# Combination of Erlotinib and Naproxen Employing Pulsatile or Intermittent Dosing Profoundly Inhibits Urinary Bladder Cancers

Altaf Mohammed[1], Mark Steven Miller[1], Ronald A. Lubet[1], Chen S. Suen[1], Shizuko Sei[1], Robert H. Shoemaker[1], Margaret M. Juliana[2], Fariba L. Moeinpour[2], and Clinton J. Grubbs[2]

## ABSTRACT

Daily dosing of either NSAIDs or EGFR inhibitors has been shown to prevent bladder cancer development in a N-butyl-(4-hydroxybutyl)nitrosamine (OH-BBN)-induced rat model. However, these inhibitors cause gastrointestinal ulceration and acneiform rash, respectively, limiting their continuous use in a clinical prevention setting. We studied chemopreventive efficacy of pulsatile dosing of EGFR inhibitor erlotinib (42 mg/kg BW, once/week) combined with intermittent or continuous low doses of the NSAID naproxen (30 mg/kg BW/day, 3 weeks on/off or 128 ppm daily in diet) in the OH-BBN induced rat bladder cancer model. The interventions were started either at 1 or 4 weeks (early intervention) or 3 months (delayed intervention) after the last OH-BBN treatment, by which time the rats had developed microscopic bladder lesions. All combination regimens tested as early versus late intervention led to the reduction of the average bladder tumor weights (54%–82%; $P < 0.01$ to $P < 0.0001$), a decrease in tumor multiplicity (65%–85%; $P < 0.01$ to $P < 0.0001$), and a decrease in the number of rats with large palpable tumors (>200 mg; 83%–90%; $P < 0.01$ to $P < 0.0001$). Levels of signal transduction markers, Ki-67, cyclin D1, IL1β, pSTAT3, and pERK, were significantly ($P < 0.05$ to $P < 0.001$) reduced in the treated tumors, demonstrating their potential utility as predictive markers for efficacy. These findings demonstrate that significant chemopreventive efficacy could be achieved with alternative intervention regimens designed to reduce the toxicity of agents, and that starting erlotinib and/or naproxen treatments at the time microscopic tumors were present still conferred the efficacy.

## Introduction

Urinary bladder cancer, the fifth most common cancer in humans, is the most expensive cancer to treat because of high rates of recurrence (1, 2). The American Cancer Society's estimate for new bladder cancer cases in the United States for 2019 is 80,470 (1). More than half of all bladder tumors are first found at the nonmuscle invasive stage (i.e., *in situ*) in which lesions are found only in the inner layer of the bladder wall (3). The majority of patients diagnosed with muscle-invasive transitional cell carcinoma have a low 5-year survival rate of only 5%, particularly in cases with difficult to treat distant metastasis. Recent human genomic analysis of urinary bladder cancers revealed consistent overexpression of EGFR at the RNA and protein level (4–6). Along with EGFR, inflammation is another major pathway that is altered in bladder cancers. Many studies have reported an inverse association with bladder cancer risk for individuals who reported regular use of NSAIDs (7, 8). However, anti-inflammatory NSAIDs are known to cause gastrointestinal-associated toxicities upon long-term administration, whereas EGFR inhibitors such as gefitinib and erlotinib tend to cause skin toxicities (e.g. acneiform rash), making these drugs difficult to employ in a prevention setting (9, 10). Among the available preclinical animal models to evaluate the chemopreventive potential of drugs, treatment of rats with the urinary bladder–specific carcinogen, hydroxybutyl(butyl)nitrosamine (OH-BBN), induces highly invasive bladder tumors that appear to be histologically similar to human transitional cell carcinoma (11). Gene expression profiling of these tumors showed overlap both at the pathway and gene levels to invasive human breast cancer (12, 13). This model has been extensively utilized and shown to be a valid model to determine preventive activity of several agents, including NSAIDs and EGFR inhibitors (12).

Safety and toxicity profiling of drugs are primary factors for selecting suitable agents for cancer chemoprevention, as these agents will be administered to high-risk, asymptomatic individuals for long periods of time (14). To decrease drug toxicity while retaining chemopreventive efficacy, several approaches

[1]Chemopreventive Agent Development Research Group, Division of Cancer Prevention, National Cancer Institute, Rockville, Maryland. [2]Department of Surgery, University of Alabama at Birmingham, Birmingham, Alabama.

**Note:** Supplementary data for this article are available at Cancer Prevention Research Online (http://cancerprevres.aacrjournals.org/).

**Corresponding Author:** Clinton J. Grubbs, University of Alabama at Birmingham, 1670 University Blvd., Volker Hall G78D, Birmingham, AL 35294. Phone: 205-934-6384; Fax: 205-975-5082; E-mail: clintongrubbs@uabmc.edu

Cancer Prev Res 2020;13:273–82

**doi:** 10.1158/1940-6207.CAPR-19-0339

©2019 American Association for Cancer Research.



Downloaded from http://aacrjournals.org/cancerpreventionresearch/article-pdf/13/3/273/2246079/273.pdf by guest on 22 February 2024

are currently being explored (14). It has been postulated that by employing pulsatile and intermittent dosing with an EGFR inhibitor and an NSAID, the toxicities associated with these agents (EGFR inhibitor, rash and diarrhea; NSAID, gastric toxicity) should be greatly reduced. The utility of weekly pulsatile dosing of an EGFR inhibitor is further bolstered by clinical observations that in humans, weekly dosing of erlotinib was associated with a decrease in the incidence of acneiform rash as compared with daily dosing (15, 16). In our prior studies, we showed that intermittent dosing (3 weeks on/ 3weeks off) with naproxen was equally effective as daily dosing in the rat bladder cancer model (17). In addition, we showed that pulsatile dosing with erlotinib (once per week dosing at 7× the daily dose) was equally effective as daily dosing (18).

In this study, the combination of pulsatile dose of erlotinib (once weekly) plus intermittent dose of naproxen (3 weeks on/ 3 weeks off) was administered before or after microscopic bladder tumors were formed to model strategies to both prevent disease from progression at early stages and to prevent recurrence in bladder cancer survivors.

# Materials and Methods

## Animal model

All animal experiments were conducted in accordance with, and with the approval of Institutional Animal Care and Use Committee (IACUC). The hydroxybutyl(butyl) nitrosamine (OH-BBN) model for urinary bladder cancer has been used extensively during the last 30 years for the evaluation of compounds for chemopreventive activity (11, 17, 19–21). Female Fischer-344 rats were received from Envigo at 28 days of age and placed on Teklad (4% fat; Envigo) mash diet for the duration of the study.

## Experimental design

Beginning at 8 weeks of age, the rats dosed with the carcinogen received, by gavage, the first of 16 doses of OH-BBN over an 8-week period (2×/week; **Fig. 1A**). The carcinogen was diluted with ethanol–water (1:4, v/v), so that each dose (150 mg) was contained in a volume of 0.5 mL. The rats were palpated for bladder masses 2×/week and observed daily for bloody urine. Any rat that became moribund was sacrificed. Each study was terminated approximately 10 to 11 months after the initial dosing with OH-BBN. All rats were sacrificed by $CO_2$ asphyxiation. At necropsy, the urinary bladder of all rats was removed and weighed. All grossly observed lesions in the urinary bladder of the rats were processed for histologic classification. In addition, many of the urinary bladder tumors were also fixed for IHC.

## Chemoprevention efficacy

Three different long-term efficacy studies were performed to determine the chemopreventive efficacy of different intermittent dosing regimens of erlotinib and naproxen against bladder cancer.

### Protocol 1.

Agents were given on an intermittent schedule, which began 1 week after the final OH-BBN treatment (before microscopic bladder tumors were observed; **Fig. 1A**). The OH-BBN–treated groups were: group 1, erlotinib, 42 mg/kg BW (1×/week); group 2, naproxen, 30 mg/kg BW/day (3 weeks on/3 weeks off); group 3, erlotinib + naproxen (dosing as indicated in groups 1 and 2), and group 4, none (**Table 1**). Additional groups (groups 5–8) did not receive the carcinogen, but only the chemopreventive agents as indicated for groups 1 to 4 (**Table 1**). Both erlotinib and naproxen were administered

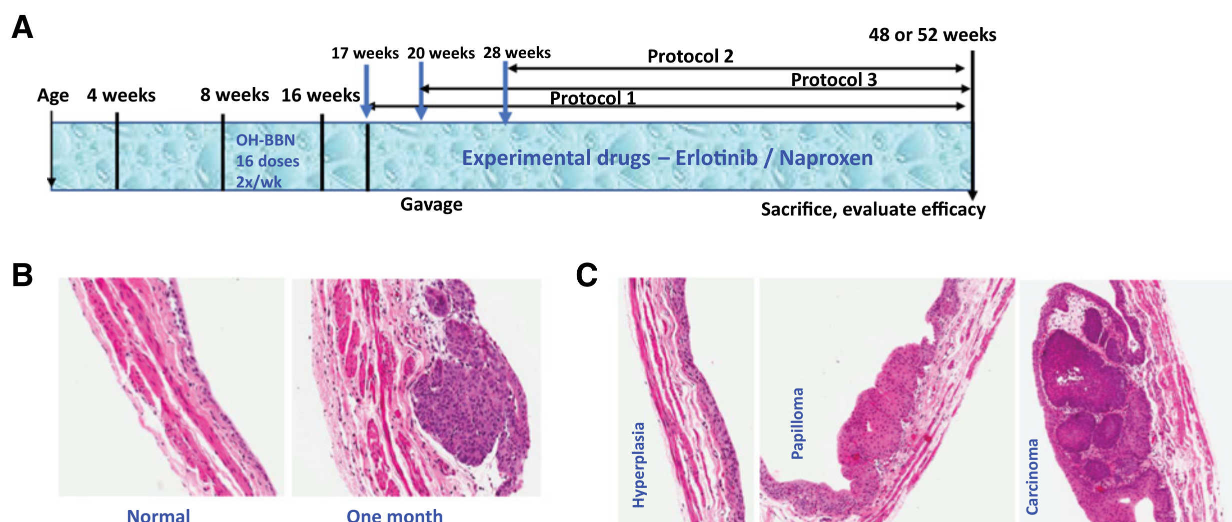


**Figure 1.**
Experimental design, dosing regimens, and OH-BBN induced rat tumorigenesis. **A,** Experimental design to evaluate the chemopreventive effects of erlotinib and naproxen in rat urinary bladder cancer model. **B,** OH-BBN induced bladder cancer progression in rats. **C,** Progression of bladder cancer in OH-BBN–treated rats.

Downloaded from http://aacrjournals.org/cancerpreventionresearch/article-pdf/13/3/273/2246079/273.pdf by guest on 22 February 2024

**Table 1.** Experimental dosing regimens for protocols 1 and 2.

| Group | No. of rats / group | OH-BBN | Treatment | Regimen |
|---|---|---|---|---|
| 1 | 25 | + | Erlotinib 42 mg/kg BW | 1×/week |
| 2 | 25 | + | Naproxen 30 mg/kg BW/day | 3 weeks on / 3 weeks off |
| 3 | 25 | + | Erlotinib + Naproxen | As in groups 1 and 2 |
| 4 | 25 | + | None | — |
| 5–8 | 10 | – | As in groups 1–4 | As in groups 1–4 |

by gavage (0.5 mL/gavage); the vehicle for erlotinib was corn oil and the vehicle for naproxen was saline. The rats were palpated for urinary bladder tumors 2×/week beginning 1 month after the final OH-BBN treatment and were sacrificed 10 months after the initial OH-BBN treatment.

**Protocol 2.**

The agents were given on an intermittent schedule that began 3 months after the final OH-BBN treatment (at a time when microscopic urinary bladder tumors were present). The OH-BBN–treated groups were similar to that in protocol 1 (**Table 1**). All urinary bladder tumors were collected from rats for histologic classification at the termination of the study (11 months after the initial OH-BBN treatment).

**Protocol 3.**

The agents were given on an intermittent schedule that began 4 weeks (1 month) after the final OH-BBN treatment. The OH-BBN–treated groups ($N = 21$) were: group 1, erlotinib (42 mg/kg BW), 1×/week; group 2, naproxen (128 mg/kg diet), daily; group 3, erlotinib + naproxen as indicated for groups 1 and 2; and group 4, none (**Table 2**). Additional groups did not receive the carcinogen, but only the chemopreventive agents (groups 5–8; **Table 2**). Erlotinib was administered by gavage (0.5 mg/gavage) and naproxen was mixed into powdered (Teklad, 4% fat) diet using a Patterson–Kelly blender with intensifier bar. Diets were prepared every 2 weeks and stored in a cold room until fed to the rats (new food added 3×/week). Control rats received only the powdered diet. The rats were palpated for urinary bladder tumors 2×/week (beginning 2 months after the final OH-BBN treatment). The study was terminated 10 months after the initial OH-BBN treatment.

## Histology of bladder tumors

The gross and histologic examination of bladders is critical in studies using OH-BBN as the carcinogen. Multiple tumors often occur, and they are not always observed by gross examination. Briefly, our procedure for histologic processing of the rat urinary bladder was as follows: at necropsy, the empty urinary bladder was tied off, weighed, and inflated with 10% formalin. After fixation, the bladder was held next to a high-intensity light and grossly observed lesions were noted and removed. The approximate location and size of each lesion was recorded on a diagram of the bladder attached to the necropsy sheet for each animal. Each bladder lesion was separately embedded in a block with its identifying number. Two sections (5-μm) from two different levels were cut from each lesion and stained with hematoxylin and eosin. At diagnosis, the slides from each individual animal were read as a set by the pathologist blinded to the identity of treatment groups. Endpoints were cancer incidence, multiplicity, and weight.

## IHC

After embedding in paraffin blocks, sections (4-μm thick) were placed on positive microscopic slides. The tissues were deparaffinized with xylene and placed in ethanol. Antigen retrieval used sodium citrate (pH 6.0) and boiling for 20 minutes. Slides were then cooled to room temperature and placed in a humidity chamber. The tissues were covered with peroxidase block for 15 minutes, and then washed with TRIS buffer. The tissues were then incubated with primary antibody for phospho-STAT3, Ki67, pP38 MAPK, cyclin D1, pERK, or IL1β (Abcam) for 1 hour at room temperature. Processing and staining of tissues were performed according to the manufacturer's instructions (DAKO Envision+ kits). Tissues were then washed and dehydrated in ethanol and xylene. The images were captured and counted using the Aperio Scan Scope imaging system (Aperio Imaging). For counting the cells, each area containing cancer cells was randomly circled and analyzed (stained cells divided by total cells counted) by the program within the scan scope. A total of 1,000 to 5,000 cells were usually counted.

**Table 2.** Experimental dosing regimens for protocol 3.

| Group | No. of rats / group | OH-BBN | Treatment | Regimen |
|---|---|---|---|---|
| 1 | 21 | + | Erlotinib 42 mg/kg BW | 1×/week |
| 2 | 21 | + | Naproxen 128 mg/kg diet | Daily |
| 3 | 21 | + | Erlotinib + Naproxen | As in groups 1 and 2 |
| 4 | 21 | + | None | - |
| 5–8 | 8 | – | As in groups 1–4 | As in groups 1–4 |

Downloaded from http://aacrjournals.org/cancerpreventionresearch/article-pdf/13/3/273/2246079/273.pdf by guest on 22 February 2024

**Table 3.** Effects of erlotinib and/or naproxen in the prevention of bladder cancer when agents were started 1 week after final OH-BBN treatment.

| | | | | Urinary bladder cancers | | | |
|---|---|---|---|---|---|---|---|
| Group | Number of rats | Carcinogen | Treatment | Incidence | Multiplicity | Weight (mg) | Number greater than 200 mg |
| 1 | 25 | OH-BBN | Erlotinib, 42 mg/kg BW, 1×/week | 76% (24%↓) | 1.48 (47%↓)[b] | 272 (8%↓)[a,c] | 35% (40%↓) |
| 2 | 25 | OH-BBN | Naproxen, 30 mg/kg BW/day (3 weeks on / 3 weeks off) | 72% (28%↓)[a] | 1.20 (57%↓)[b] | 213 (28%↓)[a,c] | 39% (33%↓)[c] |
| 3 | 24 | OH-BBN | Erlotinib + Naproxen (as indicated for groups 1 and 2) | 58% (42%↓)[b] | 0.96 (66%↓)[b] | 136 (54%↓)[b] | 9% (84%↓)[b] |
| 4 | 23 | OH-BBN | None | 100% | 2.79 | 295 | 58% |

Note: Female Fischer-344 rats received OH-BBN for 8 weeks beginning at 56 days of age. Administration of Erlotinib and/or Naproxen initiated 1 week after final OH-BBN treatment. Study terminated 10 months after the initial OH-BBN treatment.
[a]Statistically significant from the control group (group 4) at $P \leq 0.05$.
[b]Statistically significant from the control group (group 4) at $P \leq 0.01$.
[c]Statistically significant from the combination group (group 3) at $P \leq 0.05$.

Downloaded from http://aacrjournals.org/cancerpreventionresearch/article-pdf/13/3/273/2246079/273.pdf by guest on 22 February 2024

### Statistical analyses

The following statistical analyses were performed: bladder cancers, log-rank for incidence and Poisson for multiplicity; bladder weights, Wilcoxon rank sums; biomarkers, bladder cancers greater than 200 mg, Student *t* test.

## Results

### General health of animals

The experimental design and protocols for evaluating chemopreventive efficacy are summarized in **Fig. 1A** and **Tables 1** and **2**. Development of bladder cancer in rats treated with OH-BBN is shown in **Fig. 1B** and **C**. All the rats treated with experimental drugs or vehicle had similar body weight gains. As shown in Supplementary Figs. S1A to S1C, there was no significant difference in body weight between the rats treated with and without experimental drugs. No gross observable toxicity was seen in the drug treatment groups. Further histologic evaluation of stomach, colon, spleen, and kidney did not show significant toxicities in the drug treatment groups compared with untreated animals indicating that the doses applied in this study seem to be safe and devoid of toxicities (Supplementary Table S1). The treatment with either agent alone or in combination resulted in the increased incidence and multiplicity of premalignant lesions (Supplementary Tables S2–S4) whereas the control group had a higher incidence of progressive disease than treatment groups indicating arrest of the disease progression (**Tables 3–5**).

### Chemopreventive efficacy of erlotinib and/or naproxen administered 1 week after the final carcinogen treatment (Protocol 1).

**Figure 2A** shows the effect of erlotinib and naproxen on the survival of rats receiving OH-BBN. **Table 3** shows the incidence, multiplicity, and weights of urinary bladder tumors in the various groups of rats during the study. The mean urinary bladder cancer weights were group 1 (erlotinib), 272 mg; group 2 (naproxen), 213 mg; group 3 (erlotinib + naproxen), 136 mg;

**Table 4.** Effects of erlotinib and/or naproxen in the prevention of bladder cancer when agents were started 3 months after final OH-BBN treatment.

| | | | | Urinary bladder cancers | | | |
|---|---|---|---|---|---|---|---|
| Group | Number of rats | Carcinogen | Treatment | Incidence | Multiplicity | Weight (mg) | Number greater than 200 mg |
| 1 | 25 | OH-BBN | Erlotinib, 42 mg/kg BW, 1×/week | 84% (5%↓) | 1.88 (31%↓)[c] | 490 (33%↓)[d] | 58% (29%↓)[d] |
| 2 | 25 | OH-BBN | Naproxen, 30 mg/kg BW/day (3 weeks on / 3 weeks off) | 84% (5%↓) | 2.68 (1%↓)[d] | 234 (68%↓)[b,d] | 44% (46%↓)[a,d] |
| 3 | 25 | OH-BBN | Erlotinib + Naproxen (as indicated for groups 1 and 2) | 52% (41%↓)[b] | 0.96 (65%↓)[b] | 138 (81%↓)[b] | 8% (90%↓)[b] |
| 4 | 24 | OH-BBN | None | 88% | 2.71 | 732 | 82% |

Note: Female Fischer-344 rats received OH-BBN for 8 weeks beginning at 56 days of age. Administration of Erlotinib and/or Naproxen initiated three months after final OH-BBN treatment. Study terminated 11 months after the initial OH-BBN treatment. Numbers in parentheses represent percent decrease from the control group (group 4).
[a]Statistically significant from the control group (group 4) at $P \leq 0.05$.
[b]Statistically significant from the control group (group 4) at $P \leq 0.01$.
[c]Statistically significant from group 3 ($P = 0.0458$)
[d]Statistically significant from the combination group (group 3) at $P \leq 0.01$.

**Table 5.** Effects of erlotinib and/or naproxen in the prevention of bladder cancer when agents were started 1 month after final OH-BBN treatment.

| Group | Number of rats | Carcinogen | Treatment | Urinary bladder cancers: Incidence | Multiplicity | Weight (mg) | Number greater than 200 mg |
|---|---|---|---|---|---|---|---|
| 1 | 21 | OH-BBN | Erlotinib, 42 mg/kg BW, 1x/week | 86%(14%↓)[a,b] | 2.38 (56%↓)[c,d] | 475 (43%↓)[a,d,e] | 57% (37%↓)[a,b] |
| 2 | 21 | OH-BBN | Naproxen, 128 mg/kg diet | 95%(5%↓)[a,b] | 2.67 (50%↓)[c,d] | 186 (78%↓)[b,c] | 29% (68%↓)[c] |
| 3 | 20 | OH-BBN | Erlotinib + Naproxen (as indicated for groups 1 and 2) | 50%(50%↓)[c] | 0.80 (85%↓)[c] | 146 (82%↓)[c] | 15% (83%↓)[c] |
| 4 | 21 | OH-BBN | None | 100% | 5.38 | 832 | 90% |

Note: Female Fischer-344 rats received OH-BBN for 8 weeks beginning at 56 days of age. Administration of Erlotinib and/or Naproxen initiated 1 month after final OH-BBN treatment. Study terminated 10 months after the initial OH-BBN treatment.

[a]Statistically significant from the control group (group 4) at $P \leq 0.05$.

[b]Statistically significant from the combination group (group 3) at $P \leq 0.01$.

[c]Statistically significant from the control group (group 4) at $P \leq 0.0001$.

[d]Statistically significant from the combination group (group 3) at $P \leq 0.001$.

[d]Statistically significant from group 2 ($P = 0.0452$).

and group 4 (controls), 295 mg (**Table 3**). As we have done in previous efficacy studies of different agents in this model (17), we determined the number of rats with large urinary bladder tumors (i.e., 200 mg or greater) in each of the groups. As shown in **Table 3** and **Fig. 2B**, we observed a 35% (erlotinib), 39% (naproxen), 9% (erlotinib + naproxen), and 58% (controls) incidence of rats with large bladder tumors (≥200 mg). Individually, erlotinib and naproxen showed 8% ($P < 0.05$) and 28% ($P < 0.05$) decreases in the total tumor weights and reduced the number of rats with large bladder tumors by 40% and 33%, respectively (**Table 3**). Importantly, a significant decrease in the total tumor weights (54%; $P < 0.01$) and number of rats with large bladder tumors (84%; $P < 0.01$) was observed in the combination treatment groups compared with controls (**Table 3**). Thus, the treatment regimens used to reduce toxicity were effective in decreasing the size of the urinary bladder tumors.

Supplementary Table S2 shows the effects of the agents on various lesions (hyperplasia and papilloma) of the urinary bladder following histologic evaluations. As indicated, the compounds did not greatly alter the incidences of hyperplasia and papilloma (although increases were observed). It appears that the agents prevented the conversion of benign lesions into carcinomas. Further, tumor multiplicity in untreated controls was 2.79 whereas erlotinib, naproxen, and erlotinib+naproxen showed tumor multiplicities of 1.48, 1.2, and 0.96, respectively. The incidence and multiplicity of transitional cell carcinomas were decreased by 42% and 66% ($P < 0.01$) by the combination of agents (**Table 3**). Overall, all four criteria (incidence, multiplicity, weight, and large cancers) used to indicate efficacy of agents were greatly reduced by the combination of erlotinib and naproxen when administered early during the carcinogenic process (**Table 3**). Of note, the combination of the two agents was more effective than either agent alone in reducing the total tumor weights (**Table 3**). The urinary bladder weights of the rats not receiving OH-BBN were approximately 90 mg, with no differences between groups.

Because of the large decrease in the size of the urinary bladder cancers, we performed an IHC study to measure the cell proliferation rate in the treated and untreated tumors. As shown in Fig. **2C** and Supplementary Fig. S2A, the rate of cell proliferation was significantly reduced ($P < 0.05$) in the urinary bladder cancers of the treated rats. The combination of agents significantly reduced the expression of inflammatory marker IL1β as shown in **Fig. 2D** and Supplementary Fig. S2F. The effect of the combination of agents on pSTAT3 expression is shown in **Fig. 2E** and Supplementary Fig. S2B. As indicated, STAT3 activation was significantly decreased ($P < 0.001$; **Fig. 2E**; Supplementary Fig. S2B). The combination, however, did not significantly alter p38 activation (**Fig. 2F**; Supplementary Fig. S2C), suggesting a lack of effect of this treatment combination on the MAP kinase pathway. Further we observed a significant decrease ($P < 0.05$) in cyclin D1 and pERK in the treatment groups compared with the untreated control group (**Fig. 2G** and **H**; Supplementary Fig. S2D and S2E).

**Delayed treatment with erlotinib and/or naproxen 3 months after the final carcinogen treatment (Protocol 2).**

Body weights of the various groups were similar and there were no signs of toxicity during the study although rats receiving erlotinib + naproxen did show varying degrees of body weight loss (3–6%) after starting each "3 weeks naproxen on" treatment (Supplementary Fig. S1B). The urinary bladder weights of the rats not receiving OH-BBN were 100 to 110 mg with no differences between groups. There were no other signs of toxicity.

Supplementary Table S3 shows the effects of the agents on various lesions (hyperplasia and papilloma) of the urinary bladder following histologic evaluations. As was observed in Protocol 1, the compounds either alone or in combination caused varying increases in the number of benign lesions (hyperplasia and papilloma), while decreasing the incidence of invasive bladder cancer, suggesting that the treatment

Downloaded from http://aacrjournals.org/cancerpreventionresearch/article-pdf/13/3/273/2246079/273.pdf by guest on 22 February 2024

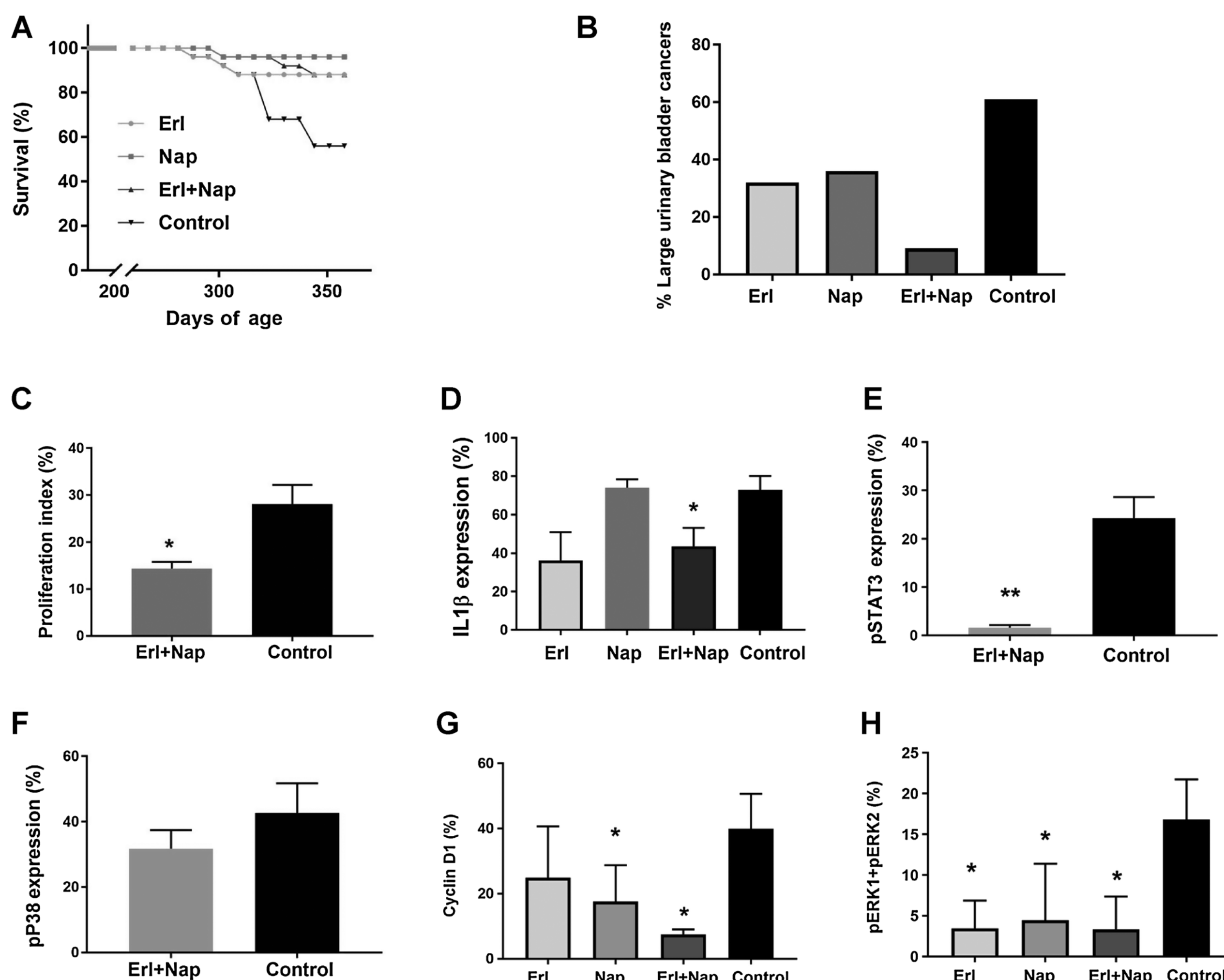


**Figure 2.**

Chemopreventive efficacy of erlotinib and/or naproxen in protocol 1. **A,** Survival of rats receiving erlotinib and/or naproxen 1 week after final carcinogen treatment during the chemoprevention study. **B,** Effect of erlotinib and/or naproxen on the incidence of rats with larger bladder tumors. Individually erlotinib and naproxen showed 40% and 33% inhibition of large bladder cancers whereas the combination treatment reduced the large cancers by 84% ($P < 0.01$). **C,** Effect of erlotinib and naproxen on cell proliferation and proliferative index. The Ki67-positive proliferation index (PI) was determined by counting the cells where each area containing cancer cells was randomly circled and analyzed and counted for stained cells divided by total cells counted by the program within the scan scope. A total of 1,000 to 5,000 cells were usually counted. **D–H,** Effect of erlotinib and/or naproxen on expression of IL1β (**D**), pSTAT3 (**E**), pP38 (**F**), cyclin D1 (**G**), and pERK (**H**; *, $P < 0.05$; **, $P < 0.001$).

regimens likely arrested the progression of premalignant lesions to carcinoma (Supplementary Table S3; **Table 4**). All four criteria (incidence, multiplicity, weight, and large cancers) were significantly reduced by the combination of erlotinib and naproxen (**Table 4**). As indicted, the control group (group 4) had an incidence of urinary bladder tumors of 88% (**Table 4**). The groups receiving the agents had palpable urinary bladder tumor incidences of 84% (erlotinib), 84% (naproxen), and 52% (erlotinib+naproxen; **Table 4**). The tumor multiplicity in untreated controls was 2.71 whereas erlotinib, naproxen, and erlotinib+naproxen showed tumor multiplicities of 1.88, 2.68, and 0.96, respectively. The combination treatment group showed 65% ($P < 0.01$) inhibition of tumor multiplicity compared with the control group. The mean bladder tumor weights were 490 mg (erlotinib), 234 mg (naproxen), 138 mg (erlotinib + naproxen), and 732 mg for Controls (**Table 4**). Individually, only the naproxen treated group demonstrated a statistically significant decrease (68%; $P < 0.01$) in the total tumor weight. A significant 81% ($P < 0.01$) reduction in the tumor weights was observed with the combination treatment as well. We also determined the number of rats with large urinary bladder tumors (i.e., 200 mg or greater) in each of the groups (**Table 4**; **Fig. 3A**) and observed statistically significant inhibitions of large tumor growth of 90% ($P < 0.01$) and 46% ($P < 0.05$) by the combination and naproxen treatment groups, respectively. The combination treatment showed significantly higher inhibition of large bladder tumors compared with individual treatment groups ($P < 0.01$; **Table 4**). Thus, initiation of drug treatment late during the carcinogenic process, when microscopic tumors are known to be present,

Downloaded from http://aacrjournals.org/cancerpreventionresearch/article-pdf/13/3/273/2246079/273.pdf by guest on 22 February 2024

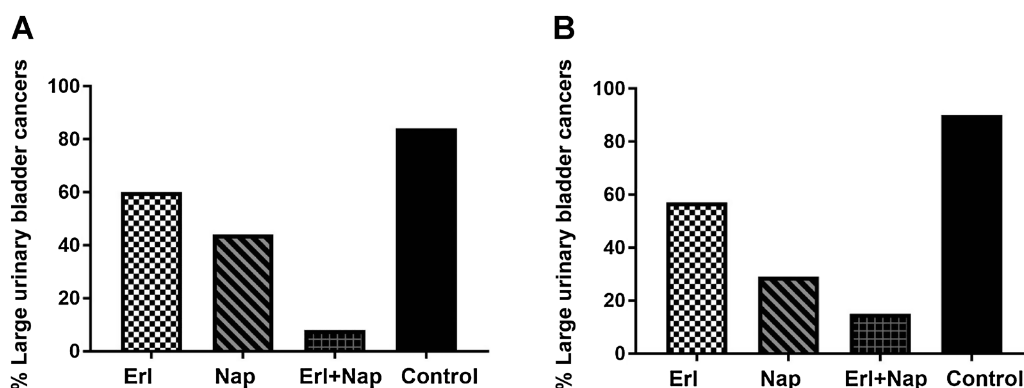


**Figure 3.**
Chemopreventive efficacy of erlotinib and/or naproxen in protocols 2 and 3. **A,** Effect of erlotinib and/or naproxen on the incidence of rats with larger bladder tumors (protocol 2). Individually erlotinib and naproxen showed 29% and 46% inhibition of large bladder cancers whereas the combination treatment reduced the large cancers by 90% ($P < 0.05$). **B,** Effect of erlotinib and/or naproxen on the incidence of rats with larger bladder tumors one month after final carcinogen treatment during the chemoprevention study (protocol 3). Individually erlotinib and naproxen showed 37% ($P < 0.05$) and 68% ($P < 0.0001$) inhibition of large bladder cancers whereas the combination treatment reduced the large cancers by 83% ($P < 0.0001$).

Downloaded from http://aacrjournals.org/cancerpreventionresearch/article-pdf/13/3/273/2246079/273.pdf by guest on 22 February 2024

demonstrated preventive efficacy on urinary bladder cancer progression.

**Chemopreventive efficacy of erlotinib and/or naproxen administered 4 weeks (1 month) after the final carcinogen treatment (Protocol 3).**

In this protocol, erlotinib was administered by oral gavage and naproxen was administered through diet. The dietary naproxen dose (128 ppm) chosen was roughly one-third of the gavage dose. As shown in Supplementary Fig. S1C, body weights of the rats treated with OH-BBN and naproxen and/or erlotinib were similar to those of the controls. There were no signs of toxicity of the agents during the study.

**Table 5** shows the incidence of palpable urinary bladder tumors in the various groups. The control group exhibited a 100% incidence of bladder tumor. The tumor incidence in erlotinib, naproxen, and erlotinib+naproxen treated rats was 86%, 95%, and 50%, respectively. Many of the rats had small lesions in the urinary bladder at necropsy. Supplementary Table S4 shows the effects of the agents on various lesions (hyperplasia and papilloma) of the urinary bladder following histologic evaluation. As observed with Protocols 1 and 2, the compounds caused small increases in the incidences and multiplicities of hyperplasia and papillomas, which reached statistical significance for some of the treatment groups (Supplementary Table S4). Again, it appears that the agents may be preventing the progression of benign or small invasive lesions into large palpable lesions.

The weights of the urinary bladders in each of the OH-BBN–treated groups are presented in **Table 5**. The mean weights were 475 mg (erlotinib), 186 mg (naproxen), 146 mg (erlotinib + naproxen), and 832 mg (controls). The number of rats with large urinary bladder tumors in each of the groups were 57% (erlotinib), 29% (naproxen), 15% (erlotinib+naproxen), and 90% of the Controls (**Table 5**; **Fig. 3B**). Individually, erlotinib and naproxen showed 43% ($P < 0.05$) and 78% ($P < 0.0001$) decreases in the total tumor weights and reduced rats with large bladder tumors by 37% ($P < 0.05$) and 68% ($P < 0.0001$), respectively (**Table 5**). The combination of agents reduced the total and large tumor weights by 82% and 83% ($P < 0.0001$), respectively. Further, tumor multiplicity in untreated controls was 5.38 whereas erlotinib, naproxen, and erlotinib+naproxen treated rats showed tumor multiplicity of 2.38, 2.67, and 0.8, respectively. The combination treatment group showed 85% ($P < 0.0001$) inhibition of tumor multiplicity compared with the control group.

## Discussion

We have previously shown that single-agent regimens with erlotinib or naproxen, when given continuously, were highly effective in the prevention of OH-BBN–induced urinary bladder cancers in rats (11, 22). However, because long-term continuous exposure to EGFR inhibitors leads to skin toxicities and diarrhea while continuous exposure to NSAIDs can cause gastrointestinal bleeding and strokes, more optimal cancer chemoprevention regimens have been actively explored through preclinical studies. Some have shown considerable efficacy with reduced toxicity when alternative dosing regimens were utilized in preclinical animal models (14). Better tolerated chemoprevention regimens can be obtained by reducing doses and frequency of administration. Low-dose combinations may also achieve substantial efficacy with minimal toxicity by targeting complementary pathways. The combination treatment examined in this study was more efficacious than either agent alone, as has been observed in the recent clinical trial in patients with familial adenomatous polyposis (23, 24). Previously, we have shown that intermittent dosing of naproxen provided similar chemopreventive efficacy as standard daily treatment regimens. In that study, daily, 1 week on/1 week off, or 3 weeks on/3 weeks off administration of naproxen resulted

in palpable bladder tumors in 27%, 22%, and 19% of the treated rats, respectively, compared with a 96% incidence of palpable tumors in vehicle-treated rats ($P < 0.01$; ref. 17). Thus, these studies suggested that the chemopreventive efficacy of naproxen can be maintained with dosing regimens that reduces the gastrointestinal toxicity of NSAIDs. Further we showed that a large weekly dose of erlotinib (42 or 21 mg/kg BW administered by gavage) was effective in inhibiting mammary cancer incidence and multiplicity, and was comparable with daily 6 mg/kg BW/day dosing of erlotinib (18).

In this study, doses of erlotinib and naproxen were chosen based on earlier preclinical *in vivo* studies that are clinically relevant. Particularly, the 42 mg/kg BW pulsatile erlotinib dose in rats is equivalent to a dose of ~75 mg/day in human patients, which is half of the clinical dose (18). Naproxen was administered either by oral gavage (protocols 1 and 2) at 30 mg/kg (3 weeks on/off) or continuously in the diet (protocol 3). The dose of naproxen in the diet of 128 ppm is equivalent to a dose of 160 mg in a human weighing 80 kg (25). This dose is significantly lower than naproxen dosing in humans, in which the over-the-counter adult dose for fever/pain relief is 220 mg orally every 8 to 12 hours, resulting in a maximum dose of 660 mg in a 24-hour period (25). The naproxen dose (30 mg/kg) employed in protocols 1 and 2 is also lower than the human equivalent dose of 40 mg/kg (17) and was administered intermittently (3 weeks on / 3 weeks off) to reduce the toxicity profile. It should also be noted that naproxen has a better safety profile than other NSAIDs with regards to cardiovascular toxicity (26). We evaluated the efficacy of agents individually and in combination in early (1 week or 1 month after last carcinogen treatment) and delayed interventions (3 months after final carcinogen treatment (**Fig. 1A**; **Tables 1** and **2**). No signs of toxicity were observed in rats treated individually or with combinations of the agents (Supplementary Fig. S1). Early intervention by combination treatments (protocols 1 and 3) significantly reduced tumor weights up to 82% and was accompanied by an increase in tumor latency and a decrease in tumor multiplicity (66–85%; $P < 0.01$–$0.0001$; **Tables 3** and **5**). In addition, the number of rats with large (>200 mg) tumors (83%–84%; $P < 0.01$–$0.0001$) was also significantly reduced (**Tables 3** and **5**). Delayed initiation of the combination treatment at the stage when microscopic TCC were visible was highly effective in reducing tumor burden with no observable toxicity and with a reduction in tumor incidence (41%, $P < 0.01$), multiplicity (65%, $P < 0.01$), and tumor weights (81%, $P < 0.01$; **Table 4**), similar to what was seen with early intervention Protocol 1 (**Table 3**).

To understand the downstream effects of EGFR and inflammation inhibition in this model, we examined the levels of proliferation, phosphorylated ERK, pSTAT3, and pP38 expression in tumors of rats treated with the combination of erlotinib and naproxen. A significant reduction in Ki67 ($P < 0.04$), IL1β, cyclin D1, pERK, and pSTAT3 ($P < 0.006$) expression was observed compared with control tumors (**Fig. 2C–H**; Supplementary Fig. S2). The results suggest a trend towards the inhibition of tumor cell proliferation with the combination treatment, although there was little effect on pP38 expression (**Fig. 2F**). This biomarker data correlate with results from earlier studies showing the effects of these drugs on inflammation and EGFR downstream pathways (17, 18, 27). Importantly, inflammation and EGFR pathways are known to synergistically activate oncogenic signaling. The EGFR and inflammatory pathways interact at several levels, and are involved in carcinogenesis, angiogenesis, and chemoresistance. Studies showed that the activation of the EGFR pathway promotes transcription of the inflammatory genes (28, 29). Likewise, the inflammatory signaling pathway activates EGFR phosphorylation (30) and EGFR transcription (31). Prostaglandins transactivate the EGFR by induction of phosphorylation of the EGFR and extracellular signal-regulated kinase (30). Many studies have shown that combination of lower doses of agents demonstrate greater chemopreventive efficacy than individual agents in several organ site cancers (32–39). Previous studies demonstrated that simultaneous targeting of EGFR and inflammatory pathways delays progression of pancreatic cancers (40). In the biomarker analysis, the erlotinib and naproxen combination at pulsatile or intermittent dosing inhibited the expression of pERK, pSTAT3, cyclin D1, Ki67, and IL1β (**Fig. 2**; Supplementary Fig. S2). Further studies are warranted to evaluate the exact and in-depth mechanism of action of this combination intermittent dosing treatments.

These studies clearly show that pulsatile and intermittent dosing regimens of erlotinib and naproxen at the tested doses provide significant chemopreventive efficacy against OH-BBN–induced rat bladder tumors with little or no serious side-effects. Also, it is evident from our studies that continuous administration of agents is not necessary for desirable chemopreventive effects, thus intermittent and combinational approaches can maintain efficacy with reduced toxicity. Although the half-life of naproxen and erlotinib is limited to 3 and ≤8 hours, respectively, in rats, the weekly treatment of erlotinib yielded effective serum levels for up to 48 hours (17, 18), chemopreventive efficacy was observed when naproxen was administered with either a 3 weeks on/3 weeks off or once a week regimen for erlotinib. Importantly, the lower naproxen dose of 128 ppm given to rats in the diet was observed to be more effective than gavage dosing. It appears that the effects of these drugs on pharmacodynamic markers might be longer than the drug half-life, as seen with our recent studies (41). In this study, pERK was inhibited in Pirc colon polyps for up to 10 days after discontinuing erlotinib treatment, with full recovery on or around day 14 (41), indicating that erlotinib showed prolonged effects on pharmacodynamic biomarkers with an intermittent dosing regimen.

In summary, a significant chemopreventive efficacy was seen when a low-dose drug combination was intermittently administered 1 week, 3 months, or 1-month after carcinogen

Downloaded from http://aacrjournals.org/cancerpreventionresearch/article-pdf/13/3/273/2246079/273.pdf by guest on 22 February 2024

treatment. The combination of the agents reduced tumor incidence, multiplicity, and tumor weights even when administered 3 months after OH-BBN treatment, when microscopic tumors are known to be present in this model. Further studies in other animal models and in-depth toxicity evaluations are warranted to move this combination regimen to the clinic for the treatment of patients with nonmuscle invasive transitional cell carcinoma.

## Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

## Authors' Contributions

**Conception and design:** A. Mohammed, M.S. Miller, R.A. Lubet, C.S. Suen, S. Sei, R.H. Shoemaker, C.J. Grubbs
**Development of methodology:** A. Mohammed, F.L. Moeinpour, C.J. Grubbs
**Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.):** F.L. Moeinpour, C.J. Grubbs
**Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis):** R.H. Shoemaker, F.L. Moeinpour, C.J. Grubbs
**Writing, review, and/or revision of the manuscript:** A. Mohammed, M.S. Miller, R.A. Lubet, S. Sei, R.H. Shoemaker, C.J. Grubbs
**Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases):** A. Mohammed, F.L. Moeinpour, C.J. Grubbs
**Study supervision:** A. Mohammed, S. Sei, C.J. Grubbs
**Other (pathologist on this study):** M.M. Juliana

## Acknowledgments

This work was supported by NCI contract number HHSN261201500036I, Task Order HHSN26100002. The authors thank Diane Cooper, MS, NIH Library Writing Center, for manuscript editing assistance.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked *advertisement* in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

Received July 8, 2019; revised October 28, 2019; accepted December 4, 2019; published first December 9, 2019.

Downloaded from http://aacrjournals.org/cancerpreventionresearch/article-pdf/13/3/273/2246079/273.pdf by guest on 22 February 2024

## References

1. Siegel RL, Miller KD, Jemal A. Cancer statistics, 2019. CA Cancer J Clin 2019;69:7–34.
2. Sievert KD, Amend B, Nagele U, Schilling D, Bedke J, Horstmann M, et al. Economic aspects of bladder cancer: what are the benefits and costs? World J Urol 2009;27:295–300.
3. Pasin E, Josephson DY, Mitra AP, Cote RJ, Stein JP. Superficial bladder cancer: an update on etiology, molecular development, classification, and natural history. Rev Urol 2008;10:31–43.
4. Dadhania V, Zhang M, Zhang L, Bondaruk J, Majewski T, Siefker-Radtke A, et al. Meta-analysis of the luminal and basal subtypes of bladder cancer and the identification of signature immunohistochemical markers for clinical use. EBioMedicine 2016;12:105–17.
5. De Boer WI, Houtsmuller AB, Izadifar V, Muscatelli-Groux B, Van der Kwast TH, Chopin DK. Expression and functions of EGF, FGF and TGFbeta-growth-factor family members and their receptors in invasive human transitional-cell-carcinoma cells. Int J Cancer 1997;71: 284–91.
6. Comprehensive molecular characterization of urothelial bladder carcinoma. Nature 2014;507:315–22.
7. Daugherty SE, Pfeiffer RM, Sigurdson AJ, Hayes RB, Leitzmann M, Schatzkin A, et al. Nonsteroidal antiinflammatory drugs and bladder cancer: a pooled analysis. Am J Epidemiol 2011;173:721–30.
8. Shih C, Hotaling JM, Wright JL, White E. Long-term NSAID use and incident urothelial cell carcinoma in the VITamins and Lifestyle (VITAL) study. Urol Oncol 2013;31:1689–95.
9. Guttman-Yassky E, Mita A, De Jonge M, Matthews L, McCarthy S, Iwata KK, et al. Characterisation of the cutaneous pathology in non-small cell lung cancer (NSCLC) patients treated with the EGFR tyrosine kinase inhibitor erlotinib. Eur J Cancer 2010;46: 2010–9.
10. Scheiman JM, Fendrick AM.Summing the risk of NSAID therapy. Lancet 2007;369:1580–1.
11. Lubet RA, Steele VE, Juliana MM, Grubbs CJ. Screening agents for preventive efficacy in a bladder cancer model: study design, end points, and gefitinib and naproxen efficacy. J Urol 2010;183:1598–603.
12. Lu Y, Liu P, Wen W, Grubbs CJ, Townsend RR, Malone JP, et al. Cross-species comparison of orthologous gene expression in human bladder cancer and carcinogen-induced rodent models. Am J Transl Res 2010; 3:8–27.
13. Williams PD, Lee JK, Theodorescu D. Molecular credentialing of rodent bladder carcinogenesis models. Neoplasia 2008;10:838–46.
14. Mohammed A, Fox JT, Miller MS. cancer chemoprevention: preclinical *in vivo* alternate dosing strategies to reduce drug toxicities. Toxicol Sci 2019;170:251–9.
15. Milton DT, Azzoli CG, Heelan RT, Venkatraman E, Gomez JE, Kris MG, et al. A phase I/II study of weekly high-dose erlotinib in previously treated patients with nonsmall cell lung cancer. Cancer 2006;107:1034–41.
16. Grommes C, Oxnard GR, Kris MG, Miller VA, Pao W, Holodny AI, et al. "Pulsatile" high-dose weekly erlotinib for CNS metastases from EGFR mutant non-small cell lung cancer. Neuro Oncol 2011;13: 1364–9.
17. Lubet RA, Scheiman JM, Bode A, White J, Minasian L, Juliana MM, et al. Prevention of chemically induced urinary bladder cancers by naproxen: protocols to reduce gastric toxicity in humans do not alter preventive efficacy. Cancer Prev Res 2015;8:296–302.
18. Lubet RA, Szabo E, Iwata KK, Gill SC, Tucker C, Bode A, et al. Effect of intermittent dosing regimens of erlotinib on methylnitrosourea-induced mammary carcinogenesis. Cancer Prev Res 2013;6:448–54.
19. Thompson HJ, Becci PJ, Grubbs CJ, Shealy YF, Stanek EJ, Brown CC, et al. Inhibition of urinary bladder cancer by N-(ethyl)-all-trans-retinamide and N-(2-hydroxyethyl)-all-trans-retinamide in rats and mice. Cancer Res 1981;41:933–6.
20. Grubbs CJ, Juliana MM, Eto I, Casebolt T, Whitaker LM, Canfield GJ, et al. Chemoprevention by indomethacin of N-butyl-N-(4-hydroxybutyl)-nitrosamine-induced urinary bladder tumors. Anticancer Res 1993;13:33–6.
21. Grubbs CJ, Lubet RA, Koki AT, Leahy KM, Masferrer JL, Steele VE, et al. Celecoxib inhibits N-butyl-N-(4-hydroxybutyl)-nitrosamine-induced urinary bladder cancers in male B6D2F1 mice and female Fischer-344 rats. Cancer Res 2000;60:5599–602.
22. Lubet RA, Steele VE, Shoemaker RH, Grubbs CJ. Screening of chemopreventive agents in animal models: results on reproducibility,

agents of a given class, and agents tested during tumor progression. Cancer Prev Res 2018;11:595–606.
23. Samadder NJ, Neklason DW, Boucher KM, Byrne KR, Kanth P, Samowitz W, et al. Effect of sulindac and erlotinib vs placebo on duodenal neoplasia in familial adenomatous polyposis: a randomized clinical trial. JAMA 2016;315:1266–75.
24. Samadder NJ, Kuwada SK, Boucher KM, Byrne K, Kanth P, Samowitz W, et al. Association of sulindac and erlotinib vs placebo with colorectal neoplasia in familial adenomatous polyposis: secondary analysis of a randomized clinical trial. JAMA Oncol 2018;4:671–7.
25. Steele VE, Rao CV, Zhang Y, Patlolla J, Boring D, Kopelovich L, et al. Chemopreventive efficacy of naproxen and nitric oxide-naproxen in rodent models of colon, urinary bladder, and mammary cancers. Cancer Prev Res 2009;2:951–6.
26. Bhala N, Emberson J, Merhi A, Abramson S, Arber N, Baron JA, et al. Vascular and upper gastrointestinal effects of non-steroidal anti-inflammatory drugs: meta-analyses of individual participant data from randomised trials. Lancet 2013;382:769–79.
27. Kim MS, Kim JE, Lim DY, Huang Z, Chen H, Langfald A, et al. Naproxen induces cell-cycle arrest and apoptosis in human urinary bladder cancer cell lines and chemically induced cancers by targeting PI3K. Cancer Prev Res 2014;7:236–45.
28. Elder DJ, Halton DE, Playle LC, Paraskeva C. The MEK/ERK pathway mediates COX-2-selective NSAID-induced apoptosis and induced COX-2 protein expression in colorectal carcinoma cells. Int J Cancer 2002;99:323–7.
29. Sheng H, Shao J, DuBois RN. Akt/PKB activity is required for Ha-Ras-mediated transformation of intestinal epithelial cells. J Biol Chem 2001;276:14498–504.
30. Pai R, Soreghan B, Szabo IL, Pavelka M, Baatar D, Tarnawski AS. Prostaglandin E2 transactivates EGF receptor: a novel mechanism for promoting colon cancer growth and gastrointestinal hypertrophy. Nat Med 2002;8:289–93.
31. Kinoshita T, Takahashi Y, Sakashita T, Inoue H, Tanabe T, Yoshimoto T. Growth stimulation and induction of epidermal growth factor receptor by overexpression of cyclooxygenases 1 and 2 in human colon carcinoma cells. Biochim Biophys Acta 1999;1438:120–30.
32. Janakiram NB, Mohammed A, Bryant T, Zhang Y, Brewer M, Duff A, et al. Potentiating NK cell activity by combination of Rosuvastatin and Difluoromethylornithine for effective chemopreventive efficacy against colon cancer. Sci Rep 2016;6:37046.
33. Madka V, Mohammed A, Li Q, Zhang Y, Biddick L, Patlolla JM, et al. Targeting mTOR and p53 signaling inhibits muscle invasive bladder cancer *in vivo*. Cancer Prev Res 2016;9:53–62.
34. Madka V, Mohammed A, Li Q, Zhang Y, Kumar G, Lightfoot S, et al. TP53 modulating agent, CP-31398 enhances antitumor effects of ODC inhibitor in mouse model of urinary bladder transitional cell carcinoma. Am J Cancer Res 2015;5:3030–41.
35. Suh N, Reddy BS, DeCastro A, Paul S, Lee HJ, Smolarek AK, et al. Combination of atorvastatin with sulindac or naproxen profoundly inhibits colonic adenocarcinomas by suppressing the p65/beta-catenin/cyclin D1 signaling pathway in rats. Cancer Prev Res 2011;4:1895–902.
36. Mohammed A, Janakiram NB, Brewer M, Vedala K, Steele VE, Rao CV. Multitargeted low-dose GLAD combination chemoprevention: a novel and promising approach to combat colon carcinogenesis. Neoplasia 2013;15:481–90.
37. Nicastro HL, Grubbs CJ, Margaret Juliana M, Bode AM, Kim MS, Lu Y, et al. Preventive effects of NSAIDs, NO-NSAIDs, and NSAIDs plus difluoromethylornithine in a chemically induced urinary bladder cancer model. Cancer Prev Res 2014;7:246–54.
38. Pereira MA, Warner BM, Knobloch TJ, Weghorst CM, Lubet RA, Steele VE, et al. Chemoprevention of mouse lung and colon tumors by suberoylanilide hydroxamic acid and atorvastatin. Int J Cancer 2012; 131:1277–86.
39. Lubet RA, Szabo E, Christov K, Bode AM, Ericson ME, Steele VE, et al. Effects of gefitinib (Iressa) on mammary cancers: preventive studies with varied dosages, combinations with vorozole or targretin, and biomarker changes. Mol Cancer Ther 2008;7:972–9.
40. Rao CV, Janakiram NB, Madka V, Devarkonda V, Brewer M, Biddick L, et al. Simultaneous targeting of 5-LOX-COX and EGFR blocks progression of pancreatic ductal adenocarcinoma. Oncotarget 2015;6: 33290–305.
41. Ulusan A, Rajendran P, Dashwood W-M, Mohammed A, Sei S, Brown PH, et al. Abstract 5074: Optimizing erlotinib plus sulindac dosing regimens in a preclinical model of FAP. Cancer Res 2019;79(13 Suppl): 5074.

Downloaded from http://aacrjournals.org/cancerpreventionresearch/article-pdf/13/3/273/2246079/273.pdf by guest on 22 February 2024