(PS1582) SYNERGISTIC ANTI-LEUKEMIC EFFECTS OF HOMOHARRINGTONINE COMBINED WITH CLIFUTINIB IN FLT3-ITD MUTATED ACUTE MYELOID LEUKEMIA

Topic: 03. Acute myeloid leukemia - Biology & translational research

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Background

FLT3-ITD mutation is one of the most aggressive molecular subtype in acute myeloid leukemia (AML) and is associated with poor prognosis and high relapse rates. Although FLT3 inhibitors have improved clinical outcomes, resistance and limited durability remain major challenges. Therefore, novel combination strategies targeting multiple oncogenic pathways are urgently needed. Homoharringtonine (HTT) and Clifutinib are both demonstrated as anti-leukemic properties through distinct mechanisms of action, making their combination a rational and promising candidate for multi-targeted therapy; however, their synergistic potential and the underlying molecular mechanisms remain unexplored.

Aims

This study aimed to investigate the synergistic anti-leukemic effects of HTT combined with Clifutinib in FLT3-ITD mutated AML and to elucidate the underlying molecular mechanisms both *in vitro* and *in vivo*.

Methods

FLT3-ITD mutated leukemia cell lines were treated with HTT and Clifutinib singly or in combination. Cell viability was assessed, and synergistic effects were evaluated using the combination index (CI) method. Apoptosis was analyzed by flow cytometry. Network pharmacology was employed to predict potential signaling pathways involved in the combined treatment. Western blotting was performed to validate the inhibition of key signaling pathways. *In vivo* efficacy was evaluated using a zebrafish xenograft model and a mouse tail-vein injection leukemia model to assess tumor burden and overall survival.

Results

The combination of HTT and Clifutinib demonstrated potent and synergistic anti-leukemic activity in FLT3-ITD mutated cell lines. Synergism was confirmed by combination index analysis after 48h treatment, with CI values of 0.77 at ED_{75}, and 0.56 at ED_{90}. Mechanistically, the combined treatment synergistically induced apoptosis in FLT3-ITD mutated cells. Network pharmacology analysis and subsequent western blot validation revealed that the combination concurrently inhibited multiple survival pathways, including PI3K-AKT, and JAK-STAT signaling, which are central to FLT3-ITD-driven disease progression and therapeutic resistance. Importantly, *in vivo* studies demonstrated that the combination therapy significantly reduced leukemic burden in the zebrafish model and markedly prolonged survival in leukemia-bearing mice compared with monotherapy or controls, underscoring its translational potential as a novel combinational strategy for FLT3-ITD mutated AML.

Summary/Conclusion

HTT combined with Clifutinib exerts synergistic anti-leukemic effects against FLT3-ITD mutated AML by inducing apoptosis and simultaneously inhibiting multiple oncogenic signaling pathways, including PI3K-AKT and JAK-STAT. These findings provide a strong preclinical rationale for further investigation of this combination strategy as a potential therapeutic approach for FLT3-ITD mutated AML.

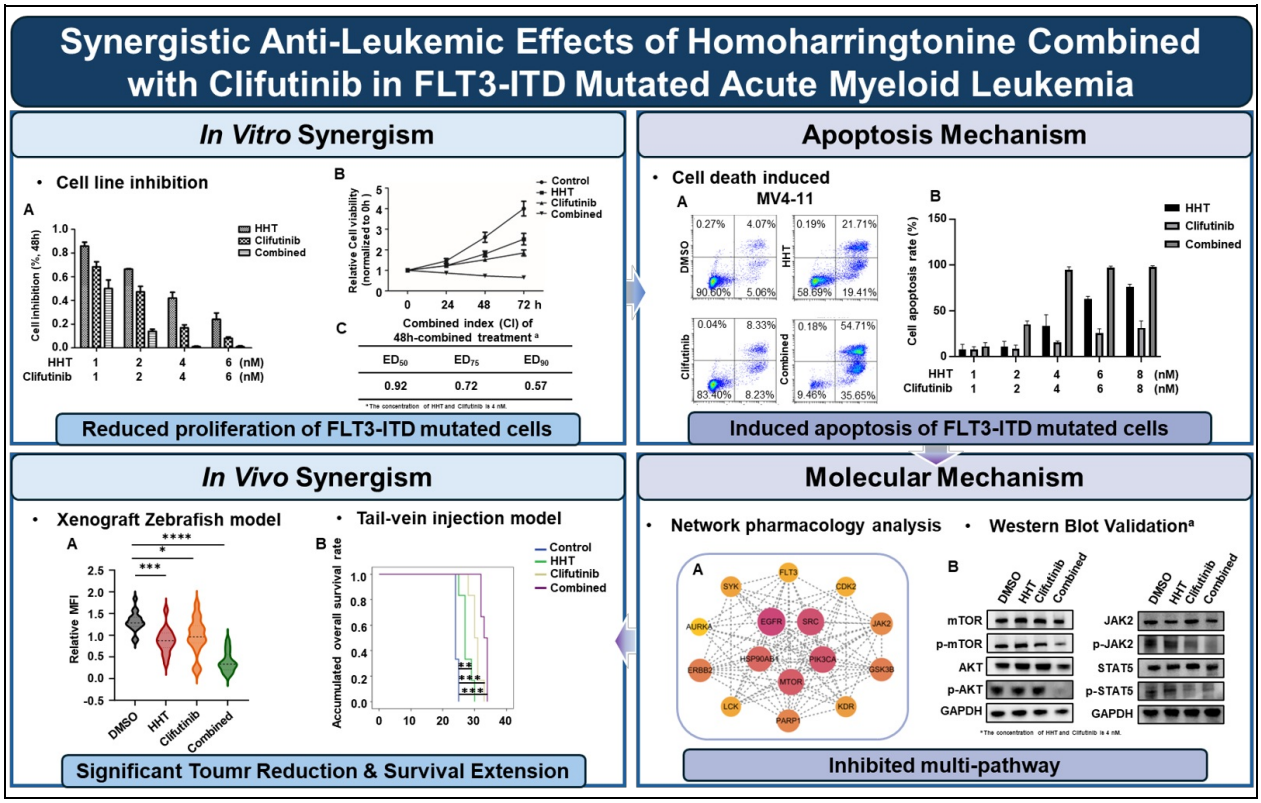

Figure 1. Synergistic efficacy and mechanisms of HHT and Clifutinib combination in FLT3-ITD AML. (A) In vitro synergistic cytotoxicity (CI < 1) in mutant cell lines. (B) Enhanced apoptosis induction by the combination treatment. (C) Mechanism: concurrent suppression of PI3K-AKT, MAPK, and JAK-STAT pathways. (D) In vivo efficacy: reduced tumor burden (zebrafish) and prolonged overall survival (mice).