

ACTIVITY OF TG01, A SELECTIVE COX-2 INHIBITOR, ALONE AND IN COMBINATION WITH STANDARD AGENTS IN HUMAN BREAST CARCINOMA XENOGRAFTS (Abstract # 5662)

Sara L Zaknoen¹, Tracy L Lawhon¹, Andrew J Wong², Kathryn R Meshaw², Mary Ann Meade³, WR Leopold, III³ and Randall K Johnson¹

Tragara Pharmaceuticals¹, San Diego, CA, Piedmont Research Center², Morrisville, NC and MIR Preclinical Services³, Ann Arbor, MI

ABSTRACT

TG01 is an oral, potent and selective inhibitor of COX-2 which is in Phase I development for the treatment of solid tumors in combination with standard agents. The COX-2 pathway has been demonstrated to impact several pathways involved in signaling in cancer including the erbB family of receptors. The initial indications which will be explored clinically are recurrent metastatic breast cancer and relapsed NSCLC. TG01 was evaluated in a number of human breast tumor xenograft models. The compound was administered orally on a continuous daily regimen in female athymic nude mice bearing established sc-implanted xenografts. The models evaluated included BT474 (erbB2+), MX1 (ER-, erbB2-, MCF7 (ER+) breast carcinomas and MDA-MB-435 (now known to be melanoma). In each tumor model, TG01 was compared over a broad dosage range with celecoxib. In addition to monotherapy, combinations of the COX-2 inhibitors with trastuzumab were evaluated in BT474 breast carcinoma and combinations with pemetrexed were evaluated in MX1 breast carcinoma. The experiments used a tumor growth delay (TGD) endpoint based on median time to endpoint, i.e., 750-1000 cu mm, and treatments were compared to untreated controls using the Logrank test for statistical significance.

Neither TG01 nor celecoxib produced significant tumor growth delay when used as monotherapy in any of the three breast tumor models or MDA-MB-435. Trastuzumab alone produced a significant TGD of 18.6 days (49%, p = .017) with 1 CR/10 in BT474. The combination of TG01 with trastuzumab was significantly more effective than trastuzumab alone with a TGD of 39.9 days (104%, p = .0003) and 1 PR + 2 CR/10. In contrast, celecoxib plus trastuzumab was no more effective than trastuzumab alone (TGD of 17.2 days and no regressions, p = .88). Pemetrexed alone produced no TGD in the MX1 model. However, the combination of pemetrexed plus TG01 significantly delayed tumor growth with a TGD of 11.9 days (44%, p = .0028). A similar therapeutic benefit was evident with pemetrexed plus celecoxib in MX1 (TGD of 13.2 days [49%, p = .0103]). These results would support the evaluation of combinations of TG01 with standard agents, particularly trastuzumab, in patients with breast cancer.

BACKGROUND-COX-2 and BREAST CANCER

The cyclooxygenase (COX) pathway has been implicated in carcinogenesis since the 1970s when increased concentrations of prostaglandins were detected in neoplastic tissues, especially colon cancer. Epidemiological studies have documented a decreased incidence of colorectal and breast cancer in patients taking non-specific COX inhibitors such as aspirin and non-steroidal anti-inflammatory drugs (NSAIDs). COX occurs in 2 isoforms: COX-1 is expressed constitutively in most tissues and is thought to serve a function in maintaining the integrity of the gastrointestinal (GI) tract and the renal medulla. COX-2 is in general not detectable in normal tissues and is upregulated in the presence of inflammation and neoplasia. COX-2 is consistently over expressed in a large percentage and variety of human tumors. The presence of elevated COX-2 in tumor tissue represents a poor prognostic factor in a number of tumors including breast cancer.

There are considerable preclinical and clinical data showing that COX-2 plays an important role in the pathogenesis of breast cancer (Mazhar et al. 2006). Moderate to strong expression of COX-2 was reported in 34% to 43% of cases of invasive breast cancer (Ristimäki et al. 2002, Half et al. 2002). Expression of COX-2 correlated with large tumor size, high histological grade, negative hormone status, high proliferative rate, and the presence of HER2/neu amplification (Ristimäki et al. 2002). Selective COX-2 inhibitors have been shown to inhibit the growth of breast cancer cell lines (Nakatsugie et al. 2000, Abou-Issa 2001). Celecoxib, a COX-2 selective compound, was able to significantly delay the onset of HER2/neu-induced tumors in vivo (Howe et al. 2002). In addition, COX-2 knock-out mice had reduced numbers of HER2/neu tumors and angiogenesis (Howe et al. 2005). There is evidence that the epidermal growth factor receptor (EGFR) family including HER2/neu and COX-2 have related signaling pathways that can interact to regulate cellular proliferation, migration, and invasion (Rimawi et al. 2006, Dang et al. 2004, Subbaramaiah et al. 2002).

BACKGROUND-APRICOXIB

Apricoxib (TG01) is a novel oral nonsteroidal anti-inflammatory drug, selective for the cyclooxygenase-2 (COX-2) isoform of cyclooxygenase.

Apricoxib has an *in vitro* IC₅₀ for COX-2 of 36nM and a COX-2:COX-1 selectivity ratio that ensures that at clinically relevant doses COX-1 inhibition is minimal. Initial data with apricoxib show potent anti-inflammatory and analgesic effects in standard animal models used for NSAIDs. *In vivo* data using human tumor xenografts implanted in mice demonstrate tumor growth inhibition superior to celecoxib used in the same experiments.

In initial clinical studies in normal volunteers apricoxib was well tolerated. Pharmacokinetics demonstrate dose proportional increases in exposure, a T_{max} of 1-2 hours and a T_{1/2} of 15-17 supporting once daily dosing.

METHODOLOGY

- Female nude mice, 10 mice per group
- Cells for implantation harvested during log phase growth and injected at 1 x 10⁷ cells in 0.2 mL
- Monitored tumors until reached 100 – 200 mm³ before dosing began
- Apricoxib and celecoxib given once daily by gavage
- Trastuzumab, cyclophosphamide and pemetrexed given ip

Protocol Designs

- BT474
 - Group 1: no treatment
 - Group 2: trastuzumab 15 mg/kg biweekly X 3
 - Groups 3-5: apricoxib at 10, 30 and 100 mg/kg daily
 - Groups 6-8: celecoxib at 30, 100 and 300 mg/kg daily
 - Groups 9-10: apricoxib at 10 and 30 mg/kg and trastuzumab at 15 mg/kg
 - Groups 11-12: celecoxib at 30 and 100 mg/kg and trastuzumab at 15 mg/kg
- MCF-7
 - Group 1: no treatment
 - Group 2: cyclophosphamide 112 mg/kg Q4D X 3
 - Groups 3-7: apricoxib at 0.3, 1, 3, 10 and 30 mg/kg daily
 - Groups 8-12: celecoxib at 1, 3, 10, 30 and 100 mg/kg daily
- MX-1
 - Group 1: no treatment
 - Group 2: pemetrexed 320mg/kg QD X 7
 - Groups 3-5: apricoxib at 10, 30 and 100 mg/kg daily
 - Groups 6-8: celecoxib at 30, 100 and 300 mg/kg daily
 - Groups 9-10: apricoxib at 10, 30 mg/kg and pemetrexed at 320 mg/kg
 - Groups 11-12: celecoxib at 30, 100 mg/kg and pemetrexed at 320 mg/kg

Endpoints

- Tumors were assessed biweekly and animals euthanized when tumors reached the protocol defined size
- The time-to-endpoint (TTE) for each mouse was calculated from the following equation:

$$TTE(\text{days}) = [\log^{10}(\text{endpoint vol, mm}^3) - b] \div m$$

b = intercept and m = slope of the line obtained by linear regression of a log-transformed tumor growth data set

- Treatment outcome was determined from tumor growth delay (TGD), defined as the increase in the median TTE in a treatment group compared to a control group:

$$TGD = T - C,$$

expressed in days, or as a percentage of the median TTE of the control group:

$$\%TGD = \frac{T - C}{C} \times 100$$

where:

T = median TTE for a treatment group,
C = median TTE for the control group (Group 1).

RESULTS

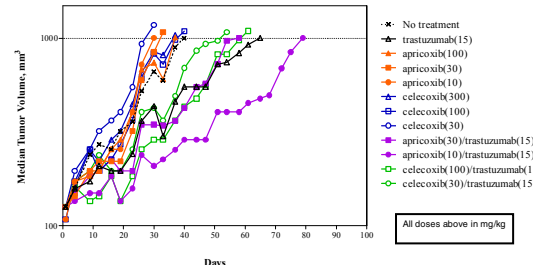
BT474

- Neither apricoxib or celecoxib were active as single agents
- Celecoxib in combination with trastuzumab was no more effective than trastuzumab alone
- Apricoxib at 10 mg/kg in combination with trastuzumab significantly prolonged the TTE compared to trastuzumab alone p < .001, the TTE with 30 mg/kg dose in combination with trastuzumab was not significant
 - Repeat of this study demonstrated the same trend for the 10 mg/kg dose
- Apricoxib at 10 mg/kg in combination with trastuzumab resulted in more tumor regressions than trastuzumab alone
- Apricoxib and celecoxib were well-tolerated as single agents and in combination with trastuzumab

Response Summary for BT474

n	Treatment Regimen 1			Treatment Regimen 2			Median TTE	T/C	%TGD	Statistical Significance				MTV (n)	Regression	
	Agent	mg/kg	Schedule	Agent	mg/kg	Schedule				vs G1	vs G2	Day #	PR			CR
10	No treatment	-	-	-	-	-	38.2	---	---	---	---	---	---	0	0	0
10	trastuzumab	15	ip	bivk x3	-	-	56.8	18.6	49	*	---	---	1 (1)	0	1	1
10	apricoxib	100	po	qd x3	-	-	32.7	-5.5	-14	ns	---	---	---	0	0	0
10	apricoxib	30	po	qd x3	-	-	32.0	-6.2	-16	ns	---	---	---	0	0	0
10	apricoxib	10	po	qd x3	-	-	29.7	-8.5	-22	ns	---	---	---	0	0	0
10	celecoxib	300	po	qd x3	-	-	36.6	-1.6	-4	ns	---	---	---	0	0	0
10	celecoxib	100	po	qd x3	-	-	36.8	-1.4	-4	ns	---	---	---	0	0	0
10	celecoxib	30	po	qd x3	-	-	27.5	-10.7	-28	**	---	---	---	0	0	0
10	apricoxib	30	po	qd x3	trastuzumab	15	ip	bivk x3	54.4	16.2	42	**	---	100 (1)	0	0
10	apricoxib	10	po	qd x3	trastuzumab	15	ip	bivk x3	78.1	39.9	104	***	---	0 (1)	1	2
10	celecoxib	100	po	qd x3	trastuzumab	15	ip	bivk x3	55.4	17.2	45	ns	---	289 (2)	0	0
10	celecoxib	30	po	qd x3	trastuzumab	15	ip	bivk x3	50.0	11.8	31	ns	---	120 (1)	0	0

Tumor Growth Delay in BT474



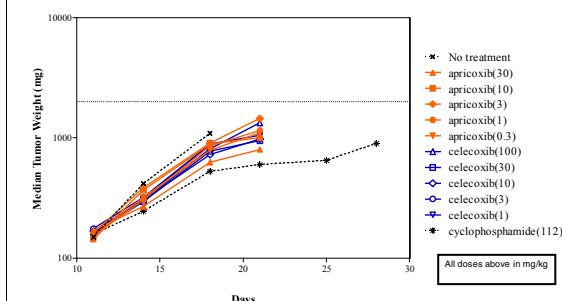
MCF-7

- Neither apricoxib or celecoxib was active as a single agent in MCF-7
- Apricoxib and celecoxib were well-tolerated

Response Summary for MCF-7

n	Treatment Regimen		TTE (Days)	TGD (Days)	Statistical Significance
	Agent	mg/kg			
10	No treatment	-	5.5	---	---
10	cyclophosphamide	112	14.7	9.2	ns
10	apricoxib	30	8.0	2.5	ns
10	apricoxib	10	7.3	1.8	ns
10	apricoxib	3	6.8	1.3	ns
10	apricoxib	1	7.1	1.6	ns
10	apricoxib	0.3	7.5	2.0	ns
10	celecoxib	100	7.1	1.6	ns
10	celecoxib	30	7.6	2.1	ns
10	celecoxib	10	7.6	2.1	ns
10	celecoxib	3	6.9	1.4	ns
10	celecoxib	1	7.2	1.7	ns

Tumor Growth Delay in MCF-7



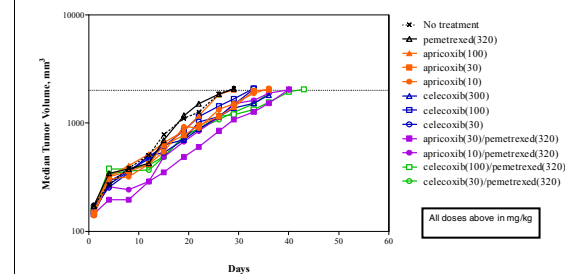
MX-1

- Neither apricoxib or celecoxib was active as a single agent in MX-1
- Pemetrexed as a single agent was not active in MX-1 despite evidence of activity in the literature
- Apricoxib at both doses tested in combination with pemetrexed significantly prolonged the TTE compared to either pemetrexed alone or the untreated control (p < .01 and p < .05), the higher dose of celecoxib in combination with pemetrexed also prolonged TTE (p < .05)
- Apricoxib and celecoxib were well-tolerated well as single agents and in combination with pemetrexed

Response Summary for MX-1

n	Treatment Regimen 1			Treatment Regimen 2			Median TTE	T/C	%TGD	Statistical Significance				MTV (n)	Regression		
	Agent	mg/kg	Schedule	Agent	mg/kg	Schedule				vs G1	vs G2	vs G3	vs G4			Day #	PR
10	No treatment	-	-	No treatment	-	-	27.1	---	---	---	---	---	---	---	0	0	
10	pemetrexed	320	ip	qd x7	-	-	25.8	-1.3	-5	ns	---	---	---	---	0	0	
10	apricoxib	100	po	qd to end	-	-	27.8	0.7	3	ns	---	---	---	---	0	0	
10	apricoxib	30	po	qd to end	-	-	31.2	4.1	23	ns	---	---	---	40 (1)	0	0	
10	apricoxib	10	po	qd to end	-	-	31.3	4.2	23	ns	---	---	---	---	0	0	
10	celecoxib	300	po	qd to end	-	-	32.9	5.8	21	ns	---	---	---	---	0	0	
10	celecoxib	100	po	qd to end	-	-	30.8	3.7	14	ns	---	---	---	---	0	0	
10	celecoxib	30	po	qd to end	-	-	31.0	3.9	22	ns	---	---	---	---	0	0	
10	apricoxib	30	po	qd to end	pemetrexed	320	ip	qd x7	39.0	11.9	44	**	---	---	100 (1)	0	0
10	apricoxib	10	po	qd to end	pemetrexed	320	ip	qd x7	37.0	9.9	37	*	---	---	---	0	0
10	celecoxib	100	po	qd to end	pemetrexed	320	ip	qd x7	40.3	13.2	49	**	---	---	---	0	0
10	celecoxib	30	po	qd to end	pemetrexed	320	ip	qd x7	37.0	9.9	37	*	---	---	---	0	0

Tumor Growth Delay in MX-1



CONCLUSIONS

- Apricoxib in combination with trastuzumab significantly prolonged TTE in BT474 breast cancer, celecoxib in combination with trastuzumab was no better than trastuzumab alone
- Apricoxib in combination with pemetrexed significantly prolonged TTE in triple negative MX-1 breast cancer
- Activity of apricoxib in combination with trastuzumab supports findings reported by Howe et al and Danenberg et al that the COX-2 pathway and the HER2/neu pathway interact in breast cancer and down regulation of both pathways may offer increased clinical activity
- The mechanism whereby apricoxib in combination with pemetrexed in MX-1 prolongs TTE may be a result of suppression of the COX-2 upregulation seen in response to chemotherapy treatment, however this remains speculative
- In active combinations survival of the animals was prolonged (data not shown)
- Apricoxib was well tolerated in all models both as a single agent and in combination
- These results are currently being investigated in a Phase II study in HER2/neu+ breast cancer patients

REFERENCES

- Mazhar D, Ang R, Waxman J. COX inhibitors and breast cancer. *Brit J Cancer*. 2006;94:346-350.
- Ristimäki A, Sivula A, Lundin J, et al. Prognostic significance of elevated cyclooxygenase-2 expression in breast cancer. *Cancer Res*. 2002;62:632-635.
- Nakatsugi S, Ohta T, Kawamori T, et al. Chemoprevention by nimesulide, a selective cyclooxygenase-2 inhibitor, of 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine (PhIP)-induced mammary gland carcinogenesis in rats. *Jpn J Cancer Res*. 2000;91:886-892.
- Abou-Issa HM, Alshafie GA, Seibert K, Koki AT, Masferrer JL, Harris RE. Dose-response effects of the COX-2 inhibitor, celecoxib, on the chemoprevention of mammary carcinogenesis. *Anticancer Res*. 2001;21:3425-3432.
- Howe LR, Subbaramaiah K, Patel J, et al. Celecoxib, a selective cyclooxygenase 2 inhibitor, protects against human epidermal growth factor receptor 2 (HER-2)/neu-induced breast cancer. *Cancer Res*. 2002;62:5405-5407.
- Howe LR, Chang SH, Tolle KC, et al. HER2/neu-induced mammary tumorigenesis and angiogenesis are reduced in cyclooxygenase-2 knockout mice. *Cancer Res*. 2005;65:10113-10119.
- Rimawi MF, Weiss HL, Bhatia WP, Chammess G, Elledge RM. EGFR expression in breast cancer: Association with biologic phenotype, prognosis, and resistance to adjuvant therapy. *J Clin Oncol*. (Meeting abstracts). 2006;24:513.
- Dang CT, Dannenberg AJ, Subbaramaiah K, et al. Phase II study of celecoxib and trastuzumab in metastatic breast cancer patients who have progressed after prior trastuzumab-based treatments. *Clin Cancer Res*. 2004;10:4062-4067.
- Subbaramaiah K, Norton L, Gerald W, Dannenberg AJ. Cyclooxygenase-2 is overexpressed in HER-2/neu-positive breast cancer: evidence for involvement of AP-1 and PEA3. *J Bio Chem*. 2002;277:18649-18657.