In vivo activity of novel capecitabine regimens alone and with bevacizumab and oxaliplatin in colorectal cancer xenograft models

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Abstract

Modifying the capecitabine dosing schedule from 14 days on, 7 days off (14/7) to 7 days on, 7 days off (7/7) may enable higher doses and improved antitumor efficacy in colorectal cancer xenografts. Capecitabine 14/7 (267 or 400 mg/kg) and 7/7 (467 or 700 mg/kg) schedules in doublet and triplet combinations with optimally dosed bevacizumab (5 mg/kg) and oxaliplatin (6.7 mg/kg) were studied in female athymic nude mice bearing HT29 colorectal xenografts. Additional studies of suboptimally dosed bevacizumab (2.5 mg/kg) and capecitabine 7/7 (360 mg/kg) were done in a similar Colo205 tumor xenograft model. Monotherapy and combination regimens were administered to groups of 10 animals and compared with vehicle controls. In the HT29 model, tumor growth inhibition and increase in life span (ILS) were significantly greater with capecitabine 7/7 than with 14/7 ($P < 0.05$). The additional benefit of capecitabine 7/7 versus 14/7 was biologically significant according to National Cancer Institute criteria (>25% ILS). Adding bevacizumab to capecitabine 7/7 resulted in significantly greater survival relative to either agent alone ($P < 0.0001$). When oxaliplatin was added, efficacy was significantly better with the triplet combination including capecitabine 7/7 (tumor growth inhibition >100% and ILS 234%) compared with 14/7 (95% and 81%, respectively). In the Colo205 model, combination therapy with capecitabine 7/7 plus bevacizumab resulted in significantly greater survival relative to either agent alone ($P < 0.0001$). In conclusion, in athymic nude mice bearing moderately thymidine phosphorylase–expressing HT29 or Colo205 colorectal xenografts, a capecitabine 7/7 schedule permits increased drug delivery compared with traditional 14/7 regimens, greatly improving monotherapy activity without major toxicity. [Mol Cancer Ther 2009;8(1):75–82]

Introduction

Fluoropyrimidine-based regimens remain the backbone of therapy for colorectal cancer.[6] Although the addition of new agents (oxaliplatin, irinotecan, bevacizumab, and cetuximab) has improved patient outcomes (1), there remains a need for novel regimens and refinement of existing regimens to extend survival and decrease treatment-related toxicity.

The p.o. administered fluoropyrimidine capecitabine is preferentially converted to 5-fluorouracil by thymidine phosphorylase (TP) in tumors (2–4) and has shown antitumor activity in xenograft models, including colorectal cancer (5–7). Randomized controlled trials have shown that in the management of colorectal cancer, capecitabine can safely and effectively replace i.v. 5-fluorouracil/leucovorin as monotherapy (8–10) and in combination with oxaliplatin, with or without bevacizumab (11–17). Bevacizumab is a recombinant, humanized monoclonal antibody directed against vascular endothelial growth factor (18). Bevacizumab, which significantly inhibits human colon cancer growth by disrupting tumor angiogenesis (19), significantly extends survival when added to fluoropyrimidine-based regimens including irinotecan (20) or oxaliplatin, a platinum-based chemotherapy often given in combination with fluorouracil and leucovorin, in patients with metastatic colorectal cancer (21).

The conventional, approved dosing schedule for capecitabine is 14 days of therapy followed by 7 days of rest (14/7; refs. 8–10, 22). In a breast cancer xenograft model, administration of capecitabine 14/7 at the maximum tolerated dose (MTD) reduced the percentage change in tumor volume compared with control, but did not cause tumor regression (23). Mathematical methods applied to such breast cancer xenograft models suggested that the maximum effect of capecitabine therapy occurs after ~7 days of treatment, with doses beyond 7 days adding

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[6] NCCN Practice Guidelines in Oncology—Colon Cancer v.1.2008 (available at: http://www.nccn.org/professionals/physician_gls/PDF/colon.pdf).

toxicity without additional antitumor benefit (24). Based on these findings, a series of experiments were conducted to test the hypothesis that a capecitabine dosing schedule of 7 days of treatment followed by 7 days of rest (7/7) will enable delivery of higher doses and improve efficacy in colorectal cancer xenografts. The specific objectives of the study were to determine the antitumor activity and tolerability of capecitabine at MTD and 2/3 MTD administered using either the traditional schedule (14/7) or the 7/7 schedule alone and in doublet and triplet combinations with optimally dosed bevacizumab and oxaliplatin (at 2/3 MTD) in female nude mice bearing HT29 colorectal xenografts. Additional *in vivo* studies were done to characterize the effects of bevacizumab and capecitabine combination therapy on tumor growth in the Colo205 colorectal cancer xenograft model.

Materials and Methods

Animals

Athymic nude mice (Crl:NU-Foxn1nu), 13 to 14 wk old and weighing ~23 to 25 g, were purchased from Charles River Laboratories. The health of all animals was monitored daily by gross observation and analysis of blood samples of sentinel animals. All animals were allowed to acclimatize and recover from any shipping-related stress for a minimum of 72 h before experimental use. Autoclaved water and irradiated food (5058-ms Pico Chow, Purina) were provided *ad libitum*, and the animals were maintained on a 12-h light and dark cycle. Cages, bedding, and water bottles were autoclaved before use and were changed weekly.

All animal experiments were done in accordance with protocols approved by the Institutional Animal Care and Use Committees.

Tumors

HT29 (American Type Culture Collection) and Colo205 (American Type Culture Collection) colorectal cancer cells were chosen based on their moderate activity of TP and moderate sensitivity to the traditional continuous daily administration regimen of capecitabine (6, 7). HT29 cells were cultured using McCoy's 5A 10% (v/v) heat-inactivated fetal bovine serum and 1% (v/v) 2 mmol/L L-glutamine, and Colo205 were cultured in RPMI 1640 with 10% (v/v) heat-inactivated fetal bovine serum and 1% (v/v) 2 mmol/L L-glutamine.

Test Agents and Study Design

Capecitabine, Oxaliplatin, and Bevacizumab in the HT29 Model. HT29 cells (3×10^6) in 0.2 mL PBS were injected into the right lateral flank of test mice. Capecitabine (Xeloda, Roche Laboratories) was formulated as a suspension and administered p.o. at the MTD previously determined in KPL-4 tumor–bearing mice (23). The MTDs were 400 mg/kg per day (qd) for the 14/7 schedule (2/3 MTD = 267 mg/kg) and 700 mg/kg qd for the 7/7 schedule (2/3 MTD = 467 mg/kg). Clinical-grade bevacizumab (Avastin, Genentech, Inc.) was obtained as a stock solution of 25 mg/mL, diluted with sterile saline, and given i.p. at the optimal dose (5 mg/kg twice/wk). Clinical-grade oxaliplatin (Eloxatin, Sanofi-Aventis U.S. LLC) was obtained as a stock solution of 5 mg/mL, diluted with sterile water, and given i.p. at 2/3 MTD (6.7 mg/kg, days 1, 7, and 14).

Different treatment schemes were tested to compare traditional and novel regimens of capecitabine alone or combined with bevacizumab and oxaliplatin: (*a*) monotherapy with capecitabine 14/7 (at MTD), oxaliplatin, or bevacizumab; (*b*) monotherapy with capecitabine (at MTD and 2/3 MTD) administered using either 7/7 or 14/7 schedules; (*c*) capecitabine (at MTD and 2/3 MTD) administered using either 7/7 or 14/7 schedules in combination with bevacizumab; or (*d*) combination therapy with capecitabine (at 2/3 MTD) administered using either 7/7 or 14/7 schedules plus oxaliplatin, with or without bevacizumab. All treatments were compared with appropriate vehicle controls and started ~14 d after HT29 cell implantation. There were 10 animals per treatment group. Tumor volume and weights were recorded twice to thrice a week and health status was checked daily by veterinary staff.

Capecitabine and Bevacizumab in the Colo205 Model. Athymic nude mice were injected s.c. on the right dorsal flank with 5×10^6 Colo205 cells suspended in 0.2 mL HBSS. Submaximum effective doses of capecitabine and bevacizumab were studied so that any additional inhibitory effect resulting from combination therapy could be observed. The dose of capecitabine was selected on the basis of an initial dose escalation monotherapy study in the Colo205 xenograft model (doses of 90, 180, 360, 540, and 900 mg/kg p.o. for 7 d; $N = 10$) showing that 360 mg/kg achieved ~50% tumor growth inhibition (TGI) compared with vehicle control. The MTD was 900 mg/kg. A 2.5 mg/kg dose of bevacizumab was selected because it resulted in significant but partial inhibition of Colo205 tumor growth. Treated mice ($N = 10$ per group) received capecitabine (360 mg/kg qd, days 0–6, p.o.), bevacizumab (2.5 mg/kg, i.p., days 0 and 3), concurrent capecitabine and bevacizumab (doses as per monotherapy), or vehicle control.

Efficacy Measures

Similar efficacy measures were used to express treatment effects in HT29 and Colo205 experiments. TGI was calculated from percent change in mean tumor volume. Survival was calculated using a cutoff of 1,500 mm^3, and the mean increase in life span (ILS) was calculated using the formula

$$[(\text{Median day of death in treated tumorbearing mice}) - (\text{median day of death in control tumorbearing mice})]/\text{Median day of death in control tumorbearing mice}$$

Tolerability Measures

Total body weight was used as a surrogate end point for tolerability in all studies.

Measurement of TP

Tumor samples were fixed by immersion in 10% zinc formalin, processed in a Tissue-Tek VIP (Sakura Finetek),

and embedded in paraffin. Sections for immunohistochemistry were cut at 5 μm. For evaluation of morphology, sections were stained with H&E. TP detection was done as previously described (25). Briefly, antigen retrieval was done by immersing sections in Target Retrieval Solution pH 9.0 (DakoCytomation) and heating to 95°C in a steamer (Black & Decker) for 20 min. Endogenous peroxidase activity was quenched by incubation in 3.0% H_2O_2 in methanol for 5 min. For detection of total TP, sections were incubated for 1 h at room temperature with a mouse monoclonal anti-TP antibody (Vector Lab), 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.

Statistical Methods

Statistical analysis was determined by Mann-Whitney rank sum test, one-way ANOVA, and post hoc Bonferroni *t* test (SigmaStat, version 2.0, Jandel Scientific). Survival was analyzed by Kaplan-Meier method. Treated groups were compared with the vehicle group by log-rank test, and survival comparisons between groups were analyzed by the Breslow-Gehan-Wilcoxon test (StatView, SAS). Differences between groups were considered significant when $P \leq 0.05$.

Results

Capecitabine, Oxaliplatin, and Bevacizumab in the HT29 Model

Monotherapy with Capecitabine 14/7, Oxaliplatin, and Bevacizumab. Capecitabine 14/7 (MTD = 400 mg/kg per d qd; 60% TGI) and bevacizumab (69% TGI) showed moderate monotherapy activity. Bevacizumab (5 mg/kg) for 1 week did not cause an induction of TP in the HT29 model (data not shown), which is consistent with data previously generated in the KPL-4 breast xenograft model (25). Oxaliplatin monotherapy was inactive at MTD (10 mg/kg every week for 3 cycles) and, therefore, assumed to be inactive at 2/3 MTD (data not shown).

Monotherapy with Capecitabine 7/7 versus 14/7 Schedules. Capecitabine 7/7 significantly improved TGI compared with vehicle control and 14/7 schedules (Table 1; Fig. 1A). The 7/7 schedule was significantly superior to vehicle control when administered at MTD (700 mg/kg qd; 92% TGI; $P < 0.01$) and 2/3 MTD (467 mg/kg qd; 77% TGI; $P < 0.01$). Furthermore, the 7/7 schedule was superior to the 14/7 schedule when given at MTD (700 mg/kg qd; $P < 0.05$) or at 2/3 MTD (467 mg/kg qd; $P < 0.05$). The TGI values of capecitabine 7/7 given at 2/3 MTD (467 mg/kg qd) and MTD (700 mg/kg qd) were not significantly different. ILS was significantly increased in comparison with vehicle control with the capecitabine 7/7 schedule

Table 1. Statistical comparisons (treatment 1 versus 2) of different capecitabine schedules administered as monotherapy or combined with bevacizumab or oxaliplatin in athymic mice bearing HT29 or Colo205 colorectal cancer xenografts

Schedules*	Treatment 1	Treatment 2	TGI, P †	ILS, P ‡
HT29 colorectal cancer xenografts				
Capecitabine 7/7 and 14/7	14/7 at 2/3 MTD	14/7 at MTD	<0.05	<0.0001
	14/7 at 2/3 MTD	7/7 at 2/3 MTD	<0.05	0.0001
	14/7 at 2/3 MTD	7/7 at MTD	<0.05	<0.0001
	14/7 at MTD	7/7 at 2/3 MTD	<0.05	0.5873
	14/7 at MTD	7/7 at MTD	<0.05	0.0006
	7/7 at 2/3 MTD	7/7 at MTD	>0.05	0.0006
Capecitabine 7/7 and 14/7 + bevacizumab 5 mg/kg	14/7 at 2/3 MTD	14/7 at MTD	<0.05	0.1917
	14/7 at 2/3 MTD	7/7 at 2/3 MTD	<0.05	0.0348
	14/7 at 2/3 MTD	7/7 at MTD	<0.05	0.0016
	14/7 at MTD	7/7 at 2/3 MTD	>0.05	0.5117
	14/7 at MTD	7/7 at MTD	>0.05	0.0729
	7/7 at 2/3 MTD	7/7 at MTD	>0.05	0.1316
Capecitabine 7/7 and 14/7 + oxaliplatin 6.7 mg/kg ± bevacizumab 5 mg/kg (+B)	14/7 at 2/3 MTD	7/7 at 2/3 MTD	<0.05	<0.0001
	14/7 at 2/3 MTD	14/7 at 2/3 MTD (+B)	<0.05	<0.0001
	14/7 at 2/3 MTD	7/7 at 2/3 MTD (+B)	<0.05	<0.0001
	7/7 at 2/3 MTD	14/7 at 2/3 MTD (+B)	<0.05	0.0111
	7/7 at 2/3 MTD	7/7 at 2/3 MTD (+B)	<0.05	<0.001
	14/7 at 2/3 MTD (+B)	7/7 at 2/3 MTD (+B)	<0.05	0.0015
Colo205 colorectal cancer xenografts				
Capecitabine 7/7 ± bevacizumab 2.5 mg/kg	Capecitabine	Bevacizumab	<0.05	0.0044
	Capecitabine	Combination	>0.05	0.0025
	Bevacizumab	Combination	<0.05	<0.0001

*Capecitabine 14/7 at MTD = 400 mg/kg qd; 14/7 at 2/3 MTD = 267 mg/kg qd; 7/7 at MTD = 700 mg/kg qd; 7/7 at 2/3 MTD = 467 mg/kg qd.
†One-way ANOVA, post hoc Bonferroni.
‡Breslow-Gehan-Wilcoxon.

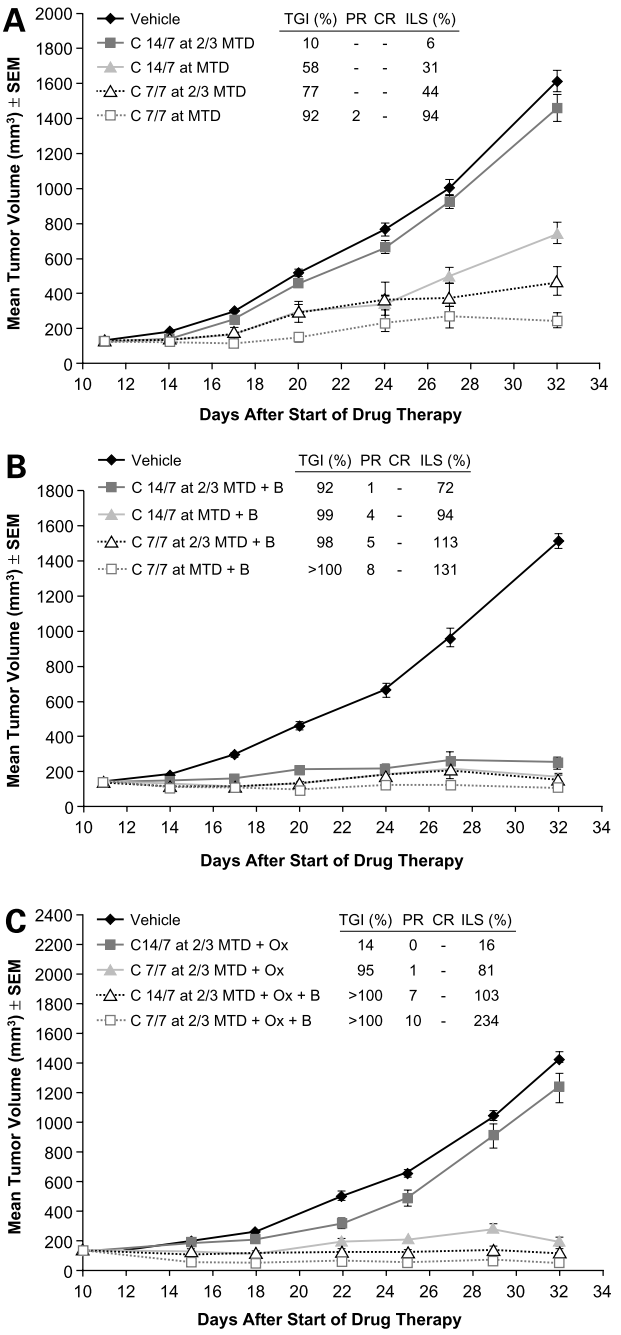

Figure 1. Antitumor activity of capecitabine 7/7 and 14/7 schedules administered as monotherapy or combined with bevacizumab or oxaliplatin in athymic mice bearing HT29 colorectal cancer xenografts. Capecitabine 14/7 at MTD = 400 mg/kg qd; 14/7 at 2/3 MTD = 267 mg/kg qd; 7/7 at MTD = 700 mg/kg qd; 7/7 at 2/3 MTD = 467 mg/kg qd. HT29 was treated 7/7 for 21 d. **A,** capecitabine (*C*) 7/7 and 14/7 schedules (all groups significantly different from vehicle control; $P < 0.01$ for %TGI and $P < 0.0001$ for %ILS). **B,** capecitabine (*C*) + bevacizumab (*B*) 5 mg/kg (all groups significantly different from vehicle control; $P < 0.01$ for %TGI and %ILS). **C,** capecitabine (*C*) + oxaliplatin (*Ox*) 6.7 mg/kg ± bevacizumab (*B*) 5 mg/kg (all groups significantly different from vehicle control except for capecitabine 14/7 + oxaliplatin; $P < 0.01$ for %TGI and %ILS).

(94% at MTD and 44% at 2/3 MTD; both $P < 0.0001$) and the 14/7 schedule (31% at MTD; $P < 0.0001$). The capecitabine 7/7 schedule also significantly improved survival compared with the 14/7 schedule (Table 1; Fig. 2A). The survival of mice treated with capecitabine 7/7 at 2/3 MTD (467 mg/kg qd) was significantly better than in those treated with capecitabine 14/7 at 2/3 MTD (267 mg/kg qd), although not significantly different from those treated with capecitabine 14/7 at MTD (400 mg/kg qd). In addition, survival was significantly better with capecitabine 7/7 administered at the MTD (700 mg/kg qd) than with the 14/7 schedule at MTD (400 mg/kg qd) or the 7/7 schedule at 2/3 MTD (467 mg/kg qd; Fig. 2A).

Capecitabine 7/7 and 14/7 in Combination with Bevacizumab. TGI was significantly improved ($P < 0.001$) with all doublet combinations of capecitabine and bevacizumab compared with vehicle control (Table 1; Fig. 1B). TGI values with capecitabine 14/7 at MTD (400 mg/kg qd) plus bevacizumab, capecitabine 7/7 at 2/3 MTD (467 mg/kg qd) plus bevacizumab, and capecitabine 7/7 at MTD (700 mg/kg qd) plus bevacizumab were statistically equivalent (99%, 98%, and >100%, respectively), although a trend for increased regressions and survival was seen with increased dosage of the 7/7 regimen. TGI with capecitabine 14/7 at 2/3 MTD (267 mg/kg qd) plus bevacizumab was statistically inferior to all other doublets ($P < 0.05$) and survival was also inferior to all regimens, except for capecitabine 14/7 at MTD (400 mg/kg qd) plus bevacizumab.

Survival with all doublet combinations was significantly ($P < 0.001$) better than vehicle control as shown by ILS, which ranged from 72% for capecitabine 14/7 at 2/3 MTD (267 mg/kg qd) plus bevacizumab to 131% for capecitabine 7/7 at MTD (700 mg/kg qd) plus bevacizumab (Fig. 1B). The survival advantage for doublets was also confirmed by Kaplan-Meier estimates (Fig. 2B).

Capecitabine 7/7 and 14/7 in Combination with Oxaliplatin ± Bevacizumab. Both TGI and ILS were significantly improved ($P < 0.001$) compared with vehicle control in all groups, except for capecitabine 14/7 at 2/3 MTD (267 mg/kg qd) plus oxaliplatin (Fig. 1C). Between-group comparisons (Table 1) showed that TGI and ILS were significantly better with the triplet combinations than with either doublet and significantly better with the triplet combination administered using the 7/7 schedule of capecitabine (TGI >100% and ILS 234%) compared with the 14/7 schedule (95% and 81%, respectively). Survival was inferior with capecitabine 14/7 at 2/3 MTD (267 mg/kg qd) plus oxaliplatin compared with all other groups (Fig. 2C).

Capecitabine and Bevacizumab in the Colo205 Model

To confirm the results seen in the HT29 model, a second set of experiments further explored capecitabine 7/7 ± bevacizumab in another model of metastatic colorectal cancer. Combining bevacizumab (2.5 mg/kg) with capecitabine 7/7 (360 mg/kg) resulted in greater tumor cell killing activity compared with either agent alone (Table 1; Fig. 3). TGI values for capecitabine 7/7 administered as monotherapy (83%) or combined with bevacizumab

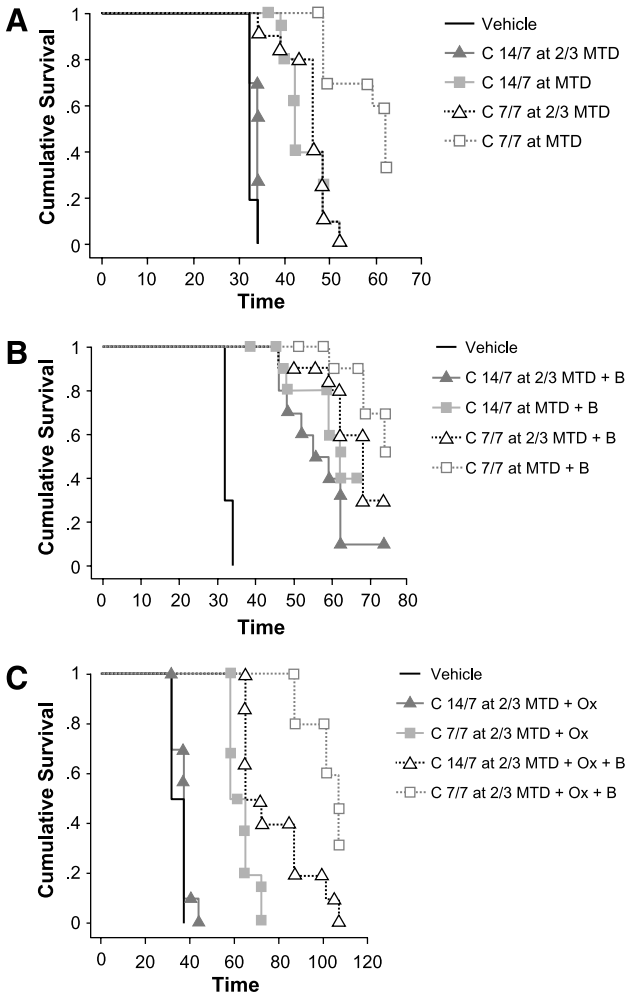

Figure 2. Kaplan-Meier estimates of cumulative survival with capecitabine 7/7 versus 14/7 administered as monotherapy or combined with bevacizumab or oxaliplatin in athymic mice bearing HT29 colorectal cancer xenografts. Capecitabine 14/7 at MTD = 400 mg/kg qd; 14/7 at 2/3 MTD = 267 mg/kg qd; 7/7 at MTD = 700 mg/kg qd; 7/7 at 2/3 MTD = 467 mg/kg qd. **A,** capecitabine (*C*) 7/7 and 14/7 schedules. **B,** capecitabine (*C*) + bevacizumab (*B*) 5 mg/kg. **C,** capecitabine (*C*) + oxaliplatin (*Ox*) 6.7 mg/kg ± bevacizumab (*B*) 5 mg/kg.

(>100%) were significantly different from vehicle control ($P < 0.001$); however, bevacizumab monotherapy (TGI 32%) was not significantly different from control ($P = 0.097$). The TGI of capecitabine 7/7 plus bevacizumab was also superior to bevacizumab alone ($P < 0.05$). In addition, combination therapy with capecitabine 7/7 plus bevacizumab resulted in significantly greater survival relative to either agent alone (Fig. 4). Median survival for animals treated with capecitabine 7/7 plus bevacizumab was 48 days, compared with 29 days for capecitabine alone and 22.5 days for bevacizumab alone.

Tolerability

There was no evidence of toxicity with any of the capecitabine 7/7 and 14/7 monotherapy or combination regimens tested in athymic mice bearing HT29 or Colo205 xenografts. Data for average percent weight change showed no meaningful changes during the treatment course and no significant differences between the treatment groups (data not shown).

Discussion

HT29 and Colo205 xenograft models were used to investigate two potential strategies for increasing the antitumor activity of capecitabine, specifically, schedule modification from 14/7 to 7/7 (a dose densification strategy) and combination with oxaliplatin and/or bevacizumab. Results from the HT29 model support the hypothesis that modifying the standard schedule of capecitabine from 14/7 to 7/7 improves antitumor efficacy of monotherapy and combination regimens in nude mice bearing HT29 colorectal xenografts. High TP expression has been associated with a favorable response to therapy whereas low TP expression has been associated with poor outcomes (26). Consequently, the HT29 and Colo205 xenografts used in these studies were selected because they moderately express TP (6, 7). When the human colon cancer cell line, WiDr, was incubated with paclitaxel, docetaxel, or mitomycin C, or tumor necrosis factor-related apoptosis-inducing ligand, each agent greatly induced TP (27). However, a decrease in TP activity in xenografts compared with primary tumors means that care is needed when extrapolating findings to the clinical setting (28).

As monotherapy, capecitabine administered using the 7/7 schedule had greater efficacy than the traditional 14/7 schedule, which showed moderate antitumor activity (60% TGI) in this model, which is similar to previously published results in colon cancer xenograft models (CXF280, Colo205, and HT29; refs. 6, 7). Although the TGI values of capecitabine 7/7 given at 2/3 MTD and MTD were not different, survival was significantly better at

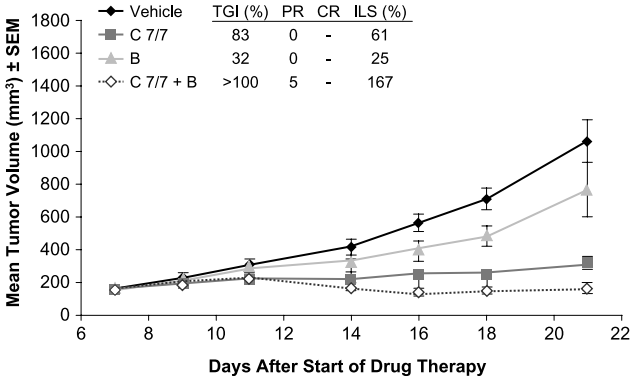

Figure 3. Antitumor activity of capecitabine (*C*) 360 mg/kg 7/7 qd and bevacizumab (*B*) 2.5 mg/kg monotherapy compared with concurrent capecitabine 7/7 qd and bevacizumab doublets in the Colo205 colorectal cancer xenograft model. Colo205 was treated 7/7 for 14 d.

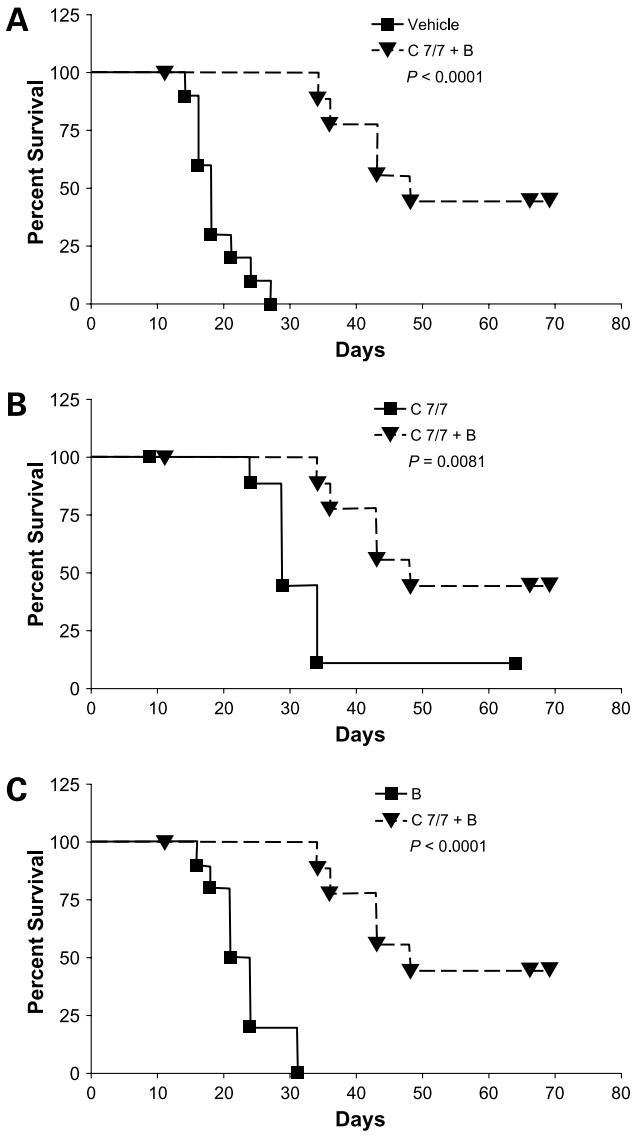

Figure 4. Survival curves for capecitabine 360 mg/kg 7/7 qd or bevacizumab 2.5 mg/kg monotherapy and concurrent capecitabine 7/7 qd and bevacizumab doublets in the Colo205 colorectal cancer xenograft model. **A,** capecitabine (*C*) + bevacizumab (*B*) versus control. **B,** capecitabine (*C*) + bevacizumab (*B*) versus capecitabine. **C,** capecitabine (*C*) + bevacizumab (*B*) versus bevacizumab.

MTD. The additional benefit of capecitabine administered using the 7/7 schedule compared with the 14/7 schedule was biologically significant according to National Cancer Institute criteria that require a >25% ILS (29).

Adding bevacizumab to capecitabine increased efficacy in both the HT29 and Colo205 models. In the HT29 model, the greatest effect of the capecitabine plus bevacizumab doublet on TGI and survival was achieved with the 7/7 schedule, which delivers a greater dosage. The 7/7 doublet achieved a biologically significant increase in survival, and there was a trend for increased regressions and percent ILS with greater dosage. Capecitabine (14/7) + bevacizumab is statistically similar to capecitabine (7/7) + bevacizumab, but this may be because the gains seen with 7/7 and 14/7 are masked by the beneficial effect of bevacizumab. In the colorectal cancer model reported here, treatment with the optimal dose of bevacizumab (5 mg/kg) for 1 week did not cause an induction of TP (25), suggesting additive results from the dual antiangiogenic and cytotoxic mechanisms of action of the combination(s). Jain has proposed that drugs that induce vascular normalization, such as bevacizumab and other antiangiogenic agents, can alleviate hypoxia and, therefore, increase the efficacy of conventional therapies if both are carefully scheduled (30, 31).

In the Colo205 model, the capecitabine and bevacizumab doublet also resulted in greater inhibition of tumor regrowth than that observed with either agent alone. Suboptimal doses of capecitabine plus bevacizumab (360 and 2.5 mg/kg, respectively) resulted in a significantly greater duration of tumor inhibition (beyond 7 weeks) than with either agent alone. Although monotherapy resulted in significant TGI, tumor regrowth occurred 2 to 3 weeks after cessation of treatment. In the preliminary dose-finding studies for the Colo205 model, it was notable that single doses of capecitabine up to 900 mg/kg were achievable compared with the MTD of capecitabine (7/7) of 700 mg/kg in the HT29 studies; however, capecitabine was administered for 1 cycle in the Colo205 studies and for 2 cycles in the HT29 studies, which may account for the difference in tolerated dose. Like the HT29 xenograft model, Colo205 is moderately responsive to the traditional 14/7 capecitabine regimen and the 7-day dose-dense regimen greatly increased antitumor activity in both xenograft models. Therefore, data from the HT29 and Colo205 models suggest that capecitabine dose densification (using the 7/7 schedule) and combination with bevacizumab result in prolonged survival compared with traditional capecitabine monotherapy schedules.

Although oxaliplatin was inactive as a single agent in the HT29 model, its addition to capecitabine, with or without bevacizumab, improved efficacy and there were trends in increased survival with capecitabine plus oxaliplatin, with or without bevacizumab (Fig. 1A and C). The triplet combinations were statistically more effective than doublets. In addition, the increases in TGI and survival with triplet combinations were greater when capecitabine was administered using the 7/7 schedule (at 2/3 MTD) compared with the 14/7 schedule (at 2/3 MTD). Other investigators have also observed additivity between capecitabine and oxaliplatin in colorectal cancer models (CXF280 and COL-05-JCK), which was attributed to upregulation of TP by oxaliplatin (32). Importantly, one study has shown that coadministration of capecitabine, oxaliplatin, and bevacizumab does not result in clinically relevant pharmacokinetic interactions (33).

The results of these xenograft studies suggest that the 7/7 schedule should be further investigated in clinical practice to test the hypothesis that delivery of a greater dosage will safely improve outcomes. A preliminary study of weekly capecitabine plus oxaliplatin in patients

with advanced colorectal cancer has been reported (34, 35). The weekly schedule, consisting of capecitabine 1,750 mg/m^2 twice daily (bd) on days 1 to 7 and days 14 to 21 every 4 weeks plus oxaliplatin 85 mg/m^2 days 1 and 14, allowed an increase in capecitabine dose administered to 105 to 131, 25% compared with a conventional schedule (capecitabine 1,000 mg/m^2 bd on days 1–14 every 3 weeks plus oxaliplatin 130 mg/m^2 day 1). A significantly longer median progression-free survival time (10.5 versus 6.0 months; $P = 0.0013$) was achieved with the weekly schedule, and there was no difference in hematologic or symptomatic toxicities despite the increase in dosage. Of note, patients had a longer total drug-free period with the weekly schedule compared with the traditional schedule (6 versus 4 weeks in each 12-week period, respectively).

A capecitabine 7/7 schedule has also been investigated in patients with metastatic breast cancer (36). This study showed that the capecitabine 7/7 schedule was well tolerated and enabled safe delivery of higher daily doses than routinely used in practice. The ability to increase dosage without exacerbating toxicity is potentially an important advantage for the 7/7 schedule, particularly for U.S. patient populations in whom fluoropyrimidines may be less well tolerated than in European or Asian patients (37). In the nonclinical study reported here, there was no evidence of toxicity with any of the regimens studied. Whereas this model may be somewhat predictive, it should be borne in mind that the primary objective of this study was to investigate the antitumor effects of these agents with dosages that are lower than those that would be used in the clinical setting. Thus, translating these results into the clinical setting should be done cautiously. Clearly, further analyses in animal safety models to better understand toxicity and clinical studies are needed to determine the appropriate dose to be used in patients. Published clinical studies have shown that combining the standard 14/7 schedule of capecitabine with oxaliplatin (XELOX), with or without bevacizumab, is clinically effective and well tolerated, with no unexpected toxicities (11, 14–17, 38–40). Future studies of the XELOX regimens with or without bevacizumab should, therefore, also investigate the ability of the 7/7 capecitabine schedule to improve therapeutic ratio.

In conclusion, these studies in athymic nude mice bearing moderately TP-expressing HT29 or Colo205 colorectal xenografts showed that capecitabine administered using a 7/7 schedule permits increased drug delivery compared with the traditional 14/7 regimen, greatly improving monotherapy activity without major toxicity. Addition of bevacizumab significantly potentiated the activity of capecitabine in both models, whereas the addition of oxaliplatin significantly improved TGI and trended toward improved survival in the HT29 model. In this xenograft model, the capecitabine 7/7 schedule seems to be promising alone and in combination with oxaliplatin and bevacizumab, providing support for further clinical testing. Moreover, other studies investigating the capecitabine 7/7 regimen in combination with other applicable agents to colorectal cancer, such as irinotecan and cetuximab, along with newer targeted agents, are also under way and will be reported separately.

Disclosure of Potential Conflicts of Interest

K. Kolinsky, Y-E. Zhang, J. Kohles, U. Dugan, D. Heimbrook, K. Packman, and B. Higgins: employees of Hoffman-La Roche, Roche Group. B-Q. Shen and T.F. Zioncheck: employees of Genentech. No other potential conflicts of interest were disclosed.

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