Supplemental Methods and Results not presented in Manuscript

Statistical Considerations

The effects of PTC299 on serum and plasma VEGF, VEGFR and cytokine profiles were evaluated comparing Day 28 values with pre-treatment values and with the lowest value subsequent to Day 28, and differences tested with Wilcoxon signed rank tests. Tumor IHC expression for the target molecule expression was counted as number of positive cells per high power field and an index value derived from 10 fields. A 50% change was considered a significant biologic response. The proportion of participants with biologic response was expressed as a binomial proportion with exact 95% CIs. Effects of PTC299 on tumor cell proliferation via Ki67 IHC were assessed with the Wilcoxon sign test. Changes in circulating concentrations of angiogenic factors, cytokines, HIV and KSHV viral loads, and T-lymphocyte subsets from pre-treatment to the pre-specified sampling times were evaluated using general estimating equations (GEE) adjusted for intra-patient variation after normalizing transformations.

KS Tumor Biopsies

Biopsies of non-indicator KS lesions were performed at baseline and during week 4 of cycle 1 and were considered mandatory. Samples were evaluated by immunohistochemistry (IHC) for expression of VEGF (Dako), VEGFr2 and CD31 (Cell Signaling), phospho-Akt (Novocastra), KSHV LANA (Leica), p53 (Santa Cruz), and HIF-1α (R&D Systems). Ki-67 and phospho-Akt were labeled with ImmPRESS HRP anti-mouse IgG and all other antibodies were labeled with the appropriate Vectastain ABC Kit (Vector Laboratories). Tumor cell proliferation was assessed by Ki-67 staining, quantified as percentage of tumor cells staining positively. Tumor biopsies were also evaluated for KSHV DNA using RT-PCR (Taqman, Applied Biosystems Inc., Foster City, CA) and with a targeted array of 96 mRNAs shown to be involved in angiogenesis and endothelial cell remodeling (DP Dittmer, unpublished).

Effects on tumor expression of VEGF, VEGFR-2/3, phospho-Akt, p53, HIF-1α, and KI-67

There was no significant change in expression of any tumor markers we evaluated by immunohistochemistry before and after the first cycle of study drug ($p>0.05$ for all markers).

Effects on in viral gene expression and cellular gene transcription in tumor biopsies

Comparing median expression levels before and during drug treatment, there was no significant change (unadjusted $p>0.05$ for all markers) in mRNA expression of any tumor markers we evaluated by RT-qPCR.

Pharmacokinetics

Serial blood samples for pharmacokinetic analysis were collected immediately preceding and at 1, 2, 3, 4, 5, 6, and 8 hours following the morning administration of PTC299 on Days 1 and 28 of Cycle 1. Additional trough samples were obtained on Day 15 of Cycle 1 and Day 28 of Cycle 2. Total plasma concentrations of PTC299 and its metabolite, des-methyl PTC299, were determined using a validated liquid chromatography-tandem mass spectrometry (LC/MS/MS) method by Covance Laboratories, Inc (Madison, WI). Analytes were detected by electrospray tandem mass spectrometry. Individual pharmacokinetic parameters were estimated by standard non-compartmental analysis using Phoneix WinNonlin 6.3 (Pharsight Corporation, Cary, NC). We estimated area under the plasma concentration-time curve from zero to 8 hours ($AUC_{0\text{-}8h}$) on Day 1 and Day 28, peak plasma concentration (C_{max}), time to C_{max} (T_{max}), minimal plasma concentrations at steady-state ($C_{min,ss}$), half-life ($T_{1/2}$), and the relative systemic exposure to PTC299 for the metabolite. Dose-normalized C_{max}, $C_{min,ss}$ (trough measurement), and AUC values were calculated. Pharmacokinetic parameters were summarized using descriptive statistics according to the ability of the co-administered antiretroviral regimen to induce or inhibit CYP2C19, one of the major cytochrome P450 enzymes thought to metabolize PTC299 [1, 2]. Differences in dose-normalized pharmacokinetic parameters between days were evaluated using a two-sided Kruskal-Wallis test. Correlations between pharmacokinetic parameters and response was performed by Kruskal-Wallis analysis of variance by ranks.

Pharmacokinetic analysis on 15 patients was available (Supplemental Table 4). Two patients were excluded due to sample processing errors. The inter-patient variability of PTC299 was less than 45% for single-dose and steady-state C_{max} and $AUC_{0\text{-}8h}$, and increased to 63% for $C_{min,ss}$. However, the inter-patient variability of des-methyl PTC299 was significantly higher, with up to 190% variability noted in single-dose C_{max} and $AUC_{0\text{-}8h}$ but only 61% for $C_{min,ss}$. While the trial was not designed with stratification based on ART regimen, we performed a post-hoc analysis categorizing participants on whether the ART regimen was known to induce CYP2C19 (ritonavir) or inhibit CYP2C19 (efavirenz) [1]. There were no statistically significant alterations in PTC299 pharmacokinetics between these groups. However, there were significant alterations by CYP2C19 strata in the des-methyl PTC299 metabolite exposure only for the steady-state assessments. At steady-state, participants on CYP2C19 inducers showed a 3% increase compared to those on a CYP2C19 non-inducer/non-inhibitor (i.e., neutral) regimens, whereas regimens with CYP2C19 inhibitors were associated with a 58% decrease in the C_{max} of the des-methyl PTC299 metabolite, respectively (p=0.025). A similar trend was noted for the $AUC_{0\text{-}8h}$, with only a 7% increase associated with CYP2C19 inducers, and 55% decrease with CYP2C19 inhibitors (p=0.024). However, CYP2C19 inhibitors and inducers did not alter the des-methyl PTC299 $C_{min,ss}$ (p=0.17). The differences in des-methyl PTC299 concentrations in turn significantly altered the ratio of metabolite to parent drug (p=0.022 for $AUC_{0\text{-}8h}$) but not the sum total $AUC_{0\text{-}8h}$ (p=0.63) when assessed on a dose-normalized basis. Half-life of PTC299 ranged between 2.3 and 6.5

hours depending on CYP2C19 induction/inhibition status and dosage. The half-life of the metabolite was not reportable due to statistical concerns (see Supplemental Table 4).

Effects of PTC299 on Serum/Plasma Biologic markers

KSHV DNA in plasma was quantified at baseline, cycles 2 and 5, and treatment discontinuation, using competitive DNA PCR as previously described [3]. CD4+ T cell counts in whole blood and quantitative HIV RNA in plasma were determined at these same time points.

Levels of VEGF-A and IL-6 were quantified by ELISA (Quest Laboratories, San Juan Capistrano, CA) in both serum and plasma on the first day of cycles 1-6 as well as Cycle1, Day 15 and at treatment discontinuation. Day 1, cycle 1 levels of serum VEGF were significantly higher than levels at all later time points (Figure S1); plasma VEGF showed a less sustained decrease that was significant only at intermediate time points. No changes were observed in serum or plasma IL-6. The median KSHV viral load in PBMCs before treatment was 1.78$\log_{10}$ copies/μL (interquartile range (IQR) -0.26, 3.81), was 0.99 $\log_{10}$ copies/μL (IQR 0.64, 2.83) during cycle 1, and was 0 copies/μL during cycle 2 (IQR 0 copies, 1.83$\log_{10}$ copies). The trends in KSHV viral load were not significant between baseline and cycle 1 (p=0.564) or cycle 2 (p=0.617). Absolute and percent CD4 counts are shown in Table 2; changes from baseline to cycle 2 or cycle 5 were not statistically significant.

1. Rudek, M.A., C. Flexner, and R.F. Ambinder, *Use of antineoplastic agents in patients with cancer who have HIV/AIDS.* Lancet Oncol, 2011. **12**(9): p. 905-12.
2. PTC Therapeutics, I., *PTC 299 Investigator Brochure.* **Ver 6, 12,** .
3. Lin, L., et al., *Effects of chemotherapy in AIDS-associated non-Hodgkin's lymphoma on Kaposi's sarcoma herpesvirus DNA in blood.* J Clin Oncol, 2009. **27**(15): p. 2496-502.

Supplemental Table 2: Summary of therapy received and best response

	40mg BID N=3	80mg BID N=3	100mg BID N=11	Total N=17
Cycles Received –median (range)	7 (6,13)	3 (2,5)	4 (1,10)	5 (1,13)
Best response N (%)				
Partial response	1 (33)	-	2 (18)	3 (18)
Stable disease	1 (33)	3 (100%)	7 (64)	11 (65)
Progression		-	1 (9)	1 (6)
Unevaluable[a]	1 (33)	-	1 (9)	2 (12)
Reasons for drug discontinuation				
Completion of protocol[b]	2 (67)	1 (33)	2 (18)	5 (29)
Participant withdrawn	1 (33)	1 (33)	2 (18)	4 (36)
Disease progression	-	1 (33)	5 (45)	6 (35)
Alternative therapies pursued	-		1 (9)	1 (6)
Death on study			1 (9)	1 (6)
Treatment delays			2	2 (12)
Constipation/nausea	1 (33)	-	-	1 (6)
Myalgias (dose reduced)			1 (9)	1 (6)

[a]The participant who died during the study did so prior to the first follow-up visit, and therefore had no KS response evaluated. The second participant withdrew from the study after 3 weeks, not completing a minimum of 1 cycle of PTC299 and therefore also unevaluable.

[b] Participants were considered to have completed the study protocol if they: 1) achieved CR and then received 2 further cycles of study drug; 2) received 12 cycles of study drug if they achieved a PR; or 3) had documented stable disease for 6 months, at which time they were removed from the study

Supplemental Table 3: Adverse Events by Study Product Dosage

Event Category	40mg BID N=3	80mg BID N=3	100mg BID N=11	Total N=17
Persons with Events N (%)				
Anemia	1	-	1	2 (12)
Abdominal Pain/Distention	-	-	2	2 (12)
Constipation	1	-	1	2 (12)
Diarrhea	-	-	4	4 (24)
Nausea	2	-	4	6 (35)
Vomiting	1	-	1	2 (12)
Fatigue	-	-	4	4 (24)
Skin/Soft tissue Infection	-	-	1	1 (6)
Elevated Creatinine	1	1	1	3 (18)
Elevated AST/ALT	-	1	1	2 (12)
Elevated cholesterol	-	-	2	2 (12)
Elevated Triglycerides	1	-	3	4 (24)
Decreased neutrophil count	-	-	1	1 (6)
Pain in Extremity	-	-	2	2 (12)
Myalgias			1	1 (6)
Proteinuria	1		1	2 (12)
Dyspnea	1	1	-	2 (12)
Events N				
Any Adverse Event	24	10	116	150
Grade 1 (Mild) Events	16	7	86	109
Grade 2 (Moderate) Events	5	2	22	29
Grade 3 (Severe) Events	3	1	7	11
Serious Adverse Events (SAE)	1	-	2	3
Events prompting early termination	-	-	1	1

The upper portion of the table presents person-level (prevalent) events for the most frequently reported events that were considered “probably related,” “possibly related” or “unlikely related” to the study drug: unrelated events not displayed. The bottom portion of the table lists total numbers of all related and unrelated events (event-level, or incident events).

Supplemental Table 4. Plasma pharmacokinetics parameters of PTC299 and des-methyl PTC299[1]

Group/Dose (mg/day)	n	C_{max} (ng/mL)		T_{max} (h)		AUC (ng*h/mL)		des-methyl PTC299: PTC-299 AUC Ratio (%)		$T_{1/2}$ (h)		$C_{min,ss}$ (ng/mL)
		C1D1	C1D28	C1D1	C1D28	C1D1	C1D28	C1D1	C1D28	C1D1	C1D28	
PTC299												
CYP2C19 inducer/40	3	410 ± 153	688 ± 135	3.0 (2.0-5.0)	4.0 (3.0-5.0)	1684 ± 653	4147 ± 544	4.3 ± 6.1[2]	38 ± 20	2.3[3]	2.5[3]	372 ± 53
CYP2C19 inhibitor/80	1	359	1070	5.0	5.0	1874	6649	0.0[2]	12	N.R.[3]	N.R.[3]	1034
CYP2C19 inducer/80	2	773, 1230	1020, 1985	3.0, 4.0	0.0, 5.0	3211, 6142	6054, 11353	3.0, 3.0	26, 37	4.1[3]	4.4[3]	300, 1943
CYP2C19 neutral/100	3	968 ± 88	2080, 3000[4]	3.0 (2.0-4.0)	5.0, 6.0[4]	4688 ± 904	13178, 15678[4]	4.5 ± 2.1	20, 32[4]	4.4 ± 2.0	N.R.[3,4]	1017 ± 449
CYP2C19 inhibitor/100	3[4]	1175 ± 429	3567 ± 1212	2.0 (2.0-4.0)	5.0 (2.0-8.0)	6064 ± 2090	23213 ± 9564	1.3 ± 0.5	9.6 ± 3.6	3.8[3]	N.R.[3,4]	2423 ± 1313
CYP2C19 inducer/100	3	892 ± 425	2740[4]	5.0 (2.0-5.0)	5.0[4]	3708 ± 1468	18236[4]	2.9 ± 1.6	31[4]	6.6[3]	N.R.[3,4]	1908[4]
des-methyl PTC299												
CYP2C19 inducer/40	3	8, 188[2]	236 ± 156	5.0, 8.0[2]	5.0 (5.0-6.0)	30, 242[2]	1615 ± 984	N.A.	N.A.	N.R.	N.R.	226 ± 152
CYP2C19 inhibitor/80	1	BLQ[2]	118	N.A.	8.0	BLQ[2]	809	N.A.	N.A.	N.R.[3]	N.R.[3]	141
CYP2C19 inducer/80	2	20, 40	343, 552	8.0, 8.0	0.0, 5.0	95, 175	2228, 2937	N.A.	N.A.	N.R.[3]	N.R.[3]	215, 587
CYP2C19 neutral/100	3	50 ± 37	363, 834[4]	6.0 (6.0-6.0)	6.0, 6.0[4]	220 ± 140	2572, 4985[4]	N.A.	N.A.	N.R.[3]	N.R.[3,4]	465 ± 249
CYP2C19 inhibitor/100	3[4]	16 ± 1	286 ± 51	6.0 (6.0-8.0)	6.0 (6.0-8.0)	72 ± 8	1921 ± 335	N.A.	N.A.	N.R.[3]	N.R.[3,4]	268 ± 60
CYP2C19 inducer/100	3	28 ± 24	805[4]	8.0 (8.0-8.0)	4.0[4]	119 ± 98	5687[4]	N.A.	N.A.	N.R.[3]	N.R.[3,4]	830[4]

Abbreviations: AUC area under the plasma concentration-time curve to 8 h; C_{max} peak plasma concentration; $C_{min,ss}$ average plasma trough concentration; N.A. not applicable; N.R. not reportable; $T_{1/2}$ half-life; T_{max} time to peak concentration.

[1]Data are presented in the table as mean±SD(n). T_{max} is presented as median (range, n). If n<3, the actual values are reported.

[2]One patient has undetectable concentrations on C1D1. For the AUC ratio, a value of 0 was utilized.

[3]The $T_{1/2}$ could not be estimated or the r^2 was <0.9 for at least one patient. Therefore, the $T_{1/2}$ was not reported.

[4]A full day pharmacokinetic profile was not obtained on at least one patient.

Supplemental Figure S2
Average PTC-299 (A, C, E) and its metabolite, des-methyl PTC-299 (B, D, F) plasma concentration versus time profiles in patients after a single dose (closed symbols) or multiple dose (open symbols) administration of 40 mg (A, B), 80 mg (C, D), and 100 mg (E, F). The antiretroviral regimens with neutral (circle), induction (square), and inhibition (triangle) of CYP2C19 are depicted on each graph.