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Selective Polo-like Kinase 1 (PLK1) Inhibitor PCM-075 is Highly Active Alone and Shows Synergy When Combined with FLT3 Inhibitors in Models of Acute Myeloid Leukemia (AML)

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Karena Kosco[1], Maya Ridinger[1], Penn Whitley[1], Antonella Ciavolella[2], Peter Croucher[1], Barbara Valsasina[2], Jeffrey N. Miner[1], Mark Erlander[1]
[1]Trovagene, San Diego, CA, [2]Nerviano Medical Sciences, 20014 Nerviano, MI, Italy

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Background

- Acute Myeloid Leukemia (**AML**) is an aggressive hematological disease that is characterized by the accumulation of immature myeloid precursor cells. Treatment options for patients are selected based on factors such as age, comorbidities, and cytogenetics. For patients deemed ineligible for standard intensive induction chemotherapy (~40%), treatments include either low dose cytarabine (LDAC) or hypomethylating agents (e.g., azacitidine and decitabine) but when used as single agents, relapses are inevitable.
- First generation **FLT3** inhibitors have been useful in treating AML (sorafenib, midostaurin) and recently the FDA approved midostaurin (RYDAPT, Novartis Pharmaceuticals Corp.) for the treatment of adult patients with newly diagnosed AML who are FLT3 mutation-positive in combination with standard cytarabine and daunorubicin induction and cytarabine consolidation. Second generation inhibitors with more selectivity are currently in Phase 3 trials in AML (quizartinib, gilteritinib). Resistance to FLT3 inhibitors and disease progression while on FLT3 inhibitors remains an important problem for patient care.
- Polo-like Kinase 1 (**PLK1**), a serine/threonine kinase that is a master regulator of cell-cycle progression, is overexpressed in a number of cancer types including AML. Depletion of PLK1 preferentially induces cell death in tumor versus normal cells. PCM-075 is a potent, highly-selective adenosine triphosphate competitive inhibitor of PLK1. PCM-075 is highly active in blocking proliferation and inducing G2/M arrest in multiple AML cell lines. In a therapeutic model, PCM-075 was capable of inducing significant tumor growth inhibition (TGI), and increasing survival in an in vivo disseminated leukemia model (AML-NS8 Cells)[1].
- Synergistic interactions between PCM-075 and either the FLT3 tyrosine kinase inhibitor sorafenib or chemotherapeutic agents were examined in cell culture models. In AML models, we analyzed the activity of PCM-075 both alone and in combination with various chemotherapies and targeted therapeutics, including FLT3 inhibitors.
- Orally administered PCM-075 is currently in clinical trials for the treatment of AML (NCT03303339) and Metastatic Castration-Resistant Prostate Cancer (NCT03414034).

1. Casolaro et al., PLoS One. 2013;8(3):e58424

Results

In Vitro, PCM-075 Displays Anti-Proliferative Activity as a Single Agent in Cell Lines Derived From Hematological Tumors

- PCM-075 was tested for antiproliferative activity on a panel of human tumor cell lines derived from hematological tumors.
- Different concentrations of each compound were added to the cultured cells and incubated for 72h
- Proliferation was measured using CellTiter Glo (Promega)

Leukemic Cell Type	Cell Line	IC_{50} (µM)
AML (monocytic)	MOLM-13	0.023
AML (monocytic)	THP-1	0.032
AML (monocytic)	KG-1	0.058
AML (myelomonocytic)	MV-4-11	0.058
AML (monocytic)	SKM-1	0.060
AML (promyelocytic)	PL-21	0.022
AML (promyelocytic)	HL60	0.072
CML	KU812	0.020
CML	K-562	0.097
Erythroleukemia	HEL	0.048
Erythroleukemia	TF-1	0.440
Erythroleukemia	TF1a	1.777
B cell Leukemia	697	0.026
B cell Leukemia	RS4-11	0.043
B cell Leukemia	NALM-6	0.061
B cell Leukemia	MEC-1	0.112
T Cell Leukemia	MOLT-4	0.045
T Cell Leukemia	Jurkat	0.048
T Cell Leukemia	KARPAS-299	0.054
T Cell Leukemia	CCRF-CEM	0.055
Megakaryoblastic	M-07e	0.066
Megakaryoblastic	SET-2	0.145

Results

In Vitro, PCM-075 Exhibits Synergistic Anti-Proliferative Effects When Combined with Chemotherapy Agents and the FLT3 Inhibitor Sorafenib

Compound	Cell line	Combination Ratio	CI	Schedule
Paclitaxel	HL60 Promyelocytic leukemia	1:1	0.73	simultaneous
		2:1	0.60	
		4:1	0.62	
Paclitaxel	HL60 Promyelocytic leukemia	0.5:1	0.50	sequential
		1:1	0.55	
		2:1	0.55	
Sorafenib	KMS-11 Multiple myeloma	25:1	0.59	sequential
		50:1	0.77	
		100:1	0.61	
		200:1	0.76	
Doxorubicin	HL60 Promyelocytic leukemia	5:1	0.75	simultaneous
		10:1	0.52	
		20:1	0.62	
		40:1	0.61	
Cytarabine	HL60 Promyelocytic leukemia	50:1	0.59	sequential
		4:1	0.67	
		0.005:1	0.47	
		0.025:1	0.64	
		0.02:1	0.49	

CI, Combination index

- Cell lines were treated with PCM-075 in combination therapy with several anti-cancer drugs. The combining agent was given simultaneously with PCM-075 or 24 hours before treatment with PCM-075.
- Cell proliferation inhibition was determined 72h after treatment (CellTiter Glo, Promega) and the combination index was calculated using the equation of Chou-Talalay method for mutually non exclusive drugs[2].
- PCM-075 was found to have synergistic antiproliferative effects (combination index: 0.3-0.8) when combined with DNA replication inhibitors, proteasome inhibitors, mitotic inhibitors, and kinase inhibitors (some data not shown).

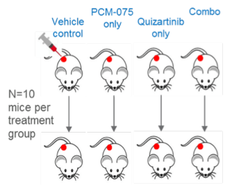
2. Chou TC. Drug combination studies and their synergy quantification using the Chou-Talalay method. Cancer Res; 70(2); 440–6.

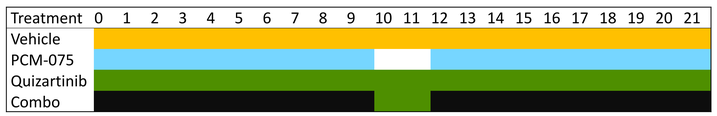
Evaluation of the In Vivo Efficacy of PCM-075 in the Treatment of Subcutaneous MV-4-11 Human AML Xenograft Model in NOD.SCID Mice

MV-4-11 cells in an exponential growth phase were harvested and counted for tumor inoculation. The treatments were started when the mean tumor size reached approximately 200 mm^3. Each mouse was inoculated subcutaneously at the right flank region with MV-4-11 tumor. The date of tumor cell inoculation was denoted as day 0. Tumor volumes were measured twice weekly. Mouse weight was recorded daily.

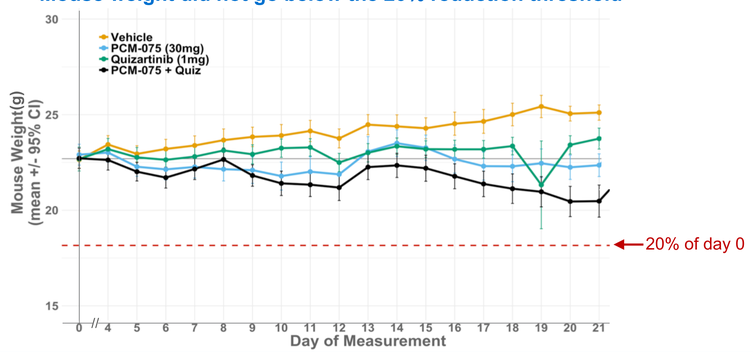
Dosing Schedule

Group	N	Treatment	Dose (mg/kg)	Dosing Route	Revised Schedule
1	10	Vehicle	-	oral	Daily (Days 0-21)
2	10	PCM-075	30	oral	Daily (Days 0-9,12-21)
3	10	Quizartinib (AC220)	1	oral	Daily (Days 0-21)
4	10	PCM-075+ AC220	30/1	oral	Daily (Days 0-9,12-21)/ Daily (Days 0-21)





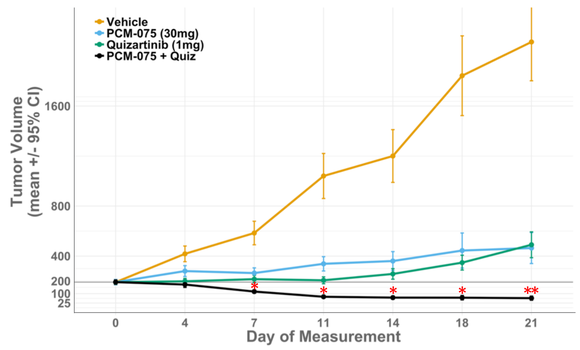
Mouse weight did not go below the 20% reduction threshold



Results

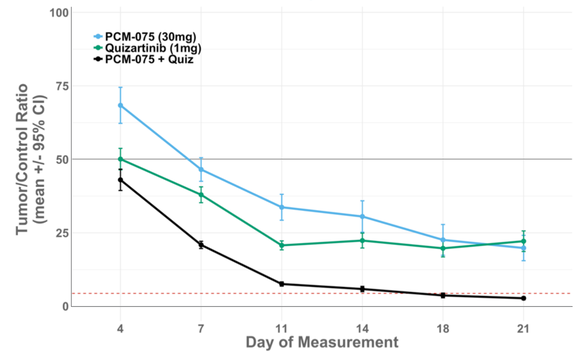
Combination Studies in an AML FLT3 Mutant 21-Day Treatment Xenograft Model Reveal Synergistic Interactions Between Quizartinib and PCM-075

Raw Tumor Volume



- PCM-075 (dosed orally 30 mg/kg QD for days 1-10 and 12-21) in combination with Quizartinib (1 mg/kg QD for 21 days) resulted in 97.3% (± 1.1%) tumor growth inhibition (TGI), compared to 77.9% (± 7.9%) with Quizartinib and 80.2% (± 9.8%) with PCM-075 as monotherapy.
- Tumor volume for the PCM-075/Quizartinib combination mice was significantly ($p < 0.05\%$) reduced compared with vehicle (D7-D21; *) or both single agents (**).

Percent Tumor/Vehicle Control



Expected Ratio: 0.044
(0.221 X 0.198 = 0.0438)

Observed Ratio: 0.027
(95% CI 0.016 - 0.038)

Based on the Bliss independence model, if the Tumor/Control ratio for the combination is less than the product of the two individual drugs, the effect of the combination is supra-additive. Therefore, the PCM-075/Quizartinib drug combination is supra-additive effect (e.g. shows synergy).

Conclusions

- The combination of PCM-075 and Quizartinib resulted in 97% tumor growth inhibition and regression in FLT3 AML xenograft model
- Combination therapies targeting PLK1 and FLT3 could extend the duration of response to FLT3 inhibitors prior to developing resistance
- This data warrants future investigation of the combination of PCM-075 with FLT3 inhibitors in clinical studies

Mark Erlander, PhD,
Trovagene, Inc.
11055 Flintkote Ave.
San Diego, CA 92121