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 activated in a wide variety of tumor types. We have shown (AACC 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 tumor growth 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 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 with the benchmark compound. ARRY-334543 has entered clinical development with Phase 1 studies to commence in late 2005.

**Methods**

**Tumor Growth Studies**

1. **A431 Epidermoid Carcinoma**
   - Tumor cells (1x10^6) were implanted in nude mice (NcrNo/No, Taconic Laboratories, Inc.) subcutaneously in the flank, and the tumors were allowed to grow to 150-200mg in size.
   - 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.

2. **Calu-3 NSCL Carcinoma**
   - Tumor cells (1x10^6) were implanted in nude mice (NcrNo/No, Taconic Laboratories, Inc.) subcutaneously in the flank, and the tumors were allowed to grow to 150-200mg in size.
   - Tumor cells (1x10^6) were implanted in nude mice (NcrNo/No, Taconic Laboratories, Inc.) subcutaneously in the flank, and the tumors were allowed to grow to 150-200mg in size.

3. **MDA-MB-453 Breast Carcinoma**
   - Tumor cells (1x10^6) were implanted in nude mice (NcrNo/No, Taconic Laboratories, Inc.) subcutaneously in the flank, and the tumors were allowed to grow to 150-200mg in size.

4. **BT474 Breast Carcinoma**
   - Tumor cells (1x10^6) were implanted in nude mice (NcrNo/No, Taconic Laboratories, Inc.) subcutaneously in the flank, and the tumors were allowed to grow to 150-200mg in size.

**Results**

**Tumor Growth Studies**

- **A431 Epidermoid Carcinoma**
  - ARRY-334543 showed dose-related inhibition of MDA-MB-453 tumor growth (0-100 mg/kg, BID).
  - Dose of 50 mg/kg, BID resulted in greater than 50% tumor growth inhibition.

- **Calu-3 NSCL Carcinoma**
  - ARRY-334543 showed dose-related inhibition of Calu-3 tumor growth (0-100 mg/kg, BID).
  - Dose of 50 mg/kg, BID resulted in greater than 50% tumor growth inhibition.

- **MDA-MB-453 Breast Carcinoma**
  - ARRY-334543 showed significant inhibition of BT474 tumor growth (0-100 mg/kg, BID).
  - Dose of 50 mg/kg, BID resulted in greater than 50% tumor growth inhibition.

- **BT474 Breast Carcinoma**
  - ARRY-334543 showed significant inhibition of BT474 tumor growth (0-100 mg/kg, BID).
  - Dose of 50 mg/kg, BID resulted in greater than 50% tumor growth inhibition.

**Conclusion**

ARRY-334543, a potent EGFR/ErbB2 inhibitor, has been shown to inhibit tumor growth in several human xenograft models in the mouse. These include a A431 - epithelial carcinoma, a Calu-3 - NSCL carcinoma, o A549 - 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:
- Calu-3 (8/8 animals)
- H1650 (2/8 animals)
- BT474 (6/8 animals)

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

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