

PR-104 plus sorafenib in patients with advanced hepatocellular carcinoma

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Abstract

Purpose PR-104 is activated by reductases under hypoxia or by aldo–keto reductase 1C3 (AKR1C3) to form cytotoxic nitrogen mustards. Hepatocellular carcinoma (HCC) displays extensive hypoxia and expresses AKR1C3. This study evaluated the safety and efficacy of PR-104 plus sorafenib in HCC.

Methods Patients with advanced-stage HCC, Child-Pugh A cirrhosis, and adequate organ function, were assigned to dose escalating cohorts of monthly PR-104 in combination with twice daily sorafenib. The plasma pharmacokinetics (PK) of PR-104 and its metabolites were evaluated.

Results Fourteen (11 men, 3 women) HCC patients: median age 60 years, ECOG 0–1, received PR-104 at two dose levels plus sorafenib. Six patients were treated at starting cohort of 770 mg/m². In view of one DLT of

febrile neutropenia and prolonged thrombocytopenia, a lower PR-104 dose cohort (550 mg/m²) was added and accrued 8 patients. One patient had a partial response and three had stable disease of ≥ 8 weeks in the 770 mg/m² cohort. Three patients at the 550 mg/m² had stable disease. There were no differences in PK of PR-104 or its metabolites with or without sorafenib, but the PR-104A AUC was twofold higher ($P < 0.003$) than in previous phase I studies at equivalent dose.

Conclusions PR-104 plus sorafenib was poorly tolerated in patients with advanced HCC, possibly because of compromised clearance of PR-104A in this patient population. Thrombocytopenia mainly and neutropenia were the most clinically significant toxicities and led to discontinuation of the study. PR-104 plus sorafenib is unlikely to be suitable for development in this setting.

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Introduction

PR-104 is a prodrug that undergoes a step-wise activation to form cytotoxic nitrogen mustards. PR-104 is rapidly converted to the alcohol PR-104A, which then diffuses into cells and is metabolically reduced to several active cytotoxins, predominantly the hydroxylamine PR-104H and amine PR-104M, which exert their cytotoxic effect through the formation of DNA inter-strand cross-links [1–3]. The activation of PR-104A to PR-104H and M occurs at pathologic levels of hypoxia ($pO_2 < 1$ mmHg) [4] through reduction by ubiquitous 1-electron reductases (NADPH: cytochrome P-450 oxidoreductase, iNOS, and others) [5]. PR-104A also gets activated to PR-104H and M, independently of hypoxia by the two electron reductase enzyme aldo–keto reductase 1C3 (AKR1C3) which is highly expressed in some tumors [6].

The first phase I study of PR-104 was initiated in patients with solid tumors using a 1-h intravenous infusion once every 3 weeks and determined 1,100 mg/m² as the maximum tolerated dose (MTD) with fatigue, neutropenic sepsis, and infection with normal neutrophil counts identified as the dose-limiting toxicities (DLTs). Dose-dependent myelosuppression, primarily neutropenia and thrombocytopenia, was considered the most significant toxicity of PR-104 [7].

Hepatocellular carcinoma (HCC) displays a high degree of hypoxia [8] and expresses high levels of AKR1C3 [6]. AKR1C3 expression is regulated by the *nrf2/keap1* pathway which also regulates a number of genes responsible for resistance to many anticancer agents [9]. The expression of these genes in HCC may explain, in part, the poor response of HCC to conventional anticancer therapies and at the same time may render these tumors particularly sensitive to PR-104A.

Sorafenib, an oral multi-kinase inhibitor [10], is considered the standard of care therapy for advanced HCC based on improvement in survival in two double-blind, randomized phase III trials in patients with advanced HCC and Child-Pugh A cirrhosis against placebo [11, 12]. PR-104 and sorafenib have demonstrated a synergistic anti-tumor effect in HCC xenograft models [13]. Sorafenib has been shown to induce hypoxia in xenograft models of renal cell carcinoma, suggesting a possible synergy between PR-104 and sorafenib [14].

Based on the above and the lack of overlapping toxicities between PR-104 and sorafenib, a phase I study designed to evaluate the safety and efficacy of the

combination of PR-104 and multi-kinase inhibitor sorafenib in HCC is reported herein.

Methods

This was a multi-institutional study. The Human Investigation Review Board or Committee of each accruing institution reviewed and approved both the protocol and informed consent. Informed consent was obtained from each patient.

Patients' eligibility

Men and women ≥ 18 years of age, of any racial or ethnic background with advanced-stage, histologically confirmed HCC with at least one measurable lesion by RECIST 1.1 [15], that was suitable for treatment with sorafenib, but had not received any prior systemic therapy were eligible. Patients were required to have an ECOG performance status of 0 or 1; a Child-Pugh score A; adequate hematologic function (ANC $\geq 1.5 \times 10^9/L$; platelet count $\geq 100 \times 10^9/L$; hemoglobin ≥ 8.5 g/dL maintained in the absence of red blood cell transfusions; and international normalized ratio ≤ 1.7 ; or prothrombin time ≤ 2 s above control); adequate hepatic function (albumin ≥ 2.8 g/dL; total bilirubin ≤ 2 mg/dL; and alanine aminotransferase and aspartate aminotransferase ≤ 5 times the upper limit of the normal range); and adequate renal function (serum creatinine ≤ 1.5 times the upper limit of the normal range or creatinine clearance ≥ 60 mL/min). Patients with serious inter-current illnesses were excluded.

Treatment plan

The starting dose of PR-104 was 770 mg/m² administered intravenously over 1-h every 4 weeks. Sorafenib was prescribed at the standard dose of 400 mg PO twice daily and was commenced after the first dose of PR-104.

PR-104 dose escalation rules

The MTD was defined as a dose level of PR-104 at which one or fewer patients in six exhibited dose-limiting toxicity (DLT), and for which the next highest dose level of PR-104 demonstrated two or more of six patients with DLT. A safety committee consisting of the investigators and the medical monitor could modify the dose escalation algorithm to add patients to a dose level (not to exceed 12 patients per dose level) in order to better define toxicity at that dose level and add an intermediate dose level to define an MTD closer to the dose level which exhibited DLT. In the event, the start of a cycle was delayed for toxicity

and patients were allowed to receive PR-104 at a reduced dose. Each dose reduction for PR-104 was at least 25% (e.g., no more than 75% of the current dose). Each patient was allowed a maximum of two dose reductions for PR-104. Any patient who required a third dose reduction for PR-104 or >4-week delay (e.g., unable to start a subsequent cycle, 8 weeks from the start of the prior cycle) in the start of a cycle was removed from study. At the discretion of the treating physician, a dose delay and/or reduction for any other toxicity experienced during the prior cycle was permitted.

Sorafenib dosing

Sorafenib dose was adjusted as per package insert. If patients continued to demonstrate unacceptable grade 3 or grade 4 adverse events thought to be related to sorafenib while receiving the lowest dose of 400 mg every 2 days, patients were removed from study.

Dose-limiting toxicity

DLT was assessed during the first cycle of PR-104 and defined as any one of the following: grade 4 thrombocytopenia (platelets < 25 K/mm³) of any duration, grade 4 hematologic toxicity (excluding thrombocytopenia) that lasts for ≥5 days (ANC ≤ 0.5 K/mm³, leukocytes < 1 K/mm³, hemoglobin < 6.5 g/dL), non-hematologic toxicity ≥ grade 3 despite appropriate supportive care, neutropenic fever, and grade 2 or higher neurotoxicity of ≥1-week duration. Any toxicity of grade 2 or higher that had not resolved within 4 weeks of the end of cycle 1 (except grade 2 alopecia) was reviewed by the safety committee for consideration of DLT. Toxicity was assessed in accordance with the National Cancer Institute Common Toxicity Criteria for Adverse Events (version 3.0).

Pharmacokinetics

During cycle 1, pharmacokinetics for PR-104 and PR-104 metabolites were evaluated prior to the start of sorafenib. Using a limited sampling strategy, blood samples were obtained before PR-104 administration, 30 min into the infusion, at end of the infusion, and at 30 min, 1 h, and 2 h after the end of the infusion. Sorafenib was started with the evening dose on the same day.

During cycle 2, pharmacokinetics for sorafenib, PR-104, and PR-104 metabolites were performed. Blood samples for sorafenib pharmacokinetics were obtained at least 3 h following the last administered dose of sorafenib. Blood samples for PR-104 pharmacokinetics were obtained at the same times as in cycle 1.

Sample preparation and analysis of PR-104 and its metabolites were undertaken using a validated LC/MS/MS method [16]. Non-compartmental pharmacokinetic variables were estimated using WinNonlin (v5.0).

Response

Disease assessment using computed tomography or magnetic resonance imaging was performed after every two cycles. Response was assessed by the investigators and confirmed by an independent radiologist using RECIST 1.1 [15].

Statistical analyses

Data were analyzed using descriptive statistics. The number of patients accrued on the study depended on the MTD and the toxicity observed. It was estimated that this would require from 9 to 15 patients. A paired t-test was used for comparison of cycle 1 and 2 (before and after in the same patient), for the pharmacokinetics of PR-104 and PR-104 metabolites.

Results

Demographics

Between April 30 and December 17, 2009, 14 patients were enrolled: 7 from Taiwan, 4 from Hong Kong, and 3 from the United States of America. All patients had Child-Pugh A score as per eligibility criteria. Half of the patients had some form or a combination of different loco-regional therapies, including surgery, chemoembolization, and ethanol injection. Demographics are shown in Table 1.

Treatment

Six patients were treated at the PR-104 770 mg/m² dose level. A total of 14 cycles were administered, with a median of 2 cycles (range 1–4). A dose de-escalation to 550 mg/m² followed. Eight patients were treated with a total of 19 cycles at that dose level, with a median of 3 cycles (range 1–3). At both dose levels of PR-104, the median daily dose of sorafenib was 400 mg (range, 200–800 mg).

Safety and tolerability

All 14 patients were evaluable for safety. With the first PR-104 dose cohort of 770 mg/m², one patient developed grade 3 hyperbilirubinemia. While this was not considered a DLT, the cohort was expanded to six patients to gain

Table 1 Demographics ($n = 14$)

Characteristic	No.	%
Age, years		
Median	60	
Range	27–79	
Sex		
Male	11	79
Female	3	21
Race		
Pacific Islander, Asian	12	86
White	2	14
ECOG performance status		
0	8	57
1	6	43
AFP > ULN		
Yes	11	79
No	3	21
Positive hepatitis status		
Hepatitis B	10	71
Hepatitis C	0	0
Alcohol	1	7
Unknown	3	21

additional experience at this dose. In the expanded cohort, one DLT of febrile neutropenia occurred during cycle 1. Despite the lack of any further DLTs, two patients, following administration of a second cycle, experienced grade 3 or 4 neutropenia lasting 7–14 days. In addition, three patients experienced grade 3 ($n = 2$) or 4 ($n = 1$) thrombocytopenia after receiving 2–4 cycles of PR-104 that continued for a median of 33 days (range 7–56 days) with recovery to only grade 2, but not to their baseline level. The patient with grade 4 thrombocytopenia required platelet transfusion support for nearly 1 month. One patient (17%) had cycle 2 dose reduced by 25% due to grade 3 thrombocytopenia and prolonged grade 3 neutropenia during the prior cycle.

In view of the worsening myelosuppression with repeated cycles, the safety committee determined that the 770 mg/m² dose exceeded the MTD, and PR-104 was subsequently de-escalated to a 550 mg/m² cohort of eight patients. There were no DLTs during cycle 1. Following treatment with a second cycle, two patients experienced grade 3 neutropenia (nadir at days 15 and 22 from start of cycle 2) with recovery in 7 days to a grade 1 or within normal limits. In addition, one patient experienced grade 3 thrombocytopenia following administration of one cycle of PR-104 and recovered to grade 2 after 21 days. Another patient had grade 4 thrombocytopenia after 3 cycles of PR-104. This thrombocytopenia had not recovered at the time

of last follow-up visit (30 days after administration of cycle 3). Three patients (38%) required at least a 1-week delay in the start of the next cycle and 25% dose reduction in PR-104 due to thrombocytopenia ($n = 2$) and liver enzyme elevations ($n = 1$).

Sorafenib required dose reductions in 4 patients (67%) on the 770 mg/m² PR-104 cohort. All eight patients on the 550 mg/m² PR-104 cohort required dose reduction of the sorafenib. Dose reductions in sorafenib were due to elevated liver function tests (aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase, and bilirubin), myelosuppression (neutropenia, thrombocytopenia, anemia), hand–foot skin reaction, fatigue, and hypokalemia.

Grade 3 or 4 events that occurred in $\geq 10\%$ on both cohorts are noted in Table 2. No deaths occurred while patients were active on the study.

Pharmacokinetics

Seven patients (2 at 770 mg/m² and 5 at 550 mg/m²) consented to PK sampling. The PK parameters for PR-104 and its major plasma metabolites for the 770 mg/m² cohort are summarized in Table 3, and for the 550 mg/m² cohort in Table 4. As previously reported in a general oncology population [17], the major plasma metabolites were PR-104A and its *O*-glucuronide PR-104G, with lower area under the curve (AUC) for the semi-mustard metabolite PR-104S1 (arising from oxidative debromoethylation of the nitrogen mustard moiety of PR-104A) and its activated reduced metabolites PR-104H and PR-104M. As shown in the Tables, there was no significant difference in the PK parameters for PR-104 or any of its metabolites between the first and second cycles (prior to and 1 month following commencement of sorafenib). The PK parameter considered to be of greatest pharmacodynamic significance is the AUC of PR-104A [18]; the value in cycle 1 determined here at 550 mg/m² (mean 19.7, SD 9.7 $\mu\text{g h/mL}$, Table 4) was twofold higher than for 12 patients treated at the same dose level in phase I studies in non-HCC cancer patients using the same analytical method [16], and calculating AUC using only data from the same sampling times as in the present study (mean 9.8, SD 6.2 $\mu\text{g h/mL}$). This difference was statistically significant ($P = 0.003$, *t*-test). Values of C_{max} and $T_{1/2}$ for PR-104A were also significantly higher in the HCC patients ($P = 0.002$ and $P < 0.001$ respectively).

Response

Based on independent radiology review, there were one partial response, and four stable disease of ≥ 8 weeks, in the 770 mg/m² PR-104 cohort. The partial response was commensurate with a major drop in alpha-fetoprotein

Table 2 Grade 3 or 4 events with $\geq 10\%$ occurrence

Adverse event (preferred term by organ system class)	PR-104 770 mg/m ² + sorafenib (<i>n</i> = 6)		PR-104 550 mg/m ² + sorafenib (<i>n</i> = 8)	
	Grade <i>n</i> (%)		Grade <i>n</i> (%)	
	3	4	3	4
Blood and lymphatic system				
Leukopenia	2 (33)		3 (38)	
Anemia			1 (13)	
Neutropenia	1 (17)	1 (17)	2 (25)	
Thrombocytopenia	2 (33)	1 (17)	1 (13)	1 (13)
Metabolism and nutrition				
Hypoalbuminemia			2 (25)	
Hyponatremia	3 (50)		1 (13)	
Hypokalemia		1 (17)	1 (13)	
Investigations				
Aspartate aminotransferase increased	1 (17)		4 (50)	
Alanine aminotransferase increased	2 (33)		3 (38)	
Blood alkaline phosphatase increased			2 (25)	
General disorders				
Fatigue			1 (13)	
Skin and subcutaneous tissue				
Palmar-plantar syndrome	2 (33)		1 (13)	
Hepatobiliary				
Hyperbilirubinemia			1 (13)	

Table 3 Plasma pharmacokinetics of PR-104 and its metabolites at 770 mg/m²

Parameter	N	Cycle	PR-104	PR-104A	PR-104G	PR-104S1	PR-104H	PR-104M
<i>C</i> _{max} (μg/mL)	2	1	7.1 ± 3.2	7.6 ± 1.9	9.6 ± 2.3	0.17 ± 0.15	0.21 ± 0.11	0.04 ± 0.02
	2	2	6.0 ± 3.3	7.6 ± 1.0	10.4 ± 2.2	0.18 ± 0.07	0.20 ± 0.05	0.04 ± 0.02
<i>P</i> value*			0.76	0.98	0.74	0.90	0.92	0.98
<i>T</i> _{1/2} (h)	2	1	0.15 ± 0.10	0.75 ± 0.35	0.62 ± 0.08	0.87 ± 0.11	1.37 ± 0.18	3.57 ± 0.82
	2	2	0.17 ± 0.11	0.85 ± 0.20	0.67 ± 0.17	0.79 ± 0.14	1.05 ± 0.35	02.93 ± 2.48
<i>P</i> value*			0.86	0.77	0.73	0.43	0.37	0.76
AUC (μg h/mL)	2	1	5.1 ± 3.2	11.0 ± 4.4	12.3 ± 1.3	0.26 ± 0.23	0.47 ± 0.27	0.22 ± 0.09
	2	2	4.3 ± 2.9	10.6 ± 0.1	15.8 ± 2.9	0.28 ± 0.10	0.42 ± 0.04	0.19 ± 0.17
<i>P</i> value*			0.8	0.92	0.26	0.93	0.80	0.87

Values are mean and SD

* Cycle 1 versus cycle 2

(AFP) from 1,238.1 ng/mL at baseline to a nadir of 50.56 ng/mL. Similar AFP reduction was noted in two other patients with stable disease. Due to study discontinuation, independent radiology review of the eight patients on the 550 mg/m² PR-104 cohort was not performed. Nonetheless, there were no partial responses, although five had stable disease lasting ≥ 8 weeks by the investigators' assessment. At the time of study closure, 4 of 14 patients had died; none were considered related to an adverse event. Survival status was not followed after the study closed.

Discussion

While only one DLT of febrile neutropenia occurred at the 770 mg/m² PR-104 dose level, its prolonged course and that of grade 3 and 4 thrombocytopenia seen in 3 of 6 patients in the cohort led the decision to evaluate the lower dose level of 550 mg/m² of PR-104 as per protocol. While no DLTs occurred at the 550 mg/m² dose level of PR-104, grade 3 neutropenia in two patients and grade 3 and 4 thrombocytopenia in 2 of 8 patients accrued to the cohort

Table 4 Plasma pharmacokinetics of PR-104 and its metabolites at 550 mg/m²

Parameter	N	Cycle	PR-104	PR-104A	PR-104G	PR-104S1	PR-104H	PR-104 M
$C_{\max}$ (μg/mL)	5	1	3.2 ± 1.1	15.8 ± 8.9	4.9	0.21 ± 0.18	0.72 ± 0.80	0.12 ± 0.12
	5	2	4.3 ± 0.7	14.6 ± 10.4	5.8	0.17 ± 0.17	0.69 ± 0.78	0.16 ± 0.13
<i>P</i> value*			0.31	0.85	ND [†]	0.73	0.95	0.91
$T_{1/2}$ (h)	5	1	0.10 ± 0.01	0.68 ± 0.04	0.53	0.69 ± 0.20	0.84 ± 0.34	1.02 ± 0.42
	5	2	0.09 ± 0.01	0.79 ± 0.23	0.50	0.90 ± 0.44	0.91 ± 0.25	0.99 ± 0.52
<i>P</i> value*			0.26	0.34	ND [†]	0.36	0.77	0.93
AUC (μg h/mL)	5	1	2.4 ± 0.8	19.7 ± 9.7	6.4	0.30 ± 0.23	0.81 ± 0.71	0.18 ± 0.13
	5	2	3.3 ± 0.6	22.7 ± 18.2	7.6	0.31 ± 0.21	1.07 ± 1.02	0.25 ± 0.18
<i>P</i> value*			0.27	0.76	ND [†]	0.97	0.67	0.52

Values are mean and SD

* Cycle 1 versus cycle 2

[†] Not determined for PR-104G due to the availability of only one dataset from each cycle

led to the decision to discontinue the study. The extent and significance of protracted and sometimes irreversible thrombocytopenia may have been underestimated in the initial phase I study evaluating PR-104 as a single agent [7], where DLT evaluation was limited to a scheduled 3-week observation for adverse events.

Neutropenia occurred earlier in the course of treatment and was more reversible than thrombocytopenia.

While the small number of patients may limit the interpretation of these important observations, one may wonder about the contribution of the hypoxic niche in bone marrow to the activation of PR-104A, analogous to the reported sensitivity of primitive hemopoietic stem cells sensitive to the hypoxia-activated benzotriazine N-oxide prodrug tirapazamine [19].

There are no data reported on a differential response of platelets and neutrophils to the hypoxia in the bone marrow, but one may consider that the presumed hypoxia-induced environment in HCC may also contribute to thrombocytopenia, in view of the usual abundance of platelets in the liver and spleen.

Comparison of plasma pharmacokinetic parameters for all analytes showed no statistically significant differences between cycle 1 and 2 ($P > 0.05$) at either dose level, suggesting no significant sorafenib effect on the plasma pharmacokinetics of PR-104. The PK results interpretation remains limited considering that the sorafenib PK was not analyzed once a decision was made to discontinue the study.

The metabolite profile was broadly similar in the predominantly Asian HCC population of this study compared with prior PR-104 phase I trials in a general oncology population [17]. A substantial and statistically significant twofold increased exposure to PR-104A in the HCC population compared to the populations of previous studies was noted, suggesting that the increased toxicity of PR-104

in the HCC population reported herein may reflect in part compromised clearance of PR-104A.

Despite the multiple intriguing scientific questions, safety concerns led to the halt of the development of PR-104 in combination with sorafenib in HCC.

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