Pan-ErbB Inhibition by ARRY-334543 is Superior to Selective ErbB Inhibition in a Preclinical Model that Signals Through Multiple ErbB Receptors

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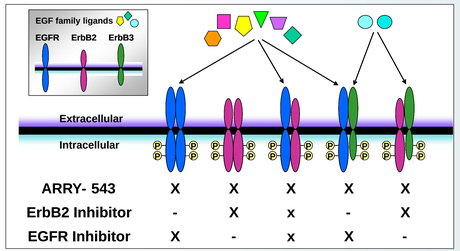
INTRODUCTION

ARRY-334543 (ARRY-543) and Pan-ErbB Inhibition

- ARRY-543 is an oral TKI that targets all ErbB family kinases
- ARRY-543 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-543 should have broader activity than selective ErbB inhibitors in tumors that signal through redundant ErbB dimers

Figure 1. ErbB Family Signaling



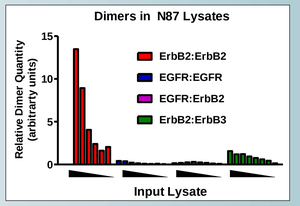
METHODS

- **ErbB2 inhibition *in vitro***
  - Serum starved N87 gastric carcinoma cells were incubated with increasing concentrations of inhibitor for 2 hours followed by the presence or absence of EGF stimulation for 10 minutes.
  - Cells were lysed and ErbB2 was captured on plates coated with an anti-ErbB2 antibody. The plates were incubated with an anti-phosphotyrosine-HRP antibody and pErbB2 was detected spectrophotometrically after the addition of TMB substrate. pErbB2 levels were normalized to DMSO-treated control cells.
- **Growth inhibition *in vitro***
  - Actively proliferating N87 cells were incubated with inhibitors for 3 days in the presence or absence of EGF. Cell viability was determined using CellTiter-Blue and normalized to DMSO-treated control cells.
- **Tumor Growth inhibition *in vivo*: N87 Gastric Carcinoma Model**
  - 5x10^6 tumor cells were implanted in nude mice (Athymic Ncr:Nu/Nu, Taconic Laboratories, Inc.) subcutaneously in the flank, and the tumors were allowed to grow to 150-200 mm^3 in size. Mice were then randomized into the following treatment groups:
    - Vehicle: 10 mL/kg, BID, PO
    - ARRY-543: 50 or 100 mg/kg, BID, PO
    - ARRY-380 (ErbB2 selective inhibitor): 25 or 50 mg/kg, QD, PO
    - Erlotinib (EGFR selective): 100 mg/kg, QD, PO
    - Combination: ARRY-380 25 mg/kg, QD, PO + erlotinib 100 mg/kg, QD, PO
    - Note: 100 mg/kg erlotinib was not well tolerated. Dose was dropped to 50 mg/kg on day 8 and this was tolerated.
  - Dosing for all groups continued for 21 days.
  - All *in vivo* studies were performed in accordance with IACUC guidelines and in harmony with the Guide for Laboratory Animal Care and Use.
- **ErbB Dimer Analysis**
  - ErbB dimers were evaluated in N87 lysates at Monogram Biosciences using the *VeraTag*™ Assay System (www.MonogramBio.com). Total input lysate ranged from 0-9.4 µg (1:1.7 dilutions) for ErbB2 homodimer detection and 0-130 µg (1:2 dilutions) for detection of all other dimers.

RESULTS

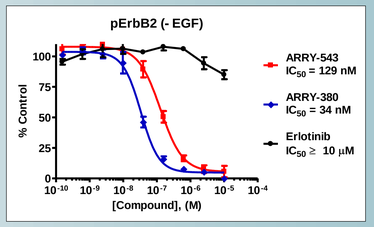
N87 Cells are ErbB2-Dependent in the Absence of EGF

Figure 2. ErbB2 homodimers are predominant in N87 cells



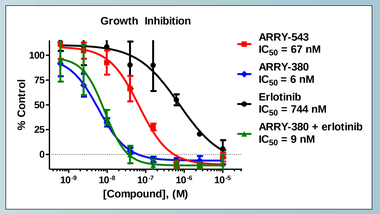
- N87 is a gastric carcinoma cell line that expresses high levels of constitutively active ErbB2 and low levels of EGFR
- ErbB2 homodimers are predominant in N87 cell lysates *in vitro*

Figure 3. ErbB2 inhibition by pan-ErbB or ErbB-selective molecules



- Under basal conditions, ARRY-543 (pan-ErbB) and ARRY-380 (ErbB2-selective) significantly inhibited ErbB2 phosphorylation
- Erlotinib (EGFR-selective) did not inhibit ErbB2 phosphorylation, as expected
- EGFR phosphorylation was undetectable in the absence of EGF, consistent with ErbB2-driven biology

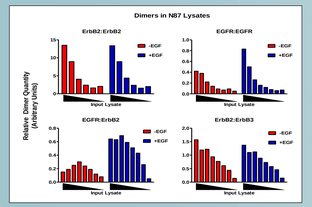
Figure 4. N87 cell growth is ErbB2-dependent in the absence of EGF



- N87 cell proliferation was significantly inhibited by treatment with ARRY-543 (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 growth conditions *in vitro*

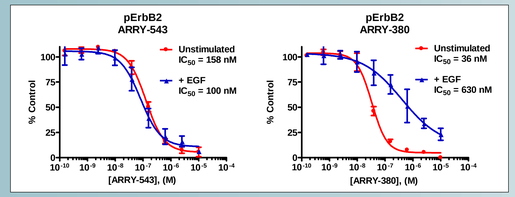
N87 Cells are Dual-Responsive in the Presence of EGF

Figure 5. EGFR-containing dimers are formed upon EGF stimulation



- In the presence of EGF, an increase in EGFR homodimers and EGFR:ErbB2 heterodimers was observed
- High levels of ErbB2 homodimers remained in the presence of EGF
- ErbB2:ErbB3 heterodimers were low and unchanged in response to EGF stimulation

Figure 6. Activity of ARRY-543 is maintained in the presence of EGFR:ErbB2 Heterodimers



- In the presence of EGF and increased EGFR:ErbB2 dimers, inhibition of ErbB2 phosphorylation by ARRY-380 (ErbB2-selective) was reduced 18-fold
- ARRY-543 (pan-ErbB) was equipotent for inhibiting ErbB2 signaling in the presence of EGF (EGFR:ErbB2 and ErbB2:ErbB2 dimers) and in the absence of EGF (ErbB2 homodimers)

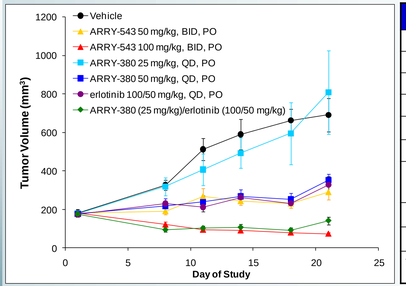
Table 1. Pan-ErbB inhibition is superior for blocking growth of N87 cells in the presence of EGF

Growth Inhibition (n=3)				
Compound	Target	Average IC_{50} (nM)		IC_{50} Fold Shift
		-EGF	+EGF	
ARRY-543	EGFR ErbB2	59	640	11
erlotinib	EGFR	945	714	0.8
ARRY-380	ErbB2	5	309	62
ARRY-380 + erlotinib	EGFR ErbB2	8	72	9

- The IC_{50} for ErbB2-selective growth inhibition by ARRY-380 increased 62-fold in the presence of EGF while the IC_{50} for erlotinib was relatively unchanged, suggesting a loss of dependence on ErbB2 signaling alone
- Dual EGFR/ErbB2 inhibition by ARRY-543 or the combination of ARRY-380 and erlotinib largely maintained potency with only a modest increase in IC_{50}

N87 Cells are Dual-Responsive *In Vivo*

Figure 7. N87 Tumor Xenograft Model



Group	TGI	Regr
ErbB2 selective		
25 mg/kg ARRY-380	0%	0%
50 mg/kg ARRY-380	49%	0%
EGFR selective		
100 mg/kg erlotinib	53%	0%
ErbB2 + EGFR		
25 mg/kg ARRY-380 50 mg/kg erlotinib	80%	48%
50 mg/kg ARRY-543	**58%**	**0%**
100 mg/kg ARRY-543	**90%**	**60%**

TGI: Tumor Growth Inhibition
Regr: Regression, ≥ 50% decrease in tumor size

Note: 100 mg/kg erlotinib was not well tolerated. The dose was dropped to 50 mg/kg on day 8 and this was well tolerated alone and in combination.

- Dual-response was observed *in vivo* where EGFR and ErbB2 homodimers were detected in N87 xenograft samples (data not shown)
- ARRY-543 was highly active with 90% tumor growth inhibition in the 100 mg/kg BID group
- Treatment with the combination of ARRY-380 and erlotinib had similar activity with 80% TGI
- Treatment with erlotinib or ARRY-380 alone was less efficacious than simultaneous EGFR and ErbB2 inhibition

CONCLUSIONS

- **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 ErbB2 inhibition**
  - **EGF stimulation causes the formation of EGFR containing dimers, which diminishes cellular responses to selective ErbB2 inhibition but has minimal effect on dual EGFR/ErbB2 inhibition**
  - **Blocking *both* EGFR and ErbB2 *in vivo* resulted in tumor growth inhibition that was superior to selective EGFR or ErbB2 inhibition**
- **These data suggest that ARRY-543 can be differentiated from selective ErbB inhibitors by targeting tumors that signal through multiple ErbB receptors**
  - **A wide range of tumors has been shown to activate multiple ErbB receptors and clinical evaluation of this hypothesis is ongoing**

Acknowledgement

We would like to thank Monogram Biosciences, Inc. for evaluating ErbB family dimers in N87 cell lysates and xenograft samples.