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CLINICAL TRIAL REPORT

A phase I trial of PR-104, a nitrogen mustard prodrug activated by both hypoxia and aldo-keto reductase 1C3, in patients with solid tumors

Michael B. Jameson · Danny Rischin · Mark Pegram · John Gutheil · Adam V. Patterson · William A. Denny · William R. Wilson

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Abstract
Purpose PR-104 is a "pre-prodrug" designed to be activated to a dinitrobenzamide nitrogen mustard cytotoxin by nitroreduction in hypoxic regions of tumors. This study was conducted to establish the maximum tolerated dose (MTD), dose-limiting toxicity (DLT), safety, and pharmacokinetics (PK) of PR-104 in patients with advanced solid tumors.
Methods Patients with solid tumors refractory or not amenable to conventional treatment were evaluated in a dose-escalation trial of PR-104 administered as a 1-h intravenous (IV) infusion every 3 weeks. The plasma PK of PR-104 and its primary metabolite, PR-104A, were evaluated.
Results Twenty-seven patients received a median of two cycles of PR-104 in doses ranging from 135 to 1,400 mg/m^2. The MTD of PR-104 as a single-dose infusion every 3 weeks was established as 1,100 mg/m^2. One of six patients treated at 1,100 mg/m^2 experienced DLT of grade 3 fatigue. Above the MTD, the DLTs at 1,400 mg/m^2 were febrile neutropenia and infection with normal absolute neutrophil count. No objective responses were observed, although reductions in tumor size were observed in patients treated at doses $\geq$550 mg/m^2. The plasma PK of PR-104 demonstrated rapid conversion to PR-104A, with approximately dose-linear PK of both species.
Conclusions PR-104 was well tolerated at a dose of 1,100 mg/m^2 administered as an IV infusion every 3 weeks. The area under the PR-104A plasma concentration–time curve at this dose exceeded that required for activity in human tumor cell cultures and xenograft models. The recommended dose of PR-104 as a single agent for phase II trials is 1,100 mg/m^2 and further trials are underway.

Keywords PR-104 · Hypoxia · Prodrug · Dinitrobenzamide nitrogen mustard · Phase I

M. B. Jameson (✉)
Regional Cancer Centre, Waikato Hospital, Private Bag 3200, Hamilton 3240, New Zealand
e-mail: jameson@waikatodhb.govt.nz

D. Rischin
Peter MacCallum Cancer Centre, Melbourne, Australia

M. Pegram
University of California, Los Angeles, Los Angeles, CA, USA

J. Gutheil
Proacta Inc., San Diego, CA, USA

A. V. Patterson · W. A. Denny · W. R. Wilson
Auckland Cancer Society Research Centre, The University of Auckland, Auckland, New Zealand

Introduction

Hypoxia is a common feature of many solid tumors, where it is typically more extreme than in normal tissues, and this difference can potentially be exploited to provide selective drug targeting in cancer [1]. Tumor hypoxia is commonly associated with aggressive, rapidly growing tumors and has been shown to be a poor prognostic indicator in prostate, head and neck, and cervical cancers [2]. It is also associated with resistance to both radiotherapy and chemotherapy [3], and several mechanisms may contribute to this. These include decreased proliferation of hypoxic cells [4], decreased drug exposure of hypoxic cells due to their distance from blood vessels [5, 6], hypoxia-induced

selection of p53-defective tumor cells [7], requirement of oxygen for optimal drug or radiation-induced DNA damage [8–10], and hypoxia-induced up-regulation of genes involved in drug resistance [11–14]. Tumor hypoxia is also associated with genotypic and phenotypic changes that lead to enhanced potential for invasion and metastasis [15–19]. Thus, tumor hypoxia appears to be an important limitation for current cancer treatment modalities. Antineoplastic agents capable of targeting and destroying hypoxic tumor cells may therefore represent an important new class of therapy for patients with cancer. These physiologically-targeted drugs may improve outcomes for patients with prostate, head and neck, cervical, and other hypoxic tumors, especially in combination with standard anti-cancer treatments that are less effective against hypoxic cells.

Several prodrugs capable of being activated selectively by bioreduction in hypoxic cells have been evaluated clinically [3, 20]. The prototype quinone bioreductive DNA crosslinking agent, mitomycin C, has documented activity in single-agent and combination settings against a broad spectrum of tumors, but its hypoxic selectivity is modest because of facile activation by oxygen-insensitive reductases such as DT-diaphorase (NQO-1) [21]. An analog with superior hypoxic selectivity in vitro, porfiromycin (Promycin; Vion Pharmaceuticals Inc., New Haven, CT), was evaluated clinically but was inferior to mitomycin C with radiation in a randomized phase III trial in head and neck cancer [22] and has not been developed further. Another quinone bioreductive prodrug, apaziquone (EO9), has shown good activity as an intravesical treatment for superficial bladder cancer [23] but also appears to be primarily an NQO-1-activated prodrug rather than a hypoxia-selective agent [24].The aziridinyldinitrobenzamide CB1954 is also capable of generating DNA interstrand crosslinks on bioreduction [25], but again the primary clinical focus is on its activation by oxygen-insensitive reductases such as NQO-2 [26], or the *Escherichia coli nfsB* nitroreductase in a gene therapy context [27–29].

The most intensively studied hypoxia-activated prodrug, the benzotriazine *N*-oxide tirapazamine (TPZ), improved overall survival of advanced non-small cell lung cancer (NSCLC) in a randomized phase III trial when combined with cisplatin [30], but TPZ plus cisplatin was inferior to cisplatin plus etoposide in a subsequent study [31]. Of concern, TPZ increased toxicity without improved efficacy when combined with paclitaxel and carboplatin in advanced NSCLC [32]. Furthermore, addition of TPZ to cisplatin and radiation failed to improve overall survival in advanced head and neck cancer, although a trend for improved loco-regional control in favor of the experimental arm was seen when patients receiving substandard radiotherapy were excluded from the analysis [33]. Notably, in a single institution substudy, TPZ improved loco-regional control in patients with more hypoxic tumors as assessed by PET imaging with F-MISO or FAZA [34].

While the overall clinical experience with TPZ supports the concept of targeting tumor hypoxia, its low therapeutic ratio calls for improved hypoxia-activated prodrugs. Preclinical studies have identified several limitations of TPZ including inefficient penetration into hypoxic tissue [6], lack of a bystander effect [35], and activation at relatively high oxygen concentrations characteristic of physiologically hypoxic niches in normal tissues [36, 37] which may contribute to its toxicity [38, 39]. We have developed dinitrobenzamide mustard prodrugs, exemplified by the initial lead compound SN 23862 in which hypoxia-selective reduction of a nitro group acts as an electronic switch to activate a nitrogen mustard moiety [40]. Activation of SN 23862 requires more severe hypoxia for activation than does tirapazamine [35], lacks the retinal toxicity of TPZ [36], and elicits a marked bystander effect in multicellular layer cultures [35].

PR-104 is a dinitrobenzamide mustard with marked antitumor activity in xenograft models, both as monotherapy and in combination with radiotherapy or chemotherapy [41–43]. PR-104 is well tolerated in preclinical species, with a maximum-tolerated dose (MTD) 7.5-fold higher (on a molar basis) than TPZ in CD-1 nude mice [41]. Myelotoxicity was dose-limiting in rats and dogs. As illustrated in Fig. 1, PR-104 itself is a phosphate ester pre-prodrug which is rapidly hydrolyzed systemically to the corresponding alcohol, PR-104A, in preclinical species [41, 44]. PR-104A penetrates into hypoxic tumor tissue more efficiently than TPZ, as determined using the multicellular layer model [42]. PR-104A is activated, at tenfold lower oxygen concentrations than for TPZ [42], via reduction to the corresponding hydroxylamine (PR-104H) and amine (PR-104M) [41, 43] by NADPH:cytochrome P450 oxidoreductase and other one-electron reductases in hypoxic cells [45] (Fig. 1). These reactive nitrogen mustards provide hypoxia-selective cytotoxicity via DNA crosslinking in a wide range of cell lines [43, 46]. PR-104H appears to have sufficient stability to elicit bystander killing of cells at higher oxygen concentrations in tumors [41, 42, 47], which may contribute to the surprising monotherapy activity of PR-104. However, the large variation in aerobic cytotoxicity (and metabolic reduction to PR-104H) between different human tumor cell lines [43] has recently led to the identification of aldo-keto reductase 1C3 (AKR1C3, also known as prostaglandin F synthase) as an aerobic PR-104A reductase [48]. AKR1C3 catalyses the formation of steroid hormones (testosterone, 17β-estradiol) and prostaglandins (PDH2, 9α,11β-PGF2, 15d-PGJ2) [49], but has not previously been reported as a nitroreductase. PR-104A is the first prodrug to be described as a substrate for AKR1C3, which does not reduce other classes of

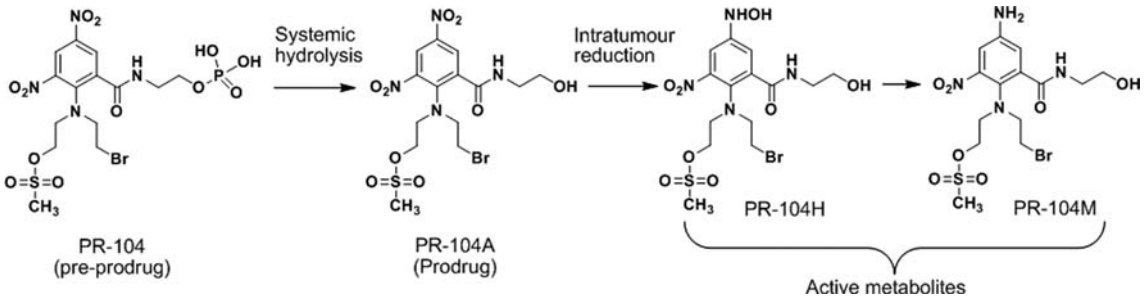

Fig. 1 Structure of the pre-prodrug PR-104 and its alcohol metabolite PR-104A. PR-104A is a prodrug activated by metabolic reduction in tumors to the hydroxylamine PR-104H and amine PR-104M, both of which are reactive nitrogen mustard DNA crosslinking agents

bioreductive drugs [48]. AKR1C3 has been reported to be upregulated in a range of carcinomas [50–52], which we have confirmed in a recent tissue microarray study with surgical biopsies of 24 tumor types [48]. Thus, certain tumors may also activate PR-104 in a hypoxia-independent manner as a consequence of elevated AKR1C3 expression, adding to its hypoxic activation and increasing overall single agent activity in vivo.

This phase I clinical trial was the first evaluation of the safety and tolerability of PR-104, a first-in-class hypoxia-activated nitrogen mustard prodrug, in human subjects. Based on anticipated limited toxicity from PR-104, a 1-h intravenous infusion time was chosen to facilitate administration of high doses in the later cohorts. The major objectives were to determine the MTD and dose-limiting toxicities (DLT) of PR-104 using a once every three week schedule, and to evaluate plasma PK (including conversion to PR-104A) in the first cycle.

Materials and methods

Patient selection

Patients ≥18 years old with pathologically confirmed solid tumors (no hematological malignancies) refractory or not amenable to standard therapy were eligible for this trial. Other eligibility criteria included a Karnofsky performance status of ≥70%; a projected life expectancy of >3 months; measurable or evaluable disease; adequate bone marrow (with a hemoglobin level maintained in the absence of red blood cell transfusion), renal, and hepatic function; >4 weeks since major surgery, radiotherapy, or last treatment with chemotherapy (>6 weeks for prior nitrosoureas or mitomycin C); and >2 weeks since hormonal therapy (ongoing androgen deprivation therapy was permitted). Steroid use was allowed if the dose of corticosteroids was stable for the prior 2 weeks with a stable clinical condition for 1 month. Patients were ineligible if they received prior radiotherapy to more than 25% of bone marrow; previous high-dose chemotherapy; prior treatment with >3 myelosuppressive chemotherapy regimens; ongoing coagulopathy; or if they had not recovered from all acute toxic effects of prior anticancer therapy. Patients were also ineligible if they were pregnant, breast-feeding, or planning to become pregnant; had a history of significant cardiac comorbidity; HIV or hepatitis B infection; hepatitis C with abnormal liver function tests; or had a known allergy to non-platinum-containing alkylating agents.

Institutional review board or ethics committee approval and written informed consent were obtained for all patients prior to initiation of any trial-related procedures, and the study complied with the provisions of the Declaration of Helsinki. The trial was registered with http://www.clinicaltrials.gov, identifier NCT00349167.

Trial design

This was a single-arm, phase I, multi-center, open-label, uncontrolled, dose-escalation study designed to establish the MTD of PR-104. Dose levels consisted of a minimum of three evaluable patients with an expansion to six patients if DLT was observed. Intrapatient dose escalation was allowed after completing two cycles at their assigned dose, no toxicity ≥grade 2 occurred at their current dose level, and at least one patient had safely received PR-104 in the absence of DLT at the next dose level. The MTD was defined as one dose level below that at which two of six patients experienced a DLT.

Drug administration and dose escalation procedure

Drug administration

A lyophilized cake of 400 mg PR-104 was reconstituted in 2 mL of water for injection (WFI), further diluted in 250 mL of 5% dextrose in water, and administered as an intravenous infusion over 1 h once every 3 weeks (1 cycle = 21 days).

Definition of dose-limiting toxicity

Toxicity was assessed according to the National Cancer Institute Common Toxicity Criteria for Adverse Events (version 3.0). DLT was assessed during cycle 1 and was defined as any of the following: grade 4 hematological toxicity (platelets $\leq$25,000/mm^3, hemoglobin <6.5 g/dL, absolute granulocyte count or absolute neutrophil count (ANC) $\leq$500/mm^3 for $\geq$5 days); $\geq$grade 3 nonhematological toxicity (excluding nausea or vomiting); $\geq$grade 2 treatment-emergent neurotoxicity or allergy.

Dose escalation procedure

The starting dose for PR-104 of 135 mg/m^2 was based on results of toxicology studies in rats, the most sensitive species to PR-104 (MTD 1,328 and 2,678 mg/m^2 in rats and dogs, respectively, by weekly IV dosing for four consecutive weeks). Seven dose levels of PR-104 were explored: 135, 216, 346, 550, 770, 1,100, and 1,400 mg/m^2.

Patient evaluation and follow-up

Baseline evaluation included a history and physical examination, assessment of performance status (Karnofsky), complete blood count (CBC), blood chemistry profile, coagulation studies (PTT and INR), urinalysis, pregnancy test, and serum tumor markers. Vital signs and electrocardiogram were taken before, during, and after the administration of the first dose of PR-104. With each cycle, weekly assessments included performance status, symptom-directed physical examination, laboratory (CBC, coagulation studies, serum chemistry, and urinalysis), intercurrent adverse events, and concomitant medication use. Extent of disease was determined by chest radiograph and computed tomography or magnetic resonance scans prior to enrollment and scans to assess tumor response were repeated every two cycles. Efficacy was assessed using Response Evaluation Criteria in Solid Tumors (RECIST) criteria whenever possible [53].

Pharmacokinetic analyses

Blood samples to determine plasma concentrations of PR-104 and PR-104A were collected in EDTA vacutainer tubes predose, 45 min into the infusion, immediately following completion of the infusion and at 5, 10, 20, 30, 45, 60, 120, and 240 min and 24 h post-infusion of the first dose. Blood samples were centrifuged for 5 min; the plasma was immediately deproteinised by addition of nine volumes of methanol:ammonium acetate:acetic acid (1,000:3.5:0.2; v/w/v) and stored at –70°C. Extracts were assayed by a validated high-performance liquid chromatography method [44] using triple quadrupole mass spectrometric detection with tetradeuterated internal standards [54] by MicroConstants, Inc. (San Diego). The descriptive pharmacokinetic parameters AUC(0-inf) (area under the concentration time curve extrapolated to infinity), CL (clearance), V_{ss} (volume of distribution at steady state), T_{max} (time of maximum concentration, from start of infusion), and $T_{1/2}$ (terminal half life) were determined by standard noncompartmental methods with WinNonLin v4.0.1, using actual infusion times (1–1.25 h) and doses.

Results

Patient characteristics

Between January 2006 and June 2007, 29 patients were enrolled in this clinical trial. Two were excluded from analysis because they never received PR-104 due to a change in their eligibility status. Characteristics of the 27 evaluable patients who received at least one dose of PR-104 are listed in Table 1; none of the patients had hematological malignancies.

Safety and tolerability

Twenty-seven patients were assessable for safety. Patients received a median of two cycles of PR-104 (range 1–15). Two patients developed DLT (grade 3 dehydration and grade 3 fatigue) that led to cohort expansions at the 135 mg/m^2 and the 1,100 mg/m^2 dose levels, respectively. The number of patients treated at each dose level and observed DLTs is shown in Table 2. The MTD of PR-104 was determined to be 1,100 mg/m^2. The major toxicity was dose-related myelosuppression, predominantly neutropenia. The relationship between dose and rate of neutropenia is illustrated in Fig. 2.

At the MTD, six patients received a median of two cycles of PR-104 (range 2–3). Grade 3 or 4 neutropenia was observed during cycle 1 in three patients, but this did not qualify as DLT as it persisted for fewer than 5 days. Grade 2 thrombocytopenia was observed in a single patient during cycle 1. After repeated PR-104 dosing, four patients developed thrombocytopenia. Three of the four patients had low-grade (grade 1 or 2) thrombocytopenia that persisted beyond cessation of PR104 administration through the 30-day follow-up period. A fourth patient with breast carcinoma and bone metastases developed grade 4 thrombocytopenia following three cycles of PR-104. In this patient, further evaluation of prolonged thrombocytopenia demonstrated metastatic tumor involving the bone marrow. Three patients had cycle 2 delayed by 1 week for either neutropenia ($n = 2$) or thrombocytopenia ($n = 1$). A single

Table 1 Patient characteristics

Characteristic	Patients ($n = 27$)
Median age, years (range)	54 (28–72)
Males, *n* (%)	15 (56)
Race, *n* (%)	
White	21 (78)
New Zealand Maori	3 (11)
Other Pacific Islander/Asian	3 (11)
Karnofsky Performance Status, median (range)	90% (70–100%)
Diagnosis, *n* (%)	
Gastrointestinal	6 (22)
Breast	4 (15)
Head and neck	4 (15)
Non-small cell lung cancer	3 (11)
Other	10 (37)
Prior chemotherapy, *n* (%)	23 (85)
Median no. of prior chemotherapy regimens, *n* (range)	2 (1–5)

Table 2 Dose escalation scheme and dose-limiting toxicities observed at all doses in cycle 1 of PR-104 treatment

Dose (mg/m^2)	No. of patients	Dose-limiting toxicities
135	6	Grade 3 dehydration ($n = 1$)
216	3	
346	3	
550	3	
770	3	
1,100	6	Grade 3 fatigue ($n = 1$)
1,400	3	Grade 3 febrile neutropenia ($n = 1$)
		Grade 3 infection with normal ANC ($n = 1$)

ANC absolute neutrophil count

patient at MTD received a dose reduction (825 mg/m^2) in cycle 3 due to grade 4 neutropenia during cycle 2.

Above the MTD, two of three patients who received 1,400 mg/m^2 of PR-104 developed DLT or unacceptable toxicity. One patient, a 66-year-old woman with stage IV colon carcinoma with liver metastases, received two cycles of PR-104. On day 16 of cycle 1, she developed a DLT of grade 3 febrile neutropenia (ANC = 500/mm^3) and grade 3 anemia (hemoglobin = 7.6 g/dL) requiring RBC transfusion. This event resulted in a 1-week delay in the start of cycle 2 and a 20% dose reduction (to 1,120 mg/m^2). On day 9 of cycle 2, the patient developed grade 4 anemia, and on day 15 she developed grade 4 neutropenia along with grade 2 thrombocytopenia. Treatment in this patient was discontinued after two cycles due to progressive disease.

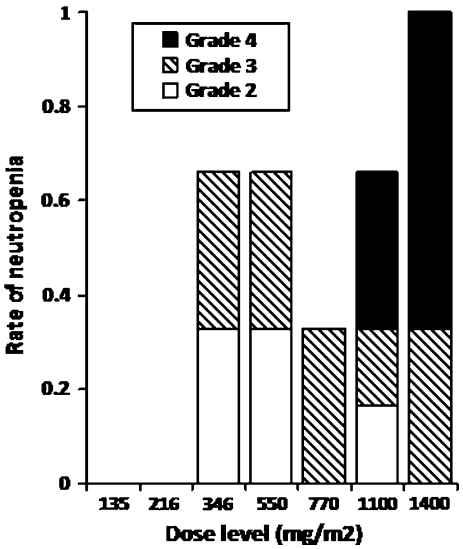

Fig. 2 Proportion of patients at each dose level experiencing ≥grade 2 neutropenia during cycle 1 of PR-104 treatment. The grade of neutropenia was determined by the nadir absolute neutrophil count for each patient during cycle 1

A 69-year-old man with metastatic cholangiocarcinoma who received two cycles of PR-104 at 1,400 mg/m^2 developed a grade 3 infection (cholangitis) with a normal ANC on day 2 of cycle 1, considered unrelated to PR-104 treatment. On day 21, he developed grade 4 neutropenia (ANC = 400/mm^3) lasting less than 5 days resulting in a 2-week delay in the start of cycle 2. Treatment in this patient was discontinued after two cycles due to progressive disease. Grade 4 thrombocytopenia was observed at the 30-day follow-up visit. While this did not fulfill the formal definition of DLT, the requirement for a 2-week delay following cycle 1, and the onset of progressive thrombocytopenia after cycle 2 was considered to be unacceptable toxicity.

Below the MTD, neutropenia and/or thrombocytopenia that precluded further therapy was observed in two patients, one after a single dose of PR-104 (216 mg/m^2) and another after prolonged dosing (15 cycles at 135 mg/m^2).

The majority of the patients discontinued PR-104 administration due to disease progression ($n = 18$). Four patients discontinued PR-104 due to adverse events [thrombocytopenia ($n = 2$) and neutropenia/thrombocytopenia ($n = 2$)].

PR-104 was well tolerated at or below the MTD. Most adverse events were grade 1 or 2 in severity. Sixty-one adverse events (AEs) of grade 2 or higher were considered related (possibly, probably, or definitely) to treatment with PR-104. The most common treatment-related AEs (≥grade 2) observed in the 27 evaluable patients were fatigue (52%), anemia (26%), neutropenia (26%), nausea (22%), thrombocytopenia (19%), vomiting (15%), leukopenia (11%), and

Table 3 Most frequent treatment-related adverse events (≥grade 2) occurring with PR-104 treatment, presented as the highest grade observed for each patient

Adverse event, *N*	Doses < 1,100 mg/m^2 ($n = 18$)				Doses ≥1,100 mg/m^2 ($n = 9$)			
	Max grade				Max grade			
	2	3	4	Total	2	3	4	Total
Fatigue	9	–	–	9	3	2	–	5
Anemia	1	1	–	2	1	2	2	5
Neutropenia	–	2	–	2	1	1	3	5
Nausea	3	1	–	4	2	–	–	2
Thrombocytopenia	2	–	–	2	–	2	1	3
Vomiting	2	2	–	4	–	–	–	–
Leukopenia	1	1	–	2	–	1	–	1
Febrile neutropenia	–	–	–	–	–	2	–	2
Abdominal pain	1	–	–	1	–	–	–	–
Cholangitis	–	–	–	–	–	1	–	1
Dehydration	–	1	–	1	–	–	–	–
Dysgeusia	1	–	–	1	–	–	–	–
Dysphagia	–	–	–	–	–	1	–	1
Hemoptysis	–	1	–	1	–	–	–	–
Hiccups	1	–	–	1	–	–	–	–
Indigestion	–	–	–	–	1	–	–	1
Oral pain	1	–	–	1	–	–	–	–
Palmar–plantar syndrome	1	–	–	1	–	–	–	–
Pneumonia	–	–	–	–	–	1	–	1
Postural dizziness	1	–	–	1	–	–	–	–
Wound pain	–	–	–	–	1	–	–	1

febrile neutropenia (7%) (Table 3). Prophylactic anti-emetics were not administered to patients in cohort 1 due to uncertainty as to whether nausea and vomiting would occur with this pre-prodrug. When it became apparent that it caused this toxicity, these and all subsequent patients received prophylactic anti-emetics (steroids and $5HT_3$ antagonists).

A total of 29 serious adverse events (SAEs) were reported. Seven were considered related to PR-104 (infection, vomiting, dehydration, hemorrhage secondary to disease progression in the presence of thrombocytopenia, infection with normal ANC, febrile neutropenia, and anemia).

Three patients died while on study or within 30 days of being removed from the study. All of these deaths were attributed to underlying disease and none were considered related to PR-104 treatment.

Pharmacokinetics

PK data for PR-104 (Table 4) and PR-104A (Table 5) were obtained for all 27 patients during cycle 1 PR-104 administration. Plasma concentrations of PR-104 declined rapidly from the end of infusion, with a mean terminal half-life of 8 min, V_{ss} of 29 L/m^2, and clearance of 105 L/h/m^2 at the MTD (1,100 mg/m^2). This was accompanied by extensive conversion to PR-104A, which had a mean terminal half-life of 47 min. The AUC of PR-104 and PR-104A at the MTD were 11 and 20 μg h/mL, respectively. The dose dependence of the C_{max} and AUC for PR-104 and PR-104A are shown in Fig. 3. Although there was substantial inter-patient variability, the data were broadly consistent with dose-linear pharmacokinetics, as is also the case in mice, rats, and dogs (manuscript in preparation, Patel K, Holford N.H.G, Hicks K.O and Wilson W.R.).

Anti-tumor effects

No objective tumor responses were observed, although reductions in tumor size and symptomatic improvement were seen. Interestingly, the majority of patients with symptomatic improvement and reductions in tumor size occurred at PR-104 doses ≥550 mg/m^2. The change in greatest tumor dimension for the 17 patients evaluable for efficacy (defined as having at least one post-baseline tumor assessment) is illustrated in Fig. 4 where the dark histogram bars represent patients who received PR-104

Table 4 PR-104 plasma pharmacokinetic parameters, cycle 1

Parameter	PR-104 dose, mg/m^2							
	135	187[a]	216	346[a]	550	770[b]	1,100	1,400
N	6	1	3	2	3	2	6	3
C_{max}, μg/mL	2.06 (1.41)	1.32	2.82 (1.59)	4.44 (1.31)	10.19 (6.17)	8.18 (0.37)	18.0 (4.51)	28.5 (25.1)
AUC(0-inf), μg.h/mL	1.35 (0.75)	0.88	1.77 (1.30)	2.96 (0.02)	6.99 (3.32)	5.84 (0.54)	10.7 (1.60)	18.2 (16.6)
CL, L/h/m^2	124 (65)	213	167 (96)	116 (3)	93 (49)	132 (13)	105 (18)	122 (76)
V_{ss}, L/m^2	43.9 (22.9)	72	42.5 (25.3)	37.2 (4.4)	31.3 (17.6)	40.4 (4.8)	28.9 (10.5)	36.2 (21.7)
$T_{1/2}$, h	0.039 (0.009)	0.037	0.061 (0.028)	0.100 (0.040)	0.147 (0.076)	0.155 (0.066)	0.136 (0.043)	0.109 (0.021)

Values are arithmetic means (standard deviations in brackets)

[a] A dosing error resulted in one patient receiving an initial dose of 187 mg/m^2, with the balance of the planned 346 mg/m^2 dose administered as a second 1 h infusion 7 h later. Pharmacokinetics was assessed after the 187 mg/m^2 dose only

[b] One patient excluded because an interruption in the infusion made PR-104 concentrations uninformative

Table 5 PR-104A plasma pharmacokinetic parameters, cycle 1

Parameter	PR-104 dose, mg/m^2							
	135	187	216	346	550	770	1100	1400
N	6	1	3	2	3	3	6	3
C_{max}, μg/mL	1.89 (0.16)	1.67	3.56 (1.05)	3.74 (1.09)	14.2 (8.6)	8.57 (1.5)	15.4 (3.4)	26.4 (12.3)
T_{max},[a] h	1.06 (0.08)	1.08	0.78 (0.22)	0.99 (0.34)	1.03 (0.27)	0.92 (0.29)	0.88 (0.20)	0.86 (0.19)
AUC(0-inf), μg h/mL	1.91 (0.35)	1.50	3.48 (0.83)	4.06 (0.82)	14.3 (9.8)	8.40 (1.56)	19.5 (6.7)	29.7 (12.1)
$T_{1/2}$, h	0.55 (0.14)	0.56	0.61 (0.33)	0.75 (0.28)	0.72 (0.33)	0.59 (0.13)	0.78 (0.08)	0.75 (0.05)

Values are arithmetic means (standard deviations in brackets)

[a] Time of maximal concentration, from start of infusion

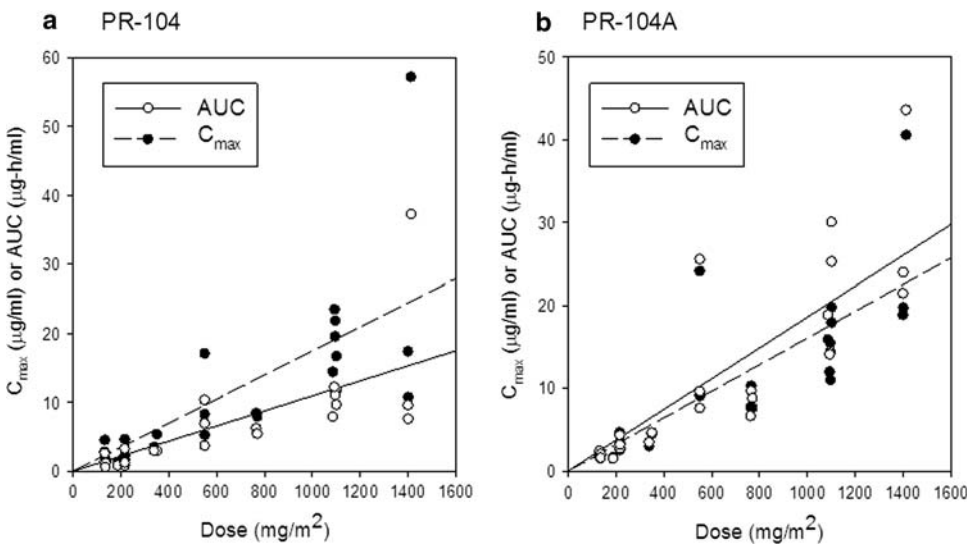

Fig. 3 Dose dependence of PR-104 plasma pharmacokinetic parameters following a 1 h intravenous infusion. **a** AUC and C_{max} of PR-104. **b** AUC and C_{max} of PR-104A. Values are for individual patients, and lines are linear regressions through the origin

doses ≥550 mg/m^2. Five patients (three with head and neck cancer and one each with sarcoma and vulvar adenocarcinoma) had stable disease maintained for at least 4.0 months (range 4.0–14.0 months). Symptomatic improvement was reported in three patients [head and neck cancer ($n = 2$), breast cancer ($n = 1$)].

Discussion

Drugs that selectively target hypoxic areas of tumors may overcome an important limitation in current cancer therapy by destroying cells that are often resistant to conventional treatments. PR-104 is a novel prodrug with selective

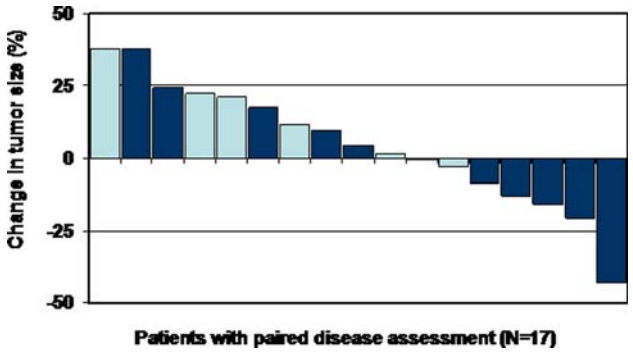

Fig. 4 Best reduction or smallest increase from baseline in tumor size for the 17 patients with at least one post-baseline disease assessment. *Dark bars* represent patients who received at least 550 mg/m^2 of PR-104. Despite a reduction in tumor size of 43%, the patient with the greatest reduction in tumor size developed a new lesion which signified progressive disease

cytotoxicity under hypoxic conditions in preclinical models and augments antitumor activity when used in combination with conventional therapies [41, 42]. Although not known at the time of this phase I study, in addition to its activation by one-electron reductases in hypoxic cells [45], PR-104 is also activated in tumor cells that express the two-electron reductase AKR1C3 [48], which is likely to augment its single agent activity in such tumors.

This phase I trial demonstrates that PR-104 can safely be administered to humans as a 1-h infusion every 3 weeks, at a MTD of 1,100 mg/m^2. Importantly, this dose of PR-104 achieved a mean plasma AUC for PR-104A (20 μg h/mL) that provides significant tumor cell killing in human tumor xenografts; from the reported plasma PK in mice [41], the estimated PR-104A AUC required for 90% clonogenic kill of SiHa xenografts [42] is approximately 15 μg h/mL. H460, and A2780 tumor xenografts have similar PR-104 monotherapy sensitivity to SiHa [41], although other xenografts (H1299, MiaPaCa-2 and C33A) showed little response indicating that a spectrum of sensitivities can be expected. This is consistent with indications of monotherapy antitumor activity in some tumors at higher doses of PR-104 (Fig. 4) and suggests that PR-104 at 1,100 mg/m^2 warrants additional investigation in the phase II setting. However, PR-104 would be expected to have its greatest utility when used in combination with existing therapies, such as radiation or cytotoxic drugs that tend to spare hypoxic cells. PR-104, at doses as low as 10% of its MTD, showed significant activity against SiHa cervical tumors when administered after a single dose of radiation [42], and had additive or super-additive efficacy when combined with docetaxel and gemcitabine [41], both of which have been suggested to spare hypoxic cells in xenograft models [55, 56].

The major toxicity observed in this trial was dose-dependent myelosuppression, expressed primarily as neutropenia. Some patients demonstrated increased bone marrow toxicity with subsequent cycles that precluded further therapy and, in some cases, neutropenia and thrombocytopenia persisted well beyond the cessation of PR-104 administration. This cumulative myelosuppression will need to be more fully evaluated in future trials. Overall, PR-104 was well tolerated, with most drug-related adverse events being low grade and manageable with outpatient care.

Although consistent with preclinical studies in rats and dogs, the mechanism of dose-limiting myelosuppression by PR-104 is not yet clear. Several possibilities are currently under evaluation in nonclinical species, including whether PR-104A is activated in the hypoxic niche in bone marrow recently shown to harbor primitive hemopoietic stem cells sensitive to TPZ [39]. Myelosuppression due to systemic toxicity of circulating PR-104A or its reduced metabolites seems less likely given the lack of other normal tissue toxicities typical of nitrogen mustards such as mucositis or diarrhea. Another possibility is that PR-104A is activated in the marrow by AKR1C3, as suggested by preliminary evidence for expression of this enzyme in human CD34 +ve myeloid progenitor cells [57]. Optimal clinical development of PR-104 may require an understanding of (and ability to monitor) the tumor-specific features that are likely to determine its activity, not just its toxicity profile. These include the severity and distribution of hypoxia, expression of PR-104A reductases (AKR1C3, NADPH: cytochrome P450 oxidoreductase and others), and intrinsic sensitivity of the tumor cells to the activated nitrogen mustard metabolites, especially as determined by DNA crosslink repair phenotype [46].

Hypoxia imaging with positron emission tomography (PET) scans using 2-nitroimidazole imaging agents, e.g., ^{18}F-fluoromisonidazole (^{18}F-MISO), ^{18}F-EF5, and fluoroazomycin arabinoside (^{18}F-AZA) is a promising tool for identifying tumor hypoxia. In recent reports tumor hypoxia detected by ^{18}F-MISO identified patients with head and neck cancer who benefited from the addition of TPZ to chemoradiation [34]. Utilization of this type of imaging may serve a valuable role with PR-104 as a patient/treatment selection technique. Use of ^{18}F-MISO PET has been incorporated into ongoing and future clinical studies of PR-104, as has the evaluation of endogenous hypoxia markers [58] and AKR1C3 expression by immunostaining of diagnostic tumor biopsies.

In summary, this was the first investigation in humans of PR-104, a novel pre-prodrug designed to become activated in areas of tumor hypoxia and thereby spare normal tissues. This study established that PR-104 is well tolerated on an every 3 week schedule with an MTD of 1,100 mg/m^2. Dose-related myelosuppression, predominately neutropenia, was the primary toxicity. PR-104 exposure reached levels similar to those associated with antitumor activity in

animal models. This trial provides a basis for future single-agent, combination, and multimodality studies with PR-104, with phase II randomized add-on trials underway in advanced non-small cell lung carcinoma (second-line with docetaxel) and advanced hepatocellular carcinoma (first-line with sorafenib), and a phase I/II trial in acute myeloid leukemia.

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