Phase I/II study of the hypoxia-activated prodrug PR104 in refractory/relapsed acute myeloid leukemia and acute lymphoblastic leukemia

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Supplemental Information

Methods

Patients and methods

A Phase I/II study of PR104 monotherapy was conducted at The University of Texas MD Anderson Cancer Center (MD Anderson) and Fred Hutchinson Cancer Research Center (FHCRC) between February 2010 and May 2012. Institutional review board approval was obtained at each participating center and all patients provided informed consent (ClinicalTrials.gov Identifier: NCT 01037556).

Response to treatment was assessed by International Working Group response criteria.[18] Morphologic leukemia-free state (MLFS) required a BM blast level of <5%. For CR, the BM blasts had to be <5% with no leukemic blasts in peripheral blood and no evidence of extramedullary disease, neutrophils $>1\times10^9$/L, and platelets $\geq100\times10^9$/L. If recovery of platelets was incomplete, the response was CRp.

Statistical considerations

The dose-finding portion of the study utilized the covariate-adjusted outcome-adaptive Bayesian method of Thall *et al*.[19] to determine a recommended dose for specific subsets of patients as determined by 3 prognostic covariates: first CR duration (<52 weeks versus ≥52 weeks), number of prior induction regimens (1 versus 2), and age (as continuous variable). The 3 prognostic covariates were determined from an analysis of historical data on patients with AML at MD Anderson.

Toxicity was assessed throughout the study. During the course of the study, efficacy and toxicity data generated from each subject were used to update the posterior of the Bayesian model for determining the best dose of PR104 for each patient subgroup.

Unadjusted overall survival (OS) time distributions were estimated by using the method of Kaplan and Meier,[20] stratified by age (older than vs. younger than median age), CR1 duration (>6 months vs. ≤6 months), or dose (4 vs. 3 vs. ≤2.2 g/m^2). OS and progression-free survival (PFS) were compared between subgroups using the log rank test.[21] Associations between each response (CR, CRp, or MLFS) and covariates were assessed by Bayesian logistic regression, with computations carried out using the Bayesian subroutine of the software package SAS Proc

Logistic.[22] The Fisher exact test[23] and its generalizations[24] were used to assess associations between categorical variables, and the Wilcoxon rank sum test or Kruskal-Wallis test[25] was used to assess associations between continuous variables and categorical variables.

Biomarker studies

Biomarkers studies were optional and performed only in samples from patients consenting to these studies. Pimonidazole (PIMO) is approved under Investigative New Drug status as a diagnostic tracer for human tumor hypoxia. PIMO is a 2-nitroimidazole that undergoes metabolic reduction in hypoxic cells to generate stable intracellular adducts that can be detected by immunohistochemical staining.[1] For this study, PIMO was dissolved in 0.9% saline solution and infused over 20 minutes at a dosage of 0.5 g/m^2 16 ($\pm$6) hours prior to (BM) biopsy.

The expression of PIMO, Hypoxia-inducible factor 1 alpha (HIF-1α), and carbonic anhydrase IX (CAIX) was assessed in formalin-fixed, paraffin-embedded tissue of bone marrow (BM) biopsy specimens by using Hypoxyprobe-1 MAb1 (HPI, Cat #HP2-100kit), anti-HIF-1α (Novus, NB100-105) or CAIX (Novus, NB100-417) antibodies, respectively, by immunohistochemical methods as described previously.[2] The stained slides were reviewed independently by 2 authors (SK and CEB). Positive and negative cells were counted in 5 random high-power fields ($\times$400) and averaged. A tumor was considered positive if 10% or more of cells demonstrated nuclear (HIF) or membrane (CAIX) staining.

Enzymatic activity of aldo-keto reductase 1C3 (AKR1C3) was measured in peripheral blood samples or BM aspirates from subjects enrolled on the study by a method based on its ability to reduce the nonfluorescent probe coumberone to fluorescent coumberol as described elsewhere.[3,4] Because coumberone is also reduced by other members of the AKR1C family, the AKR1C3-specific inhibitor SN34037 was used to define the contribution of AKR1C3 to total coumberone reductase activity. SN34037-sensitive coumberol formation (i.e., AKR1C3-dependent coumberone metabolism) was determined as previously[4], calculated as the difference in coumberol formation without and with SN34037.

Supplemental Table 1. Overall summary of adverse effects reported by ≥15% of patients regardless of attribution by dose levels and by maximum grades

		Dose Levels			
Toxic Effect	**Grade**	**1.1-2.2 g/m^2 (N=8)**	**3.0 g/m^2 (N=20)**	**4.0 g/m^2 (N=22)**	**Total (N=50)**
		N (%)	**N (%)**	**N (%)**	**N (%)**
Hypotension	Grade 1/2	0	8 (40)	7 (32)	15 (30)
	Grade ≥3	0	2 (10)	2 (9)	4 (8)
Abdominal pain	Grade 1/2	0	3 (15)	4 (18)	7 (14)
	Grade ≥3	0	3 (15)	1 (5)	4 (8)
Anorexia	Grade 1/2	1 (12)	4 (20)	5 (23)	10 (20)
	Grade ≥3	0	1 (5)	0	1 (2)
Constipation	Grade 1/2	1 (12)	4 (20)	3 (14)	8 (16)
	Grade ≥3	0	0	0	0
Cough	Grade 1/2	2 (25)	5 (25)	3 (14)	10 (20)
	Grade ≥3	0	0	0	0
Dehydration	Grade 1/2	1 (12)	4 (20)	3 (14)	8 (16)
	Grade ≥3	0	0	1 (5)	1 (2)
Diarrhea	Grade 1/2	5 (62)	9 (45)	15 (68)	29 (58)
	Grade ≥3	0	2 (10)	1 (5)	3 (6)
Dysgeusia	Grade 1/2	2 (25)	2 (10)	5 (23)	9 (18)
	Grade ≥3	0	0	0	0
Dyspnea	Grade 1/2	2 (25)	4 (20)	2 (9)	8 (16)
	Grade ≥3	0	0	0	0
Edema, limbs	Grade 1/2	2 (25)	8 (40)	6 (27)	16 (32)
	Grade ≥3	1 (12)	1 (5)	0	2 (4)
Fatigue	Grade 1/2	3 (38)	4 (20)	10 (45)	17 (34)
	Grade ≥3	1 (12)	1 (5)	1 (5)	3 (6)
Febrile neutropenia	Grade 1/2	0	0	0	0
	Grade ≥3	5 (62)	12 (60)	10 (45)	27 (54)
Generalized muscle weakness	Grade 1/2	1 (12)	3 (15)	4 (18)	8 (16)
	Grade ≥3	0	0	0	0
Infections	Grade 1/2	0	1 (5)	0	1 (2)
	Grade ≥3	1 (12)	3 (15)	5 (23)	9 (18)
Infusion-related reaction	Grade 1/2	3 (38)	3 (15)	5 (23)	11 (22)
	Grade ≥3	0	0	0	0
Lung infection	Grade 1/2	2 (25)	0	1 (5)	3 (6)
	Grade ≥3	2 (25)	9 (45)	6 (27)	17 (34)
Nausea	Grade 1/2	3 (38)	9 (45)	10 (45)	22 (44)
	Grade ≥3	0	0	1 (5)	1 (2)
Sepsis	Grade 1/2	0	0	1 (5)	1 (2)

	Grade ≥3	1 (12)	4 (20)	2 (9)	7 (14)
Vomiting	Grade 1/2	3 (38)	6 (30)	9 (41)	18 (36)
	Grade ≥3	1 (12)	0	0	1 (2)
Weight loss	Grade 1/2	3 (38)	6 (30)	0	9 (18)
	Grade ≥3	0	0	0	0
Anemia	Grade 1/2	2 (25)	1 (5)	3 (14)	6 (12)
	Grade ≥3	2 (25)	16 (80)	15 (68)	33 (66)
Neutrophil count decreased	Grade 1/2	0	0	1 (5)	1 (2)
	Grade ≥3	4 (50)	13 (65)	12 (55)	29 (58)
Platelet count decreased	Grade 1/2	0	0	1 (5)	1 (2)
	Grade ≥3	3 (38)	13 (65)	10 (45)	26 (52)
White blood cell count decreased	Grade 1/2	0	0	0	0
	Grade ≥3	6 (75)	15 (75)	17 (77)	38 (76)
Lymphocyte count decreased	Grade 1/2	1 (12)	0	1 (5)	2 (4)
	Grade ≥3	5 (62)	15 (75)	18 (82)	38 (76)
Bilirubin increased	Grade 1/2	4 (50)	7 (35)	11 (50)	22 (44)
	Grade ≥3	0	2 (10)	2 (9)	4 (8)
Creatinine increased	Grade 1/2	5 (62)	5 (25)	6 (27)	16 (32)
	Grade ≥3	0	2 (10)	0	2 (4)
Hypoalbuminemia	Grade 1/2	6 (75)	9 (45)	16 (73)	31 (62)
	Grade ≥3	1 (12)	3 (15)	3 (14)	7 (14)
Hyperglycemia	Grade 1/2	4 (50)	11 (55)	14 (64)	29 (58)
	Grade ≥3	0	4 (20)	1 (5)	5 (10)

Supplemental Table 2. Summary of all treatment-related adverse effects by dose level and by grade (grade 1/2 vs. grade 3/4/5)

		Dose Level			
Adverse Effect	**Grade**	**1.1-2.2 g/m^2 (N=8)**	**3 g/m^2 (N=20)**	**4 g/m^2 (N=22)**	**Total (N=50)**
		N (%)	**N (%)**	**N (%)**	**N (%)**
Gastrointestinal disorders					
Nausea	Grade 1/2	1 (12)	6 (30)	6 (27)	13 (26)
	Grade ≥3	0 (0)	0 (0)	1 (5)	1 (2)
Vomiting	Grade 1/2	2 (25)	4 (20)	5 (23)	11 (22)
	Grade ≥3	1 (12)	0 (0)	0 (0)	1 (2)
Diarrhea	Grade 1/2	2 (25)	7 (35)	9 (41)	18 (36)
	Grade ≥3	0 (0)	2 (10)	1 (5)	3 (6)
Mucositis, oral	Grade 1/2	0 (0)	1 (5)	1 (5)	2 (4)
	Grade ≥3	0 (0)	0 (0)	0 (0)	0 (0)
Esophagitis	Grade 1/2	0 (0)	0 (0)	0 (0)	0 (0)
	Grade ≥3	0 (0)	0 (0)	1 (5)	1 (2)
Gastritis	Grade 1/2	0 (0)	0 (0)	0 (0)	0 (0)
	Grade ≥3	0 (0)	0 (0)	1 (5)	1 (2)
Enteritis	Grade 1/2	0 (0)	0 (0)	0 (0)	0 (0)
	Grade ≥3	0 (0)	3 (15)	1 (5)	4 (8)
Colitis	Grade 1/2	0 (0)	0 (0)	0 (0)	0 (0)
	Grade ≥3	0 (0)	0 (0)	1 (5)	1 (2)
Enterocolitis	Grade 1/2	0 (0)	0 (0)	0 (0)	0 (0)
	Grade ≥3	0 (0)	0 (0)	1 (5)	1 (2)
Small intestine obstruction	Grade 1/2	0 (0)	0 (0)	0 (0)	0 (0)
	Grade ≥3	0 (0)	0 (0)	1 (5)	1 (2)
Hepatic failure	Grade 1/2	0 (0)	0 (0)	0 (0)	0 (0)
	Grade ≥3	0 (0)	0 (0)	1 (5)	1 (2)
Abdominal pain	Grade 1/2	0 (0)	1 (5)	2 (9)	3 (6)
	Grade ≥3	0 (0)	1 (5)	0 (0)	1 (2)
Anorexia	Grade 1/2	1 (12)	2 (10)	1 (5)	4 (8)
	Grade ≥3	0 (0)	1 (5)	0 (0)	1 (2)
Dysgeusia	Grade 1/2	2 (25)	2 (10)	3 (14)	7 (14)
	Grade ≥3	0 (0)	0 (0)	0 (0)	0 (0)
Dyspepsia	Grade 1/2	0 (0)	0 (0)	2 (9)	2 (4)
	Grade ≥3	0 (0)	0 (0)	0 (0)	0 (0)
Constipation	Grade 1/2	0 (0)	1 (5)	1 (5)	2 (4)
	Grade ≥3	0 (0)	0 (0)	0 (0)	0 (0)
Infections					
Febrile neutropenia	Grade 1/2	0 (0)	0 (0)	0 (0)	0 (0)

	Grade ≥3	2 (25)	3 (15)	5 (23)	10 (20)
Sepsis	Grade 1/2	0 (0)	0 (0)	0 (0)	0 (0)
	Grade ≥3	0 (0)	1 (5)	1 (5)	2 (4)
Lung infection	Grade 1/2	0 (0)	0 (0)	0 (0)	0 (0)
	Grade ≥3	1 (12)	1 (5)	2 (9)	4 (8)
Mucosal infection	Grade 1/2	1 (12)	1 (5)	0 (0)	2 (4)
	Grade ≥3	1 (12)	0 (0)	0 (0)	1 (2)
Catheter-related infection	Grade 1/2	1 (12)	0 (0)	0 (0)	1 (2)
	Grade ≥3	0 (0)	0 (0)	0 (0)	0 (0)
Other infection	Grade 1/2	0 (0)	0 (0)	0 (0)	0 (0)
	Grade ≥3	1 (12)	0 (0)	1 (5)	2 (4)
Other					
Fever	Grade 1/2	0 (0)	0 (0)	0 (0)	0 (0)
	Grade ≥3	0 (0)	1 (5)	0 (0)	1 (2)
Rash	Grade 1/2	0 (0)	1 (5)	1 (5)	2 (4)
	Grade ≥3	0 (0)	0 (0)	1 (5)	1 (2)
Pruritis	Grade 1/2	0 (0)	1 (5)	0 (0)	1 (2)
	Grade ≥3	0 (0)	0 (0)	0 (0)	0 (0)
Alopecia	Grade 1/2	0 (0)	1 (5)	0 (0)	1 (2)
	Grade ≥3	0 (0)	0 (0)	0 (0)	0 (0)
Dehydration	Grade 1/2	1 (12)	1 (5)	1 (5)	3 (6)
	Grade ≥3	0 (0)	0 (0)	0 (0)	0 (0)
Anxiety	Grade 1/2	0 (0)	0 (0)	1 (5)	1 (2)
	Grade ≥3	0 (0)	0 (0)	0 (0)	0 (0)
Back pain	Grade 1/2	1 (12)	0 (0)	0 (0)	1 (2)
	Grade ≥3	0 (0)	0 (0)	0 (0)	0 (0)
Bone marrow hypocellular	Grade 1/2	0 (0)	0 (0)	0 (0)	0 (0)
	Grade ≥3	0 (0)	3 (15)	0 (0)	3 (6)
Chills	Grade 1/2	0 (0)	0 (0)	0 (0)	0 (0)
	Grade ≥3	0 (0)	1 (5)	0 (0)	1 (2)
Cough	Grade 1/2	0 (0)	1 (5)	0 (0)	1 (2)
	Grade ≥3	0 (0)	0 (0)	0 (0)	0 (0)
Dizziness	Grade 1/2	1 (12)	0 (0)	2 (9)	3 (6)
	Grade ≥3	0 (0)	0 (0)	0 (0)	0 (0)
Dyspnea	Grade 1/2	1 (12)	0 (0)	0 (0)	1 (2)
	Grade ≥3	0 (0)	0 (0)	0 (0)	0 (0)
Edema, limbs	Grade 1/2	0 (0)	1 (5)	0 (0)	1 (2)
	Grade ≥3	0 (0)	0 (0)	0 (0)	0 (0)
Epistaxis	Grade 1/2	0 (0)	1 (5)	0 (0)	1 (2)
	Grade ≥3	0 (0)	0 (0)	0 (0)	0 (0)
Fatigue	Grade 1/2	3 (38)	2 (10)	6 (27)	11 (22)

	Grade ≥3	0 (0)	1 (5)	1 (5)	2 (4)
Flu-like symptoms	Grade 1/2	1 (12)	0 (0)	1 (5)	2 (4)
	Grade ≥3	0 (0)	0 (0)	0 (0)	0 (0)
Flushing	Grade 1/2	0 (0)	0 (0)	1 (5)	1 (2)
	Grade ≥3	0 (0)	0 (0)	0 (0)	0 (0)
Generalized muscle weakness	Grade 1/2	1 (12)	1 (5)	1 (5)	3 (6)
	Grade ≥3	0 (0)	0 (0)	0 (0)	0 (0)
Headache	Grade 1/2	0 (0)	2 (10)	1 (5)	3 (6)
	Grade ≥3	0 (0)	0 (0)	0 (0)	0 (0)
Hematuria	Grade 1/2	0 (0)	0 (0)	1 (5)	1 (2)
	Grade ≥3	0 (0)	0 (0)	0 (0)	0 (0)
Hypoxia	Grade 1/2	0 (0)	0 (0)	0 (0)	0 (0)
	Grade ≥3	0 (0)	0 (0)	1 (5)	1 (2)
Infusion-related reaction	Grade 1/2	3 (38)	1 (5)	5 (23)	9 (18)
	Grade ≥3	0 (0)	0 (0)	0 (0)	0 (0)
Nervous system disorders	Grade 1/2	0 (0)	1 (5)	1 (5)	2 (4)
	Grade ≥3	0 (0)	0 (0)	0 (0)	0 (0)
Noncardiac chest pain	Grade 1/2	0 (0)	1 (5)	0 (0)	1 (2)
	Grade ≥3	0 (0)	0 (0)	0 (0)	0 (0)
Pain in extremity	Grade 1/2	0 (0)	0 (0)	1 (5)	1 (2)
	Grade ≥3	0 (0)	0 (0)	0 (0)	0 (0)
Pleural effusion	Grade 1/2	0 (0)	1 (5)	0 (0)	1 (2)
	Grade ≥3	0 (0)	0 (0)	0 (0)	0 (0)
Skin and subcutaneous tissue disorders	Grade 1/2	0 (0)	0 (0)	1 (5)	1 (2)
	Grade ≥3	0 (0)	0 (0)	0 (0)	0 (0)
Syncope	Grade 1/2	0 (0)	0 (0)	0 (0)	0 (0)
	Grade ≥3	0 (0)	1 (5)	0 (0)	1 (2)
Urine output decreased	Grade 1/2	0 (0)	1 (5)	0 (0)	1 (2)
	Grade ≥3	0 (0)	0 (0)	0 (0)	0 (0)
Vaginal hemorrhage	Grade 1/2	0 (0)	1 (5)	0 (0)	1 (2)
	Grade ≥3	0 (0)	0 (0)	0 (0)	0 (0)
Weight loss	Grade 1/2	1 (12)	1 (5)	0 (0)	2 (4)
	Grade ≥3	0 (0)	0 (0)	0 (0)	0 (0)

Supplemental Table 3. Analysis of the association between neutropenia and covariates using Bayesian logistic regression model. The number of prior induction therapies was significantly associated with the incidence of neutropenia.

Analysis of Maximum Likelihood Parameter Estimates							
Parameter	**DF**	**Estimate**	**Standard Error**	**Likelihood Ratio 95% Confidence Limits**		**Wald Chi-Square**	**Pr > ChiSq**
Intercept1	1	-6.0634	6.9597	-20.4373	7.5571	0.76	0.3836
Intercept2	1	-5.9672	6.9594	-20.3406	7.6530	0.74	0.3912
Intercept3	1	-5.8677	6.9576	-20.2376	7.7499	0.71	0.3990
Logdose	1	0.4700	0.7692	-1.0706	2.0275	0.37	0.5412
Age	1	0.0065	0.0234	-0.0400	0.0536	0.08	0.7817
first_CR_duration	1	-0.0189	0.0209	-0.0629	0.0245	0.81	0.3672
Prior_induction	1	1.2800	0.5642	0.3463	2.5707	5.15	0.0233
Scale	0	1.0000	0.0000	1.0000	1.0000		

DF, degrees of freedom; CR, complete remission.

Supplemental Table 4. Characteristics of Responding Patients (N=12)

Pt. No.	Diagnosis, Cytogenetics	Age, years and Sex	No. Prior Regimens	Prior SCT	CR1 Duration (weeks)	PR104 Dose (g/m^2)	No. Cycles	PR104 Response
182-1009	AML (de novo) Del(20)	58 male	HD cytarabine + anthracycline	Busulfan/ fludarabine	<52	3.0	1	CRp (SCT)
183-1005	AML (de novo)	52 female	SD cytarabine + anthracycline	Mini-TBI; treosulfan + fludarabine	<52	3.0	2	MLFS
183-1009	AML (high-risk MDS)	45 male	1) SD cytarabine + anthracycline; 2) G-CLAC (G-CSF, clofarabine, cytarabine)	None	<52	4.0	1	MLFS
183-1010	AML (de novo)	66 male	1) SD cytarabine + anthracycline 2) SD cytarabine + anthracycline	None	>52	4.0	1	CRp
183-1011	AML (high-risk MDS)	56 female	1) HD cytarabine + anthracycline + pravastatin; 2) azacitidine	None	<52	4.0	1	CRp (SCT)
182-1014	AML (secondary, h/o CMML) del(12)	64 male	HD cytarabine + anthracycline × 2 (induction-consolidation)	None	<52	4.0	1	MLFS (SCT)
182-1023	AML (high-risk MDS) +8	66 male	BIDFA (Fludarabine, cytarabine) × 1	None	<52	4.0	3*	CRp
182-1032	AML (de novo) Complex including -7	66 Female	Clofarabine+LD cytarabine × 3, decitabine × 1	CBT (fludarabine /cytarabine/TBI)	<52	3.0	2	MLFS
182-1033	AML (de novo) Del(8)	58 Female	Fludarabine, cytarabine, idarubicine × 1	None	<52	3.0	1	MLFS (SCT)
182-1034	AML (de novo) Normal	79 Male	Omacetaxine and LD cytarabine × 1	None	<52	3.0	3**	CR
182-1018	B-ALL t(9;22)	61 male	4 prior treatment regimens	None	<52	3.0	2	MLFS
182-1020	B-ALL Complex, t(9;22)	30 male	3 prior treatment regimens	Consolidation w/ SCT	>52	4.0	2*	CRp

SCT, stem cell transplantation; CR1, first complete remission; HD, high dose; CRp, complete remission without platelet recovery; SD, standard dose; TBI, total body irradiation; MLFS, morphologic leukemia-free state; MDS, myelodysplastic syndrome; CMML, chronic myelomonocytic leukemia; LD, low dose; CBT, cord blood transplant.

*1 cycle consolidation at 2.0 g/m^2

**1 induction, 2 consolidations at 1.5 g/m^2

Supplemental Table 5. Regression analysis of overall survival from the start of treatment*

	Posterior Values					
			Percentiles			
Parameter	**Mean**	**Standard Deviation**	**25%**	**50%**	**75%**	**Pr (parameter > 0 | data)**
Intercept	7.0811	2.8246	5.1980	7.0953	8.9862	-
Logdose	-0.1420	0.3337	-0.3667	-0.1436	0.0801	0.33
Age	-0.0160	0.0100	-0.0227	-0.0161	-0.00924	0.052
CR1 duration	-0.00615	0.00902	-0.0122	-0.00627	-0.00012	0.24
Number of prior inductions	-0.1481	0.1485	-0.2461	-0.1467	-0.0510	0.15
Scale	0.9779	0.1170	0.8956	0.9673	1.0490	--

*Analysis was carried out by using a Bayesian log normal survival regression model with covariates age, log(dose), first complete remission (CR1) duration (>6 months vs. ≤6 months), and number of prior inductions. Non-informative normal (mean=0, sd=10) prior distributions for the regression coefficients and a gamma (0.001, 0.001) for the variance were assumed (N=49). The fitted Bayesian log normal multivariate regression model agrees with the log rank tests in that none of the covariates were associated with overall survival, as all 95% posterior credible intervals contained 0.

Parameter	**95% Highest Posterior Density Credible Interval**	
	Lower Limit	Upper Limit
Logdose	-0.8122	0.4939
Age	-0.0354	0.00346
CR1 duration	-0.0232	0.0119
Number of prior inductions	-0.4270	0.1412
Scale	0.7684	1.2215

Supplemental Table 6. Analysis of progression-free survival from the start of treatment*

	Posterior values					
Parameter	**Mean**	**Standard Deviation**	**Percentiles**			
			25%	**50%**	**75%**	**Pr (parameter > 0 | data)**
Intercept	8.1747	2.5424	6.4864	8.1582	9.8531	-
Logdose	-0.3130	0.3003	-0.5122	-0.3121	-0.1158	0.14
Age	-0.0122	0.00870	-0.0180	-0.0122	-0.00639	0.08
CR1 duration	-0.00662	0.00787	-0.0119	-0.00665	-0.00139	0.20
Number of prior inductions	-0.1702	0.1299	-0.2555	-0.1707	-0.0826	0.09
Scale	0.8633	0.0999	0.7924	0.8536	0.9237	--

*Analysis was carried out by using a Bayesian log normal survival regression model with covariates age, log(dose), first complete remission (CR1) duration (>6 months vs. ≤6 months), and number of prior inductions. Non-informative normal (mean=0, sd=10) prior distributions for the regression coefficients and a gamma (0.001, 0.001) for the variance were assumed (N=49). The fitted Bayesian log normal multivariate regression model agrees with the log rank tests in that none of the covariates were associated with progression-free survival, as all 95% posterior credible intervals contained 0.

Parameter	**95% Highest Posterior Density Credible Interval**	
	Lower Limit	Upper Limit
Logdose	-0.9089	0.2730
Age	-0.0303	0.00416
CR1 duration	-0.0222	0.00872
Number of prior inductions	-0.4127	0.0841
Scale	0.6879	1.0692

Supplemental Table 7. Non-compartmental plasma pharmacokinetic parameters for PR-104 and its major metabolites (PR-104A and PR-104G)[a]

Patient ID	Dose (g/m^2)	PR-104			PR-104A			PR-104G		
		C_{max} (µM)	AUC (µM-h)	$T_{1/2}$ (h)	C_{max} (µM)	AUC (µM-h)	$T_{1/2}$ (h)	C_{max} (µM)	AUC (µM-h)	$T_{1/2}$ (h)
182-1003	1.1	27.42	19.15	0.148	19.49	30.88	0.52	10.61	20.97	0.58
183-1001	1.1	17.58	10.18	0.164	13.40	16.95	0.52	1.61	2.62	0.57
183-1003	1.1	43.09	33.76	0.116	16.18	22.96	0.46	4.12	6.21	0.53
	Mean	**29.36**	**21.03**	**0.14**	**16.36**	**23.59**	**0.50**	**5.44**	**9.93**	**0.56**
	SD	**12.86**	**11.90**	**0.02**	**3.05**	**6.99**	**0.04**	**4.65**	**9.73**	**0.03**
182-1004	1.6	16.79	20.19	0.081	45.40	74.64	0.52	27.97	-[b]	-[b]
182-1005	2.2	89.89	79.94	0.096	83.42	106.2	0.58	65.48	173.3	1.30
182-1007	3.0	42.79	27.22	0.132	62.05	73.13	0.50	53.94	85.14	0.70
182-1009	3.0	32.09	29.22	0.096	81.40	106.3	0.51	53.49	103.8	0.92
183-1005	3.0	121.1	81.40	0.120	74.22	119.0	0.56	31.43	50.82	0.66
183-1008	3.0	116.6	81.87	0.134	93.35	124.0	0.70	96.63	136.8	0.62
182-1016	3.0	156.5	122.5	0.107	85.12	132.8	0.71	93.65	142.49	0.78
182-1018	3.0	94.06	63.66	0.164	79.32	112.9	0.44	106.8	206.0	0.85
182-1021	3.0	10.05	9.72	0.148	54.67	63.83	0.26	135.1	190.5	0.38
182-1022	3.0	56.56	38.46	0.178	109.8	180.7	0.49	145.9	361.1	0.95
182-1031	3.0	84.95	51.56	0.423	82.04	107.0	0.87	96.24	309.5	1.55
182-1035	3.0	164.5	162.3	0.266	129.7	161.3	0.59	147.4	238.9	0.55
182-1039	3.0	45.67	40.32	0.175	73.61	149.9	0.92	86.92	236.7	1.36
	Mean	**84.08**	**64.38**	**0.18**	**84.12**	**121.0**	**0.60**	**95.21**	**187.4**	**0.85**
	SD	**51.34**	**45.36**	**0.09**	**21.03**	**35.0**	**0.19**	**38.20**	**95.5**	**0.35**
183-1009	4.0	68.24	37.53	0.159	57.45	67.53	0.61	170.37	268.6	0.81
182-1014	4.0	60.08	57.79	0.105	72.90	81.52	0.50	114.12	180.6	0.54
182-1015	4.0	69.15	38.04	0.145	71.09	78.12	0.50	129.79	129.8	0.45
182-1024	4.0	48.00	31.81	0.173	52.01	108.9	0.86	92.34	166.4	0.65
182-1025	4.0	82.53	50.09	0.503	216.9	222.0	0.83	165.3	464.9	1.32
182-1029	4.0	245.5	213.1	0.289	128.2	190.9	0.62	269.7	603.6	1.30
182-1036	4.0	210.1	158.7	0.389	298.4	337.7	0.66	131.3	267.4	0.74
182-1037	4.0	82.11	63.41	0.499	76.08	142.1	1.22	114.2	258.2	0.92
182-1041	4.0	77.17	86.33	0.169	71.63	92.32	0.46	35.74	240.6	0.51
	Mean	**104.8**	**81.86**	**0.27**	**116.1**	**146.8**	**0.70**	**135.9**	**286.7**	**0.80**
	SD	**71.2**	**62.70**	**0.16**	**85.6**	**89.3**	**0.24**	**64.1**	**152.5**	**0.32**

[a] Non-compartmental pharmacokinetic analyses was undertaken with WinNonLin

(v4.0.1) to estimate the maximum plasma concentration (Cmax), area under the plasma concentration time curve extrapolated to infinity (AUC), clearance (Cl), and elimination half-life (t1/2).

[b] insufficient data to estimate

Supplemental Table 8. Association of the area under the PR-104A concentration-time curve (AUC) with PR-104A dose.

PR-104 dose (g/m^2)	N	PR-104A AUC (μM-h)		P (versus 1.1 g/m^2)
		Mean (std)	Median (range)	
1.1	8	32.4 (13.7)	26.69 (17.0-59.6)	-
3.0	11	121.0 (33.3)	119.0	<0.05
4.0	9	146.8 (84.1)	108.9	<0.05

Values for the three PR-104 dose levels are plotted in Fig. 2, and include 5 solid tumor oncology patients treated at 1.1 g/m^2 PR-104 reported previously [5]. Statistical tests were undertaken with Sigmaplot version 12.5. The distribution of PR-104A AUC values did not satisfy the Shapiro-Wilk test for normality. Significance of associations of PR-104A AUC with PR-104 dose were therefore evaluated using Kruskal-Wallace One Way Analysis of Variance on Ranks, and pairwise comparisons tested with Dunn's method.

Supplemental Table 9. Lack of association of area under the PR-104A concentration-time curve (AUC) and response/toxicity.

Covariate	Response	N	Mean (std)	Median Range	P_KW
PK_PR104A	CR	0	. (.)	. (., .)	0.98765
	CRp	1	106.3 (.)	106.3 (106.3, 106.3)	.
	Fail	22	120.7 (93.4)	106.6 (11.0, 358.2)	.
	MLFS	5	92.0 (22.6)	81.5 (67.5, 119.0)	.

Covariate	Yes/No	N	Mean (std)	Median Range	PT2_WIL
Diarrhea	no	13	122.8 (88.1)	107.0 (16.9, 358.2)	1.000
	yes	13	117.0 (84.7)	106.3 (11.0, 337.7)	.
Febrile neutropenia	no	22	117.7 (93.0)	106.3 (11.0, 358.2)	.6779
	yes	6	105.4 (35.7)	100.3 (67.5, 161.3)	.
Nausea	no	22	117.8 (91.1)	93.9 (11.0, 358.2)	.7605
	yes	6	105.3 (52.3)	112.7 (23.0, 180.7)	.
Vomiting	no	22	123.7 (91.3)	107.9 (11.0, 358.2)	.3931
	yes	6	83.5 (35.3)	92.6 (23.0, 119.0)	.

Wilcoxon (WIL) rank sum test or Kruskal–Wallis (KW) test [6] were used to assess associations between PR104A AUC and a categorical variables. For toxicity, the analyses was limited to the 4 most frequent toxicities: diarrhea, febrile neutropenia, nausea, and vomiting.

SUPPLEMENTAL FIGURES

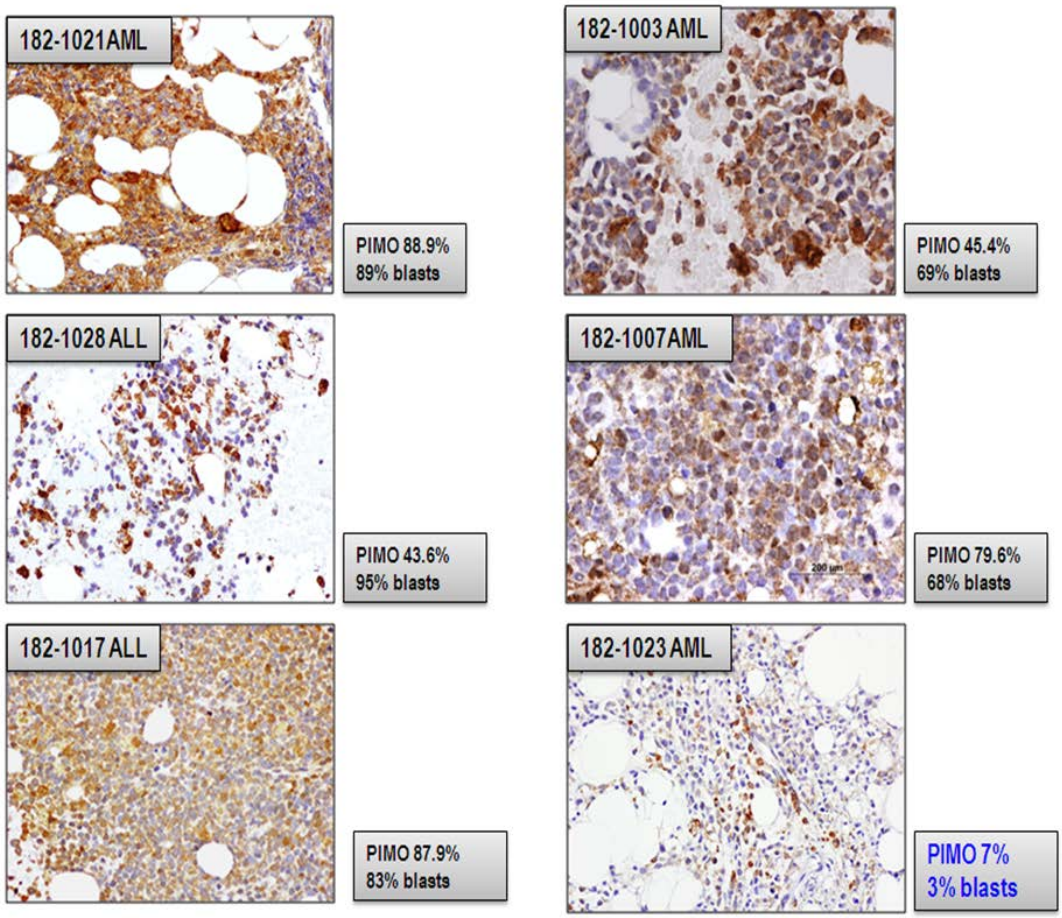
Supplemental Figure 1. Immunohistochemical detection of PIMO in BM biopsy specimens, with corresponding percentage blasts. Patient 182-1023 had a response to PR104 (specimen shown is day 42).

Supplemental Figure 2. Correlations between proportions of HIF-1α, CAIX and PIMO-positive cells and percentage BM blasts at baseline (Bl; A, B, E) or after PR104 (FU; C, D, F).

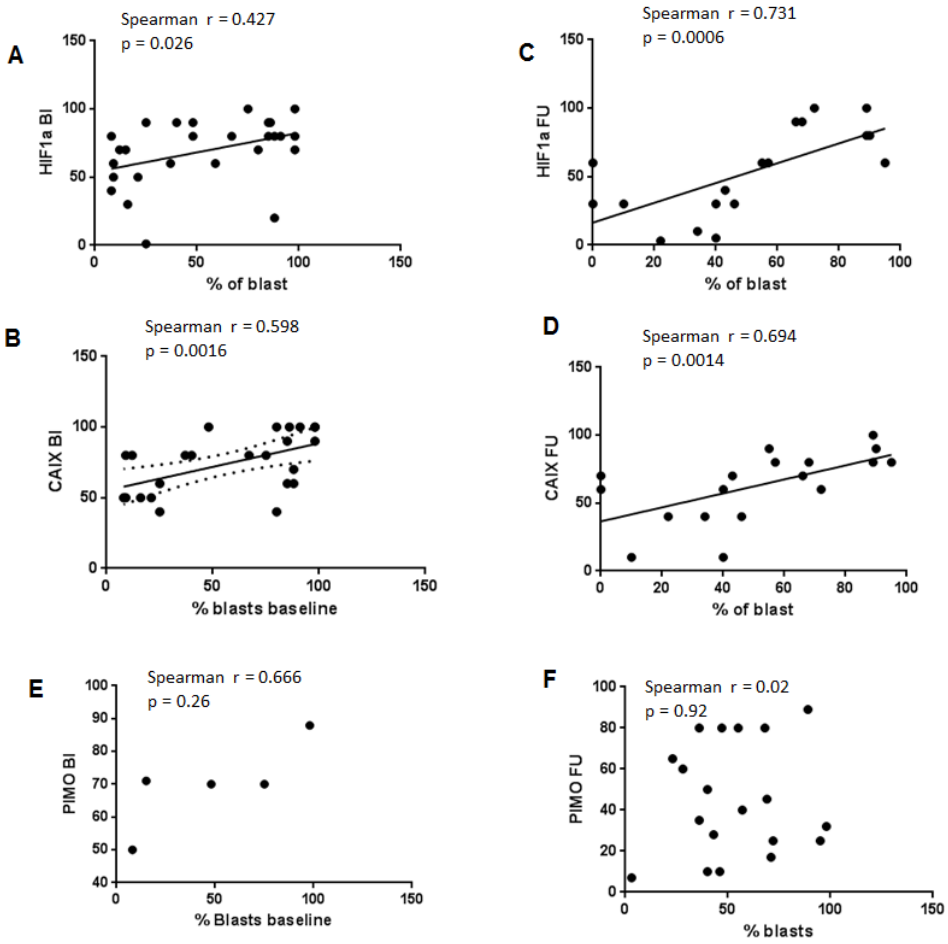
Supplemental Figure 3. Correlations between proportions of HIF-1α, CAIX and PIMO-positive cells at baseline (Bl; A, B) or after PR104 (FU; C, D).

Supplemental Figure 4. Top panel, Hematoxylin and eosin staining (HE) and PIMO immunohistochemical staining (IHC) at baseline (left) and on day 42 of PR104 (right) in a patient who achieved CRp (1023). Original magnification is shown in grey boxes. Bottom panel, proportions of HIF-1α– and CAIX-positive cells before and after PR104. BM blasts, 8% vs 3% before and after treatment.

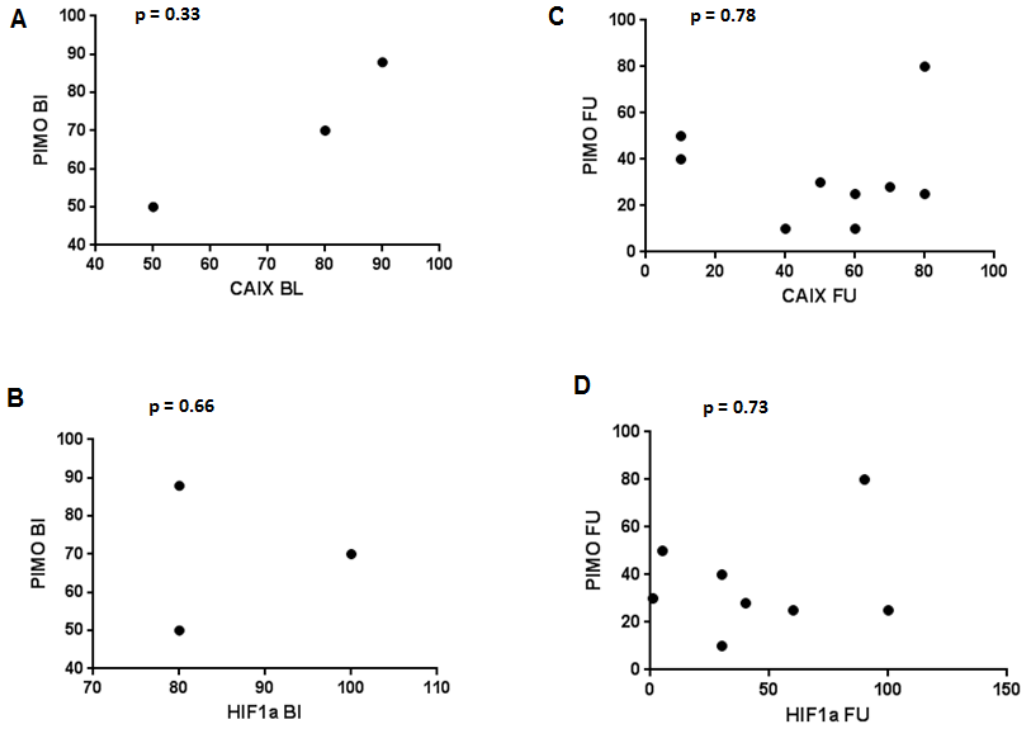
Supplemental Fig 1

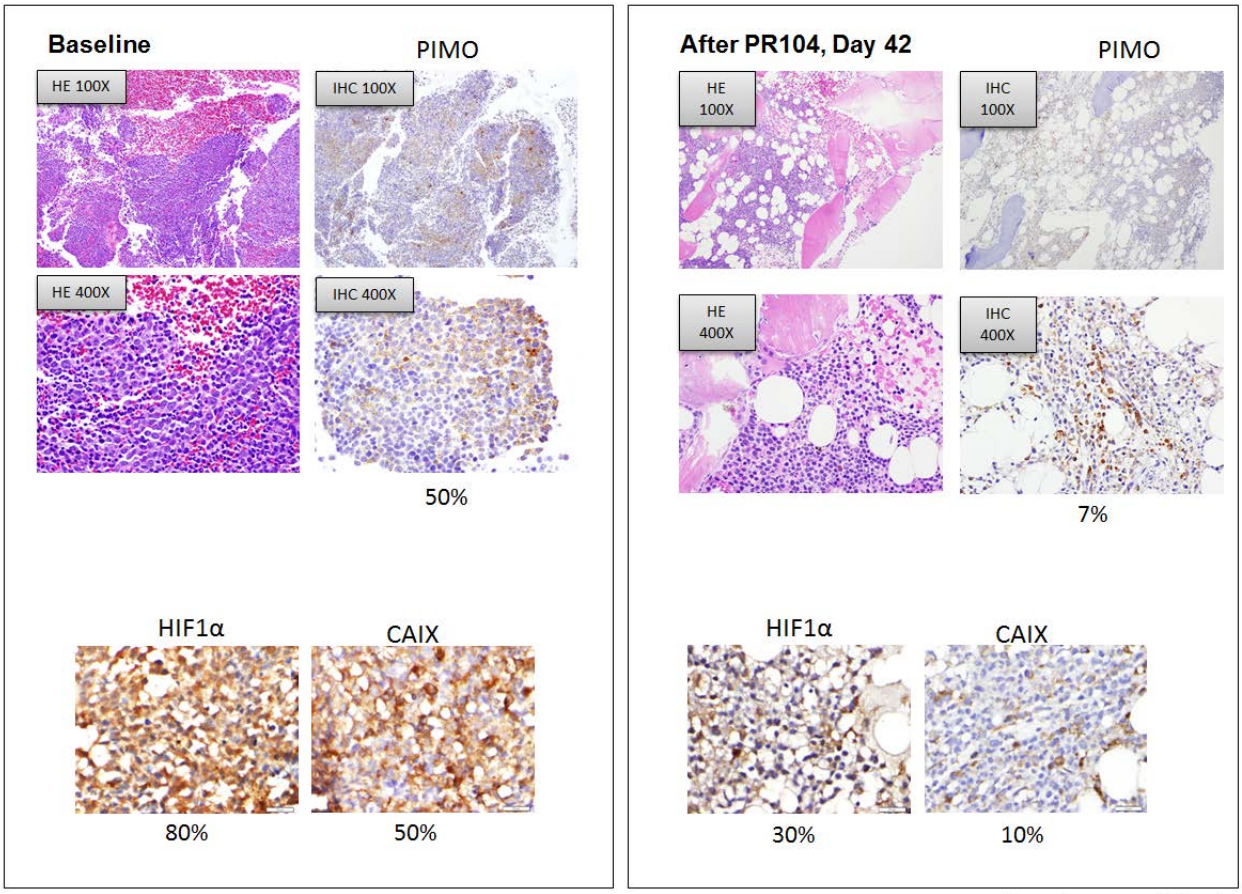


Supplemental Fig 2



Supplemental Fig 3





Supplemental Fig 4

SUPPLEMENTAL REFERENCES

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