N87 Cells are ErbB2-Dependent in the Absence of EGFR

Figure 2. ErbB homodimers are predominant in N87 cells

- N87 is a gastric carcinoma cell line that expresses high levels of constitutively active ErbB2 and low levels of EGFR.
- ErbB2 homodimers are predominant in N87 cell lysates in vitro.
- Proliferation was only modestly inhibited by selective EGFR inhibition with erlotinib in the presence of EGF.
- In the presence of EGF, an increase in EGFR homodimers and ErbB2:EGFR heterodimers was observed.
- High levels of ErbB2 homodimers remained in the presence of the EGFR inhibitor.
- ErbB2 homodimers were low and undetected in response to EGFR stimulation.

Table 1. Pan-ErbB inhibitor is superior for blocking growth of N87 cells in the presence of EGF.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Average IC50 (nM)</th>
<th>IC50 (%)</th>
<th>Regr</th>
</tr>
</thead>
<tbody>
<tr>
<td>ARRY-380</td>
<td>50</td>
<td>640</td>
<td>11</td>
</tr>
<tr>
<td>ARRY-543</td>
<td>129</td>
<td>34</td>
<td>90</td>
</tr>
<tr>
<td>Erlotinib</td>
<td>945</td>
<td>714</td>
<td>0.0</td>
</tr>
<tr>
<td>ARRY-380 + Erlotinib</td>
<td>5</td>
<td>399</td>
<td>62</td>
</tr>
<tr>
<td>ARRY-543 + Erlotinib</td>
<td>8</td>
<td>75</td>
<td>9</td>
</tr>
</tbody>
</table>

- N87 cell proliferation was significantly inhibited by treatment with ARRY-380 (pan-ErbB) and ARRY-380 (ErbB2-selective).
- Proliferation was only modestly inhibited by selective EGFR inhibition with erlotinib.
- No additional benefit was observed by combining ARRY-380 and erlotinib, consistent with the hypothesis that N87 cells are ErbB2-dependent under basal conditions in vitro.

RESULTS

- Under basal conditions, ARRY-380 (pan-ErbB) and ARRY-380 (ErbB2-selective) significantly inhibited ErbB2 phosphorylation.
- ErbB2 (ErbB2-selective) did not inhibit ErbB2 phosphorylation, as expected.
- EGFR phosphorylation was undetectable in the absence of EGFR, consistent with ErbB2-dependent biology.

INTRODUCTION

- **ARRY-34543 (ARRY-543) and Pan-ErbB Inhibition**
  - **ARRY-34543** is an oral TKI that targets all ErbB family kinases.
  - ARRY-34543 inhibits growth of EGFR- and ErbB2-dependent human tumor xenografts in vivo and is currently in clinical trials.
  - ErbB dimerization and signaling can be activated by multiple mechanisms including receptor over-expression, activating mutations and growth factor binding.
  - Ligands can stimulate formation of diverse redundant receptor dimers even when the receptor is not over-expressed.
  - Redundant ErbB family homo- and heterodimer signaling confers resistance to selective inhibitors.
  - By targeting all ErbB family kinases, ARRY-34543 should have broader activity than selective ErbB inhibitors in tumors that signal through redundant ErbB dimers.

METHODS

- **ErbB Inhibition in vitro**
  - Server-stained N87 gastric carcinoma cells were incubated with increasing concentrations of inhibitor for 2 hours followed by the presence or absence of EGFR stimulation for 15 minutes.
  - Cells were fixed and EGFR was captured on plates coated with an anti-EGFR antibody. The plates were washed, incubated with specific primary antibodies [P-ErbB2 (-EGF) and T-ErbB2 (+EGF)] and incubated with secondary antibodies.
  - The addition of TMB substrate (ψ-4440) was normalized to DMSO-treated control cells.

- **Growth Inhibition in vitro**
  - Activity of ARRY-380 was evaluated in N87 in the presence or absence of EGF. Cell viability was assessed using the CellTiter-Glo assay and normalized to DMSO-treated control cells.

- **Tumor Growth Inhibition in vivo**
  - N87 Gastric Carcinoma Model
    - N87 tumor xenografts were grown in Balb/c nu/nu mice under the care of all applicable institutional animal care guidelines and in harmony with the Guide for the Care and Use of Laboratory Animals.

- **ErbB Dimer Analysis**
  - N87 xenograft samples (data not shown) were used.
  - N87 xenograft samples were used.

- **Activation of multiple ErbB receptors confers reduced sensitivity to selective ErbB inhibitors but not pan-ErbB inhibitors**
  - In the absence of EGF, N87 gastric carcinoma cells express high levels of ErbB2 dimers and are highly sensitive to selective ErbB inhibition.
  - EGFR stimulation causes the formation of EGFR containing dimers, which diminishes cellular responses to selective ErbB inhibition but has minimal effect on dual EGFR/Erbb2 inhibition.
  - Block both EGFR and ErbB2 in vivo resulted in tumor growth inhibition that was superior to selective ErbB or EGFR inhibition.
  - These data suggest that ARRY-3453 can be differentiated from selective ErbB inhibitors by targeting tumors that signal through multiple ErbB receptors.

CONCLUSIONS

- **Pan-ErbB Inhibition by ARRY-334543 is Superior to Selective ErbB Inhibition in a Preclinical Model that Signifies Through Multiple ErbB Receptors**
  - Ryan Blackwell, Karyn Bouhana, Shannon Winski, Patrice Lee, Jim Winkler, Duncan Walker and Jennifer Garrus
  - Array BioPharma Inc. Boulder, CO

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