Homoharringtonine Synergizes with Quizartinib in FLT3-ITD Acute Myeloid Leukemia by Targeting FLT3-AKT-c-Myc Pathway

Qurais Syihab¹ and Alhana Jadira²

¹Veterinary Medicine, Airlangga University, Indonesia
²Internal Medicine, Sriwijaya University, Indonesia

Background: Acute myeloid leukemia is a group of highly heterogeneous clonal diseases with distinct clinicopathological, cytogenetic, and molecular biological characteristics. The molecular subtype of AML largely determines the clinical characteristics and prognosis. An internal tandem duplication of the FMS-like tyrosine kinase receptor gene (FLT3-ITD). FLT3-ITD AML has poor prognosis and a higher disease relapse rate, and hence, inferior disease-free and overall survival. FLT3-ITD results in constitutive activation and autophosphorylation of FLT3, which induces the activation of multiple intracellular signaling molecules, leading to autonomous cell proliferation, and thus, it plays a key role in the pathogenesis of AML. Targeted inhibition of FLT3 kinase activity is an important strategy for the treatment of AML, and numerous FLT3 inhibitors have been clinically developed. The first-generation FLT3 inhibitors midostaurin and sorafenib are multikinase inhibitors. Their targets include RAF kinase, PDGFR, VEGFR, c-KIT, and FLT3.

Materials and Method: Human AML cell lines carrying the FLT3-ITD mutation (MOLM-13 and MV4-11). Human FLT3-WT AML cell lines (THP-1 and HL60/S4). The cells were maintained in RPMI1640/Iscove's modified Dulbecco's medium (HyClone, Thermo Fisher Scientific, USA) supplemented with 10% foetal bovine serum (Gemini, USA), 100 U/ml penicillin, and 100 µg/ml streptomycin (HyClone, Thermo Fisher Scientific, USA) at 37 °C in a humidified atmosphere containing 5% CO₂. The cell lines were verified by short tandem repeat analysis and tested for mycoplasma contamination. To generate FLT3-ITD AML cells with consistent over c-Myc expression, human AML cell line MV4-11 was infected with c-Myc-OE lentivirus. Meanwhile, MV4-11 was infected with negative control lentivirus (MV4-11-CTR). After infection, transduced cells were selected and maintained in a puromycin+ culture medium. Mononuclear cells were isolated by density gradient centrifugation using Ficoll-Hypaque, then use Analysis by; Analysis of the cell cycle and apoptosis by flow cytometry and RNA-sequencing (RNA-seq).

Results: Here, we showed that HHT synergizes with a selective next-generation FLT3 inhibitor, quizartinib, to inhibit cell growth/viability and induce cell-cycle arrest and apoptosis in FLT3-ITD AML cells in vitro, significantly inhibit acute myeloid leukemia progression in vivo, and substantially prolong survival of mice-bearing human FLT3-ITD AML. Mechanistically, HHT and quizartinib cooperatively inhibit FLT3-AKT and its downstream targets GSKβ, c-Myc, and cyclin D1, cooperatively up-regulate the pro-apoptosis proteins Bim and Bax, and down-regulate the anti-apoptosis protein Mcl1. Most strikingly, HHT and quizartinib cooperatively reduce the numbers of side-population (SP) and aldehyde dehydrogenase (ALDH)-positive cells, which reportedly are rich in LSCs. In conclusion, HHT combined with quizartinib may be a promising treatment strategy for patients with FLT3-ITD AML.

Conclusion: FLT3 inhibitors combined with chemotherapy may be a promising treatment for FLT3-ITD AML. Homoharringtonine (HHT) is a classical anti-leukaemia drug with high sensitivity to FLT3-ITD AML cells. Here, we showed that HHT synergizes with a selective next-generation FLT3 inhibitor, quizartinib, to inhibit cell growth/viability and induce cell-cycle arrest and apoptosis in FLT3-ITD AML cells in vitro, significantly inhibit acute myeloid leukemia progression in vivo, and substantially prolong survival of mice-bearing human FLT3-ITD AML. Mechanistically, HHT and quizartinib cooperatively inhibit FLT3-AKT and its downstream targets GSKβ, c-Myc, and cyclin D1, cooperatively up-regulate the pro-apoptosis proteins Bim and Bax, and down-regulate the anti-apoptosis protein Mcl1. Most strikingly, HHT and quizartinib cooperatively reduce the numbers of side-population (SP) and aldehyde dehydrogenase (ALDH)-positive cells, which reportedly are rich in LSCs. In conclusion, HHT combined with quizartinib may be a promising treatment strategy for patients with FLT3-ITD AML.

Keywords: Homoharringtonine, Acute myeloid leukemia, FLT3-AKT-c-Myc