

Clinical–patient studies

Combination chemotherapy with 13-*cis*-retinoic acid and celecoxib in the treatment of glioblastoma multiforme

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Summary

In a phase II clinical trial, we sought to determine if combining celecoxib with 13-*cis*-retinoic acid (13-cRA, Accutane™) was efficacious in the treatment of recurrent (progressive) glioblastoma multiforme (GBM). In parallel, we also sought to determine to what extent the outcomes from this clinical trial correlated with the findings from studies utilizing two murine intracerebral GBM models, U87MG and U251HF, to determine the predictive value of these murine models. In the clinical trial, 25 patients were studied at recurrence. Stable disease, which occurred in 44% of the patients, was the best response. The median progression-free survival (PFS) was 8 weeks, with a PFS at 6 months of only 19%. For the patients with stable disease, the median PFS was 24 weeks. The toxicity profile was unremarkable. The modest effect on PFS seen in this study agreed with the recent findings of another study, which showed a 19% PFS at 6 months in patients treated with 13-cRA alone. Thus, the combination of 13-cRA with celecoxib is not more effective than 13-cRA in the treatment of progressive GBM. In the murine model study, we found that long-term dosing with 13-cRA or celecoxib alone or in combination did not increase survival in animals with U87MG tumors but modestly increased survival in animals with U251HF tumors. There was no evidence of synergism between the two drugs. From this, we concluded that the animal studies generally predicted that the two agents would have only a modest effect alone and no additive effect when given in combination to patients.

Abbreviations: 13-cRA – 13-*cis*-retinoic acid or isotretinoin; COX-2 – cyclooxygenase-2; EGFR – endothelial growth factor (EGF) receptor; GBM – glioblastoma multiforme; IRB – Internal review board

Introduction

The treatment of glioblastoma multiforme (GBM) has remained essentially unchanged over the past 30 years and is primarily palliative [1,2]. Thus, there is a dire need for potentially curative therapies for this tumor.

One agent that showed early promise in patients with recurrent GBM was 13-*cis*-retinoic acid (13-cRA), a synthetic analog of vitamin A [3–5]. It was further found to be more active in patients with recurrent GBM than in patients with anaplastic gliomas. Initially, this observation was incompletely understood. It is now known that retinoids such as 13-cRA have growth-inhibitory and differentiating effects on neuroblastoma and glioma cells [6,7]. Further, the mechanism of the growth inhibition in glioma cells may be partially due to alterations in epidermal growth factor receptor (EGFR) effects, which can be inhibited by 13-cRA [8,9]. Clinical studies in glioma patients to date have indicated that 13-cRA is more effective than two other retinoids – all-*trans*-retinoic acid [10] and fenretinide (Puduvalli, personal communication).

Another potential target of treatment for high-grade gliomas, including GBM, is cyclooxygenase (COX), which is elevated in these and other tumors [11,12]. Indeed, the preclinical evaluation of compounds with COX-2 inhibitor activity in GBM cell lines showed that it inhibited cell proliferation in monolayer cell cultures, the growth of glioma spheroids, and tumor cell migration in a dose-dependent fashion [13]. In addition, the COX-2 inhibitor celecoxib alone has been shown to exhibit anti-proliferative effects on glioblastoma cell lines [14].

On the further basis of *in vitro* studies showing that the treatment of human oral squamous carcinoma cells with retinoids suppressed the basal expression of COX-2 as well as EGF-mediated induction of COX-2 protein mRNA by EGF [15], we hypothesized that the combination of a retinoid with a COX-2 inhibitor would result in even further suppression of COX-2 expression in patients with GBM. We therefore performed a phase II clinical trial of 13-cRA and celecoxib, a commercially available inhibitor of COX-2, alone and in combination, in patients with recurrent GBM using progression-free survival (PFS) as the primary end-point. In parallel, we

evaluated the potential benefit of combining 13-cRA and celecoxib in the treatment of murine GBM xenograft models in order to determine the predictive value of the animal model.

Laboratory materials and methods

Cell preparation

U87MG and U251HF human glioma cell lines were grown in Dulbecco's modified Eagle's medium supplemented with 10% fetal calf serum and 1% penicillin/streptomycin and incubated at 37 °C in a humidified atmosphere containing 95% air/5% carbon dioxide. On the day of cell implantation, cultures were harvested with trypsin/ethylenediamine tetraacetic acid solution, rinsed one time with Ca²⁺- and Mg²⁺-free phosphate-buffered saline (PBS), and resuspended in PBS to yield a final concentration of 1×10⁵ cells/10 μl. Cells were then maintained on ice until inoculation.

Animal model

Four- to six-week-old, male nude mice (strain nu/nu; provided by the Department of Experimental Radiation Oncology, The University of Texas M. D. Anderson Cancer Hospital, Houston, TX) were anesthetized by intraperitoneal injection of a ketamine/xylazine cocktail (200 mg of ketamine and 20 mg of xylazine in 17 ml of saline) at a dose of 0.15 mg/10 gm body weight. Glioma cells were injected directly into the caudate nucleus in a freehand fashion at an approximate point 2 mm lateral and 1–2 mm anterior of the bregma. A 27-gauge, short-beveled needle fitted with a metal cuff 3 mm from the needle tip provided the adequate penetration depth for implantation. A Hamilton repeating dispenser (#83700; Hamilton Company, Reno, NV) afforded prompt and precise 10-μl injections. Mice were kept warm under a heating lamp until recovery and then arbitrarily sorted into new groups.

Therapeutic agents

Laboratory chow was removed from each cage on the day after tumor cell implantation and replaced with irradiated powdered feed (Research Diets, Inc.; New Brunswick, NJ). All mice were allowed a dietary acclimation period of 2–3 weeks, depending on the growth rate of the studied cell line. On a day halfway between the day of tumor cell implantation and the median day of death, determined in control tumor-bearing mice, oral treatment with 13-cRA and celecoxib was initiated. We calculated the amount of each agent to be added to feed that would achieve 5, 10, and 20 mg/kg/day, for 13-cRA, 40 and 80 mg/kg/day, for celecoxib. Because formulated 13-cRA and celecoxib were administered *ad libitum*, each animal must ingest 5.15 gm of powdered food per day to satisfy the planned dosing regimen. An average consumption rate for each group was calculated based on the recorded daily individual animal weight and consumption rate. Group consumption rates were then

averaged with other group consumption rates for animals receiving a similar single-agent dose, which yielded the *actual* dose each animal received. The survival benefit was measured using the formula T/C-1, where T represents the median survival for treated animals and C represents the median survival for control animals.

Tissue collection and treatment

In both experiments, we used 10 control animals and 6 animals per dose for the evaluation of efficacy so as to provide 0.95 power for detecting a 50% increase in median survival. An additional three animals per dose group were euthanized after 7 days of chemotherapy to be kept for future molecular or genetic studies if needed. We expected to use a Kaplan–Meier representation to depict the 'survival' of study animals and a 2-tailed *t*-test where the groups had differing variances.

Clinical materials and methods

To be eligible for the study, patients had to have a histologically proven supratentorial GBM that had recurred or progressed despite radiation therapy, as shown radiographically; were aged 18 years or older; could not have been previously treated with 13-cRA as a chemotherapeutic agent; and have normal white blood cell counts, platelet counts, hemoglobin, hematocrit, blood urea nitrogen, creatinine, bilirubin, and liver enzymes within 1.5 times normal. All patients were treated at the University of Texas M. D. Anderson Cancer Center and signed an IRB-approved informed consent for treatment with the combination of 13-cRA and celecoxib.

The statistical design was that of a single stage study of 40 patients. PFS was the end-point of the study and the statistical hypotheses to be tested was H₀: $P \leq 15\%$ vs. H₁: $P \geq 30\%$, where P is the probability of remaining alive and free from progression at 6 months. We used alpha (false positive rate) = 10%, and beta (false negative rate) = 5%. The study design called for evaluating success (alive and progression-free at 6 months) after 40 patients received treatment and declaring a success if more than 9 responses were seen out of 40 patients to yield an alpha of 7% and a beta of 20%.

Treatment

Both 13-cRA and celecoxib were given on the same days to maximize their interaction and to minimize possible systemic toxicity. 13-cRA was administered orally as Accutane (Roche Pharmaceuticals), at a dose of 100 mg/m² daily in divided doses for 21 consecutive days followed by 7 drug-free days. The doses of 13-cRA chosen were based on the usual and maximal doses used in our other clinical trials [3,5], and the dose of celecoxib was a high non-toxic one. Doses were rounded down rather than up, depending on body surface area and available 13-cRA capsule sizes. The 21 days 'on'/7 days 'off' regimen constituted one course. The regimen was repeated until treatment failure or the development of

significant toxicity. Treatment failure was defined as progressive disease (see definition in next section) or death. Medications were used as needed to control hypercholesterolemia and hypertriglyceridemia. Celecoxib (Celebrex; G.D. Searle) was administered orally at a dose of 400 mg twice daily with 13-cRA for 21 consecutive days followed by the 7-day rest period.

Evaluation and outcome measures

Patients were evaluated every 8 weeks, and this included a neurologic assessment and magnetic resonance imaging of the brain with and without gadolinium. Response to therapy was assessed according to radiographic criteria and the extent of corticosteroid use [16]. Laboratory tests including complete blood count, serum electrolyte levels, serum amylase levels, and liver function; serum cholesterol and triglyceride levels were assessed every 8 weeks or at closer intervals depending on results. Toxicity was graded according to National Cancer Institute Common Toxicity Criteria (version 2.0).

All patients were analyzed for response to treatment (complete response, partial response, stable disease, progressive disease), PFS, and toxicity to treatment. Kaplan–Meier estimates were calculated for PFS at 6 months after initiation of the 13-cRA–celecoxib combination.

Results

Murine tumor model studies

Figure 1 illustrates the Kaplan–Meier representations of survival probability for mice implanted intracerebrally with U87MG (Figure 1a) or U251HF (Figure 1b) human glioma cells and treated with 13-cRA and celecoxib alone or in combination. Animals either died from tumor or were euthanized when severe symptoms occurred, as determined by staff in the Department of Veterinary Medicine. Tables 1 and 2 summarize the survival advantage for the experimental cohorts. Neither single-agent dosing nor combination-agent dosing significantly prolonged the survival of mice with U87MG tumors. However, both 13-cRA and celecoxib were modestly active in animals with the U251HF tumors and even significantly increased survival in some groups.

Tables 1 and 2 also show the *actual* drug doses received by each experimental group. All values indicate that the *actual* doses of both 13-cRA and celecoxib never reached our planned doses. While this might be considered to be a reason why the findings in animals were disappointing and raise a question as to the reliability of the animal model in predicting clinical results for this treatment, nonetheless, we observed what we believed to be clinical

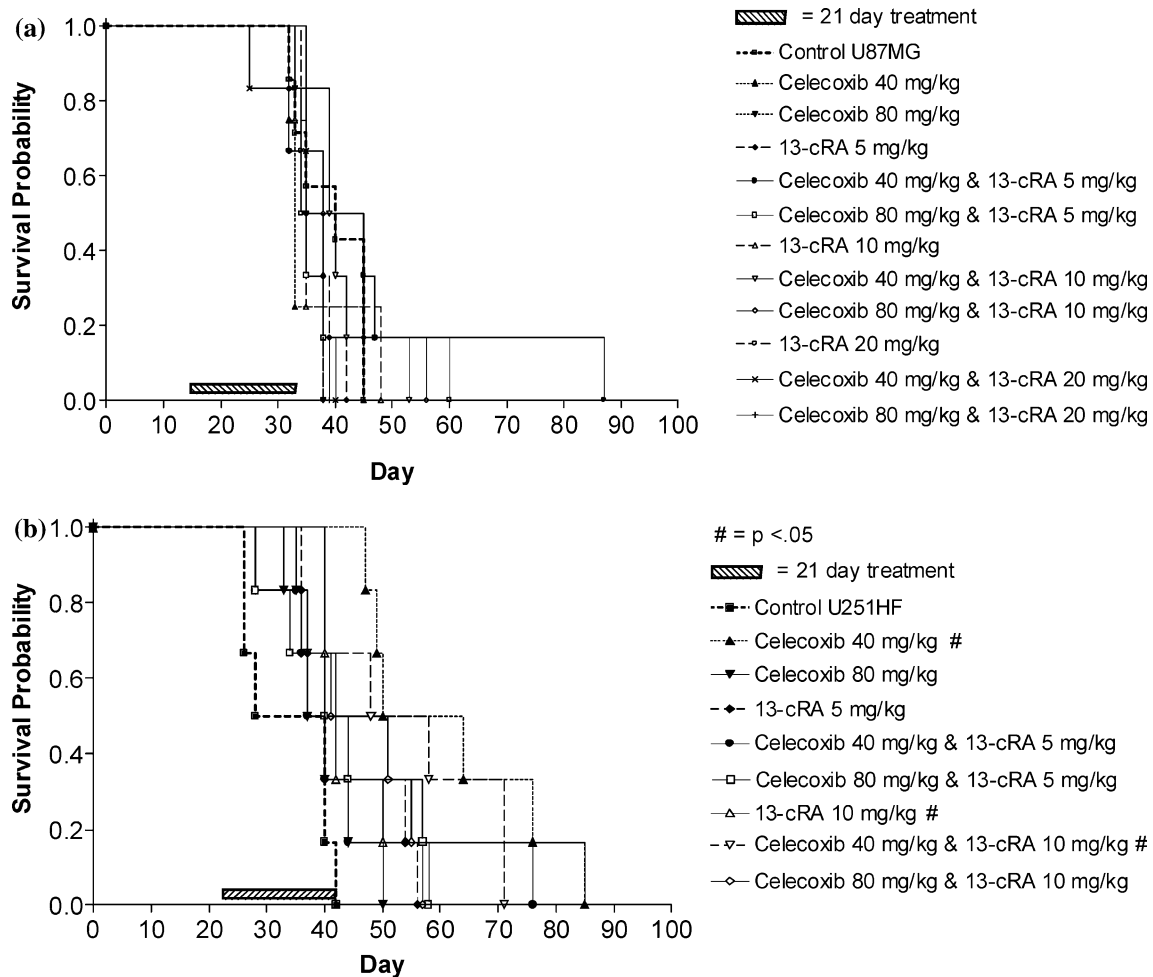


Figure 1. Kaplan–Meier representation of survival probability for mice with U87MG (a) or U251HF (b) tumors that were treated with celecoxib alone, 13-cRA alone, or both agents.

Table 1. Survival of mice with U87MG xenografts following treatment with 13-cRA or celecoxib or both

13-cRA Planned dose (mg/kg)	Actual 13-cRA dose (mg/kg)	Celecoxib planned dose (mg/kg)		
		0.00	40.00	80.00
		Actual celecoxib dose (mg/kg)		
		0.00	31.94	61.78
0.00	0.00	Control	-17.5%	-8.8%
5.00	3.86	-8.8%	0.0%	-13.8%
10.00	7.92	-12.5%	-1.3%	3.8%
20.00	15.38	-5.0%	-5.0%	-5.0%

No pairs are significantly different by *t*-test.

The data for the actual dose administered based on animal weight and consumption rate are listed next to the proposed dose. Survival benefit was calculated using the formula T/C-1, where T, treated median survival and; C, control median survival (or euthanasia date).

Table 2. Survival of mice with U87MG xenografts following treatment with 13-cRA or celecoxib or both

13-cRA Planned dose (mg/kg)	Actual 13-cRA dose (mg/kg)	Celecoxib planned dose (mg/kg)		
		0	40	80
		Actual celecoxib dose (mg/kg)		
		0	34.18	65.58
0	0	Control	67.6% (<0.01)	13.2% (NS)
5	3.77	17.6% (NS)	13.2% (NS)	23.5% (NS)
10	8.79	23.5% (NS)	55.9% (<0.05)	35.3% (NS)

Values in parenthesis are *P*-value calculated for *t*-test of T/C-1 data; NS, not significant and above *P* = 0.05.

toxicity to treatment at the highest dose of 13-cRA in the mice with U87 tumors (Table 1).

Clinical trial

Between October 11, 2002, and October 20, 2004, 27 patients were entered into this IRB-approved protocol at the University of Texas M. D. Anderson Cancer Center. Two patients refused treatment after consenting to the protocol; therefore, 25 patients were eligible for toxicity and response analysis. The study was halted at 27 patients instead of the 40 patients the study was powered for because of slow patient accrual. The characteristics of these patients are summarized in Table 3. All patients had a pathologically confirmed diagnosis of GBM, and all patients received radiation therapy and surgery. The mean number of prior chemotherapies was 1.56 ± 0.92 (range 0–3) for the 25 evaluable patients. The number of prior chemotherapies was 1.8 for the 11

patients with stable disease and 1.6 for the 14 patients with progressive disease, but the difference was not statistically significant by *t*-test. Of interest, two patients in each group had not received prior chemotherapy for recurrence before starting this protocol.

The toxicity profile was modest. Grade 3 toxicities (i.e., skin rash/desquamation, pruritus, dry skin, xerostoma, headache, and thrombocytopenia) were noted in one patient each. Grade 2 skin toxicity, such as dryness, pruritus, and rash, occurred in 12% (3/25) but did not lead to the dose reduction of either drug. The only other grade 2 toxicity of note was fatigue in 16% (4/25) patients. No cardiorespiratory or cerebrovascular toxicity was observed.

Response was measured from the on-study date to tumor progression or death, whichever occurred first. No partial response was seen. The best treatment benefit was stable disease, which was seen in 11 patients (44%). Based on a Kaplan–Meier representation, the median PFS for the 25 evaluable patients was 8 weeks (95% CI = 7,17), with a PFS at 6 months of 19% (95% CI = 8,44); for the subset of 11 patients with stable disease, the median PFS was 24 weeks (Figure 2).

Discussion

This paper reports the parallel conduct of a clinical phase II trial and intracerebral murine tumor model study of the combination of 13-cRA and celecoxib against GBM tumors. In these studies we sought to determine if combining 13-cRA and celecoxib would

Table 3. Characteristics of 25 evaluable patients

Characteristic	Value
Number	25
Male:female	16:9
Median age, years (range)	55 (31–72)
Mean Karnofsky performance score (SD)	82.8 (10.6)
Mean number of prior chemotherapies (range)	1.56 (0–3)
Mean number of prior surgeries	1
Response to study chemotherapy	
Partial response (PR)	0%
Stable disease (SD)	44% (11/25)

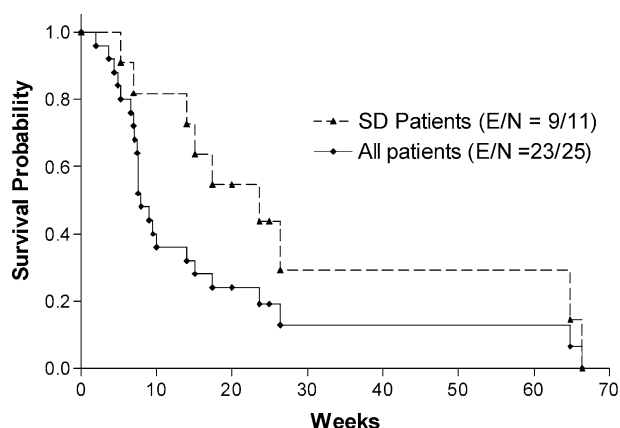


Figure 2. Kaplan-Meier representation of the probability of the 25 evaluable patients (—) being tumor progression-free and the subset of 11 patients with stable disease (---) being tumor progression-free. From this curve, it can be seen that the median progression-free survival was 8 weeks for all patients and 24 weeks for the patients with SD. E/N = events/number of patients.

increase PFS for patients with recurrent GBM when compared to historic studies of 13-cRA alone [3,5] and if the outcome could be anticipated using intracerebral murine GBM models. The rationale for the combination was based on experimental studies in the literature. *In vitro* studies have shown that treatment of human oral squamous carcinoma cells with retinoids suppressed basal expression of Cox-2 as well as EGF-mediated induction of Cox-2 protein mRNA by EGF [15]. The combination of a retinoid with a Cox-2 inhibitor would be expected to result in even further suppression of Cox-2 expression. Given the prognostic significance of Cox-2 expression in human gliomas [11,12], we hypothesized that this would be a desirable therapeutic endpoint. In addition, celecoxib alone has been shown to exhibit anti-proliferative effects on glioblastoma cell lines [14].

Although previously we showed that 13-cRA was clinically active against recurrent GBM [3,5], our experience with intracerebral xenograft models described here is disconcerting since we failed to find oral 13-cRA at 5, 10, and 20 mg/kg/day for 21 days to have significant antitumor activity in nu/nu mice with intracerebral U87MG tumors. This was also in contrast to its activity against U87MG cells in culture [17,18]. In mice in the present study, the U87MG tumors were insensitive to long-term oral dosing with 13-cRA or celecoxib alone and in combination. A slightly better, though modest, result was observed in animals with U251HF tumors treated with either drug alone (24% to 68% T/C-1), but the combination of agents showed no improved benefit (24% to 56% T/C-1). These data suggest that there is some heterogeneity of response to 13-cRA and/or celecoxib in GBM murine models. Certainly, however, there is no evidence for drug synergism against these tumors. The clinical data also indicate that the combination of 13-cRA and celecoxib has limited benefit.

There were no partial responses among the 25 evaluable patients in this phase II clinical trial, but 44% had stable disease. The median PFS for all 25 patients was 8 weeks, with a PFS at 6 months of 19%. For the patients with stable disease, the median PFS was

24 weeks. The toxicity profile for both drugs, alone and in combination, was tolerable, and no cardiorespiratory or cerebrovascular toxicity was observed.

We conclude from our findings that the use of an intracerebral murine GBM model reasonably predicted that the two agents were likely to have only modest activity alone and no additive activity when combined. Unfortunately for the patients, the clinical trial showed no improvement in PFS rate, which was the same as the 19% 6-month PFS rate seen in a recently published study of 13-cRA in patients with GBM [5]. Thus, further use of the combination of 13-cRA with celecoxib is not warranted. It is difficult to speculate why the efficacy of the combination of 13-cRA and celecoxib was somewhat inferior to that of 13-cRA alone, but it may reflect the small number of patients involved or the effect of an unidentified intracellular feedback loop that becomes operational in response to the combination of 13-cRA and celecoxib.

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