Differential Activity of ARRY-543, a Potent, Small Molecule Inhibitor of EGFR/ErbB2 Compared to Lapatinib in *In Vivo* Xenograft Models

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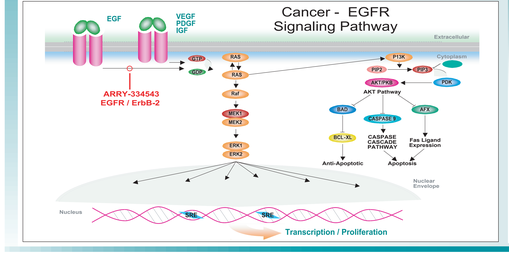
Abstract *(modified from published version)*

ARRY-543 is an orally active, potent small molecule tyrosine kinase inhibitor targeting both EGFR and ErbB2. The compound is a reversible, ATP-competitive inhibitor with nanomolar potency in both in vitro and in cell-based assays. This compound has very good *in vivo* and *in vitro* PK/ADME properties and has shown excellent activity in numerous mouse tumor models. In a series of experiments, we show that ARRY-543 has superior activity to lapatinib in several subcutaneous human tumor xenograft models selected for EGFR and ErbB2 expression patterns: A431, H1650 and N87. A431 is a wild-type EGFR-overexpressing epidermoid carcinoma cell line. H1650 is an EGFR overexpressing, lung adenocarcinoma cell line, which has the Exon 19 deletion conferring increased sensitivity to gefitinib and erlotinib. N87 is a gastric carcinoma cell line expressing high levels of both EGFR and ErbB2 and also relies on both for proliferation and survival. For all tumor studies, female nude mice were inoculated with cells subcutaneously in the flank. For tumor growth inhibition (TGI) studies, tumor size was measured at regular intervals for up to 21 days. In the A431 TGI study, ARRY-543 demonstrated significant dose-related TGI; 30, 57 and 81% at doses of 25, 50 and 100 mg/kg, BID, PO, respectively. Lapatinib administration at 50 and 100 mg/kg, BID, PO showed 33 and 52% TGI, respectively. In the H1650 TGI study, animals received oral doses of vehicle, ARRY-543 or lapatinib (100 mg/kg, BID) or erlotinib (100 mg/kg, QD). ARRY-543 demonstrated significant tumor growth inhibition (95%), with 2/8 animals showing tumor regressions. Comparable tumor growth inhibition was seen in the erlotinib group; 6/8 animals with tumor regressions. Lapatinib showed significantly less activity with respect to tumor growth inhibition (~18%; NS); no tumor regressions were observed. In the dual EGFR/ErbB2-over-expressing gastric carcinoma tumor line N87, pharmacodynamic studies were performed evaluating the effects of vehicle, ARRY-543 (100 mg/kg, once) or lapatinib (100 mg/kg, once) on pEGFR and pErb2 levels in tumors at 1, 4, 12 and 24hrs. post-dose. ARRY-543 significatly inhibited the phosphorylation of ErbB2 at all timepoints. Lapatinib showed good inhibition of ErbB2 at all timepoints but was less robust at the 1hr timepoint than ARRY-543. With respect to EGFR phosphorylation, ARRY-543 showed superior inhibition versus lapatinib over the 24hr time period. In the N87 TGI study, ARRY-543 demonstrates significant tumor growth inhibition (90%) and tumor responses (7/8 PRs) compared with lapatinib (61% TGI, 1/8 PR). These studies demonstrate that ARRY-543 has superior activity over lapatinib with respect to TGI and/or PD effects (extent and duration) on the EGFR receptors in preclinical mouse tumor models. ARRY-543 is entering Phase II trials in select cancer patient populations.



Methods

All in vivo studies were performed in accordance with IACUC guidelines and in harmony with the Guide for Laboratory Animal Care and Use.

Tumor Growth Studies

NCI-N87 Human Gastric Carcinoma

Tumor cells (5 x 10^6) were implanted in female 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. Then, mice were randomized into treatment groups:
- Vehicle: 10 mL/kg, BID, PO, n=8
- ARRY-543: 25, 50 or 100 mg/kg, BID, PO, n=8
- Lapatinib: 25, 50 or 100 mg/kg, BID, PO, n=8

Dosing for all groups continued for 21 days.

A431 Human Epidermoid Carcinoma

Tumor cells (3 x 10^6) were implanted in female 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. Then, mice were randomized into treatment groups:
- Vehicle: 10 mL/kg, BID, PO, n=8
- ARRY-543: 25, 50 or 100 mg/kg, BID, PO, n=8
- Lapatinib: 50 or 100 mg/kg BID, PO, n=8

Dosing for all groups continued for 20 days.

H1650 Human NSCL Carcinoma

Tumor cells (2.1 x 10^6) were implanted in female 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. Then, mice were randomized into treatment groups:
- Vehicle: 10 mL/kg, BID, PO, n=8
- ARRY-543: 100 mg/kg, BID, PO, n=8
- Lapatinib: 25, 50 or 100 mg/kg, BID, PO, n=8
- Erlotinib: 100 mg/kg, QD, PO, n=8

Dosing for all groups continued for 18 days.

ENDPOINTS DEFINED:

Tumors ((W2*L)/2) were measured twice weekly for all studies

%TGI = 100(1-T/C)

PR= 50% or greater decreases in tumor size

Avg. group regression = 100((T-T0)/T0)

T=average group tumor size at lowest point

T0=starting tumor size for group

Pharmacokinetic/Pharmacodynamic (PK/PD) Study

N87 Human Gastric Carcinoma

Pharmacodynamics: N87 tumor cells (5 x 10^6) were implanted subcutaneously in female nude mice in the flank and tumors were allowed to grow to 250-350 mm^3 in size. Mice then received a single PO dose of vehicle or inhibitor (ARRY-543 or lapatinib at 100 mg/kg). At the desired timepoints (1, 4, 8, 12 and 24 h), mice were euthanized by CO_2 inhalation and tumors flash frozen in liquid nitrogen. For quantification of pEGFR or pErbB2 /Total EGFR or ErbB2, the tumor fragments were thawed and homogenized in lysis buffer containing protease and phosphatase inhibitors. The tumors were processed with the Fast-Prep Lysis System and protein quantity of the lysate was determined. EGFr was immunoprecipatated using Neomarkers EGFr AB-13 antibody (MS-609-PABX). 50µg of total protein were separated on a 4-12% Bis-Tris acrylamide gel, and transferred to nitrocellulose. ErbB2 was not immunoprecipitated.

Immunoblotting was carried out using the following antibodies:
- pEGFr and pErbB2:
  - 1º anti-phosphotyrosine (PY-20) Ab. 1:1500 (Zymed #03-7720),
  - 2ºAb - Alexa 680 anti Mouse, MP#A-21057 (Molecular Probes)
- Total EGFR: 1º Ab - rabbit anti-EGFr Ab (CST #2232),
- Total ErbB2: 1° Ab- rabbit anti-ErbB2 (Upstate 06-562)

2º Ab - Rockland IR dye800 anti Rabbit, #611-132-122 (Rockland Immunochemicals)

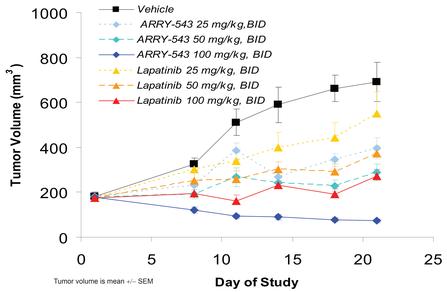
Band intensities on immunoblots were quantified using a Licor imaging system.

Pharmacokinetics:

Compound concentrations were determined in plasma samplesobtained at the designated timepoints. A robust, qualified LC-MS/MS method was utilized.

Results

NCI-N87 : Dual EGFR and ErbB2-Human Gastric Carcinoma



Dose	ARRY-543 % TGI	PR	% Avg Group Regression	lapatinib % TGI	PR	% Avg Group Regression
25 mg/kg	43	08	0	20	0/8	0
50 mg/kg	58	1/8	0	46	0/8	0
100 mg/kg	90	7/8	60	61	1/8	6

Figure 1.
Tumor Growth Inhibition: ARRY-543 showed dose-related inhibition of NCI-N87 tumor growth at all doses. Maximum inhibition (90%) was seen at doses of 100 mg/kg, BID. At this dose, 60% of the tumors regressed in animals treated with ARRY-543 while only 6% regressed in the lapatinib-treated high dose group.

Efficacy was superior to that seen with lapatinib at all doses.

Pharmacodynamics: Western Blot Data

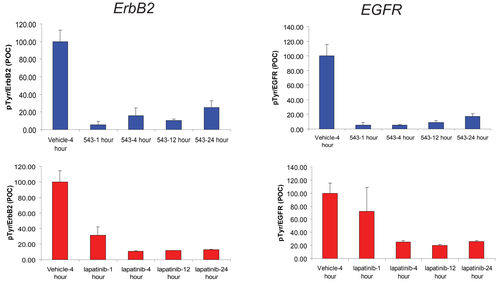
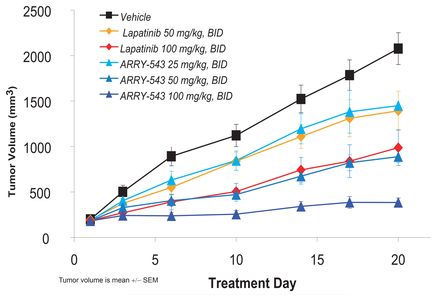


Figure 2.
ErbB2: ARRY-543 showed significant and sustained inhibition of the phosphorylation of ErbB2 at all timepoints. Lapatinib showed significant inhibition of ErbB2 at all timepoints but the magnitude of inhibition at the 1 hr timpoint was less than that seen with ARRY-543.
EGFR: ARRY-543 showed significant and sustained inhibition of the phosphorylation of EGFR at all timepoints. Lapatinib showed significant inhibition of EGFR at the 4, 12 and 24 hr timepoints but no significant inhibition at the 1 hr timepoint. Inhibition of pEGFR by ARRY-543 was superior to lapatinib over a 24 hr. period.

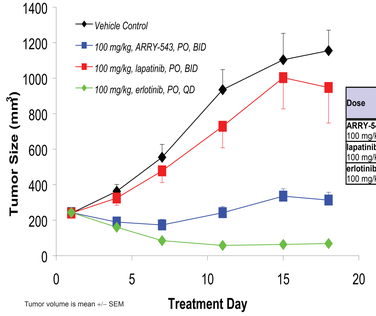
A431 : EGFR-Overexpressing Human Epidermoid Carcinoma



Dose	ARRY- 543 % TGI	lapatinib % TGI
25 mg/kg, BID	30	-
50 mg/kg, BID	57	33
100 mg/kg, BID	81	52

Figure 3.
Tumor Growth Inhibition: ARRY-543 showed dose-related inhibition of A431 tumor growth; significant at the mid and high doses. Maximum inhibition (81%) was seen at doses of 100 mg/kg, BID. Efficacy at 50 and 100 mg/kg was superior to that seen with lapatinib.

H1650 : EGFR -Overexpressing NSCLC (with exon 19 del)



Dose	% TGI	PR	% Avg Group Regression
ARRY-543 100 mg/kg, BID	73	2/8	29
lapatinib 100 mg/kg, BID	18	0/8	0
erlotinib 100 mg/kg, QD	94	8/8	76

Figure 4.
The exon 19 deletion,in the absence of T790M, has been shown to confer senstivity to EGFR TKIs such as gefitinib and erlotinib.
ARRY-543 showed signifcant inhibition of H1650 tumor growth; similar to that of erlotinib.
Lapatinib is inactive in this model.

Safety of ARRY-543

TGI studies

ARRY-543 was well tolerated at doses up to 200 mg/kg/d for up to 21 days in numerour *in vivo* studies. There were no significant changes in body weight or clinical pathology.

GLP Safety Studies

A comprehensive battery of GLP safety studies on ARRY-543 has been completed. ARRY-543 is well tolerated in rats and cynomolgus monkeys across a suitable dose-range.

Phase 1 Clinical Studies
- ARRY-543 was well tolerated up to 400 mg BID.
- ARRY-543 demonstrated excellent dose-proportional pharmacokinetics with no evidence of dose-limiting exposure
- Fatigue, nausea, anorexia, diarrhea, rash, and vomiting are the most common AEs
- There were no significant drug-related increases in liver function tests with the exception of one patient at the 200 mg BID dose level who experienced grade 3 AST and ALT elevation
- The 400 mg BID dose was determined to be the MTD in early Phase 1 Studies

Summary

ARRY-543, a potent EGFR/ErbB2 inhibitor, demonstrates excellent inhibition of EGFR and ErbB-2 phosphorylation in a number of *in vivo* tumor models.

ARRY-543 inhibits tumor growth in several human xenograft models in the mouse. These include:
- o NCI-N87 - Gastric carcinoma
- o A431 - Epidermoid carcinoma
- o H1650 - NSCL carcinoma

Activity in these models was superior to that seen with lapatinib.

In the NCI-N87 dual-ErbB2/EGFR driven gastric carcinoma:
- o ARRY-543 treatment at 100 mg/kg, BID resulted in 5 regressions, whereas lapatinib produced 1 regression.
- o PD studies in this tumor type demonstrate excellent and sustained inhibition of EGFR and ErbB2 with ARRY-543. ARRY-543 activity against EGFR is greater than that observed with lapatinib over a 24 hr period.

ARRY-543 is in clinical development with Phase 2 studies commencing in mid-2008.