Abstract

Introduction: Activating mutations within internal tandem duplication (ITD) and tyrosine kinase domain (TKD) of FLT3 have been reported as driver oncogenes in about 30% of acute myeloid leukemia (AML). FLT3 mutation AML is associated with poor prognosis with a high risk of relapse after therapy and reduced overall survival. Currently, FLT3 inhibitors have proven clinical benefits in AML patients with FLT3 mutation, but FLT3-targeted therapies can be interfered from various resistance mechanisms including acquired DSSY and FBH1, mutations in TKD. In this study, we characterized HM43239, a novel FLT3 inhibitor, and assessed its potential as a novel therapeutic agent to overcome the resistance against AML therapy.

Materials and Methods: In vitro side-directed combination binding assay was performed to measure interactions of HM43239 with FLT3 kinase. Standard kinase inhibition and cell proliferation assay, immunoblotting, and apoptosis analysis were carried out to identify the activity of HM43239 in the AML cell lines. The antileukemic activity of HM43239 was evaluated in mice models xenografted with FLT3 mutated Ba/F3 or wild type KG-1 cell line. Combination studies were performed in MOL-013 cell xenografts to mouse.

Results: HM43239 potently inhibited FLT3 along with SIK1 and JAK1 associated with AML resistance, and exhibited strong binding affinity to FLT3 kinase regardless of mutations. HM43239 effectively reduced the activity of FLT3 and downstream STAT5s, including ITD/TKD double mutations. In vitro modes using Ba/F3 cells expressing ITD and TKD double mutations, HM43239 doubly prolonged the survival time compared to control group. In LSC-like KG-1a cell model, HM43239 more effectively decreased CD34+CD38+ populations, known to leukaemic stem cell (LSC) marker and more strongly decreased expression of the cell cycle markers to G1 phase. This suggests the possibility of HM43239 for targeting LSC. HM43239 strongly inhibited the cell growth in the presence of FLT3, showing a potential for overcoming FL-induced resistance. Interestingly, HM43239 similarly inhibited the phosphorylation of FLT3 and cell growth markers, suggesting exact translation of anti-leukemia activity from cell to human. Moreover, in ex vivo leukemia model, HM43239 showed synergistic effect with various agents (e.g. DNMT or IAP inhibitor) without any significant toxicity.

Kinase and Cell Growth Inhibition

<table>
<thead>
<tr>
<th>FLT3 WT</th>
<th>FLT3 TKD</th>
<th>FLT3 ITD/TKD</th>
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<tbody>
<tr>
<td>% Inhibition</td>
<td>% Inhibition</td>
<td>% Inhibition</td>
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<tr>
<td>0.1 nM</td>
<td>50.1</td>
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<tr>
<td>10 nM</td>
<td>72.4</td>
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<td>IC50</td>
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The survival time of HM43239 was extended in female nude mice intravenously injected with Ba/F3-FLT3 (ITD/TKD) and Ba/F3-FLT3 ITD/TKD cells.

Combination Effect in AML Cell Models

Combination with DNM7 Inhibitor

A. In vitro cell growth

B. Survival time in xenograft model

Combination with IAP Inhibitor

A. In vitro cell growth

B. Survival time in xenograft model

Effect on FLT3 ITD/TKD Mutation

The effective binding of inhibition by FLT3 TKD mutations was determined in human plasma.

Effect on FLT3 Ligand (FL)

The effective growth inhibition on the FLT3 ligand was determined in human plasma.

Conclusions

1) HM43239 is a novel FLT3 inhibitor with strong antileukemia activity in vitro and in vivo.
2) HM43239 effectively inhibited the phosphorylation of FLT3 and cell growth markers, suggesting exact translation of anti-leukemia activity from cell to human.
3) HM43239 showed synergistic effect with various agents (e.g. DNMT or IAP inhibitor) without any significant toxicity.

References
2. World J Clin Oncol. 2012. 95, 90-97

Hannmi Pharm. Co., Ltd. (http://www.hannmipharm.com)