Background

• TP53 mutations in acute myeloid leukemia (AML) are associated with complex karyotype and high risk of relapse (Döhner et al., 2017; Giacomelli et al., 2018). The mechanisms responsible for response and relapse in TP53-mutant AML remain unclear and investigating novel mechanisms is critical to develop more effective therapies.

• In order to shed light on the defective p53 signaling pathways underlying TP53 mutant AML, we performed RNA-sequencing (RNA-seq) on bulk mononuclear cells or FACS-sorted leukemic stem cells (LSCs) using samples collected from TP53-mutant or TP53-wt high-risk AML patients.

• We identified a key regulator of centriole biogenesis: Polo-like kinase 4 (PLK4) as a potential target highly expressed in TP53-mutant AML samples.

• Previous publications showed that PLK4 is transcriptionally repressed by p53 and induces apoptosis upon RNAi silencing (Fischer et al., 2014; Li et al., 2005). Here we show that TP53-mutant AML samples lack the p53-dependent PLK4 repression and have higher levels of PLK4 compared to TP53-wt AML.

• Gap of knowledge: The mechanisms responsible for response and relapse in TP53-mutant AML remain unclear and there are no effective treatments against TP53-mut AML.

• We hypothesized that targeting PLK4 will triguer mitotic defects, and activate apoptosis in TP53-mut leukemia cells, making it a potential treatment approach for TP53-mut AML.

Materials and Methods

RNA sequencing datasets:
MD Anderson AML Moonshot RNA seq dataset:
Total = 44 AML samples as following:
TP53-mut samples = 19 (bulk = 12, LSC = 7)
TP53-wt samples = 25 (bulk =14, LSC =11)

Munich Leukemia Laboratory (MLL) RNA-seq dataset:
Total = 726 AML samples as following:
TP53-mut samples = 72, TP53-wt samples = 654.

PLK4 is overexpressed in primary samples from TP53-mut AML patients

PLK4 inhibition results in higher polyploidy in TP53-mut vs TP53-wt MOLM13

Figure 1: Survival analysis performed based on A) TP53 status. B) PLK4 expression levels.

Figure 2: RNA-seq datasets show a significant increase in PLK4 expression in TP53-mut AML.
A) MDACC Moonshot (bulk n=26, LSC n=18).
B) Munich Leukemia Laboratory (bulk n = 726).

PLK4 is overexpressed in TP53-mut AML MOLM13 cell lines

Figure 4: TP53-wt and TP53-mut MOLM13 cell lines were treated with 25nM CFI-400945 for 72 hours. Polyploid status shown in A) TP53-wt, B) TP53-KO, C) TP53-R248Q, and D) TP53-R273H.

Figure 5: Monitoring DNA content with the Click-IT EdU labeling. Pseudocolor plots showing the levels PI and EdU levels in TP53-wt, TP53-KO, and TP53-R244Q MOLM13 cell lines. Left column shows untreated samples. Right column shows samples treated with 25nM CFI-400945 for 48 hours. Color axis represents the levels of cleaved Caspase-3.

Figure 6: Survival analysis performed on three different PDX models.

Conclusions

• TP53-mutant AML has an overexpression of PLK4, which is a central regulator for centriole duplication.

• Targeting PLK4 results in increased levels of polyploidy in TP53-mut AML vs TP53-wt AML.

• PLK4 inhibition upregulates cleaved Caspase-3 in polyploid cells, and results in significantly higher apoptosis in TP53-mut MOLM13 cell lines in comparison to TP53-wt MOLM13 cell line.

• A clinical trial is ongoing testing the efficacy of PLK4 inhibition (CFI-400945) in AML (ID: NCT04730258, TWT-202).

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Polyploid TP53-mut MOLM13 cells have increased levels of cleaved Caspase-3

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