra were acquired from tissue water using the same coil and sequence to provide a water signal for use in subsequent SR-4554 concentration calculations, but with a TR of 5 s to avoid partial saturation effects. The frequency of SR-4554 was set relative to the ^{1}H reference spectra water signal scaled by a factor of 0.9408617.

After SR-4554 i.v. infusion, the position of the surface coil and the method of patient immobilization were noted, to enable reproducible set-up for subsequent MR studies. A second scan was performed at a time point at least two plasma half-lives ($t_{1/2}$) after the first scan. A third scan was performed the following day. If no ^{19}F signal was detected during a scan, then subsequent scans were omitted. Each MRS study took ~60–75 min.

Quantification of SR-4554 concentration in tumor was estimated using the ^{19}F and ^{1}H data described above, as:

$$[\text{SR-4554}] = \frac{^{19}\text{F peak area}}{^{1}\text{H peak area}} \times \frac{\text{n } ^{1}\text{H}}{\text{n } ^{19}\text{F}} \times (55 \times 10^{6}) \times 0.75 \times \text{GF} \times \text{SF}(^{19}\text{F}) \quad (1)$$

where [SR-4554] is the concentration of SR-4554 (μm), n ^{1}H is the number of ^{1}H atoms in H_2O, n ^{19}F is the number of ^{19}F atoms in SR-4554, 55×10^6 is molarity of water, 0.75 is the assumed water content of tissue, GF is the gain factor (dB) provided by the MR instrument, and SF is the ^{19}F saturation factor, calculated by:

$$\text{SF}(^{19}\text{F}) = \frac{1}{1 - e^{-(\text{TR}/\text{T}_1)}} \quad (2)$$

No saturation factor was required for ^{1}H, because data were acquired when ^{1}H was fully relaxed.

Results

Patient Characteristics. Eight patients were enrolled between December 2000 and December 2001. Characteristics of the eight patients who participated in the study are shown in Table 1. Seven patients had malignant disease that was stable, and one patient had locally aggressive fibromatosis. MRS studies were performed in three patients with superficially located soft tissue tumors. No patients were receiving cytotoxic treatment or radiotherapy at the time of study, or had done so in the 3 weeks (for cytotoxic treatment) or 4 weeks (radiotherapy) before study entry. The median age of patients was 70 (range, 34–77) years.

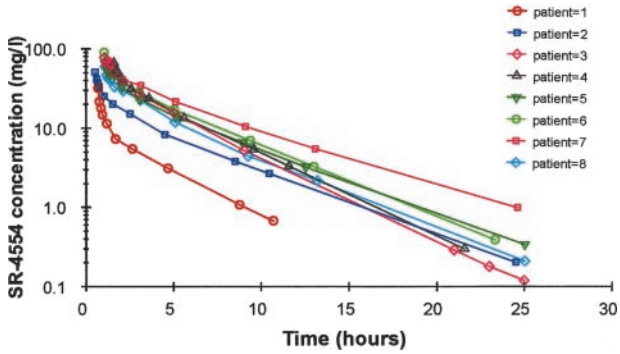

Fig. 3 Plasma concentration-time profiles in eight patients who were treated with SR-4554 at dose levels of 400 mg/m^2 (patient 1), 800 mg/m^2 (patient 2), 1200 mg/m^2 (patient 5), 1400 mg/m^2 (patients 6–8), and 1600 mg/m^2 (patients 3 and 4). Treatment and plasma sampling were performed on study day 1.

Dose-escalation Schedule and Toxicity. A summary of the dose-escalation schedule is shown in Table 2. Starting at 400 mg/m^2, one patient was treated per dose level up to 1600 mg/m^2. These three patients also underwent MRS studies. An additional patient was treated at 1600 mg/m^2. This patient experienced grade 2 gastrointestinal toxicity, manifesting as nausea and vomiting on two occasions requiring premature termination of the SR-4554 infusion, which was DLT. This defined the MTD as 800 mg/m^2. However, to optimize the sensitivity for detection of ^{19}F signal from tumor in MRS studies, a protocol amendment was approved, allowing exploration of dose levels between 800 and 1600 mg/m^2. Accordingly, one patient was treated at 1200 mg/m^2, and three patients at 1400 mg/m^2. In the absence of additional DLT, 1400 mg/m^2 were defined as the MTD. Other than the grade 2 gastrointestinal toxicity that defined DLT at

Table 3 The pharmacokinetic profile of SR-4554 in human plasma[a]

Dose (mg/m^2)	Day	C_{max}[b] (mg/liter)	T_{max} (h)	AUC_{last} (h·mg/liter)	$t_{1/2}$ (h)	V_{ss} (liter)	Cl (liter/h)
400	1	32.0	0.68	49.3	2.72	53.4	18.5
800	1	51.1	0.52	133.9	3.78	45.1	10.7
1200	1	66.7	1.08	226.5	3.79	47.9	11.0
1400	1	90.6	1.03	268.2	3.35	37.5	9.3
	1	63.8	1.25	309.7	4.58	47.1	8.4
	1	59.9	1.08	191.5	3.57	63.7	16.0
1600	1	67.0	1.58	227.0	2.88	50.8	13.8
	1	78.0	1.05	247.3	2.91	43.7	12.9
Mean ± SD					3.45 ± 0.62	48.6 ± 7.7	12.6 ± 3.4

[a] Plasma pharmacokinetic parameters in eight patients who underwent plasma sampling after SR-4554 infusion on day 1. SR-4554 concentrations were determined by HPLC-UV analysis. Pharmacokinetic data were analyzed by noncompartmental analysis.

[b] C_{max}, peak plasma concentration; T_{max}, time of peak plasma concentration; AUC_{last}, area under the concentration-time curve; $t_{1/2}$, half life; V_{ss}, volume of distribution; Cl, clearance.

1600 mg/m^2, no toxicity was encountered in any other patients. In particular, no neurological or hematological toxicity was encountered in any patients.

Pharmacokinetic Studies. All patients underwent plasma sampling for pharmacokinetic studies. Pharmacokinetic profiles and parameters of patients dosed on day 1 are shown in Fig. 3 and Table 3, respectively. Plasma time-concentration profiles indicated a rapid distribution phase (Fig. 3). SR-4554 seemed to be widely distributed, with a mean ± SD volume of distribution of 48.6 ± 7.7 l. This is larger than the vascular compartment, indicating extravascular distribution and/or metabolism. The timing of the peak plasma concentration (T_{max}) was at the end of the SR-4554 infusion (of 30 or 60 min duration). The peak plasma concentration (C_{max}) increased linearly with SR-4554 dose ($r^2 = 0.80$; $P = 0.0002$), although variation was noted between patients within the dose levels of 1400 and 1600 mg/m^2. The AUC, indicating total drug exposure, also increased linearly with SR-4554 dose ($r^2 = 0.82$; $P = 0.0001$). The terminal plasma $t_{1/2}$ was relatively short (mean ± SD, 3.45 ± 0.62 h), and plasma clearance was quite rapid (mean ± SD, 12.6 ± 3.4 liters/h). Three patients also underwent repeat plasma sampling on day 8. Plasma concentration-time profiles on days 1 and 8 for these three patients were superimposable (Fig. 4). Comparison of day 1 and day 8 pharmacokinetic parameters revealed no statistically significant differences ($P > 0.05$), indicating good intrapatient reproducibility (Table 4). Interpatient consistency of plasma pharmacokinetic profiles on both days 1 and 8 was observed, with little variation in pharmacokinetic parameters (Tables 3 and 4). For all 11 sets of pharmacokinetic parameters, the mean ± SD for the terminal $t_{1/2}$ was 3.28 ± 0.59 h (CV, 18%; range, 2.63–4.58), the mean ± SD for the volume of distribution was 47.7 ± 8.1 l (CV, 17%; range, 36.0–63.7), and the mean ± SD for the plasma clearance was 12.8 ± 3.3 liters/h (CV, 26%; range, 8.4–18.5).

All patients underwent urine collection for estimation of 24-h excretion of SR-4554. Excluding patient 1 (in whom urine collection was incomplete) and patient 8 (in whom SR-4554 infusion was terminated prematurely because of acute gastrointestinal toxicity), the urinary recovery ranged from 79.8 to 100%, with a mean ± SD of 87.4 ± 8.6% and a CV of 9.8% (Table 5). This demonstrated extensive renal clearance.

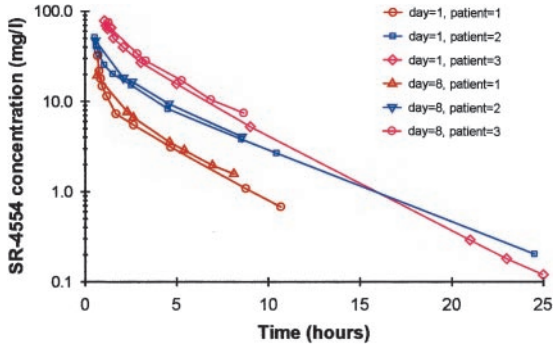

Fig. 4 Intrapatient reproducibility of plasma concentration-time profiles in three patients who were treated with SR-4554 at dose levels of 400, 800, and 1600 mg/m^2, respectively. Patients were treated and underwent plasma sampling on study days 1 and 8.

MRS Studies. Three patients underwent MRS studies after SR-4554 doses of 400, 800, and 1600 mg/m^2. One patient had fibromatosis, a benign locally aggressive sarcomatous condition, and the other two patients had malignant sarcomas (Table 6). Diagnostic MRI scans were performed on day 1, to provide current information on tumor site and size. Representative images are shown in Fig. 5. All three patients underwent unlocalized MRS studies immediately after SR-4554 infusion (Table 7). MRS studies were commenced at 1.11, 1.38, and 2.18 h after the beginning of the SR-4554 infusion for patients 1, 2, and 3 respectively. These timings reflect infusion times of 0.62, 0.53, and 1.28 h, respectively, and include the time taken for patient positioning and shimming. SR-4554-related ^{19}F signal was readily detected from the tumors of all three patients at this early time point (Fig. 6, *a* and *b*, and Fig. 7*a*). A second time point was also examined in all three patients. Timings were related to day 1 plasma $t_{1/2}$ estimates and were equivalent to 2.2, 1.8, and 2.7 times the plasma $t_{1/2}$. This indicates that ~75–85% of parent SR-4554 had been eliminated from the plasma at the time of the second MRS time point. At this second time point, ^{19}F signal was not detected in patient 1, treated at the lowest dose level. However, ^{19}F signal was reliably detected in patients 2 and 3 (Figs. 6*c* and 7*b*). A third MRS time point was examined in

Table 4 Intrapatient reproducibility of the pharmacokinetic profile of SR-4554 in human plasma[a]

Dose (mg/m^2)	Day	C_{max}[b] (mg/liter)	T_{max} (h)	AUC_{last} (h·mg/liter)	$t_{1/2}$ (h)	V_{ss} (liter)	Cl (liter/h)
400	1	32.0	0.68	49.3	2.72	53.4	18.5
	8	19.3	0.65	49.3	2.96	56.4	17.1
800	1	51.1	0.52	133.9	3.78	45.1	10.7
	8	47.3	0.57	124.9	2.96	36.0	10.2
1600	1	78.0	1.05	247.3	2.91	43.7	12.9
	8	75.0	1.28	224.4	2.63	43.7	12.7
Mean ± SD	1				3.14 ± 0.57	47.4 ± 5.3	14.0 ± 4.0
	8				2.85 ± 0.19	45.4 ± 10.3	13.3 ± 3.5
Paired Student's *t* test					$P = 0.45$	$P = 0.63$	$P = 0.17$

[a] Comparison of plasma pharmacokinetic parameters in three patients who underwent plasma sampling after SR-4554 infusions on days 1 and 8. SR-4554 concentrations were determined by HPLC-UV analysis. Pharmacokinetic data were analyzed by noncompartmental analysis.

[b] C_{max}, peak plasma concentration; T_{max}, time of peak plasma concentration; AUC_{last}, area under the concentration-time curve; $t_{1/2}$, half life; V_{ss}, volume of distribution; Cl, clearance.

Table 5 Urinary excretion of SR-4554

Patient	Dose level (mg/m^2)	Total SR-4554 dose (mg)	Percentage of SR-4554 dose recovered in urine (%)
1	400	960	9.4[a]
2	800	1440	100.0
3	1600	3200	88.9
4	1600	3200	52.4[b]
5	1200	2520	82.1
6	1400	2520	80.0
7	1400	2660	79.8
8	1400	3080	92.2
Mean ± SD			87.4 ± 8.6

[a] Data excluded as 24-h urine collection were incomplete.

[b] Data excluded as SR-4554 infusion were terminated prematurely because of gastrointestinal toxicity.

Table 6 Details of tumors scanned in MRS studies after SR-4554 infusion

Patient	Tumor type	Tumor site	Size (cm)	Perpendicular depth (cm)[a]
1	Fibromatosis	Shoulder	3.5 × 4.0 × 2.9	4.0–7.0
2	Liposarcoma	Thigh	9.7 × 7.0 × 5.1	1.0–10.0
3	Leiomyosarcoma	Thigh	8.8 × 3.5 × 7.0	2.6–8.7

[a] Measurements taken from MRI scans: the perpendicular distance from the skin surface to the most superficial and deepest parts of the tumor.

patient 3 on day 9 at a time of 27.5 h, which was equivalent to 9.5 times the plasma t½ (indicating the presence of <1% of parent SR-4554 in the plasma). No ^{19}F signal was detected from the tumor at this late time point (Fig. 7c).

In patients 2 and 3, the ^{19}F MRS data were quantified to allow comparison of concentrations of total SR-4554 in tumor (detected by MRS) and parent SR-4554 in plasma (detected by HPLC-UV; Fig. 8, *a* and *b*). The data indicate that the quantification method produces estimates of concentrations of SR-4554 that are consistent at early time points with HPLC-derived plasma concentrations. This probably reflects the distribution of parent SR-4554 from plasma into tumor. At later time points (equivalent to 1.8 and 2.7 times the plasma $t_{1/2}$, respectively), there was a trend for total SR-4554 in tumor, measured by MRS, to be present at slightly higher concentrations than parent SR-4554 in plasma, extrapolated from HPLC-UV determinations.

Discussion

We report the first Phase I study of SR-4554 in humans, assessing the plasma pharmacokinetic and toxicity profiles of SR-4554 and describing preliminary MRS studies that demonstrate the feasibility of detecting SR-4554 in human tumors.

The extensive use of nitroimidazoles as radiosensitizers in humans has provided a broad knowledge of their pharmacokinetic properties (Table 8). The P_{oct} (octanol:water partition coefficient) value, a measure of lipophilicity, has been shown in detailed studies in mice and dogs to exert a clear influence on the pharmacokinetic behavior and toxicity of these agents (38–40). A relatively high P_{oct} value, indicating greater lipophilic character, generally predicts for a long terminal plasma $t_{1/2}$, slow plasma clearance by metabolism, and, thus, a relatively prolonged drug exposure. Furthermore, lipophilic agents are associated with increased capacity for crossing the blood-brain

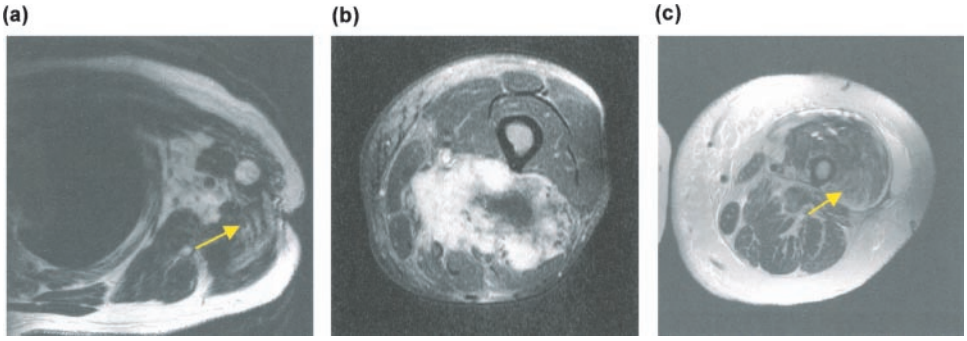

Fig. 5 Magnetic resonance images of patient 1 showing fibromatosis located in the left shoulder (*a*, *arrow*; total transverse relaxation time-weighted image), patient 2 showing a liposarcoma of the left thigh (*b*; longitudinal relaxation time-weighted fat-saturated post-contrast image), and patient 3 showing a leiomyosarcoma of the left thigh (*c*, *arrow*; total transverse relaxation time-weighted image). ^{19}F MR spectra for these tumors are shown in Figs. 6 and 7.

Table 7 Details of MRS studies after SR-4554 infusion

			MRS scan 1	MRS scan 2		MRS scan 3	
Patient	SR-4554 dose (mg/m^2)	Infusion time (h)	Time[a] (h)	Time[a] (h)	Multiple of plasma $t_{1/2}$ (h)	Time[a] (h)	Multiple of plasma $t_{1/2}$ (h)
1	400	0.62	1.11	5.87	2.2	–[b]	–
2	800	0.53	1.38	6.67	1.8	–	–
3	1600	1.28	2.18	7.98	2.7	27.5	9.5

[a] Time after the beginning of the SR-4554 infusion.
[b] –, measurement not performed.

barrier into the nervous system, thereby increasing the risk of neurotoxicity (38, 39). This has been borne out in clinical studies, in which peripheral neuropathy and acute central nervous system toxicity have been dose limiting for misonidazole (P_{oct} 0.43) and pimonidazole (P_{oct} 8.0), respectively. In contrast, lower P_{oct} values, indicating more hydrophilic character, predict for short terminal plasma $t_{1/2}$, rapid plasma clearance, and a high percentage of administered agent recovered unchanged in the urine. Thus, drug exposure is shorter, and there is less capacity for crossing of the blood-brain barrier into the nervous system and causing neurotoxicity. In clinical studies of etanidazole, the short plasma $t_{1/2}$ and rapid plasma clearance were consistent with a low P_{oct} of 0.05; as predicted, no central nervous system toxicity was observed (21, 22). However, peripheral neuropathy remained dose limiting with repeat dosing (21, 22), demonstrating that low P_{oct} values reduce but do not eliminate cumulative neurotoxicity. The 5-Nitroimidazole nimorazole, despite a relatively high P_{oct} (1.4), has been associated with little neurotoxicity (41, 42), although it is difficult to compare 5-nitroimidazoles with 2-nitroimidazoles directly because of their differing redox potentials. Nevertheless, the more hydrophilic nitroimidazoles seem to be less neurotoxic per unit dose than more lipophilic agents.

SR-4554 was designed to be used specifically as a hypoxia marker, incorporating various structural features to produce desirable properties. In particular, amide and hydroxyl groups were introduced into the side chain to increase hydrogen-bonding capability, hydrophilic character, high renal excretion, and low central nervous system penetration. The measured P_{oct} of SR-4554 (0.65) was higher than expected (11). Nevertheless, preclinical studies in mice demonstrated a short plasma $t_{1/2}$, fairly rapid plasma clearance, low brain penetration and a high percentage (68%) of administered SR-4554 recovered unchanged in the urine, all features that are characteristic of more hydrophilic nitroimidazoles such as etanidazole (11, 16). We have hypothesized that the advantageous pharmacokinetic properties relate to the local hydrogen bonding character of the side chain rather than the overall lipophilicity of the compound, as measured by P_{oct} (16).

The pharmacokinetic profile of SR-4554 in humans is similar to that demonstrated in mice (16). Specifically, after i.v. infusion, SR-4554 distributed rapidly out of the vascular compartment, as shown by the large volume of distribution. Compared with several other nitroimidazoles, the terminal plasma $t_{1/2}$ was short and plasma clearance was rapid (Table 8). The terminal $t_{1/2}$ of SR-4554 in humans (mean, 3.28 h) differed from that in mice (0.62 h) by a factor of 5.3. This is comparable with the equivalent ratio for several other nitroimidazoles including misonidazole (6.1), desmethylmisonidazole (7.7), and etanidazole (6.7). The plasma AUC increased linearly with SR-4554 dose, indicating that, over the dose range examined, the pharmacokinetic behavior was linear and the clearance mechanisms of SR-4554 were not saturated. Finally, a high percentage (87.4%) of SR-4554 was recovered unchanged in the urine, demonstrating extensive renal clearance, again consistent with findings in mice (16). Given the high renal clearance of SR-4554, it is possible that renal excretion and secretion of the drug may be occurring. If so, then drug-drug interactions are a

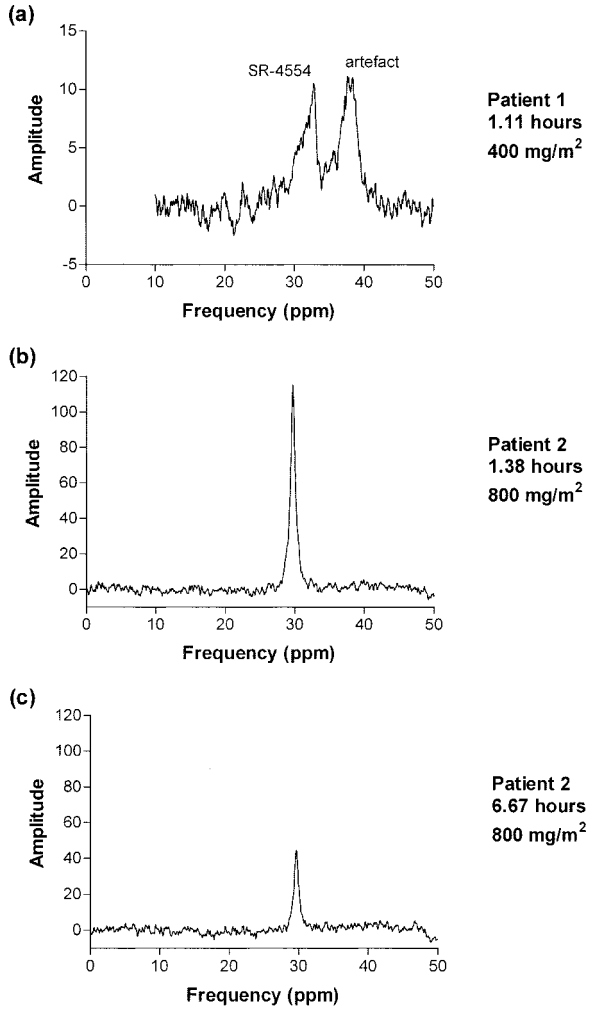

Fig. 6 ^{19}F MR spectra acquired from the tumors of patients 1 and 2. *a*, MR spectra acquired from patient 1 (fibrosarcoma of the shoulder) at 1.11 h after the start of SR-4554 infusion (400 mg/m^2). The "artifact" signal was traced to the lubricant used in the tuning capacitors in the surface coil. Spectra acquired from patient 2 (liposarcoma of the thigh) at 1.38 h (*b*) and 6.67 h (*c*) after the start of SR-4554 infusion (800 mg/m^2). Unlocalized spectra were acquired using a 16-cm (patient 1) or 10-cm (patient 2) surface coil and a 1.5 T MR system (2048 transients acquired over 34 min; TR, 1 s).

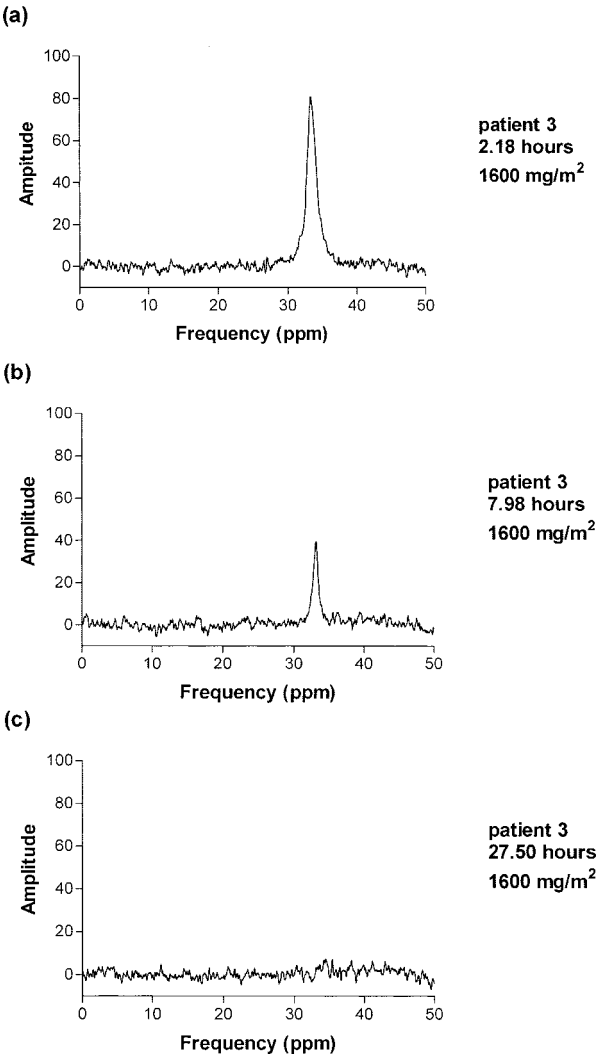

Fig. 7 ^{19}F MR spectra acquired from the tumor of patient 3. MR spectra acquired from patient 3 (leiomyosarcoma of the thigh) at 2.18 h (*a*), 7.98 h (*b*), and 27.50 h (*c*) after the start of SR-4554 infusion (1600 mg/m^2). Unlocalized spectra were acquired using a 10-cm surface coil and a 1.5 T MR system (2048 transients acquired over 34 min; TR, 1 s).

possibility, and additional studies will be required to investigate this. Importantly, pharmacokinetic behavior was very reproducible both within and between individual patients.

The pharmacokinetic profile of SR-4554 in humans is consistent with its suitability for use as a hypoxia marker. Fairly rapid clearance and reproducible pharmacokinetics are desirable for nitroimidazole hypoxia markers, because tumor hypoxia is indicated by the presence of retained SR-4554 bioreduction products, detected by ^{19}F MRS after parent SR-4554 has been eliminated from tumor and plasma (11, 12, 14, 15). The risk of neurotoxicity should also be reduced.

Diagnostic agents are generally used in large numbers of patients with no direct therapeutic benefit *per se* and as a consequence must cause only minimal or manageable toxicity. Lack of toxicity was, therefore, a priority for SR-4554. In this study, the only toxicity encountered was acute nausea and vomiting during the SR-4554 infusion in a single patient treated at 1600 mg/m^2, and MTD was finally defined as 1400 mg/m^2. This is consistent with previous Phase I studies of metronidazole (27, 28), nimorazole (29, 43), and misonidazole (44, 45), in which nausea and vomiting were frequently among the dose-limiting side effects seen. Notably, no neurological toxicity occurred, as predicted by the preclinical and clinical pharmacokinetic studies and the preclinical toxicology. No other nongastrointestinal toxicity occurred. Thus, the experience with the first eight patients suggests that SR-4554 is likely to be safe for use as a diagnostic agent. Experience with larger numbers of patients is now required to confirm this.

There are inherent challenges in the relatively unusual situation of performing a Phase I study of a diagnostic agent, in

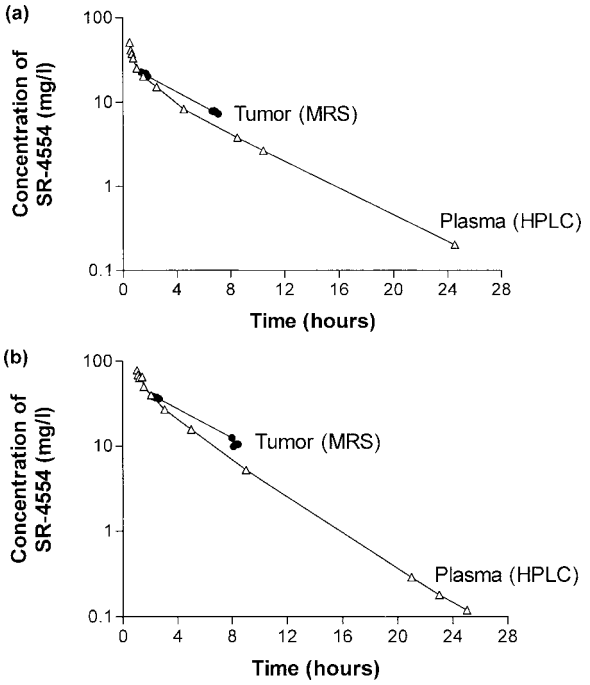

Fig. 8 Quantification of ^{19}F signal detected from tumor, comparing values of SR-4554 concentrations of total SR-4554 detection by ^{19}F MRS and parent SR-4554 in plasma detected by HPLC-UV at time points after the start of the i.v. infusion of SR-4554 in patient 2 at a dose of 800 mg/m^2 with an infusion time of 31 min (*a*) and in patient 3 at a dose of 1600 mg/m^2 with an infusion time of 63 min (*b*). Unlocalized MRS was performed using a 10-cm surface coil.

contrast with a conventional therapeutic agent. The differing requirements and priorities have introduced potential limitations in our study design. The MTD was defined by occurrence of grade1/2 toxicity, because more severe toxicity was considered to be unacceptable for a diagnostic agent. In addition, only one patient was recruited per dose level to reach the MTD rapidly, so that as many patients as possible could potentially receive a dose likely to be detectable in tumor by MRS. These study design features have resulted in the possibility that the "actual reasonably tolerated dose" of SR-4554 may have been underestimated, with a consequent limitation on the SR-4554 concentration achievable in tumor. We will not know whether this is so until a larger cohort of patients have undergone MRS studies at 1400 mg/m^2. However, if with experience it is clear that this dose is insufficient, then we will reexplore doses of 1600 mg/m^2 or greater.

Three patients underwent ^{19}F MRS studies after SR-4554 infusion, at doses of 400, 800, and 1600 mg/m^2, respectively. The remaining five patients had tumors that did not fulfil the eligibility criteria for suitability for MRS studies. The purpose of these initial examinations was to establish the feasibility of detecting SR-4554 in tumor by ^{19}F MRS at these doses. All patients underwent unlocalized spectroscopy, using a surface coil, on at least two separate occasions. ^{19}F MRS detects total SR-4554 (the sum of parent SR-4554 and retained bioreduction products). An initial MRS study was performed immediately after completion of the SR-4554 infusion. On the basis of preclinical mouse studies (15), it was estimated that after the delay necessitated by patient positioning and shimming (~30 min), MRS data acquisition should approximately coincide with the peak of parent SR-4554 in tumor. ^{19}F signal was detected in all three patients at this early time point, indicating that SR-4554 can be detected in tumors by ^{19}F MRS after administered doses of 400 mg/m^2 (total dose, 960 mg) or greater. A second MRS time point was examined in all three patients, at approximately two to three times the plasma $t_{1/2}$ after the start of the SR-4554 infusion, at which time 75–80% of parent SR-4554 had been eliminated from plasma. At this second time point, ^{19}F signal was reliably detected in patients 2 and 3, treated at 800 and 1600 mg/m^2, respectively. However, no ^{19}F signal was detected in patient 1 (treated at 400 mg/m^2), indicating that the concentration of total SR-4554 present in tumor was below the lower limit of ^{19}F MRS detection. A third, later MRS time point was examined, in patient 3 only, at approximately nine times the plasma $t_{1/2}$ after the start of the SR-4554 infusion. No ^{19}F signal was detectable. After nine times the plasma $t_{1/2}$, <1% of parent SR-4554 was present in the plasma.

These preliminary MRS measurements have established the initial feasibility of detecting SR-4554-related ^{19}F signal in human tumors. Although the feasibility has been established, care should be taken not to over-interpret the results in a small number of patients. It is possible that the optimum time for MRS measurements may be somewhere between 8 and 20 h. The optimal timing in the mouse was calculated to be 3 h, equivalent to approximately five times the plasma $t_{1/2}$ (5 × 37 min, equivalent to 185 min) of parent SR-4554 (15). Thus, a reasonable estimate of the timing of an equivalent MRS time point in humans would be at ~16 h after the start of the SR-4554 infusion (5 × 3.28 h). In the next phase of clinical development, it is intended that patients will undergo MRS studies at various time points ~16 h after SR-4554 infusion.

In patients 2 and 3, the ^{19}F MRS data were quantified to give an estimation of the concentration of total SR-4554 present in tumor at the time of data acquisition. The method used was validated in previous mouse model studies (15). This allowed comparison of concentrations of total SR-4554 in tumor with parent SR-4554 in plasma, detected by HPLC-UV, at particular time points. In both patients, evaluation at the first time point, shortly after completion of the SR-4554 infusion, indicated that estimates of total SR-4554 in tumor were very similar to estimates of parent SR-4554 in plasma. This most likely reflects the initial distribution of parent SR-4554 from plasma into tumor, when little bioreduction of SR-4554 in tumor has occurred, so that most of the acquired ^{19}F signal is from parent SR-4554. Examination of the second time point, at 1.8 and 2.7 times the plasma $t_{1/2}$ after the beginning of the SR-4554 infusion, in patients 2 and 3, respectively, showed that estimates of SR-4554 concentration by the two methods were again similar. There was a trend in both patients for the concentration of total SR-4554 in tumor to be greater than the estimated parent SR-4554 in plasma, although the difference was small. Nevertheless, this could be interpreted as providing an early indication of retention of SR-4554 bioreduction products in tumor. Similar results were

Table 8 Nitroimidazoles used in humans: comparisons of P_{oct}, plasma elimination half-life, and plasma clearance

Nitroimidazole agent	P_{oct}[a]	Plasma elimination half-life (h)	Plasma clearance (liter/h)	References
5-Nitroimidazoles				
Metronidazole	1.0	12		Urtusan *et al.* (27)
		8		Thomas *et al.* (28)
Nimorazole	1.4	3.1	13.1	Overgaard *et al.* (29)
Ornidazole (Ro 07-0207)		15.6		Okkan *et al.* (30)
2-Nitroimidazoles				
Misonidazole analogues				
Misonidazole (Ro 07-0582)	0.43	12.8		Dische *et al.* (31)
		15.0		Wasserman *et al.* (32)
			2.5	Workman *et al.* (33)
Desmethylmisonidazole (Ro 05-9963)	0.13	4.6–6.0	6.3	Coleman *et al.* (34)
Pimonidazole (Ro 03-8799)	8.0	6.1	20.2	Roberts *et al.* (35)
Etanidazole analogues				
Etanidazole (SR-2508)	0.05	5.4		Coleman *et al.* (21)
			8.1	Newman *et al.* (24)
KU-2285	0.25			Shibamoto *et al.* (26)
EF5	4.0	11.7		Koch *et al.* (23)
Doranidazole (PR-350)[b]	0.05			Oya *et al.* (36)
		4.2–4.6		Nemoto *et al.* (37)
SR-4554	0.65			Aboagye *et al.* (11)
		3.3	12.8	Present study

[a] P_{oct}, octanol:water partition coefficient.
[b] PR-350 is a 50:50 mixture of optical isomers PR-68 and PR-69.

seen in mouse tumors (15). However, given that data were available from only two patients, and were acquired by unlocalized spectroscopy, this remains speculative. Studies in additional patients using localized spectroscopy will clarify whether this initial experience is reproducible.

It is possible that SR-4554 uptake in tumor may be affected by, or may reflect, tumor blood flow. The current data are insufficient to assess this. However, the crucial issue for future studies will be to show SR-4554 retention in tumor at a late time point when plasma levels are low, and unbound parent SR-4554 has washed out of tumor. Demonstration of such retention would be indicative that retention reflects tumor hypoxia rather than merely blood flow. The next phase of clinical development will investigate this by assessing later time points and includes dynamic contrast-enhanced MRI that will evaluate parameters of tumor vascularity and permeability. As mentioned previously, higher doses of SR-4554 may need to be revisited if MRS sensitivity is inadequate at these later time points.

At this early stage in the clinical development of SR-4554, it can be concluded that the agent can be administered to patients at doses that allow detection and quantification of total SR-4554 in tumor using ^{19}F MRS. The correspondence between MRS- and HPLC-derived concentrations of total and parent SR-4554, respectively, at early time points supports the validity of the MRS quantification method. It is now planned to recruit additional patients in whom up to three MRS time points will be examined after SR-4554 infusion at a dose of 1400 mg/m^2. A number of issues will be addressed in this next phase of clinical evaluation, particularly the timing of MRS measurements, the use of localized MRS, and the reproducibility of patient positioning for sequential MRS measurements. Additional studies will then be performed to correlate the results obtained using an optimized SR-4554 protocol with established measures of tumor hypoxia.

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