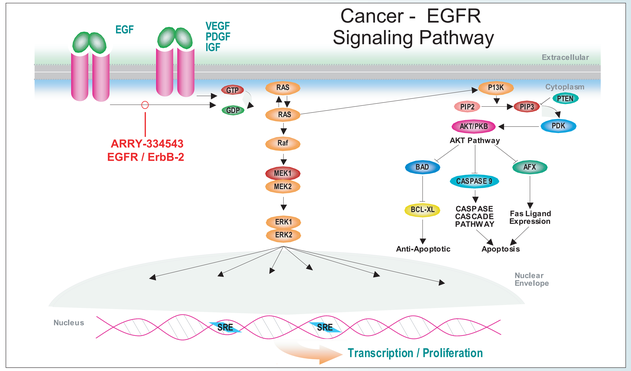
In Vivo Anti-Tumor Activity of ARRY-334543, a Small Molecule Inhibitor of EGFR and ErbB-2

Tracy Pheneger, Rich Woessner, Shannon Winski, Deborah Anderson, Joe Lyssikatos, Bryan Bernat, Greg Miknis, George Topalov, Li Ren, Eli Wallace and Patrice Lee
Array BioPharma Inc., Boulder, Colorado, 80301

Abstract

The growth factor receptor tyrosine kinases EGFR (ErbB-1) and HER-2 (ErbB-2) play major roles in controlling cell growth and differentiation. These two receptor tyrosine kinases are often over-expressed and/or abnormally active in a wide variety of tumor types. We have shown (AACR Abstract # 3399) that ARRY-334543 is an orally active, dual inhibitor of EGFR and HER-2 and that the compound is a reversible ATP-competitive inhibitor with nanomolar potency both *in vitro* and in cell-based assays using A431 and BT474 cells. In order to test *in vivo* efficacy, we examined the effects of ARRY-334543 in several human tumor xenograft models. In the A431 (EGFR-overexpressor) human epidermoid carcinoma xenograft model, dose-dependent inhibition of tumor growth was observed. Doses of 50 mg/kg, BID resulted in greater than 50% tumor growth inhibition. Activity of ARRY-334543 was evaluated in a panel of non-small cell lung cancer models including those with both wild type (A549, Calu-3) and mutant EGFR (H1650; exon 19 deletion). Dose-dependent inhibition of tumor growth was observed in the A549 model. In the Calu-3 xenograft model, significant tumor growth inhibition was seen at 100 mg/kg, BID with 8/8 animals showing regression of their tumors. In the H1650 model, inhibition of tumor growth (80%) was observed with regressions (2/8) noted at the high dose of 100 mg/kg, BID. Nude and SCID mice bearing MDA-MB-453 and BT-474 tumors, respectively, were utilized to assess activity of ARRY-334543 in HER-2 over-expressing cells. Dose-dependent inhibition of tumor growth was observed in the MDA-MB-453 model; significant tumor regressions (6/8 animals) were observed in the BT-474 model. These experiments show that ARRY-334543 has broad anti-tumor effects *in vivo* in EGFR wt and mutant cells in xenograft models, as well as in ErbB-2 driven tumor models. ARRY-334543 has entered clinical development with Phase 1 studies to commence in late 2005.



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

A431 Human Epidermoid Carcinoma

Tumor cells ($3x10^6$) were implanted in nude mice (Athymic Ncr:Nu/Nu, Taconic Laboratories, Inc.) subcutaneously in the flank, and the tumors were allowed to grow to 150-200mg in size. Then, mice were randomized into treatment groups:
- Vehicle: 10 mL/kg, BID, PO, n=8
- ARRY-334543: 25, 50 or 100 mg/kg, BID, PO, n=8
- ARRY-112432 (benchmark compound): 50 or 100 mg/kg BID, PO, n=8

Dosing for all groups continued for 20 days. Tumors ($(W^2*L)/2$) were measured twice weekly.

A549 Non-Small Cell Lung (NSCL) Carcinoma

Tumor cells ($5x10^6$) were implanted with matrigel (Becton Dickenson) in nude mice (Athymic Ncr:Nu/Nu, Taconic Laboratories, Inc.) subcutaneously in the flank, and the tumors were allowed to grow to 150-200mg in size. Then, mice were randomized into treatment groups:
- Vehicle: 10 mL/kg, BID, PO, n=8
- ARRY-334543: 25, 50 or 100 mg/kg, BID, PO, n=8
- ARRY-112432 (benchmark compound): 100 mg/kg BID, PO, n=8

Dosing for all groups continued for 21 days. Tumors ($(W^2*L)/2$) were measured twice weekly.

Calu-3 NSCL Carcinoma

Tumor cells ($4x10^6$) were implanted with matrigel (Becton Dickenson) in nude mice (Athymic Ncr:Nu/Nu, Taconic Laboratories, Inc.) subcutaneously in the flank, and the tumors were allowed to grow to 150-200mg in size. Then, mice were randomized into treatment groups:
- Vehicle: 10 mL/kg, BID, PO, n=8
- ARRY-334543: 100 mg/kg, BID, PO, n=8

Dosing for all groups continued for 21 days. Tumors ($(W^2*L)/2$) were measured twice weekly.

MDA-MB-453 Breast Carcinoma

Tumor cells ($10x10^6$) were implanted with matrigel (Becton Dickenson) in nude mice (Athymic Ncr:Nu/Nu, Taconic Laboratories, Inc.) subcutaneously in the flank, and the tumors were allowed to grow to 150-200mg in size. Then, mice were randomized into treatment groups:
- Vehicle: 10 mL/kg, BID, PO, n=8
- ARRY-334543: 50 or 100 mg/kg, BID, PO, n=8
- ARRY-112432 (benchmark compound): 100 mg/kg BID, PO, n=8

Dosing for all groups continued for 20 days. Tumors ($(W^2*L)/2$) were measured twice weekly.

H1650 NSCL Carcinoma

Tumor cells ($5x10^6$) were implanted in nude mice (Athymic Ncr:Nu/Nu, Taconic Laboratories, Inc.) subcutaneously in the flank, and the tumors were allowed to grow to 150-200mg in size. Then, mice were randomized into treatment groups:
- Vehicle: 10 mL/kg, BID, PO, n=8
- ARRY-334543: 25, 50 or 100 mg/kg, BID, PO, n=8
- ARRY-112432 (benchmark compound): 100 mg/kg BID, PO, n=8

Dosing for all groups continued for 21 days. Tumors ($(W^2*L)/2$) were measured twice weekly.

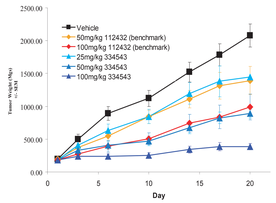
BT474 Breast Carcinoma

Estrogen and progesterone pellets (Innovative Research of America) were implanted subcutaneously seven days prior to tumor cell inoculation. Tumor cells ($1.0x10^7$) were implanted with matrigel (Becton Dickenson) in SCID-BG mice (Harlan, Inc.) subcutaneously in the flank. The tumors were allowed to grow to 150-200mg in size. Then, mice were randomized into treatment groups:
- Vehicle: 10 mL/kg, BID, PO, n=8
- ARRY-334543: 100 mg/kg, BID, PO, n=8
- ARRY-112432 (benchmark compound): 100 mg/kg BID, PO, n=8

Dosing for all groups continued for 9 days. Tumors ($(W^2*L)/2$) were measured twice weekly.

Results

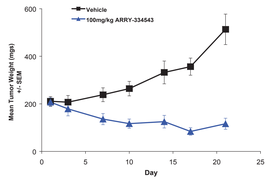
A431 Epidermoid Carcinoma



N	Agent	Dose	Route	Interval	1-T/C
8	Vehicle		PO	BID	
8	ARRY-334543	25mg/kg	PO	BID	30%
8	ARRY-334543	50mg/kg	PO	BID	57%
8	ARRY-334543	100mg/kg	PO	BID	81%
8	ARRY-112432	50mg/kg	PO	BID	33%
8	ARRY-112432	100mg/kg	PO	BID	52%

Figure 1. ARRY-334543 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 the benchmark compound.

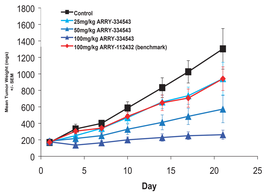
Calu-3 NSCL Carcinoma



N	Agent	Dose	Route	Interval	1-T/C
8	Vehicle		PO	BID	
8	ARRY-334543	100mg/kg	PO	BID	77%

Figure 3. ARRY-334543 showed siginficant inhibition of Calu-3 tumor growth. 77% inhibition was seen at doses of 100 mg/kg, BID.

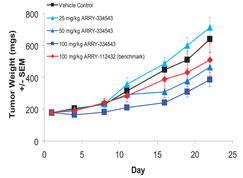
H1650 NSCL Carcinoma
(EGFR mutant-exon 19 deletion)



N	Agent	Dose	Route	Interval	1-T/C
8	Vehicle		PO	BID	
8	ARRY-334543	25mg/kg	PO	BID	28%
8	ARRY-334543	50mg/kg	PO	BID	56%
8	ARRY-334543	100mg/kg	PO	BID	80%
8	ARRY-112432	100mg/kg	PO	BID	28%

Figure 5. ARRY-334543 showed dose-related inhibition of H1650 tumor growth; significant at the mid and high doses. Maximum inhibition (80%) was seen at doses of 100 mg/kg, BID. This effect was superior to that seen with the benchmark compound.

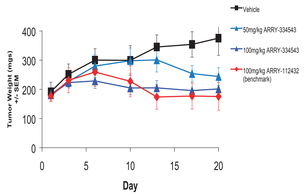
A549 NSCL Carcinoma



N	Agent	Dose	Route	Interval	1-T/C
8	Vehicle		PO	BID	
8	ARRY-334543	25mg/kg	PO	BID	-11%
8	ARRY-334543	50mg/kg	PO	BID	27%
8	ARRY-334543	100mg/kg	PO	BID	40%
8	ARRY-112432	100mg/kg	PO	BID	20%

Figure 2. ARRY-334543 showed significant inhibition of A549 tumor growth at the high dose of 100 mg/kg only (40%). Overall, activity in this cell line is very modest.

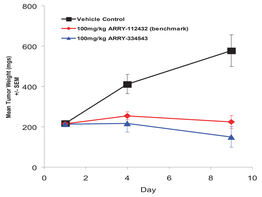
MDA-MB-453 Breast Carcinoma



N	Agent	Dose	Route	Interval	1-T/C
8	Vehicle		PO	BID	
8	ARRY-334543	50mg/kg	PO	BID	35%
8	ARRY-334543	100mg/kg	PO	BID	46%
8	ARRY-112432	100mg/kg	PO	BID	53%

Figure 4. ARRY-334543 showed dose-related inhibition of MDA-MB-453 tumor growth (maximum - 46%) which was significant at the high dose.This activity is equivalent to that seen with the benchmark compound.

BT474 Breast Carcinoma



N	Agent	Dose	Route	Interval	1-T/C
8	Vehicle		PO	BID	
8	ARRY-334543	100mg/kg	PO	BID	74%
8	ARRY-112432	100mg/kg	PO	BID	61%

Figure 6 ARRY-334543 showed significant inhibition of BT474 tumor growth (74%). This activity is equivalent to that seen with the benchmark compound.

Tolerability of ARRY-334543

In TGI studies, ARRY-334543 was well tolerated at doses up to 200 mg/kg/d for up to 21 days.

There were no siginificant changes in:
- - body weight
- - clincial chemistry
- - gross observations at necropsy

Conclusions

ARRY-334543, a potent EGFR/ErbB2 inhibitor, has been shown to inhibit tumor growth in several human xenograft models in the mouse. These include:
- o A431 - epidermoid carcinoma
- o A549 - NSCL carcinoma
- o Calu-3 - NSCL carcinoma
- o H1650 - NSCL carcinoma
- o MDA-MB-453 - breast carcinoma
- o BT474 - breast carcinoma

Activity in the EGFR mutant cell line (H1650) was exceptional and superior to that seen with the benchmark compound.

ARRY-334543 treatment at 100 mg/kg, BID resulted in regressions in several of these studies including:
- o Calu-3 (8/8 animals)
- o H1650 (2/8 animals)
- o BT474 (6/8 animals)

Other tumor models (data not shown) in which ARRY-334543 has produced tumor growth inhibition:
- o H292 - NSCL carcinoma
- o H460 - NSCL carcinoma

ARRY-334543 has entered clinical development with Phase 1 studies to commence in late 2005.