PLK4 as a Novel Therapeutic Target in *TP53*-mutant Acute Myeloid Leukemia

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Introduction

Resistance to current therapies is one of the major challenges for the cure of *TP53*-mutant (mut) acute myeloid leukemia (AML). Polo-like kinases (PLK1-PLK5) are serine/threonine kinases that have vital regulatory functions in the cell cycle.[1] Previous studies reported that *TP53*-mut cancer cells, including lung cancer and AML overexpress PLK4.[1,2] PLK4 overexpression could potentially contribute to drug resistance in these cells. Therefore, PLK4 could be a promising therapeutic target for *TP53*-mut AML and potentially improve the efficacy of current therapies.

Hypothesis

Inhibition of PLK4 may exhibit anti-leukemia activity in *TP53*-mut AML, and potentially improve the efficacy of current AML therapeutic agents.

Methods

- Human MOLM-13 AML cells with *TP53* wild-type (WT), *TP53*-knockout (KO), or *TP53*-mut were treated with PLK4 inhibitor CFI-400945 and/or venetoclax at various doses for 24 and 48 hours.
- Cell cycle and polyploidy were measured using an Edu Click-it Kit and FXCycle.
- Apoptosis was measured using FACS-flow cytometry after cells were stained with Clv-PARP antibody or AnnexinV/7AAD.
- Intracellular protein levels were measured by western blot.

Results

Increased PLK4 RNA levels in *TP53*-KO or mut compared to *TP53*-WT AML cells

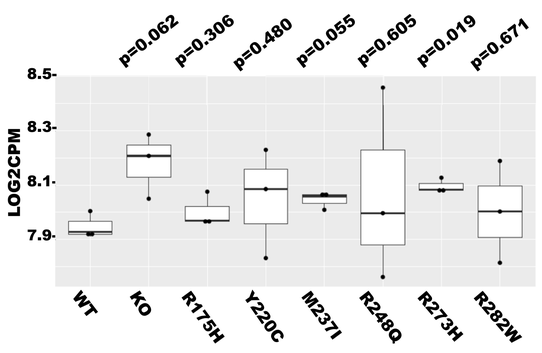
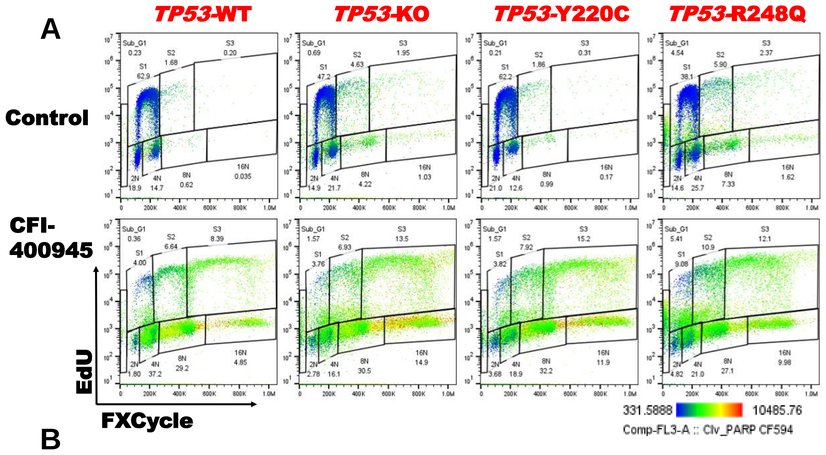


Fig 1. RNA sequencing analysis of PLK4 transcript level in MOLM-13 *TP53*-WT, *TP53*-KO, and *TP53*-mut cells.[3]

PLK4 inhibition induces polyploidy and apoptosis in *TP53*-mut AML



B

Polyploidy Population	Untreated (%)	CFI-400945 25 nM 48 hr (4N+) (%)	CFI-400945 25 nM 48 hr (8N+) (%)
TP53-WT	1.496	91.35	50.43
TP53-KO	7.34	88.12%	72.57
TP53-Y220C	2.11	87.45	68.70
TP53-R248Q	11.88	81.96	64.03

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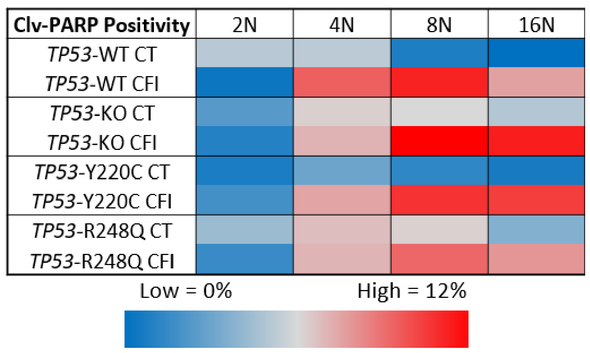


Fig 2. *TP53* isogenic MOLM-13 cells were treated with 25 nM PLK4 inhibitor (CFI-400945) for 48 hr. **A)** FACS-flow cytometry analysis of cell cycle and total DNA content in MOLM-13 *TP53* isogenic cells, also showing Clv-PARP. **B)** Polyploidy percentage in MOLM-13 *TP53* isogenic cells using the live cell population. **C)** Normalized percentage of Clv-PARP+ population within each subpopulation.

CFI-400945 decreases viability in cells from a patient *TP53*-mut AML

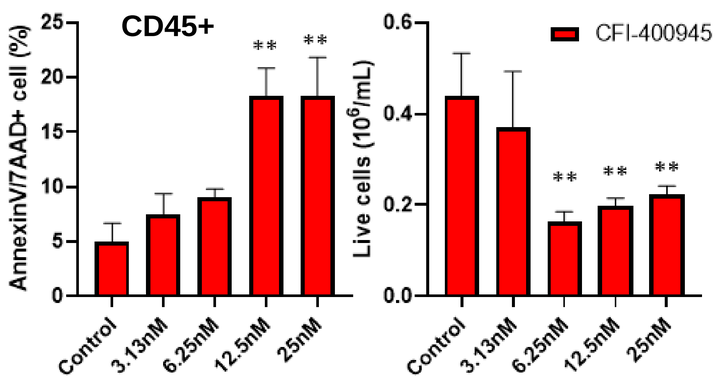
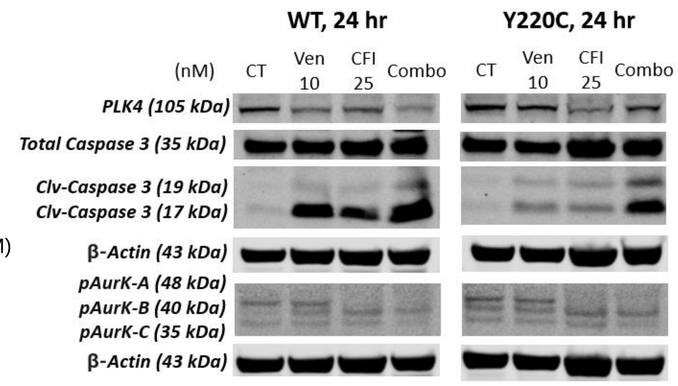


Fig 3. *TP53*-mut AML patient sample was treated with CFI-400945 (n=1) for 48 hr. Blasts 85%.

CFI-400945 induces apoptosis and enhances venetoclax efficacy in MOLM-13 Y220C *TP53*-mut cells

Fig 4. CFI-400945 and/or venetoclax treatment induces caspase 3 cleavage, and decreases PLK4 and phosphorylated aurora kinase A/B/C (pAurK-A/B/C) in MOLM-13 *TP53*-WT and *TP53*-mut isogenic cells. MOLM-13 cells were treated with CFI-400945 (25 nM) and/or venetoclax (10 nM) for 24 hr.



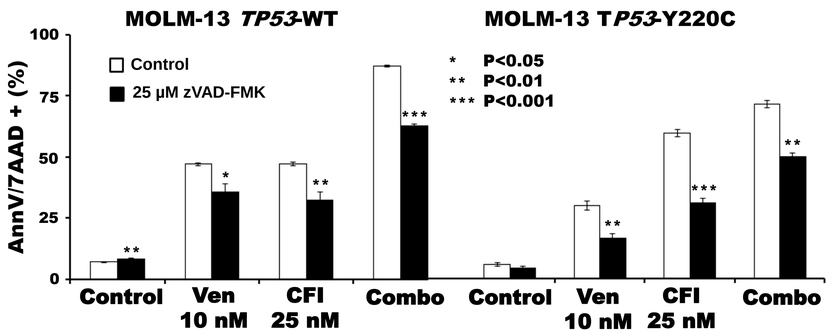
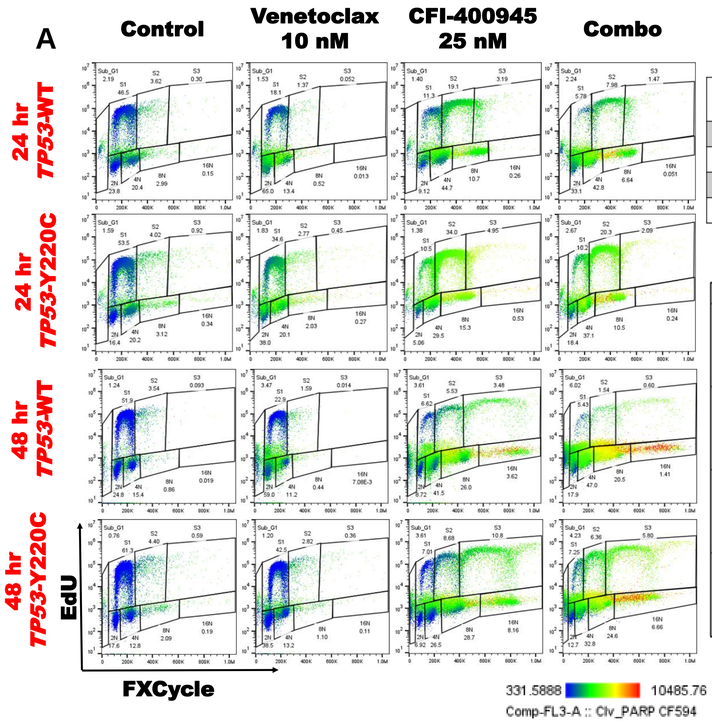


Fig 5. CFI-400945 induces, at least in part caspase-dependent apoptosis. 25 μM of z-VAD-FMK was added 1 hr before the MOLM-13 isogenic cells were treated with 10 nM venetoclax and 25 nM CFI-400945 (n=3) for 48 hr.

CFI-400945 and venetoclax combination induces more polyploidy and apoptosis in MOLM-13 *TP53*-mut compared to *TP53*-WT isogenic cells



B

Polyploidy Population	Untreated (%)	10 nM Ven (%)	25 nM CFI (%)	Combination (%)
24 hr *TP53*-WT	4.165	1.895	68.18	41.18
48 hr *TP53*-WT	2.433	1.45	77.86	61.58
24 hr *TP53*-Y220C	5.65	4.10	77.30	59.09
48 hr *TP53*-Y220C	4.67	2.443	81.37	71.63

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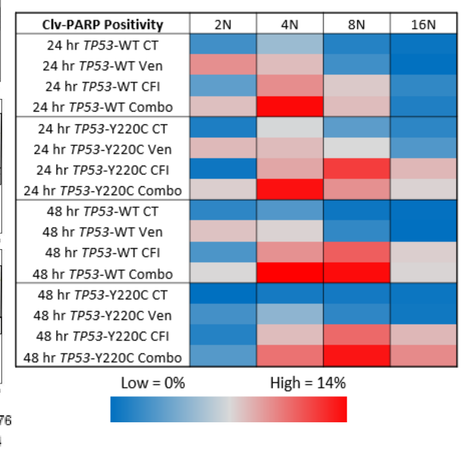

Fig 6. Cell cycle analysis of *TP53* isogenic MOLM-13 cells treated with CFI-400945 (25 nM) and/or venetoclax (10 nM) for 24 and 48 hr. **A)** FACS-flow cytometry analysis of cell cycle and total DNA content in MOLM-13 *TP53* isogenic cells, also showing Clv-PARP. **B)** Polyploidy percentage in MOLM-13 *TP53* isogenic cells using the live cell population. **C)** Normalized percentage of Clv-PARP+ population within each subpopulation.

Conclusions

- *TP53*-mut or *TP53*-KO AML cells express higher PLK4 than the isogenic *TP53*-WT cells
- Inhibition of PLK4 induces polyploidy and cell death in *TP53*-mut cells
- PLK4 inhibition-mediated cell death induction is further enhanced by combination with venetoclax
- PLK4 inhibition enhances the therapeutic efficacy of venetoclax in *TP53*-mut AML cells

References

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[2] Kawakami M., et al. Polo-like kinase 4 inhibition produces polyploidy and apoptotic death of lung cancers. *Proc Nat Acad Sci USA*. 2018 Feb 20;115(8):1913-1918

[3]Boettcher S., et al. A dominant-negative effect drives selection of *TP53* missense mutations in myeloid malignancies. *Science*. 2019 Aug 9;365(6453):599-604

Acknowledgments

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