Evaluating contribution of hyperactive c-Met and HER (ErbB) signaling to tumor progression in mouse breast tumor xenografts: an in vivo study of c-Met and HER/ErbB targeted therapies

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Background

HER2 gene (ERBB2) amplification and/or HER2 protein overexpression is detected in approximately 15–20% of breast cancers and is associated with more aggressive disease progression, metastasis, and worse prognosis.1,2 Reports demonstrating significantly improved outcomes in breast cancer patients treated with HER2-targeted therapies in clinical trials have led to the approval of multiple HER2-targeted agents.3,4 These agents have demonstrated clinical benefit in the treatment of HER2-positive breast cancers and have led to significant improvements in overall survival.5,6 HER2-targeted therapies are more effective when HER2 expression or amplification levels are high.7

HER2 expression or amplification levels are determined using IHC or FISH HER2 tests.4 However, clinical trials have indicated a weak correlation between HER2 expression or amplification levels and HER2 targeted therapy benefit.6,7 c-Met is the cognate receptor for Hepatocyte Growth Factor (HGF). MET amplification and HGF overexpression have emerged as mechanisms by which cancers become resistant to HER2-targeted therapies.14 CELx multi-pathway signaling function test.14

CELx MP Test:

Cell lines were maintained in RPMI media + 10% FBS according to ATCC recommendations and authenticated by ATCC STR profiling.

Methods

Breast Cancer Cell Lines: HCC1954, a HER2+ cell line with augmentation of both HER and c-Met signaling, and normal breast cells, a breast cancer cell line, were obtained from Celcuity Inc. 

Cell Culture: Cells were maintained in RPMI medium + 10% FBS according to ATCC recommendations and authenticated by ATCC STR profiling.

CELx MP Test: HCC1954 cells were exposed to different combination of HER/c-Met inhibitors, and their effects on pathway activation using CELx time-course curves were evaluated.

Xenograft: HCC1954 cells were activated with 0.3 nM EGF, 3 nM NRG, and 30 pM HGF ± erlotinib, tepotinib, neratinib, erlotinib + tepotinib, or neratinib + tepotinib in combination with captisol (10%).

Results

Table 1: Real-Time Live Cell CELx Multi-Pathway Signaling Inhibition Analysis of HCC1954 Cells

<table>
<thead>
<tr>
<th>HER/c-Met Inhibitors</th>
<th>Pan-HER Inhibitors</th>
<th>HER2 Inhibitors</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tepotinib (c-MeTi)</td>
<td>-8.8 ± 12.8 (n=5)</td>
<td>18.6 ± 12.8 (n=5)</td>
</tr>
<tr>
<td>Neratinib</td>
<td>-7.3 ± 12.8 (n=5)</td>
<td>31.6 ± 12.8 (n=5)</td>
</tr>
<tr>
<td>Erlotinib</td>
<td>-5.6 ± 12.8 (n=5)</td>
<td>47.6 ± 12.8 (n=5)</td>
</tr>
</tbody>
</table>

Table 2: HCC1954 Xenograft Model: Experimental Design

<table>
<thead>
<tr>
<th>Experiment Cohort</th>
<th>n</th>
<th>Drug Dose (mg/kg)</th>
<th>Dosing Frequency</th>
<th>Number of Doses</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>10</td>
<td>Vehicle 0</td>
<td>QD</td>
<td>21</td>
</tr>
<tr>
<td>B</td>
<td>9</td>
<td>Neratinib 40</td>
<td>QD</td>
<td>21</td>
</tr>
<tr>
<td>C</td>
<td>9</td>
<td>Tepotinib 50</td>
<td>QD</td>
<td>21</td>
</tr>
<tr>
<td>D</td>
<td>9</td>
<td>Neratinib + Tepotinib 40 + 50</td>
<td>QD</td>
<td>21</td>
</tr>
</tbody>
</table>

Figure 4. HCC1954 Xenograft Drug Response is Consistent with CELx MP Signaling Results

Figure 2. HER2 Abnormal Signaling Noted by CELx Test

Figure 3. HCC1954 Xenograft Model: Mouse Body Weight

Figure 1. Platform Broaden Sensitivity Equivalents Quantification of HER and c-Met Signaling Real-Time in Live Cells

Conclusions

• Hyperactive and coincident c-Met and HER signaling contributes to the progression of certain HER2-negative breast cancers.

• Combination of c-Met + HER1 inhibitors OR a pan-HER inhibitor alone can more effectively inhibit co-activation of HER and c-Met pathways, but full inhibition requires the combination of these agents.

• HER/c-Met signaling is highly responsive to the combination of c-Met + HER1 inhibitors OR a pan-HER inhibitor.

• HER/c-Met signaling is also highly responsive to the combination of HER and c-Met inhibitors.

• HER2-negative breast cancer sub-type is more responsive to treatment with a combination of c-Met inhibitor plus a pan-HER inhibitor versus a c-Met or HER1 inhibitor alone.

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