Plasma-derived Circulating Tumor DNA (ctDNA) As a Surrogate Biomarker for Treatment Response with the Polo-like Kinase 1 (PLK1) Inhibitor, Onvansertib, in Combination with LDAC or Decitabine in Acute Myeloid Leukemia (AML)

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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
- Overexpressed in solid tumors and hematological malignancies, including AML

Onvansertib (also known as PCM-075 and NMS-1286937) :

- Orally-bioavailable, highly-selective PLK1 inhibitor with a ~24-hour half-life
- Potent anti-tumor activity in AML preclinical models[1,2]
- Ongoing clinical trials for relapsed or refractory AML, metastatic castration-resistant prostate cancer and metastatic colorectal cancer

Genomics of Acute Myeloid Leukemia (AML)

- In AML, blast cells carrying a driver mutation in the bone marrow (BM) migrate into circulation in the peripheral blood (PB)
- Hematologic mutations are detectable in a majority of AML patients, and are reported to persist in over 50% of patients during complete remission (CR)[3]

Circulating Tumor DNA (ctDNA)

- Recently ctDNA has been used as a biomarker for monitoring tumor heterogeneity, treatment response, minimal residual disease and disease progression[4]

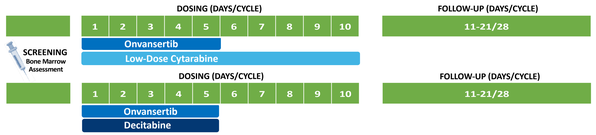
Phase 1b/2 Trial Design

Phase 1b/2 Trial (NCT03303339) of Onvansertib in Combination with either Low-Dose Cytarabine or Decitabine in Patients with Relapsed/Refractory Acute Myeloid Leukemia

Study design

- Dosing schedule:

Onvansertib x 5 days + either Low-dose Cytarabine (LDAC – 20 mg/m^2 SC qd x 10d) or Decitabine (20 mg/m^2 IV qd x 5d) in a 21 to 28 – day cycle



- Dose escalation in Phase 1b (3+3 design) with expansion cohort at the maximum tolerated dose (MTD) or recommended phase 2 dose (RP2D) for Phase 2

Study Primary Objectives

- Phase 1b: Assess safety, define dose-limiting toxicities (DLTs) and MTD/RP2D
- Phase 2: Assess safety, tolerability and preliminary anti-leukemic activity at the MTD (or RP2D)

Key Eligibility Criteria

- Patients with relapsed/refractory AML who have received ≤3 prior treatment regimens
- Treatment-related AML or APL are excluded
- ECOG performance status ≤2

Methods and Patients Characteristics

Sample Collection

- BM aspirates were collected at screening, at the end of cycles 1, 2 and then every other cycle
- Blood samples were collected on Days 1 (pre-dose), 5, 8, 15, 22 of each cycle
- Genomic DNA was extracted from peripheral blood and bone marrow mononuclear cells (PBMCs & BMMCs, respectively) and ctDNA was isolated from plasma

Molecular Profiling

- A targeted NGS assay covering 75 genes was used to identify driver mutations
- A ddPCR assay targeting an identified driver mutation was used to enumerate mutant allele frequencies in Plasma (ctDNA), PBMCs, and BMMCs

Patient Characteristics

- 40 patients enrolled in the Phase 1b were analyzed by targeted NGS, median number of genes with variants identified was 3 [range 1 - 7]
- Among them, 20 patients were chosen for subsequent ctDNA analysis
- Clinical response was evaluated by patient's pathologist using bone marrow aspirate
- **Responders** were defined as patients achieving complete response (BM blasts <5%) with or without count recovery (CR+CRi)

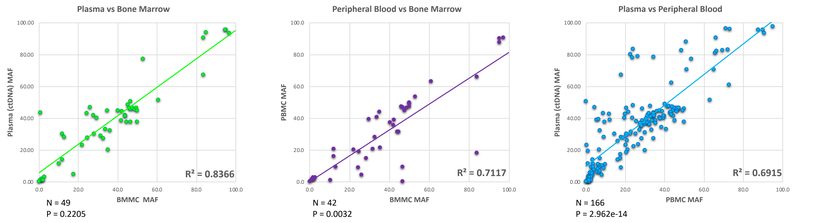
	N	(%)
Responders	6	30%
Non-Responders	14	70%

Cytogenetic Risk	N	(%)
Favorable	2	10%
Intermediate	6	30%
Adverse	9	45%
Unknown	3	15%

ctDNA as a Predictive Biomarker

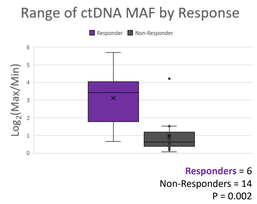
Plasma-derived ctDNA MAF Highly Correlates with MAF from Bone Marrow Mononuclear Cells

- Mutation allelic frequencies (MAF) in ctDNA from plasma, and gDNA from BMMCs and PMBCS were assessed for 20 patients across timepoints (Plasma, BM, PB).
- Linear regression, and a paired two sample t-test were performed for all timepoints with matched samples.
- When plotted, MAF from plasma and BM showed the highest linearity (R^2 = 0.8366) and no significant difference from BM (P=0.2205) when comparing 49 matched samples.
- These data suggest plasma is most representative of disease state in BM, and may be use to monitor response to treatment



Responders Have a Greater Range of ctDNA MAF than Non-responders

- As plasma was most correlative with BM, we aimed to explore if changes in plasma MAF differed between patients with **clinical response** (CR/CRi) and non-responders.
- The change in MAF over all timepoints available for each patients was log transformed: Log_2(Max/Min), where Max is highest MAF of all timepoints, and Min is the lowest MAF of all timepoints.
- A t-test was performed between **responders** (n=6) and non-responders (n=14) to determine if ctDNA range distinguished the groups
- There was a significant difference between the two groups (P=0.002), further supporting ctDNA as a biomarker for treatment response.



Plasma-derived ctDNA is a Predictive Biomarker for Treatment Response to Onvansertib

- We aimed at determining the utility of plasma MAF to predict clinical response after the 1st cycle of treatment
- MAF were assessed in ctDNA before beginning treatment (C0) and after 1 cycle of treatment (C1) when BM aspirates were collected; and change in MAF calculated as Log_2(C1/C0).
- Similarly changes in % BM blasts at end of cycle 1 versus screening were calculated as Log_2(C1/C0).
- All patients with **clinical response** (CR+CRi, n=6) showed a decrease in ctDNA MAF at cycle 1 (Log_2(C1/C0) < **-0.05**), while only 2 of the 14 non-responders (NR) showed a similar decrease.
- Measuring the changes in plasma over the first cycle predicted patient response with 90% diagnostic accuracy, 100% sensitivity and 86% specificity, with positive predictive value (75%) and negative predictive value (100%) supporting the utility of this analysis
- Conversely, the same analysis in BM Blasts at Cycle 1 were not predictive of clinical response

Patient	ctDNA MAF Log_2(C1/C0)	BM % Blasts Log_2(C1/C0)	Clinical Response
01-021	0.2193	**-0.8625**	NR
01-024	0.0489	**-0.3219**	NR
03-037	**-0.0853**	0.8301	**Responder**
05-016	0.5850	3.0704	NR
05-030	**-0.4258**	**-2.0000**	**Responder**
05-043	-0.0299	**-0.4150**	NR
07-009	**-0.3303**	-0.0468	**Responder**
07-013	0.3182	N/A	NR
07-018	0.0015	N/A	NR
07-033	-0.0200	N/A	NR
07-035	**-0.1410**	**-1.7370**	**Responder**
07-036	0.2940	**-1.0000**	NR
08-027	-0.0096	**-0.1043**	NR
08-058	**-1.4399**	0.9329	**Responder**
09-026	0.3531	0.3785	NR
09-032	0.2534	0.1454	NR
09-064	**-1.6881**	**-3.9069**	**Responder**
11-019	0.0000	0.0000	NR
11-040	**-0.3411**	0.4021	NR
12-041	**-0.1515**	0.6951	NR
	N = 20	N = 17	NR = Non-responder

Screening test using ctDNA MAF after 1 cycle

		Clinical Response: Responder	Clinical Response: NR		
Plasma Prediction	Responder	6	2	PPV	75%
	NR	0	12	NPV	100%
		100%	86%		
		Sensitivity	Specificity		

90%	Diagnostic accuracy
7.0	Positive likelihood ratio
0.0	Negative likelihood ratio

Screening test using % BM blasts after 1 cycle

		Clinical Response: Responder	Clinical Response: NR		
BM Prediction	Responder	3	5	PPV	38%
	NR	3	6	NPV	67%
		50%	55%		
		Sensitivity	Specificity		

53%	Diagnostic accuracy
1.10	Positive likelihood ratio
0.9	Negative likelihood ratio

- $Sensitivity = \frac{TP}{(TP+FN)}$
- $Specificity = \frac{TN}{(TN+FP)}$
- $Positive\ Predictive\ Value (PPV) = \frac{TP}{(TP+FP)}$
- $Negative\ Predictive\ Value (NPV) = \frac{TN}{(TP+TN)}$
- $Diagnostic\ Accuracy\ (DA) = \frac{TP+TN}{TP+TN+FP+FN}$
- $Positive\ Likelihood\ Ratio\ (PLR) = \frac{Sensitivity}{(1-Specificity)}$
- $Negative\ Likelihood\ Ration\ (NLR) = \frac{(1-Sensitivity)}{Specificity}$

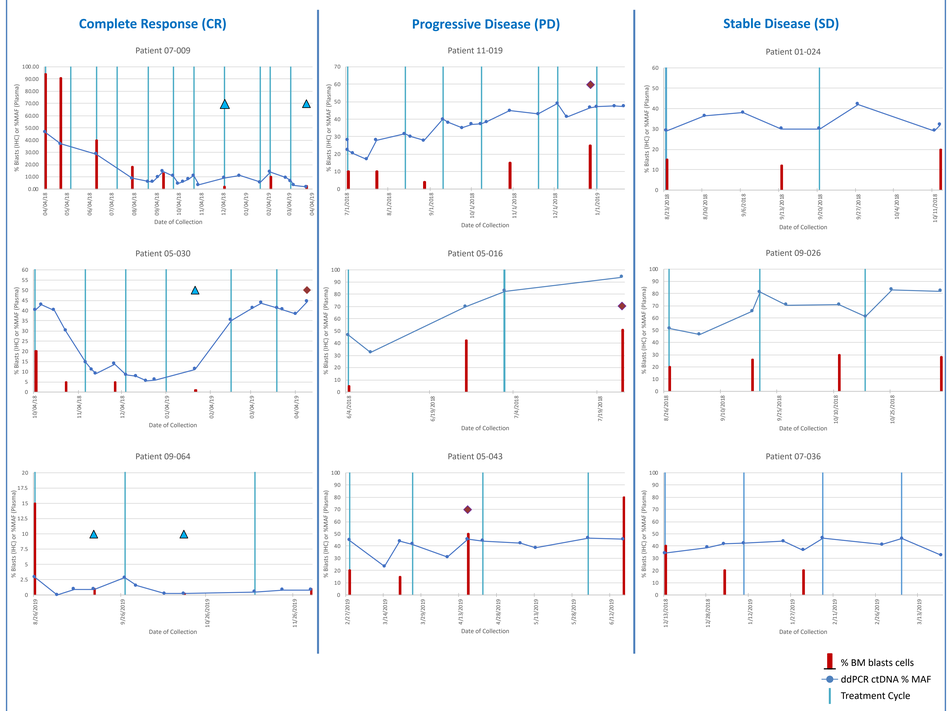
Earlier Treatment Decisions from Serial Monitoring of ctDNA

Plasma-derived ctDNA Enables Monitoring of Treatment Response and Disease Progression

- Twenty (20) patients were chosen for ctDNA monitoring.
- For each, plots were generated overlaying ctDNA MAF and %BM blasts by date of sample collection.
- Of 6 patients who reached CR/CRi (▲), all showed the lowest ctDNA MAF at or before (13-35 days) determination of clinical response.
- Similarly, 4 patients with Progressive disease (PD, ◆), displayed a maximum ctDNA MAF at or before determination of progression.
- Patient 05-030 case:
  - Minimum ctDNA MAF was reached 34 days prior to CR diagnosis
  - A spike in ctDNA MAF was detected 53 days before progression, with a maximum MAF at the time of progression.
- Patients with stable disease also show ctDNA trends resembling the bone marrow
- Patients who responded to treatment showed a ctDNA MAF trend that decreased, while those who progressed showed an increasing trend.
- ctDNA MAF is capable of monitoring both clinical response and disease progression, and often prior to the bone marrow biopsy

Patient	ctDNA MAF Min (Days before CR/CRi)
07-009	35
05-030*	34
03-037	21
09-064	13
07-035	0
08-058	0

Patient	ctDNA MAF Max (Days before PD)
11-019	24
05-043	14
05-030*	0
05-016	0



Conclusions

- Mutant allele frequency in plasma-derived ctDNA was highly correlated with MAF in gDNA from BM cells (R^2 = 0.8366)
- Patients with clinical response (CR/CRi) showed bigger changes in ctDNA MAF than non-responders, suggesting that ctDNA changes can be used as a surrogate for treatment response
- Change in ctDNA MAF after the 1st cycle of treatment was highly predictive of response to onvansertib:
  - All patients achieving CR/CRi (n=6) had a decrease in ctDNA MAF after 1 cycle (Log_2(C1/C0) < -0.05)
  - 86% of the non-responders (12 out of 14) showed no decrease in ctDNA MAF after 1 cycle
- Serial monitoring in plasma provides a less-invasive alternative to bone marrow, often enabling earlier detection of clinical response or progression:
  - Minimum ctDNA MAF was measured at or before remission, while maximum ctDNA MAF was measured at or before progression
- Patients enrolled in the Phase 2 section will be analyzed to further validate the utility of ctDNA as a biomarker for onvansertib-response and a surrogate for BM biopsy

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