ARRY-334543, a Potent, Orally Active, Small Molecule Inhibitor of EGFR and ErbB-2

Greg Miknis, Eli Wallace, Joe Lyssikatos, Patrice Lee, Qian Zhao, Jeremy Hans, George Topalov, Alex Buckmelter, Gene Tarlton, Li Ren, Josh Tullis, Bryan Bernat, Lisa Pieti Opie, Ira von Carlowitz, Janet Parry, Tony Morales, Michele Perrier, Rich Woessner, Tracy Pheneger, Keith Hoffman, Chris Borton, Jim Winkler and Kevin Koch.*

Array BioPharma Inc., Boulder, Colorado 80301

Abstract #3399

Abstract

The growth factor receptor tyrosine kinases EGFR (ErbB-1) and ErbB-2 (HER-2) play a major role in controlling cell growth and differentiation. These two receptor tyrosine kinases are often overexpressed and/or abnormally active in a wide variety of tumor types. Based on EGFR and ErbB-2's critical roles in cell proliferation, differentiation and survival, inhibition of these kinases is an attractive and promising therapeutic approach for the treatment of tumors. In a series of experiments, we show that ARRY-334543 is an orally active dual inhibitor of EGFR and ErbB-2. The compound behaves as a reversible ATP-competitive inhibitor with nanomolar potency both *in vitro* and in cell-based proliferation assays using A431 and BT-474 cells. Selectivity against a panel of kinases has been demonstrated *in vitro*. In mouse xenograft models utilizing the EGFR over expressing tumor line A431, ARRY-334543 demonstrated significant dose related tumor growth inhibition when administered orally, BID, for 21 days. Based on potency, selectivity and efficacy data, the compound is currently in development as an anti-cancer agent.

Introduction

The EGF family of receptor tyrosine kinases consists of ErbB-1 (EGFR), ErbB-2 (HER-2), ErbB-3 and ErbB-4. In response to the binding of various ligands, these kinases undergo heterodimerization and homodimerization, resulting in activation of numerous growth factor signaling pathways (1, 2). In a large variety of cancers the overexpression and/or constitutive activation of ErbB-1 and ErbB-2 is often observed and frequently correlates with poor clinical prognosis (1).

Several new cancer therapies have been developed that target the EGF family of kinases. Currently approved therapies include ErbB-1 specific small molecule inhibitors Tarceva® and the antibody Erbitux®. At the present time, the antibody Herceptin® is the only approved ErbB-2 selective inhibitor (1).

Despite the clinical success of these drugs, there still remains a need to develop more efficacious tyrosine kinase inhibitors. Due to the key role the heterodimerization of ErbB-1 and ErbB-2 plays in growth factor signaling, the simultaneous inhibition of ErbB-1 and ErbB-2 is an appealing approach for developing more efficacious cancer treatments (1).

In a series of experiments, we show that ARRY-334543 is an orally active dual inhibitor of ErbB-1 and ErbB-2. The dual activity may allow the compound to be more efficacious and broadly applicable compared to current therapies. Based on potency, selectivity and efficacy data, the compound is currently in development as an new anti-cancer agent. Ongoing experiments are underway to further demonstrate the efficacy and safety of ARRY-334543.

ARRY-334543

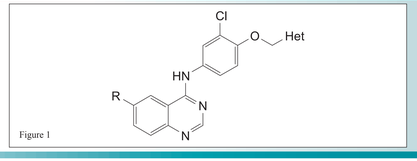
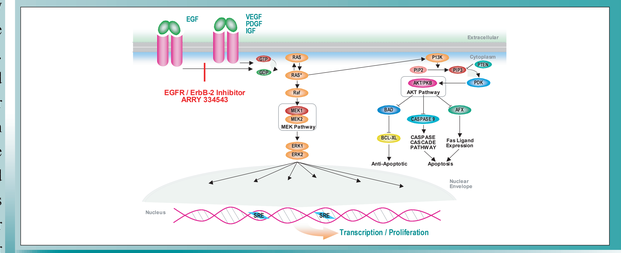


Figure 1

Cancer – EGFR Signaling Pathway



Methods & Results

ARRY-334543 Potently Inhibits ErbB-2 & ErbB-1 Phosphorylation

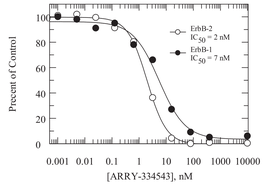


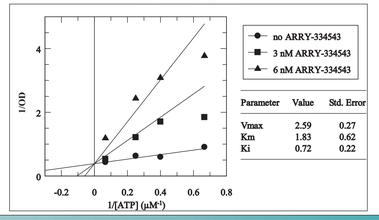
Figure 2a

104 kinases were assayed with 1 µM ARRY-334543 and 10 µM ATP

Kinase	% Inhibition
EGFR	100
ErbB4	100
Fyn	72
Lck	82
Lyn	73
Yes	35

No significant inhibition was measured with the other kinases

ARRY-334543 is an ATP-Competitive Inhibitor of ErbB-2, Ki = ~ 1 nM



Parameter	Value	Std. Error
Vmax	2.59	0.27
Km	1.83	0.62
Ki	0.72	0.22

Figure 2b

Figure 2 Enzyme Assays:

a) Purified full-length ErbB-1 (Biolmol) or histidine-tagged ErbB-2 kinase domain (residues 691-1255 purified by immobilized metal affinity chromatography) were added to reactions containing 24mM $MgCl_2$, 15 µM ATP and inhibitor in a 96-well plated coated with poly (glutamate-tyrosine). The wells were washed and incubated with an anti-phosphotyrosine monoclonal antibody conjugated to horseradish peroxidase. Quantitation of enzyme activity was completed by addition of TMB substrate and spectrophotometric detection. Kinase Selectivity Screen: Compounds were tested at a concentration of 1 µM with 10 µM ATP by Upstate using the appropriately formatted assay for each purified enzyme.

b) Enzyme assays were completed as described above (a). The ATP concentrations used were 1.5, 2, 4 and 15 µM.

ARRY-334543 Inhibits ErbB-2 and ErbB-1 Phosphorylation in Human Cancer Cells

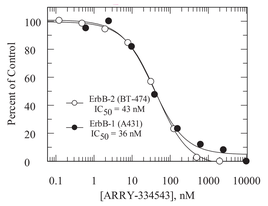


Figure 3a

ARRY-334543 Inhibits AKT Phosphorylation in Human Cancer Cells

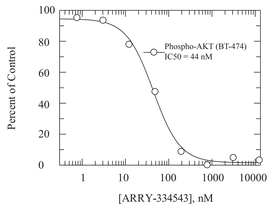


Figure 3b

Figure 3 Cellular Assays:

a) A431 cells were seeded and serum starved for 18-20 hours. Inhibitors were added for 1 hour followed by stimulation for 10 minutes with 100 ng/mL EGF. Cells were broken and the clarified lysate was added to 96-well plates coated with anti-ErbB-1 antibody. The captured protein was incubated with an anti-phosphotyrosine monoclonal antibody and quantitated as described above.

Actively proliferating BT-474 cells were washed (1X) with serum-free media before the addition of compound lasting for 2 hours. Cells were broken and the clarified lysate was added to 96-well plates coated with anti-ErbB-2 antibody. The captured protein was incubated with an anti-phosphotyrosine monoclonal antibody and quantitated as described in the enzyme assay.

b) Phospho-AKT was measured with an antibody specific for phospho-S473. Cells were also probed with a GAPDH antibody to normalize for total protein. Secondary antibodies specific for each primary antibody were used and a Licor Imaging system was used for detection.

Caspase Activation in ErbB Expressing Cells

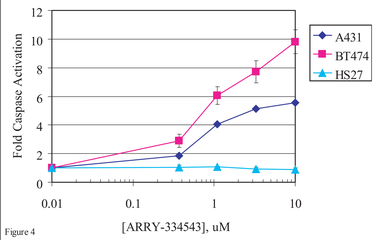


Figure 4

Figure 4 Caspase Activation: Apoptosis was induced in actively proliferating cells by treatment with compound for 48 hours. Promega's Apo-ONE Homogeneous Caspase-3/7 Buffer was added to the cells and incubated at room temperature. Fluorescent product, as a measure of Caspase 3/7 cleavage activity, was measured on a fluorescent plate reader 1 hour post-addition.

The Effect of ARRY-334543 on A431 Human Tumor Xenografts in Female Nude Mice

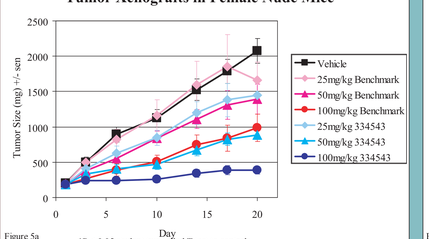


Figure 5a

*P < 0.05 student two-tailed T test to controls

The Effect of ARRY-334543 on MDA-MB-453 Tumor Xenografts on Female Nude Mice

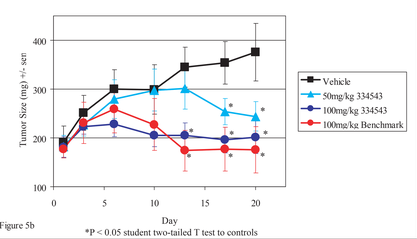


Figure 5b

*P < 0.05 student two-tailed T test to controls

Figure 5 *In vivo* studies: All *in vivo* studies were conducted in accordance with IACUC guidelines and in harmony with the Guide for the Care and Use of Laboratory Animals (National Research Council).

a) Nude mice were implanted subcutaneously in the right flank with 3 x 10^6 A431 cells and tumors were allowed to grow 150-200 mm^3. Mice bearing these established tumors were treated with 25 mg/kg, 50 mg/kg or 100 mg/kg, PO, twice daily, for 21 days (8 mice/dose). A positive control, competitor compound (benchmark) was evaluated at 25 mg/kg, 50 mg/kg or 100 mg/kg, PO, BID as well as a vehicle –treated control group of animals.

b) Nude mice were implanted subcutaneously in the right flank with 5 x 10^6 MDA-MB-453 cells with matrigel and tumors were allowed to grow 150-200 mm^3. Mice bearing these established tumors were treated with 25 mg/kg, 50 mg/kg or 100 mg/kg, PO, twice daily, for 21 days (8 mice/dose). A positive control, competitor compound (benchmark) was evaluated at 100 mg/kg, PO, BID as well as a vehicle –treated control group of animals.

Summary

Based on data provided, these experiments demonstrate:

ARRY-334543 is a selective kinase inhibitor of ErbB-1 and ErbB-2 that is competitive with ATP.

ARRY-334543 inhibits phosphorylation of ErbB-1 and ErbB-2 in cells that contain these activated receptors.

ARRY- 334543 is a potent inhibitor of the AKT pathway in cells that contain active ErbB-2 receptors.

When dosed orally, ARRY-334543 inhibits growth of human tumor xenografts that overexpress ErbB-1 (A431) or ErbB-2 (MDA-MD-453) in a dose-dependent manner. This activity is superior to that seen with a benchmark compound.

References:

1. Britten, C. (2004) *Mol. Cancer Therap.* **3**, 1335.
2. Olayioye, M., et al. (2000) *EMBO J.* **19**, 3159.