Polo-like Kinase 1 (PLK1):  
- Serine/threonine kinase, master regulator of cell-cycle progression  
- Involvement of PLK1 in cell proliferation and cell death  
- Over-expressed in various cancer types, including AML, and associated with poor patient prognosis

**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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**Background**
- AML trial design and metastatic Castration combination treatment with PCM
- Inclusion criteria: AML patients ineligible for intensive induction therapy or who have refractory or relapsed disease

**Objectives**
- Evaluate pharmacodynamic biomarkers to measure drug activity (PLK1 inhibition) and effect of combination treatment with PCM + 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 fusions**
- PCR assay (Archer) target enriched DNA 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)
- Involved in important cellular processes, such as cell growth, cell cycle progression and apoptosis
- Phosphorylated by PLK1 at Serine 46 (Cuciti C. et al., Ancan Res, 2015)

1A. PCM-075 inhibits TCTP phosphorylation at Ser46 (pTCTP) in leukemic cell lines
- M4-11 and HL-60 leukemic cell lines were treated with PCM-075 for 10min 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) and Phospho-flow (right)

2. Immuno-profiling of AML patients

2A. Normal myeloid differentiation
- Normal granulocytic development
- Normal monocytic development

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:
  - CD45-SSC gates
  - CD45-SSC profile (flow), CD34 stain (flow) and HSC

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/m² PCM 075 day 1 to day 5
- 2mg/kg cytarabine day 1 to day 10
- Cell blood count – normal ranges

3B. Treatment decreases blasts levels in blood
- Circulating leukemic cells were identified based on their CD45 and TCTP
- Normal blood cells: CD45-SSC gates

3C. Mutation allele frequencies correlate with the percentage of circulating leukemic cells
- Two mutations were detected (Archer/Plex, payer)
- SRSF2, part of the splicing machinery

AML with mutations in genes encoding chromatin and RNA-spooling regulators are associated with lower white-cell and blast counts, higher relapse rates and poor long-term clinical outcomes (Paap et al., NCGA, 2014)

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 (C022 and C022)
- Blast levels were assessed using 3 methods: CD45-SSC profile (flow), CD34 stain (flow) and HIC

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