



NOVEL THERAPEUTIC EFFECTS AND MECHANISMS OF POLO-LIKE KINASE 4 INHIBITION IN TP53 MUTATED ACUTE MYELOID LEUKEMIA

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INTRODUCTION

Acute myeloid leukaemia (AML) carrying tp53 mutation portends an extremely grave prognosis and is generally refractory to conventional chemotherapy and allogeneic hematopoietic stem cell transplantation (HSCT). Polo-Like Kinase 4 (PLK4) has been shown to regulate centrosome duplication and play a critical role in oncogenesis

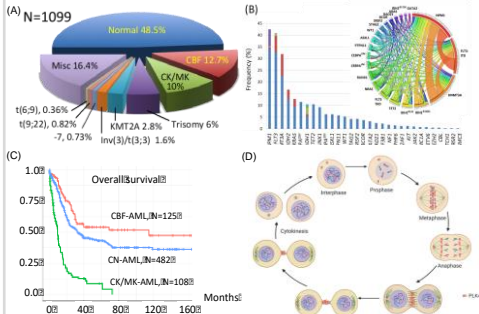


Figure 1: Genetic features of acute myeloid leukaemia (AML). (A) Cytogenetic abnormalities of AML patients (n=1099) in Hong Kong. (B) Mutation spectrum and co-occurring mutations within AML. (C) Comparison of overall survival data of AML patients in Hong Kong. (D) Schematic diagram of distribution and localization of PLK4 in cell cycle.

Hypothesis: Polo-like kinase 4 (PLK4) as a target of therapeutic intervention in tp53 mutated acute myeloid leukaemia

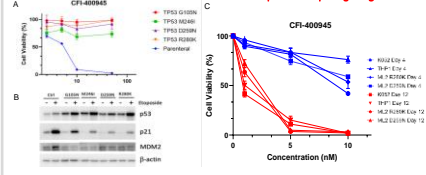
METHODOLOGY

We examined the effects of PLK4 inhibition and investigated its mechanisms of action in tp53 mutant AML. AML cell lines representative of the driver events in leukaemogenesis were used.

Anti-leukaemic effects of PLK4 inhibitor (PLK4i) were evaluated based on cellular proliferation and cell cycle analysis. Effects on centrosome duplication were examined by immunofluorescence and confocal microscopy.

DNA damage was shown by micronuclei formation and γH2AX protein and immunostaining. SA-β-Gal activity determined by cellular Senescence Analysis Assay Kit

Antileukaemic effect of PLK4i (CFI-400945) Longer-term treatment (12 days) of AML cell lines with PLK4 inhibitor suppressed leukemia growth Independent of p53 signaling.



Long-term exposure to PLK4 inhibitor induced cell cycle arrest at G2/M phase and polyploidy. Long-term exposure to PLK4 inhibitor induced defective cytokinesis

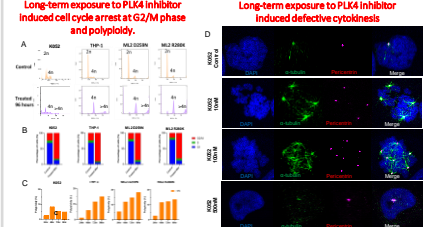


Figure 3: Effects of PLK4 inhibitors on cell cycle in human AML cell lines. (A) Cell cycle analysis of K552, THP-1 and tp53 K1 ML-2 cell treated with 10nM of CFI-400945 at 96 hours. (B) Statistic of cell cycle analysis at 96 hours. (C) Percentage of polyploidy cell (>4N) after treatment 24, 48, 72 and 96 hours. (D) Representative immunofluorescence images of K552 cell line treated with CFI-400945 for 48 hours.

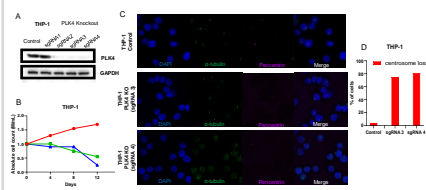


Figure 4: Effects of PLK4 knockout in human AML cell line. (A) Immunoblot analysis of PLK4 knockout efficiency in THP-1 cell. (B) Long term effect of PLK4 knockout in THP-1 cell. (C) Representative immunofluorescence images of THP-1 PLK4 knockout cell for 48 hours. (D) Statistic of number of centrosome loss in THP-1 PLK4 knockout cell.

RESULTS

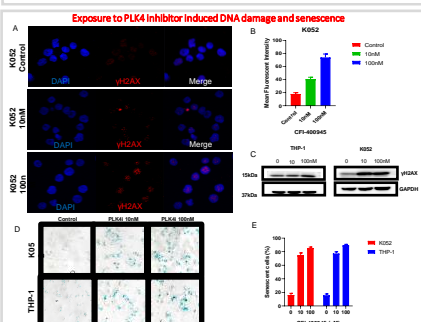
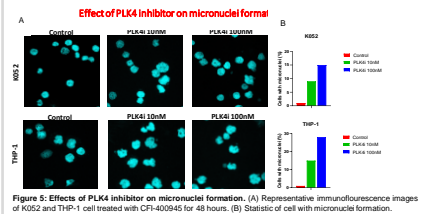


Figure 6: Effects of PLK4 inhibitors on DNA damage and senescence. (A) Representative immunofluorescence images of K552 cell treated with CFI-400945 for 24 hours. (B) Mean fluorescence intensity (MFI) of γH2AX in K552 and THP-1 cell line. (C) Immunoblot analysis of γH2AX in K552 and THP-1 cell line. (D) Senescence associated β-galactosidase staining in K552 and THP-1 cell. (E) Statistic of senescence cells.

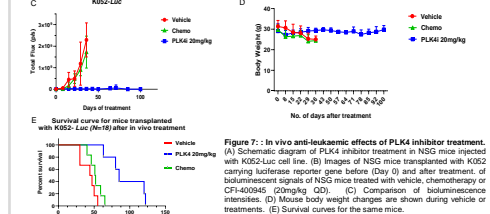
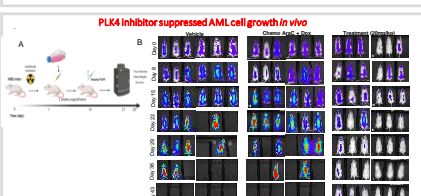


Figure 7: In vivo anti-leukaemic effects of PLK4 inhibitor treatment. (A) Schematic diagram of PLK4 inhibitor treatment in NSG mice injected with K552-Luc cell line. (B) Images of NSG mice transplanted with K552 carrying luciferase reporter gene before Day 0 and after treatment of bioluminescent signals of NSG mice treated with vehicle, chemotherapy or CFI-400945 (20mg/kg QD). (C) Comparison of bioluminescence intensities. (D) Mouse body weight changes are shown during vehicle or treatments. (E) Survival curves for the same mice.

CONCLUSIONS

TP53 mutant AML cell lines show a progressive increase in polyploidy (>4N) and number of centrosomes upon treatment with CFI-400945. Aberrant centrosome amplification was demonstrated due to defective cytokinesis. The effects of specific PLK4 knockout by CRISPR/Cas9 on tp53 mutated AML were similar to PLK4 inhibition by CFI-400945. PLK4 knockout suppressed leukemia growth after prolonged culture to 12 days.

Intriguingly, PLK4 knockout abolished centrosome formation in tp3 mutated AML cells. Besides, CFI-400945 in a dose dependent fashion induced significant increase in micronuclei and DNA damage. Following DNA damage, senescence associated β-galactosidase (SA-β-Gal) activity shows that CFI-400945 elicits a senescence phenotype in AML cells. Further, CFI-400945 significantly reduce leukemic burden in mice and prolong animal survivals without significant deleterious effects on the host.

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