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Pharmacodynamic and Tumor Biomarker Analysis of a PLK1 Inhibitor, PCM-075, in a Phase 1b/2 Trial for Acute Myeloid Leukemia

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Poster #4833

Abstract #5188

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Background

Polo-like Kinase 1 (PLK1):

- Serine/threonine kinase, master regulator of cell-cycle progression
- Inhibition of PLK1 causes mitotic arrest in prometaphase and subsequent cell death
- Over-expressed in numerous cancer types, including AML, and associated with poor patient prognosis

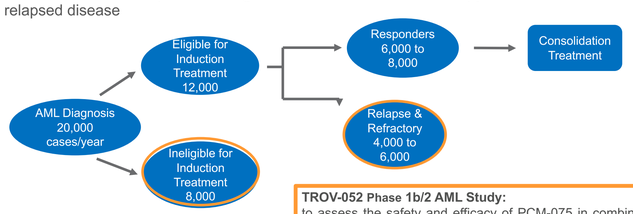
PCM-075:

- Orally-available, highly-selective PLK1 inhibitor
- Induces G2/M arrest and apoptosis in cancer cells, including leukemic cells.
- Inhibits tumor growth alone and in combination with cytarabine in xenograft tumor models

PCM-075 is currently under clinical investigation for **Acute Myeloid Leukemia (AML) – NCT03303339 and metastatic Castration-Resistant Prostate Cancer (mCRPC) – NCT03414034**

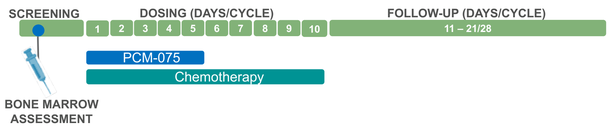
AML trial design

- Inclusion criteria: AML patients ineligible for intensive induction therapy or who have refractory or relapsed disease



TROV-052 Phase 1b/2 AML Study:
to assess the safety and efficacy of PCM-075 in combination with standard-of-care (Low-Dose Cytarabine or Decitabine)

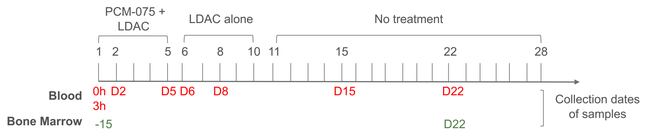
- Dose escalation (starting at 12 mg/m^2) in Phase 1b with expansion cohort at maximum tolerated dose (MTD) for Phase 2 continuation
- Dosing schedule:



Objectives

- Evaluate pharmacodynamic biomarkers to measure drug activity (PLK1 inhibition) and effect of combination treatment with PCM-075 + standard-of-care chemotherapy on leukemic cells
- Identify immuno-profiles and genetic subtypes associated with response to treatment

Schedule of Assessments



Identification of leukocyte populations
Flow cytometry analysis of cell surface markers

Detection of mutations
VariantPlex assay (Archer): target-enriched libraries from DNA to detect somatic mutations

Detection of fusions
FusionPlex assay (Archer): target-enriched cDNA libraries from RNA to characterize gene fusions

PLK1 inhibition
Flow cytometry and Western-Blot analysis of PLK1 substrate, pTCTP

Results

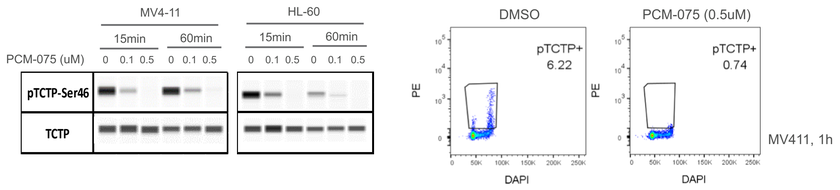
1. PLK1 activity can be assessed through TCTP phosphorylation status

Translational Control Tumor Protein (TCTP) is:

- Involved in important cellular processes, such as cell growth, cell cycle progression and apoptosis
- Phosphorylated by PLK1 at Serine-46 (Cucchi U. et al., Anticancer Res., 2010)

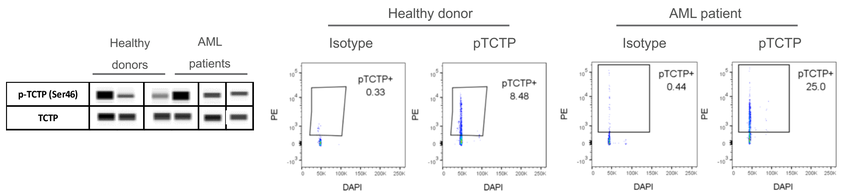
1A. PCM-075 inhibits TCTP phosphorylation at Ser46 (pTCTP) in leukemic cell lines

MV4-11 and HL-60 leukemic cell lines were treated with PCM-075 for 15min or 60min at the indicated doses
pTCTP levels were assessed by Western-Blot (left) and Phospho-flow (right)



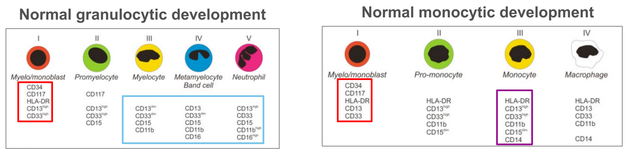
1B. pTCTP is detected in PBMC from healthy and AML donors

PBMC were isolated from CellSave blood tubes collected from either healthy donors or AML patients
pTCTP levels were assessed by Western-Blot (left) or Phospho-flow (right)



2. Immuno-profiling of AML patients

2A. Normal myeloid differentiation

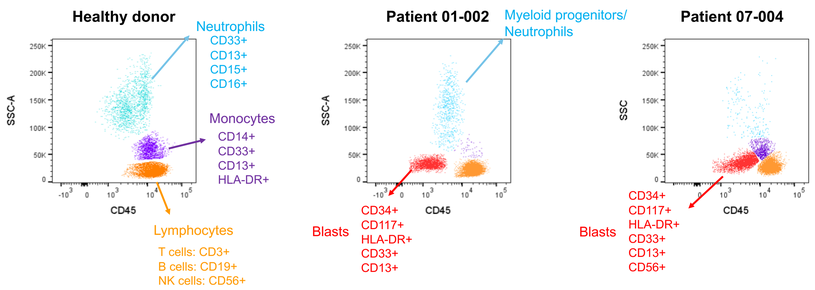


2B. Identification of leukocyte populations in AML patients

PBMC were isolated from EDTA blood tubes collected from AML patients pre-treatment and stained with the following antibodies for flow cytometry analysis:

Stains
CD45-CD13-CD14-CD33-CD15
CD45-CD34-CD117-HLADR-CD13
CD45-CD3-CD19-CD56-CD16

CD45-SSC gates
Blasts
Granulocytes
Lymphocytes
Monocytes



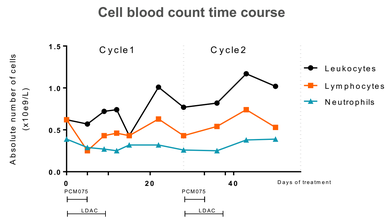
3. Trial data: patient 01-002 completed 2 cycles of PCM-075 + LDAC

3A. Treatment does not affect normal blood cells

Treatment (28day cycle):
12mg/m^2 PCM-075 day 1 to day 5
20mg/m^2 cytarabine day 1 to day 10

Cell blood count – normal ranges

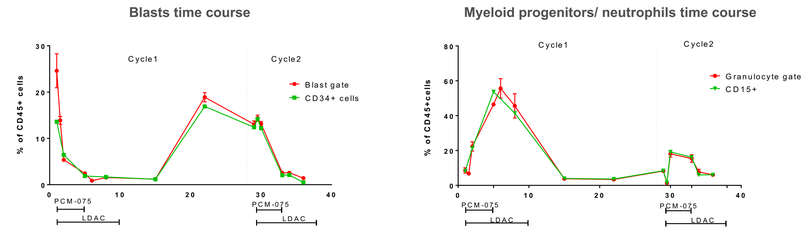
Normal ranges	Lower	Upper
Leukocytes	4	10
Neutrophils	2	8
Lymphocytes	1	4



3B. Treatment decreases blast levels in blood

Circulating leukemic cells were identified based on their CD45-SSC profile and the expression of cell surface markers (see section 2B) and followed during treatment at the indicated time points.
Two populations of leukemic cells were identified:

- Blast cells: CD45low-SSClow / CD34+
- Myeloid progenitors and neutrophils: CD45low-SSChigh / CD15+



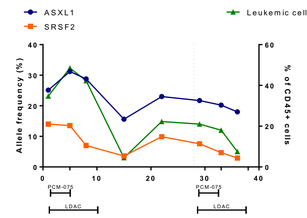
3C. Mutation allele frequencies correlate with the percentage of circulating leukemic cells

Two mutations were detected (VariantPlex, Archer)

- ASXL1: involved in chromatin remodeling
- SRSF2: part of the splicing machinery

Gene	HGVSp	HGVSc	AF
ASXL1	p.G646fs*12	c.1926_1927insG	25.1%
SRSF2	p.P95R	c.284C>G	14.0%

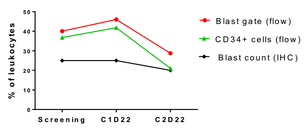
AML with mutations in genes encoding chromatin and RNA-splicing regulators are associated with: lower white-cell and blast counts, higher relapse rates and poor long-term clinical outlook (Papaemmanuil et al., NEJM, 2016)



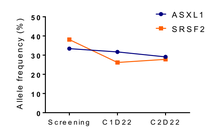
3D. Blast numbers and mutations in bone marrow

Bone marrow samples were collected before treatment (screening) and on day 22 of cycles 1 and 2 (C1D22 and C2D22)

- Blasts levels were assessed using 3 methods: CD45-SSC profile (flow), CD34 stain (flow) and IHC

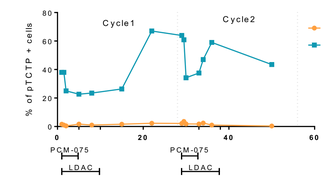


- ASXL1 and SRSF2 mutations were also detected in BM samples and the allele frequencies remain stable overtime



3E. pTCTP decreases with PCM-075 treatment

pTCTP was assessed by Phospho-flow in PBMC isolated from CellSave blood tubes at the indicated time points



Patient 01-002 Conclusions

- Treatment was well tolerated by patient and did not affect levels of normal leukocytes
- Treatment decreases the number of circulating blasts
- PLK1 target, pTCTP, is inhibited upon treatment
- Quantitative assessment of the dynamic changes of leukemic cells, genomic alterations and pTCTP levels within the course of treatment may enable a greater understanding of underlying tumor biology associated with therapy response

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