Antitumor Activity of Capecitabine and Bevacizumab Combination in a Human Estrogen Receptor-negative Breast Adenocarcinoma Xenograft Model

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Abstract. *Background: Capecitabine and bevacizumab have each been shown to inhibit tumor growth. Their combination failed to improve survival in a phase III trial of metastatic breast cancer (MBC), although it should be noted patients had been heavily pretreated with anthracyclines and taxanes. Our aim was to evaluate whether combination treatment would increase tumor growth inhibition and survival in a breast cancer model. Materials and Methods: Mice bearing KPL-4 human estrogen receptor-negative breast adenocarcinoma xenografts were given capecitabine orally daily for 14 days at the maximum tolerated dose (MTD) or half MTD, alone or with 5 mg/kg intraperitoneal bevacizumab twice weekly. Results: Tumor growth inhibition (TGI) and increased life span (ILS) were superior in the combination groups versus monotherapy ($p<0.05$). TGI and ILS were significantly improved in the high- versus low-dose capecitabine combination ($p<0.05$). Conclusion: Capecitabine in combination with bevacizumab provides a basis for pursuing the combination for first-line treatment of MBC.*

Metastatic breast cancer (MBC) accounts for about 40,000 deaths in the United States annually (1) and is largely incurable. The focus of therapy in these patients has been to improve overall survival and quality of life. Standard chemotherapy for MBC has generally utilized anthracyclines, or alternatively taxanes, in cases where resistance or toxicity was observed (2-4). Combination therapy with anthracyclines and taxanes has also been evaluated as first-line chemotherapy for metastatic disease (5, 6). The treatment options are very limited, however, for patients with tumors that are refractory to both anthracyclines and taxanes. The course of treatment can become even more complicated, as some patients cannot receive the combination regimen due to associated cardiotoxicity (7). Thus, alternative therapeutic strategies are needed to improve the overall survival of MBC patients.

Capecitabine, an oral fluoropyrimidine therapeutic, is widely used for the treatment of patients with MBC who have failed taxane- and anthracycline-containing regimens (8, 9). Clinical data confirm that capecitabine monotherapy achieves response rates of 20-26% and a median survival of >1 year in MBC patients (9). Capecitabine has also been effective in combination therapies and has been preferred due to its favorable safety profile (8).

The last decade has seen the emergence of immune-targeted therapy for cancer treatment as an alternative or adjunct to cytotoxic chemotherapy. Trastuzumab, which targets human epithelial growth factor receptor (HER)-2/neu, has been successfully used either in combination with paclitaxel as a first-line therapy or as second- and third-line monotherapy in breast cancers overexpressing the HER-2/neu growth factor receptor (10, 11). Similarly, pertuzumab, a humanized monoclonal antibody against the dimerization domain of HER-2, has shown promising results in advanced solid malignancies including MBC (12).

Bevacizumab is a humanized monoclonal antibody targeted against vascular endothelial growth factor (VEGF), a potent stimulator of tumor angiogenesis. Bevacizumab has been shown to inhibit tumor growth in animal models (13) and also has yielded promising clinical results in MBC (14). The combination of bevacizumab with the commonly used chemotherapeutic agents doxorubicin, topotecan, paclitaxel, and docetaxel in preclinical breast cancer models has demonstrated significant reductions in tumor mass when compared with monotherapy (13).

A recent phase III clinical trial comparing the efficacy and safety of capecitabine alone or in combination with bevacizumab demonstrated a significant increase in the

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overall response rates (ORR) of the combination therapy when compared with capecitabine monotherapy. This advantage was not associated with either improved progression-free survival (PFS) or improved overall survival (OS) (14, 15); however, the patients had been heavily pretreated with anthracyclines and taxanes. The potential for improved survival with capecitabine and bevacizumab combination as a first-line treatment for MBC remains untested clinically as well as preclinically. Our current studies have focused on preclinical optimization of the capecitabine and bevacizumab combination regimen to provide a rationale for clinical investigation as a first-line therapy in MBC.

The KPL-4 xenograft model was developed by Kurebayashi *et al.* (16) from a HER-2 overexpressing, estrogen receptor-negative adenocarcinoma, thus possessing characteristics of an advanced and aggressive subtype of breast cancer. This model was shown to be sensitive to trastuzumab-mediated growth inhibition, and a synergistic effect of trastuzumab and capecitabine in combination was also observed (17).

In the present study, we first confirmed the tumorigenicity and sensitivity of KPL-4 xenografts to standard therapeutic agents, including trastuzumab, paclitaxel, capecitabine and bevacizumab, which are clinically relevant to MBC. We then evaluated the antitumor activity of capecitabine at its maximum tolerated dose (MTD) and half MTD in combination with an optimal dose of bevacizumab.

Materials and Methods

Animals. Female nude mice (Crl:NU-Foxn1nu), 13 to 14 weeks old and weighing approximately 23-25 g were purchased from Charles River Laboratories (Wilmington, DE, USA). The health of all animals was monitored daily by gross observation and analyses of blood samples of sentinel animals. All animals were allowed to acclimate and recover from any shipping-related stress for a minimum of 72 hours prior to experimental use. Autoclaved water and irradiated food (5058-ms Pico Chow; Purina, Richmond, IN, USA) were provided *ad libitum*, and the animals were maintained on a 12-hour light and dark cycle. Cages, bedding and water bottles were autoclaved before use and were changed weekly. All animal experiments were performed in accordance with protocols approved by the Institutional Animal Care and Use Committee.

Tumors. KPL-4 human estrogen receptor-negative breast adenocarcinoma cells were kindly provided by Professor J. Kurebayashi. Cells were cultured in Dulbecco's modified Eagle's medium with 10% fetal bovine serum (FBS) and were routinely passaged twice weekly. For xenograft implantation, cells were harvested using 0.05% trypsin, washed and centrifuged in culture medium, and resuspended in a 1:1 mixture of phosphate-buffered saline (PBS) and Matrigel at a concentration of $2.5x10^7$ cells per mL. A volume of 0.2 mL cell suspension ($5x10^6$ cells) per mouse was implanted orthotopically in the right inguinal mammary fat pad. Tumors were allowed to grow for 10-15 days post implant when mean volume reached ~100-150 mm^3, after which animals were randomized into treatment groups.

Test agents. Capecitabine (Xeloda®; F. Hoffmann-La Roche, Nutley, NJ, USA) was formulated as a suspension in 2% Klucel LF, 0.1% Tween 80, 0.09% methylparaben, and 0.01% propylparaben. Mice were dosed with capecitabine at 200, 400, 450, or 500 mg/kg orally (*po*) *qd* for 14 days. Paclitaxel (Taxol®; Mead Johnson, Princeton, NJ) was obtained in a clinical formulation of Cremophor and ethanol and was diluted with PBS. Mice were dosed with paclitaxel at 30 mg/kg intraperitoneally (*i.p.*) *q4d*. Clinical grade trastuzumab (Herceptin®; Roche Diagnostics GmbH, Penzberg, Germany) was obtained as a lyophilized powder that was reconstituted in PBS. Mice were dosed with trastuzumab at 20 mg/kg twice weekly *i.p*. Clinical grade bevacizumab (Avastin®; Genentech, Inc., San Francisco, CA, USA) was obtained as a stock solution of 25 mg/mL and was diluted with sterile saline. Mice were dosed with bevacizumab at 5 mg/kg twice weekly *i.p*. All drugs were stored at 4°C and mixed immediately before administration.

Study design. For the preliminary MTD study with capecitabine, 5 animals per dose level (and bearing KPL-4 xenografts) were given capecitabine *po qd* for 14 days. Doses of 400-500 mg/kg were selected based on the previously reported MTD (18, 19). Animal body weights were monitored daily and a treatment was considered toxic (above the MTD) when ≥20% body weight loss was observed in ≥20% of the animals within the group. For monotherapy and combination antitumor efficacy studies, animals bearing KPL-4 xenografts were dosed according to previously established maximum tolerated or optimal dose regimens, with 10 animals per group. Tumor measurements and body weights were monitored 2-3 times per week. Both efficacy studies included a vehicle control (VC) group. For the monotherapy study, treatment began on Day 10 post tumor implant and continued until Day 28. For the combination therapy study, treatment began on Day 15 post tumor implant and continued until Day 31.

Efficacy measures. Efficacy measures were mean tumor volume, tumor regression, increased lifespan (ILS) and tumor inhibition. Tumor volumes of treated groups were presented as percentages of tumor volumes of the control groups (%T/C), using the formula: $100x((T–T_0)/(C–C_0))$, where T represents mean tumor volume of a treated group on a specific day during the experiment, T_0 represents mean tumor volume of the same treated group on the first day of treatment; C represents mean tumor volume of a control group on the specific day during the experiment, and C_0 represents mean tumor volume of the same control group on the first day of treatment. Tumor volume (in cubic millimeters) was calculated using the ellipsoid formula: $(Dx(d^2))/2$ where D represents the large diameter of the tumor and d represents the small diameter. The tumor volume obtained was mathematically correlated as described by Matar *et al.* and Yokoyama *et al.* (20, 21). Fractional tumor volumes (FTV) of capecitabine, bevacizumab and the combination were analyzed as experimental mean tumor volume/control mean tumor volume. Tumor inhibition was calculated using the formula 100–%T/C. The expected tumor volume of the combination was obtained by multiplying the mean FTV of drugs in combination. The synergistic (or non-additive) action of the combination was then calculated as expected FTV/observed FTV.

For the survival assessment, animals were evaluated for tumor regrowth following cessation of treatment on Day 31. Results were plotted as the percentage survival against days after tumor implant.

Tumor regrowth to a volume of 500 mm^3 was considered as a surrogate for death when calculating ILS. Percentage ILS was calculated as 100 x ((median survival of treated group-median survival of control group)/median survival of control group). Median survival was determined utilizing the Kaplan-Meier survival analysis method.

Histopathology. Tumor samples were fixed by immersion in 10% zinc formalin, processed in a Tissue-Tek® VIP (Sakura Finetek, Torrance, CA, USA) and embedded in paraffin. Sections for immunohistochemistry were cut at 5 μm. For evaluation of morphology, sections were stained with hematoxylin and eosin.

For thymidine phosphorylase detection, antigen retrieval was performed by immersing sections in Target Retrieval Solution pH 9.0 (DakoCytomation, Carpinteria, CA, USA) and heating to 95˚C in a steamer (Black & Decker, Towson, MD, USA) for 20 minutes. Endogenous peroxidase activity was quenched by incubation in 3.0% H_2O_2 in methanol for 5 minutes. For detection of total thymidine phosphorylase, sections were incubated for 1 hour at room temperature with a mouse monoclonal anti-thymidine phosphorylase antibody (Vector Lab, Burlingame, CA, USA), diluted 1:25 in Dako Antibody Diluent (DakoCytomation). Primary antibodies were detected using the Dako Ark Animal Research Kit Peroxidase (Dakocytomation). Vector NovaRED (Vector Laboratories) was used as the substrate. The sections were then counterstained with hematoxylin.

For CD31 detection, antigen retrieval was performed by immersing sections in Target Retrieval Solution pH 6.0 (DakoCytomation) and heating to 94˚C in a steamer (Black & Decker) for 20 minutes. Endogenous peroxidase activity was quenched by incubation in 3% H_2O_2 in methanol for 15 minutes. To block non-specific binding sites, sections were treated for 20 minutes using 10% normal serum from the species in which the secondary antibody was raised, prepared in Ultra V Block (Lab Vision, Fremont, CA, USA). Sections were then incubated overnight at room temperature with a goat polyclonal (Santa Cruz Biotechnology, Santa Cruz, CA, USA), diluted 1:800 in Dako Antibody Diluent with background reducing component (DakoCytomation). Primary antibodies were detected using a Goat IgG VECTASTAIN Elite ABC peroxidase kit, with Vector NovaRED (Vector Laboratories) as the substrate. The sections were then counterstained with hematoxylin.

Statistical methods. Statistical analysis was determined by Mann-Whitney Rank Sum Test, 1-way ANOVA, and post hoc Bonferroni *t*-test (SigmaStat, version 2.0, Jandel Scientific, San Francisco, CA, USA). Survival in treated groups was compared with the vehicle group by log-rank test, and survival comparisons between groups were analyzed by the Breslow-Gehan-Wilcoxon test (Stat View; SAS, Cary, NC, USA). Differences between groups were considered significant when the probability value (p) was ≤0.05.

Results

Maximum tolerated dose of capecitabine. Previously published preclinical studies with capecitabine utilized a gum arabic/citrate vehicle and reported an MTD of 540 mg/kg *po qd* (18, 19). For the current antitumor efficacy studies, a Klucel/Tween formulation was utilized, and thus a preliminary experiment was run to determine the MTD of capecitabine in the new formulation. Capecitabine was administered to KPL-4 bearing mice (n=5 per group) *po qd* for 2 weeks and the MTD was found to be 400 mg/kg in the Klucel/Tween formulation. This dose was the highest dose without any associated morbidity or mortality (Table I).

Table I. *Maximum tolerated dose of capecitabine in KPL-4 tumor-bearing mice.*

Treatment	Route/frequency	Morbidity[a]	Mortality	Reason for mortality
Vehicle	*po, qd* x 2 weeks	0	0	-
Capecitabine				
400 mg/kg	*po, qd* x 2 weeks	0	0	-
450 mg/kg	*po, qd* x 2 weeks	0	2	Toxicity
500 mg/kg	*po, qd* x 2 weeks	2	2	Toxicity

[a]Morbidity = ≥20% body weight loss.

Efficacy (TGI and ILS).
(i) Tumor growth inhibition (TGI) in monotherapy. The antitumor activity of paclitaxel, trastuzumab, capecitabine, or bevacizumab monotherapy was determined in KPL-4-bearing mice and was expressed as mean tumor volume ± SEM (Figure 1A). The results indicate that while paclitaxel treatment resulted in >100% TGI and 10 partial regressions (PRs), trastuzumab, capecitabine, and bevacizumab at optimal doses achieved slightly lower TGIs of 69%, 83%, and 83%, respectively ($p<0.001$ for all treatment groups *versus* VC). No body weight loss, morbidity, or mortality was associated with any of the treatment groups (data not shown).
(ii) TGI and ILS in combination therapy. The antitumor activities of capecitabine at one-half the MTD or MTD and bevacizumab at the optimal dose were evaluated alone or in combination in KPL-4-bearing mice and were expressed as mean tumor volume ± SEM (Figure 1B). Capecitabine inhibited tumor growth by 58% at 200 mg/kg *po qd* and 84% at 400 mg/kg *po qd* as compared with VC animals ($p<0.001$). Bevacizumab inhibited tumor growth by 74% at 5 mg/kg ($p<0.001$ *vs.* VC).

Following the cessation of treatment on Day 31, ILS was assessed as compared with vehicle-treated animals (Figure 2, Table II). Capecitabine extended the lifespan by 56% and 85% at one-half the MTD and MTD, respectively, and bevacizumab by 26% ($p<0.0001$ *vs.* VC). When capecitabine was dosed at one-half the MTD in combination with bevacizumab, >100% TGI was observed, with PRs observed in 7/10 animals ($p<0.001$ *vs.* VC). When capecitabine was dosed at the MTD in combination with bevacizumab, >100% TGI with 9/10 PRs and 1/10 complete regression (CR) was observed ($p<0.001$ *vs.* VC) (Figure 1B).

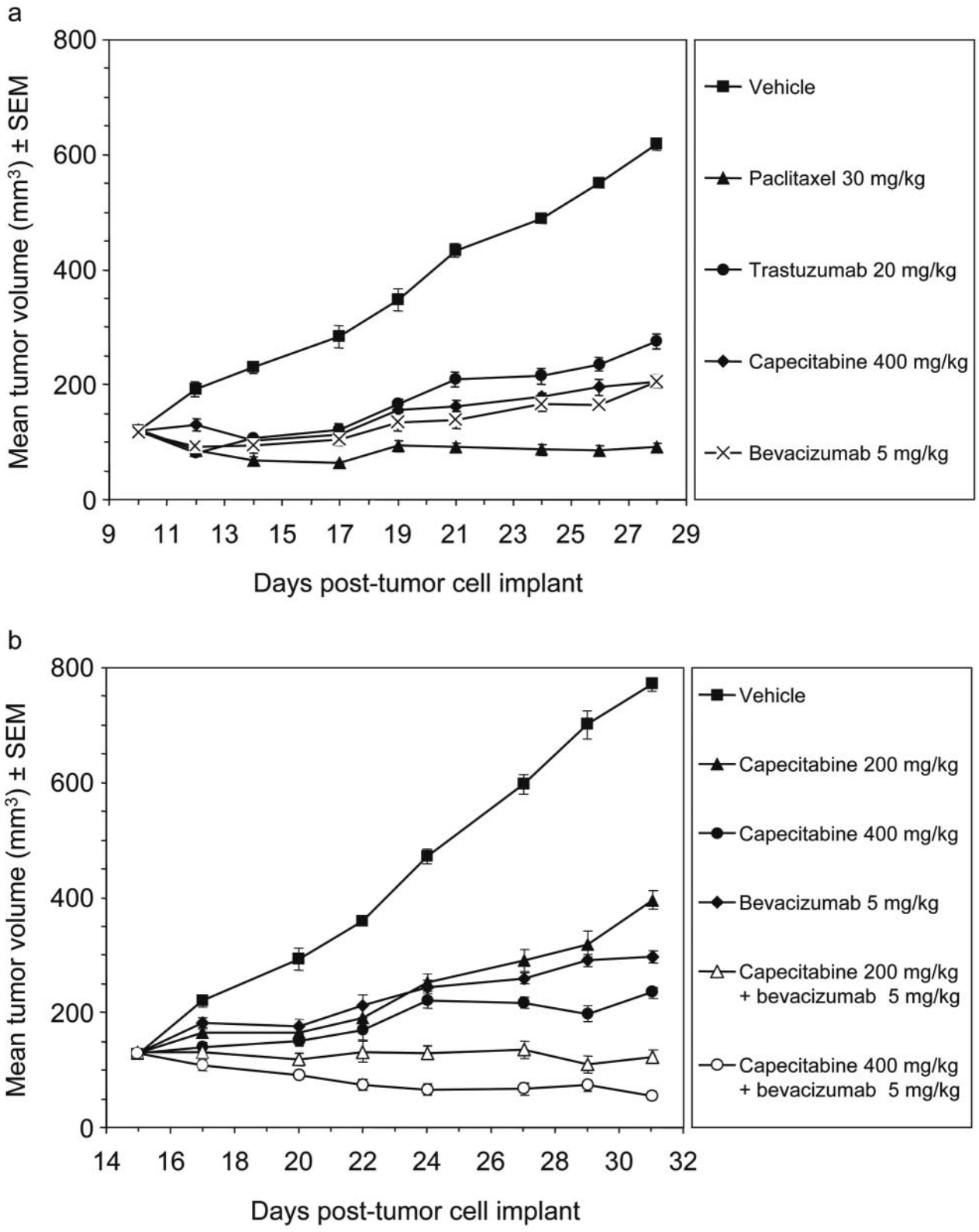

Figure 1. *Tumor growth inhibition (TGI) – monotherapy (a) and combination therapy (b).*

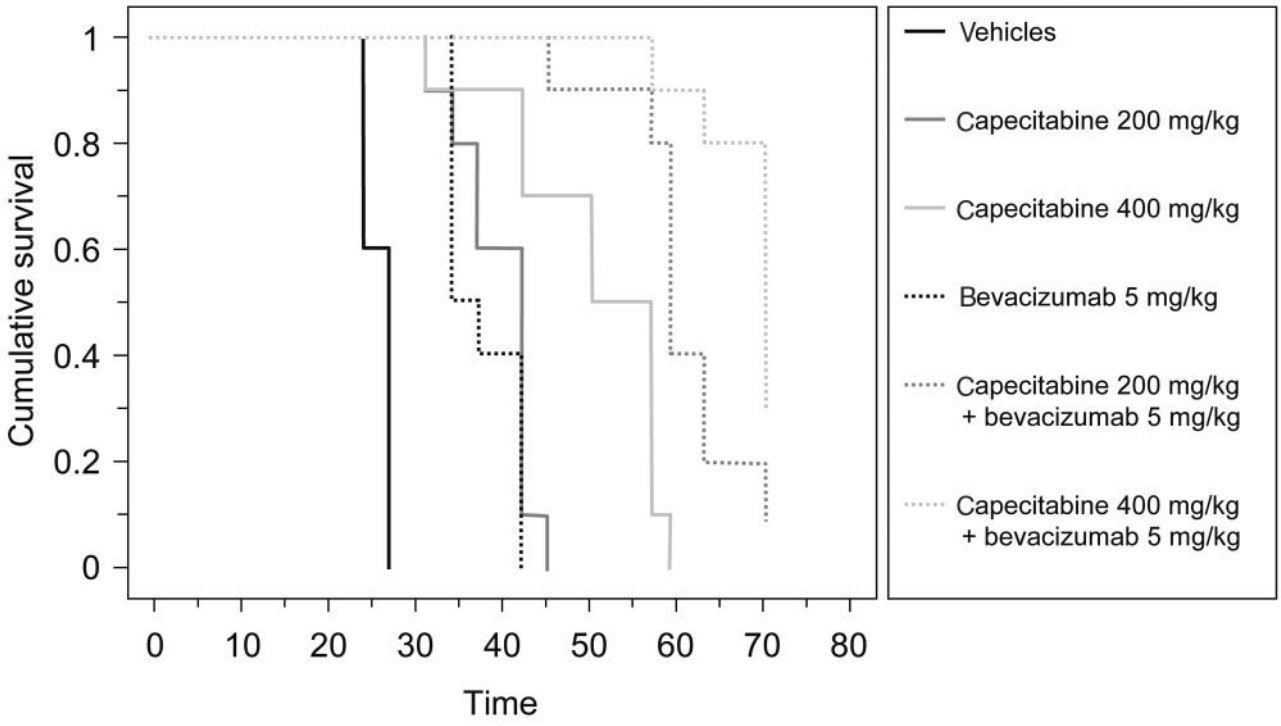

Figure 2. *Kaplan Meier survival curve – combination therapy.*

Lifespan was increased by 119% and 159% when one-half of the MTD or MTD of capecitabine was combined with bevacizumab, respectively ($p<0.05$, Figure 2, Table II). TGI and survival were significantly higher in the high-dose combination than in the low-dose combination of capecitabine and bevacizumab ($p\leq0.001$ and $p=0.019$, respectively). No body weight loss, morbidity, or mortality was associated with any of the single-agent or combination treatment groups (data not shown).

Fractional tumor volume. Tumor regression of the capecitabine plus bevacizumab combination-treated groups was further characterized by calculating the fractional tumor volume of capecitabine and bevacizumab combination relative to vehicle control. As shown in Table III, R values of 1.25 and 1.68, respectively, for combinations containing one-half MTD and the MTD of capecitabine indicate that the combinations had a synergistic effect on antitumor efficacy, with the MTD combination being more effective than the one-half MTD combination.

Histopathology. Examination of tumor morphology of H & E stained tumor sections indicated an overall increasing loss of cellularity and an increase in necrotic foci in tumors treated with bevacizumab, capecitabine, or both drugs in combination (data not shown).

Table II. *Increase in lifespan (ILS).*

Treatment	50% Treatment days	50% Vehicle days	% ILS	*p*-value
Vehicle	-	-	-	-
Capecitabine 200 mg/kg	42	27	56	<0.001
Capecitabine 400 mg/kg	50	27	85	<0.001
Bevacizumab 5 mg/kg	34	27	26	<0.001
Capecitabine 200 mg/kg plus bevacizumab	59	27	119	<0.001
Capecitabine 400 mg/kg plus bevacizumab	70	27	159	<0.001

% ILS calculated as 100 x ((median survival of treated group-median survival of control group)/median survival of control group).

Table III. *Fractional tumor volume (FTV) of capecitabine and bevacizumab combination relative to vehicle control.*

Mean FTV					
Capecitabine 200 mg/kg	Capecitabine 400 mg/kg	Bevacizumab 5 mg/kg	Expected FTV for combination[a]	Observed FTV for combination	R[b]
0.51	-	0.38	0.20	0.16	1.25
-	0.30	0.38	0.12	0.07	1.68

FTV = mean tumor volume experimental/mean tumor volume control. [a]Mean FTV of test compound 1 x mean FTV of test compound 2; [b]R=Expected FTV/observed FTV: value of 1 indicates additive, >1 indicates synergistic, <1 indicates less than additive.

When thymidine phosphorylase levels were assessed by immunohistochemistry on tumor sections from vehicle- or bevacizumab-treated animals, no difference in staining intensity was observed (Figure 3A, B). Assessment of anti-angiogenic activity was attempted by evaluation of MVD by CD31 immunohistochemistry. Due to tumor cell loss with the treatments and the resulting stromal collapse, identification of a traditional vascular "hot spot" was difficult, so the evaluation was discontinued.

Discussion

One of the objectives of the study was to establish the KPL-4 cell xenograft model for preclinical combination studies testing novel anticancer therapeutics for treating MBC. Our observations demonstrate that KPL-4 may be a relevant model due both to its inherent biology (estrogen receptor-negative, HER-2-positive), as well as its responsiveness to several conventional agents used in the treatment of MBC. In the current study, capecitabine was formulated in a Klucel/Tween formulation and dosed at the newly established MTD of 400 mg/kg daily. Comparable antitumor activity was observed with both capecitabine and trastuzumab in the KPL-4 xenograft model as compared with that reported by Fujimoto-Ouchi *et al.* and Freiss *et al.* (17, 22). Additionally, the KPL-4 model was shown to be sensitive to both paclitaxel and bevacizumab, which to our knowledge has not been previously reported.

The second but primary objective of the current studies was to assess the antitumor activity and survival of mice bearing breast cancer xenografts treated with capecitabine in combination with bevacizumab. Toxic adverse effects are commonly reported in patients undergoing chemotherapy for MBC. Previous studies have shown that bevacizumab is commonly associated with hypertension, proteinuria, thrombosis and bleeding (23, 24). For capecitabine, the predominant adverse effects have been gastrointestinal disturbances and hand-foot syndrome (25). In the present study, the combination was well tolerated, with no apparent weight changes or other gross toxicities as compared with vehicle-treated control animals, even when capecitabine was dosed up to the MTD (data not shown). This is in concordance with the phase III clinical trial, which showed no significant increase in either capecitabine- or bevacizumab-associated toxicities in patients who received the combination of capecitabine plus bevacizumab (14, 15). Furthermore, the same study reported that bevacizumab-related toxicities were largely predictable and seldom limited therapy in patients.

Our current preclinical studies demonstrated a significant synergistic effect on tumor growth inhibition as calculated by FTV and also a significant survival benefit. This result was not due to any up-regulation of thymidine phosphorylase by bevacizumab and thus represents a synergy based on the dual antiangiogenic and cytotoxic mechanisms of action. Since a significant difference was observed between the low-dose *versus* high-dose combinations of capecitabine with bevacizumab with respect to TGI and ILS, these data also emphasize the importance of maintaining capecitabine at the highest dose possible for maximal antitumor and survival benefit.

Since metastatic disease is largely incurable, the focus of therapy in these patients has been to improve OS and quality of life. Emphasis has thus been placed on better understanding the biology of the disease, which may in turn result in designing effective anticancer therapies. There is a sizeable amount of data suggesting that angiogenesis plays a role in the development, invasion, and metastasis of breast cancer (26, 27). Bevacizumab targeted against VEGF, one of the most potent regulators of angiogenesis, may be an attractive anticancer therapy. Capecitabine, on the other hand, has proven to be effective for MBC both as monotherapy and in combination with either antibody drugs (*e.g.* trastuzumab) (17) or chemical agents (*e.g.* docetaxel and vinorelbine) (8, 28). The recently concluded phase III trial, which evaluated the combination of capecitabine and bevacizumab, achieved improved response rates yet did not increase PFS as compared with capecitabine monotherapy. This is in direct contrast to our current preclinical results with the combination, where both improved response and increased lifespan were demonstrated.

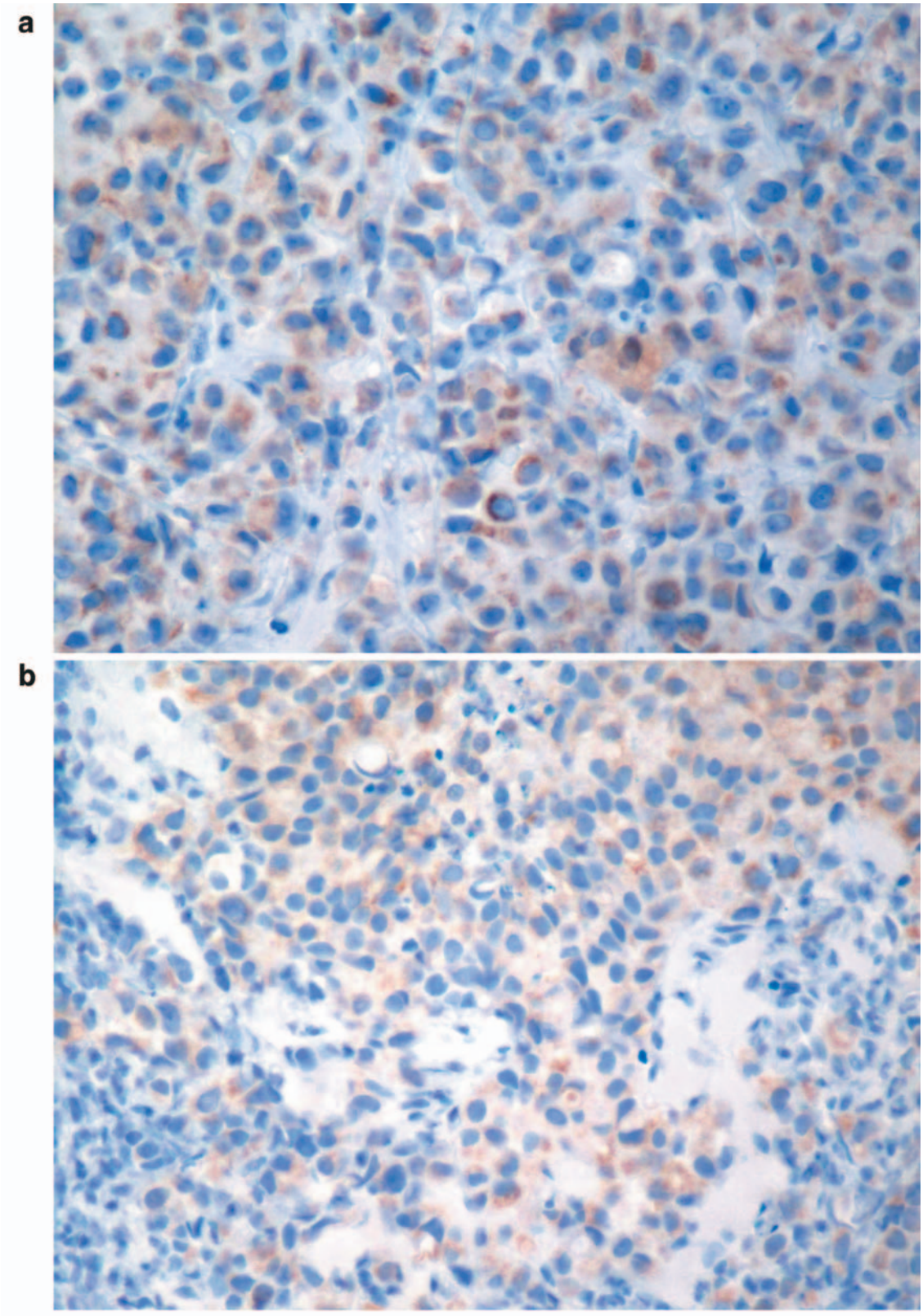

Figure 3. *Thymidine phosphorylase levels were assessed by immunohistochemistry on tumor sections from vehicle- (a) or bevacizumab-treated (b) animals. No difference in staining intensity was observed. Magnification x200.*

Several possible explanations have been put forward for the combination not extending PFS in the clinical study, which may also explain differences between preclinical *versus* clinical outcomes. As discussed by Bergsland *et al.*, the choice of the targeted population with a specific phenotype may be crucial for trials evaluating antiangiogenic therapeutics (29). Additionally, the authors suggested that bevacizumab may be more effective in early stages of tumor development, when VEGF may be the predominant angiogenic growth factor. A similar view was presented by Li *et al.* (30), who demonstrated that angiogenesis occurs far earlier in metastatic disease than previously thought, and that early intervention would be critical for successful management of tumor neovascularization. Thus, a heavily pretreated population with advanced MBC would not be ideal for maximal antitumor benefit of bevacizumab alone or in combination with capecitabine. It remains to be tested whether the combination of capecitabine and bevacizumab would confer a survival advantage in patients previously untreated with cytotoxic agents in the clinic; however, we have clearly established a survival benefit in a model of breast cancer preclinically. These data have served as a rationale for clinical studies with capecitabine and bevacizumab in the first-line treatment of MBC (31).

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Declaration of competing interests. The authors are employees of Hoffmann-La Roche, Inc.

References

1 Jemal A, Murray T, Samuels A, Ghafoor A, Ward E and Thun MJ: Cancer statistics, 2003. CA Cancer J Clin *53*: 5-26, 2003.

2 Ahn JH, Kim SB, Kim TW, Ahn SH, Kim SM, Park JM, Lee JS, Kang YK and Kim WK: Capecitabine and vinorelbine in patients with metastatic breast cancer previously treated with anthracycline and taxane. J Korean Med Sci *19*: 547-553, 2004.

3 Aapro MS and Rowinsky E: Docetaxel: assessing a range of activity. Anticancer Drugs *12(Suppl 1)*: S1-S3, 2001.

4 Perez EA: Paclitaxel in breast cancer. Oncologist *3*: 373-389, 1998.

5 Nabholtz JM, Falkson G, Campos D, Szanto J, Martin M, Chan S, Pienkowski T, Bezwoda WR, Zaluski J, Pinter T, Krzakowski M, Vorobiof D, Leonard R, Kennedy I, Azli N, Murawsky M, Riva A and Pouillartet P: A phase III trial comparing doxorubicin (A) and docetaxel (T) (AT) to doxorubicin and cyclophosphamide (AC) as first line chemotherapy for MBC. Proc Am Soc Clin Oncol *18*: 127a. Abstract 485, 1999.

6 Biganzoli L, Cufer T, Bruning P, Coleman R, Duchateau L, Calvert AH, Gamucci T, Twelves C, Fargeot P, Epelbaum R, Lohrisch C and Piccart MJ: Doxorubicin and paclitaxel *versus* doxorubicin and cyclophosphamide as first-line chemotherapy in metastatic breast cancer: the European Organization for Research and Treatment of Cancer 10961 multicenter phase III trial. J Clin Oncol *20*: 3114-3121, 2002.

7 Seidman AD, O'Shaughnessy J and Misset JL: Single-agent capecitabine: a reference treatment for taxane-pretreated metastatic breast cancer? Oncologist *7(Suppl 6)*: 20-28, 2002.

8 O'Shaughnessy J, Miles D, Vukelja S, Moiseyenko V, Ayoub JP, Cervantes G, Fumoleau P, Jones S, Lui WY, Mauriac L, Twelves C, Van Hazel G, Verma S and Leonard R: Superior survival with capecitabine plus docetaxel combination therapy in anthracycline-pretreated patients with advanced breast cancer: phase III trial results. J Clin Oncol *20*: 2812-2823, 2002.

9 Blum JL, Dieras V, Lo Russo PM, Horton J, Rutman O, Buzdar A and Osterwalder B: Multicenter, phase II study of capecitabine in taxane-pretreated metastatic breast carcinoma patients. Cancer *92*: 1759-1768, 2001.

10 Slamon DJ, Clark GM, Wong SG, Levin WJ, Ullrich A and McGuire WL: Human breast cancer: correlation of relapse and survival with amplification of the *HER-2/neu* oncogene. Science *235*: 177-182, 1987.

11 Cobleigh MA, Vogel CL, Tripathy D, Robert NJ, Scholl S, Fehrenbacher L, Wolter JM, Paton V, Shak S, Lieberman G and Slamon DJ: Multinational study of the efficacy and safety of humanized anti-HER2 monoclonal antibody in women who have HER2-overexpressing metastatic breast cancer that has progressed after chemotherapy for metastatic disease. J Clin Oncol *17*: 2639-2648, 1999.

12 Agus DB, Gordon MS, Taylor C, Natale RB, Karlan B, Mendelson DS, Press MF, Allison DE, Sliwkowski MX, Lieberman G, Kelsey FM and Fyfe G: Phase I clinical study of pertuzumab, a novel HER dimerization inhibitor, in patients with advanced cancer. J Clin Oncol *23*: 2534-2543, 2005.

13 Gerber HP and Ferrara N: Pharmacology and pharmacodynamics of bevacizumab as monotherapy or in combination with cytotoxic therapy in preclinical studies. Cancer Res *65*: 671-680, 2005.

14 Miller KD, Rugo HS and Cobleigh MA: Phase III trial of capecitabine (Xeloda®) plus bevacizumab (Avastin™) *versus* capecitabine alone in women with metastatic breast cancer (MBC) previously treated with an anthracycline and a taxane. Breast Cancer Res Treat *76(Suppl 1)*: S37, 2002.

15 Miller KD, Chap LI, Holmes FA, Cobleigh MA, Marcom PK, Fehrenbacher L, Dickler M, Overmoyer BA, Reimann JD, Sing AP, Langmuir V and Rugo HS: Randomized phase III trial of capecitabine compared with bevacizumab plus capecitabine in patients with previously treated metastatic breast cancer. J Clin Oncol *23*: 792-799, 2005.

16 Kurebayashi J, Otsuki T, Tang CK, Kurosumi M, Yamamoto S, Tanaka K, Mochizuki M, Nakamura H and Sonoo H: Isolation and characterization of a new human breast cancer cell line, KPL-4, expressing the Erb B family receptors and interleukin-6. Br J Cancer *79*: 707-717, 1999.

17 Fujimoto-Ouchi K, Sekiguchi F and Tanaka Y: Antitumor activity of combinations of anti-HER-2 antibody trastuzumab and oral fluoropyrimidines capecitabine/5'-dFUrd in human breast cancer models. Cancer Chemother Pharmacol *49*: 211-216, 2002.

18 Miwa M, Ura M, Nishida M, Sawada N, Ishikawa T, Mori K, Shimma N, Umeda I and Ishitsuka H: Design of a novel oral fluoropyrimidine carbamate, capecitabine, which generates 5-fluorouracil selectively in tumors by enzymes concentrated in human liver and cancer tissue. Eur J of Cancer *34*: 1274-1281, 1998.
19 Ishikawa T, Utoh M, Sawada N, Nishida M, Fukase Y, Sekiguchi F and Ishitsuka H: Tumor selective delivery of 5-fluorouracil by capecitabine, a new oral fluoropyrimidine carbamate, in human cancer xenografts. Biochem Pharmacol *55*: 1091-1097, 1998.
20 Matar P, Rojo F, Cassia R, Moreno-Bueno G, Di Cosimo S, Tabernero J, Guzmán M, Rodriguez S, Arribas J, Palacios J and Baselga J: Combined epidermal growth factor receptor targeting with the tyrosine kinase inhibitor gefitinib (ZD1839) and the monoclonal antibody cetuximab (IMC-C225): superiority over single-agent receptor targeting. Clin Cancer Res *10*: 6487-6501, 2004.
21 Yokoyama Y, Dhanabal M, Griffioen AW, Sukhatme VP and Ramakrishnan S: Synergy between angiostatin and endostatin: inhibition of ovarian cancer growth. Cancer Res *60*: 2190-2196, 2000.
22 Friess T, Scheuer W and Hasmann M: Superior antitumor activity after combination treatment with pertuzumab and trastuzumab against NSCLC and breast cancer xenograft tumors. Ann Oncol *17(Suppl 9)*: ix58. Abstract 96PD, 2006.
23 Gordon MS, Margolin K, Talpaz M, Sledge GW Jr, Holmgren E, Benjamin R, Stalter S, Shak S and Adelman DC: Phase I safety and pharmacokinetic study of recombinant human anti-vascular endothelial growth factor in patients with advanced cancer. J Clin Oncol *19*: 843-850, 2001.
24 Langmuir VK, Cobleigh MA, Herbst RS, Holmgren E, Hurwitz H, Kabbinavar F, Miller K and Novotny W: Successful long-term therapy with bevacizumab (Avastin) in solid tumors. Proc Am Soc Clin Oncol *21*: 9a. Abstract 32, 2002.
25 O'Shaughnessy JA, Blum J, Moiseyenko V, Jones SE, Miles D, Bell D, Rosso R, Mauriac L, Osterwalder B, Burger HU and Laws S: Randomized, open-label, phase II trial of oral capecitabine (Xeloda®) *vs.* a reference arm of intravenous CMF (cyclophosphamide, methotrexate and 5-fluorouracil) as first-line therapy for advanced/metastatic breast cancer. Ann Oncol *12*: 1247-1254, 2001.
26 McLeskey SW, Tobias CA, Vezza PR, Filie AC, Kern FG and Hanfelt J: Tumor growth of FGF or VEGF transfected MCF-7 breast carcinoma cells correlates with density of specific microvessels independent of the transfected angiogenic factor. Am J Pathol *153*: 1993-2006, 1998.
27 Weinstat-Saslow DL, Zabrenetzky VS, VanHoutte K, Frazier WA, Roberts DD and Steeg PS: Transfection of thrombospondin 1 complementary DNA into a human breast carcinoma cell line reduces primary tumor growth, metastatic potential, and angiogenesis. Cancer Res *54*: 6504-6511, 1994.
28 Welt A, von Minckwitz G, Oberhoff C, Borquez D, Schleucher R, Loibl S, Harstrick A, Kaufmann M, Seeber S and Vanhoefer U: Phase I/II study of capecitabine and vinorelbine in pretreated patients with metastatic breast cancer. Ann Oncol *16*: 64-69, 2005.
29 Bergsland E and Dickler MN: Maximizing the potential of bevacizumab in cancer treatment. Oncologist *9(Suppl 1)*: 36-42, 2004.
30 Li CY, Shan S, Huang Q, Braun RD, Lanzen J, Hu K, Lin P and Dewhirst MW: Initial stages of tumor cell-induced angiogenesis: evaluation *via* skin window chambers in rodent models. J Natl Cancer Inst *92*: 143-147, 2000.
31 Miller K, Gradishar W, Moisa C and Sledge G: Capecitabine plus bevacizumab in first line metastatic breast cancer: an interim safety and efficacy report of the first phase of Xeloda plus Avastin 1st line metastatic breast cancer trial. Breast Cancer Res Treat *100(Suppl 1)*: S103, 2006.

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