Title: Effect of Fms-like tyrosine kinase 3 (FLT3) ligand (FL) on antitumor activity of gilteritinib, a FLT3 inhibitor, in mice xenografted with FL-overexpressing cells

Therapeutic effects of FLT3 inhibitors have been reported in acute myeloid leukemia (AML) with constitutively activating FLT3 mutations, including internal tandem duplication (ITD) and point mutation, which are found in approximately onethird of AML patients. One of the critical issues of treatment with FLT3 inhibitors in FLT3-mutated AML is drug resistance. FLT3 ligand (FL) represents a mechanism of resistance to FLT3 inhibitors, including quinazolines, midostaurin, and sorafenib, in AML cells harboring both wild-type and mutant FLT3-ITD (FLT3-itd). Here, we investigated the effect of FL on the efficacy of gilteritinib, a FLT3 inhibitor, in AML-derived cells in vitro and in mice. In contrast to other FLT3 inhibitors, FL stimulation had little effect on growth inhibition or apoptosis induction by gilteritinib. The antitumor activity of gilteritinib was also comparable between xenograft mouse models injected with FL-expressing and mock MOLM-13 cells. In the FLT3 signaling analyses, gilteritinib inhibited FLT3 wt and FLT3-ITD to a similar degree in HEK293 and BaF3 cells, and similarly suppressed FLT3 downstream signaling molecules (including ERK1/2 and STAT5) in both the presence and absence of FL in MOLM-13 cells. Co-crystal structure analysis showed that gilteritinib bound to the ATP-binding pocket of FLT3. These results suggest that gilteritinib has therapeutic potential in FLT3-mutated AML patients with FL overexpression.