c-Met Inhibitor Synergizes with Tumor Necrosis Factor–Related Apoptosis-Induced Ligand to Induce Papillary Thyroid Carcinoma Cell Death

Rong Bu,1* Shahab Uddin,1* Maqbool Ahmed,1 Azhar R Hussain,1 Saif Alsobhi,2 Tarek Amin,3 Abdurahman Al-Nuaimi,3 Fouad Al-Dayel,4 Jehad Abubaker,1 Prashant Bavi,1 and Khawla S Al-Kuraya1

Online address: http://www.molmed.org

Supplementary Figure 1. Tissue microarray based immunohistochemical analysis of p-Met, TRAIL-R2 or DR5, p-AKT, BCL-XL, XIAP and HGF in PTC patients neoplastic thyroid tissue and non-neoplastic normal thyroid tissue. A: The Array spot showing high expression of p-Met (a) DR5 (b), p-AKT(c), Bcl-XL (d), XIAP (e) and HGF (f) in in PTC patients neoplastic thyroid tissue. B: The Array spot showing lower expression level of p-Met (a) DR5 (b), p-AKT(c), Bcl-XL (d), XIAP (e) and HGF in non-neoplastic normal thyroid tissue. 20 X/0.70 objective on an Olympus BX 51 microscope. (Olympus America Inc. Center Valley, PA, USA, with the inset showing a 40X 0.85 aperture magnified view of the same.)
PHA665752 treatment did not affect phosphorylation of Lyn, Stat3, Jak2 and Src. B-CPAP cells were treated with 0, 0.5, 1, 2.5, 5 and 10 μM PHA665752 for 4 h. After cell lysis, equal amounts of proteins were separated by SDS-PAGE, transferred to immobilon membrane, and immunoblotted with antibodies against p-Met, p-Lyn, p-Stat3, p-Jak2, p-Src and β-actin.
Supplementary Figure 4. A: Increase of ROS generation in PHA665752 treated PTC cells. B-CPAP and TPC-1 cells were treated with PHA665752 10\(\mu M\) for 0, 2, 4 and 6 h respectively, then loaded with 10\(\mu M\) H2DCFDA and incubated at 37\(^\circ\)C for 45 min. After washing with PBS, cells were re-suspended in PBS and immediately analyzed using flow cytometry for intracellular accumulation of ROS. B: B-CPAP and TPC-1 cells were pre-treated with 10mM NAC for two hours followed by treatment with 10\(\mu M\) PHA665752 for indicated time periods, then loaded with 10\(\mu M\) H2DCFDA and incubated at 37\(^\circ\)C for 45 min. After washing with PBS, cells were re-suspended in PBS and immediately analyzed using flow cytometry for intracellular accumulation of ROS.

Supplementary Table 1 Antibodies used for tissue microarray immunohistochemical analysis.

<table>
<thead>
<tr>
<th>Antibody</th>
<th>Clone</th>
<th>Company</th>
<th>Source</th>
<th>Dilution*</th>
<th>Antigen Retrieval</th>
<th>Subcellular Localization</th>
<th>Detection System</th>
</tr>
</thead>
<tbody>
<tr>
<td>p-MET</td>
<td>Polyclonal</td>
<td>Invitrogen</td>
<td>Rabbit</td>
<td>1:300</td>
<td>pH6, PC</td>
<td>Nuclear</td>
<td>EnVision+</td>
</tr>
<tr>
<td>DR5</td>
<td>TNFRSF10B</td>
<td>R&amp;D</td>
<td>Goat</td>
<td>1:1000</td>
<td>pH6, PC</td>
<td>Cytoplasmic</td>
<td>EnVision+</td>
</tr>
<tr>
<td>HGF</td>
<td>Polyclonal</td>
<td>SCBT</td>
<td>Rabbit</td>
<td>1:1000</td>
<td>pH9, PC</td>
<td>Cytoplasmic</td>
<td>EnVision+</td>
</tr>
<tr>
<td>P110(\alpha)</td>
<td>C73F8</td>
<td>Cell signalling</td>
<td>Rabbit</td>
<td>1:100</td>
<td>pH9, MW</td>
<td>Cytoplasmic</td>
<td>EnVision+</td>
</tr>
<tr>
<td>p-AKT</td>
<td>Ser 473</td>
<td>Cell signalling</td>
<td>Rabbit</td>
<td>1:1000</td>
<td>pH9, MW</td>
<td>Nuclear/Cytoplasmic</td>
<td>EnVision+</td>
</tr>
<tr>
<td>PTEN</td>
<td>6H2.1</td>
<td>Cascade</td>
<td>Mouse</td>
<td>1:300</td>
<td>pH9, MW</td>
<td>Cytoplasmic</td>
<td>EnVision+</td>
</tr>
<tr>
<td>XIAP</td>
<td>48</td>
<td>BD Transduction</td>
<td>Mouse monoclonal</td>
<td>1:300</td>
<td>pH9, MW</td>
<td>Cytoplasmic</td>
<td>EnVision+</td>
</tr>
<tr>
<td>BCLXL</td>
<td>54H6</td>
<td>Cell Signalling</td>
<td>Rabbit polyclonal</td>
<td>1:800</td>
<td>pH9, MW</td>
<td>Cytoplasmic</td>
<td>EnVision+</td>
</tr>
</tbody>
</table>

*Over night incubation

PC: Pressure cooker

MW: Microwave

P110\(\alpha\): Phosphatidylinositol 3 kinase catalytic subunit-110\(\alpha\)