NCI, DCPC
Chemoprevention Branch and Agent Development Committee

CLINICAL DEVELOPMENT PLAN:

PIROXICAM

DRUG IDENTIFICATION

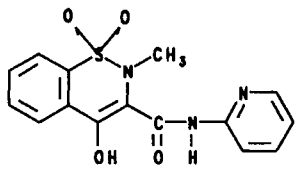
CAS Registry No.: 36322-90-4

CAS Name (9CI): 4-Hydroxy-2-methyl-*N*-2-pyridinyl-2*H*-1,2-benzothiazine-3-carboxamide 1,1-Dioxide

Synonyms: CP 16171
Feldene® (Active Ingredient)

Related Compounds:
Ampiroxicam (CAS No. 99464-64-9)
Droxicam (CAS No. 90101-16-9)

Structure:



EXECUTIVE SUMMARY

Piroxicam is under consideration for development by NCI, DCPC because of its remarkable chemopreventive activity against rat colon and mouse bladder carcinomas, and the epidemiological evidence associating non-aspirin NSAID use with decreased risk for colorectal polyps [1] and cancer [2]. Like the other NSAIDs currently being considered for further development (aspirin, ibuprofen, and sulindac), piroxicam derives its anti-inflammatory activity from repression of prostaglandin (PG) synthesis by inhibiting the cyclooxygenase activity of PGH synthase [3,4]. Since piroxicam is a potent inhibitor of cyclooxygenase, it is both a very active and fairly toxic NSAID. Gastrointestinal (GI) bleeding and ulceration, the most significant side effect of chronic administration of NSAIDs, is attributed to the lowered levels of PGs and thromboxane A_2 (TxA_2) resulting from cyclooxygenase inhibition. PGs promote protective mucin secretion and bicarbonate production in gastric mucosa, and TxA_2 is involved in platelet aggregation. These effects have been seen primarily with piroxicam doses ≥20 mg qd. Clinical development of piroxicam will concentrate on identifying an effective dosing strategy with minimal safety risk. Particularly, it will be evaluated at ≤10 mg qd doses in combination with the antiproliferative agent DFMO. In CB-sponsored studies, this combination has demonstrated synergistic chemopreventive efficacy against rat colon cancers. Based on its efficacy at these target sites in animal cancer models and the significant exposure these tissues

receive to NSAIDs, colon and bladder are the primary sites of clinical chemoprevention studies of piroxicam.

The available animal efficacy results are considered sufficient to support the clinical development of piroxicam. Besides the colon and bladder, piroxicam has demonstrated chemopreventive activity against DMBA-initiated/TPA-promoted mouse skin tumors in a CB-sponsored study. Studies reported in the literature have described its chemopreventive activity in rat colon, small intestines and tongue, and in mouse lung. The CB is sponsoring additional animal efficacy studies in mouse lung, hamster pancreas, and rat mammary gland and colon cancer models.

A significant effort in the CB program is to identify and validate intermediate biomarkers of cancer and evaluate potential for chemopreventive modulation of these markers. Piroxicam has demonstrated activity against putative biomarkers of colon cancer in rats (aberrant crypts and oncogene expression).

Since piroxicam is an approved drug for chronic use, preclinical toxicity and pharmacokinetic data on which approval was based are sufficient to support further regulatory filings. A CB-sponsored 90-day animal toxicity study of the combination of DFMO and piroxicam was completed recently. No synergistic toxicity, particularly ototoxicity or blood pathology, was observed in rats or dogs; however, a NOEL for the combination was not defined in rats due to gastric lesions in all piroxicam-treated groups. A genotoxicity study of the combination was also negative.

A CB-sponsored Phase I clinical study of the combination of DFMO and piroxicam in patients previously treated for early stage skin cancer has been initiated (Table I). Based on the results of the completed piroxicam arm (10 mg qd or qod for up to six months), a dose of 10 mg qod of piroxicam was selected for testing in combination with 0.5 g DFMO/m^2; this combination arm has started.

An NCI-sponsored Phase II efficacy trial in patients with previously resected colon adenomas is also in progress (see Table I). This study evaluates the effects of 7.5 mg piroxicam qd on proliferation of colorectal mucosa.

Piroxicam has been available from Chas. Pfizer and Co., Inc. as 10 and 20 mg capsules. For products <10 mg, bulk drug will be purchased from Pfizer or other sources for formulation.

Based on preclinical efficacy results and pharmacokinetics, the target organs for development of piroxicam as a cancer chemopreventive drug will be colon and bladder. To investigate reduction of gastric toxicity while retaining or increasing efficacy, future Phase II studies comparing piroxicam and the combination of DFMO and piroxicam in these tissues are under consideration. An alternative strategy is development of prodrugs (ampiroxicam, droxicam), which circumvent adverse gastric effects of NSAIDs by conversion to piroxicam in the small intestine.

PRECLINICAL EFFICACY STUDIES

In studies sponsored by the CB, piroxicam has demonstrated chemopreventive activity in several animal carcinogenesis models. It inhibited AOM-induced colon carcinomas in rats (25–400 mg/kg diet, *ca.* 0.004–0.06 mmol/kg-bw/day) [5,6], DMBA-intiated/TPA-promoted mouse skin tumors (≤0.0125% diet or *ca.* 0.05 μmol/kg-bw/day), and OH-BBN-induced bladder tumors in mice (15 and 30 mg/kg diet, *ca.* 0.006 and 0.011 mmol/kg-bw/day) [7]. It was not effective in an MNU-induced rat mammary cancer model. Further evidence of the chemopreventive efficacy of piroxicam comes from studies reported in the literature of the inhibition of tumor induction in rat colon [8,9] and small intestines [10], tongue [11], and mouse lung [12,13]. The animal efficacy results are adequate to support the clinical development of piroxicam. Besides the completed studies, the CB is sponsoring additional animal efficacy studies in PhIP-induced rat colon, B(*a*)P-induced mouse lung, and DMBA-induced rat mammary gland cancer models.

There is good evidence that the combination of piroxicam and DFMO will be a useful chemopreventive regimen, particularly in the colon. In CB-sponsored studies, the lowest doses of dietary piroxicam (*ca.* 0.004 mmol/kg-bw/day) and DFMO (*ca.* 0.11 mmol/kg-bw/day) tested significantly inhibited both colonic adenomas and adenocarcinomas in rats when administered subsequent to AOM. The agents alone were not efficacious at these doses and the combination clearly provided a synergistic response [14]. In a subsequent study, higher doses of this agent combination (*ca.* 0.015 mmol piroxicam/kg-bw/day and 0.27 mmol DFMO/kg-bw/day) fed continually starting two weeks prior to AOM administration inhibited the incidence and multiplicity of rat colon adenocarcinomas to a greater extent than either agent alone at the same or higher doses (*ca.* 0.03 mmol piroxicam/kg-bw/day or 0.55 mmol DFMO/kg-bw/day) [15].

In a CB-sponsored study in the mouse bladder, the combination of piroxicam (30 mg/kg diet), DFMO (1200 mg/kg diet) and 4-HPR (313 mg/kg diet) was highly efficacious compared with carcinogen controls; however, a significant decrease in survival and body weight was observed at these doses. In this experiment, profound chemopreventive activity of piroxicam alone was observed, even at lower dose levels (15 mg/kg diet) which may have masked any synergistic or additive effects of the combination [7].

A significant effort in the CB program is to identify and validate intermediate biomarkers of cancer and evaluate the potential of chemopreventive agents to modulate these markers. Piroxicam inhibited the formation of putative histological biomarkers of colon cancer in AOM-treated rats—foci of aberrant crypts, especially hexosaminidase-negative foci [16]. Concomitantly, AOM-induced *ras* oncogene expression was inhibited [17]. Currently, piroxicam's effects on additional biomarkers in rat colon (GST-π, *myc*, p53, PCNA), mouse colon (precancerous lesions, PCNA, *ras* p21), and rat bladder (dysplasia, EGFR) are being studied.

PRECLINICAL SAFETY STUDIES

Safety A CB-sponsored, 90-day toxicology study of piroxicam alone and in combination with DFMO in rats and dogs has been completed. This study investigated the potential for synergistic toxicity between the agents, particularly hearing loss or blood pathology, in preparation for carrying out a Phase I clinical trial of the combination. Effects on hearing were studied in dogs (brainstem-evoked auditory response, histopathology of auditory nuclei, and surface morphology examination of the cochlea), and blood coagulation effects were studied in both dogs and rats.

In the rat study, mucosal/transmural ulceration of the stomach was related to intragastric treatment with all doses of piroxicam (0, 1.5, and 6 mg/kg-bw/day) alone or in all possible combinations with DFMO (0, 250, and 1,000 mg/kg-bw/day). Alterations observed in hematological parameters (mild decreases in hemoglobin and hematocrit, mild leukocytosis and elevated reticulocytes) were considered secondary to the gastric lesions. Increased incidences of glomerulonephropathy or chronic nephritis of the type which occurs spontaneously in aging male rats were observed in all piroxicam treatment groups. Changes in clinical chemistry (mild increases in BUN, creatinine and serum sodium, mild decreases in serum albumin and total protein) appeared related to the renal pathology. There were no indications of synergistic toxicity due to the combinations of piroxicam and DFMO; however, a NOEL was not identified for either piroxicam or the combination due to the significant increase in gastric lesions in all piroxicam-treated groups.

In the dog study, the highest dose of piroxicam (gelatin capsule formulation, ig) was reduced from 3 to 2 mg/kg-bw/day due to increased mortality from ulceration of the stomach and/or duodenum, and associated peritonitis, inflammation, and blood loss. Combined treatment with DFMO (0, 25, and 100 mg/kg-bw/day) appeared to ameliorate piroxicam-induced (0, 0.75, and 2 mg/kg-bw/day) gastric toxicity (histopathology, melena, hematochezia) and mortality. No test article-related ophthalmic or auditory responses were observed, and the NOEL level for the combination appeared to be 0.75 mg piroxicam/kg-bw/day with 25 mg DFMO/kg-bw/day. An in-depth analysis of changes in cochlear hair cells of the dogs treated in this study has been conducted to assess possible effects on auditory function. This report is currently under review.

In contracted genotoxicity assays, the combination of piroxicam and DFMO did not significantly increase SCE in CHO cells *in vitro* or frequency of micronucleated cells in bone marrow of mice treated *in vivo*. The combination was also negative in the Ames mutagenicity assay in *Salmonella typhimurium* strains TA98, TA100, TA1535, and TA1537. No other preclinical toxicity tests of piroxicam alone have been sponsored by the CB. However, since piroxicam is an approved drug for chronic use, the available preclinical toxicity and pharmacokinetics data are considered sufficient for further regulatory filing.

ADME The kinetics and metabolism of piroxicam in laboratory animals have been extensively reviewed [18–21]. Rabbits given a single dose of 3 or 10 mg piroxicam/kg-bw by esophageal intubation or rectal dosing attained plasma peaks appreciably faster by the latter route, suggesting more rapid absorption [22]. In addition, the mean AUC after oral dosing was equivalent to that after a single iv injection of 10 mg/kg, indicating that piroxicam is virtually completely absorbed from the GI tract.

Following administration of single and repeated doses, the pharmacokinetics of piroxicam are linear, with dose-related plasma C_{max} and AUC values [20]. In rats, t_{max} were observed 2 hrs after a single oral dose of 10 mg/kg and 5.5 hrs after

rectal dosing with 3 or 10 mg/kg [22]. The plasma $t_{½}$ of piroxicam is approximately 5 hrs in mice, 3–5.5 hrs in rabbits, and 45 hrs in dogs. Because of the extensive binding (approximately 99%) to plasma proteins after absorption in both laboratory animals and humans [23], piroxicam has a small V_d and low plasma clearance [24].

Due to extensive metabolism, <1% of the agent was excreted as the parent compound in the urine of rats and monkeys, and 2.5% in the urine of dogs [25]. Biliary excretion of either the agent or the glucuronide accounted for only 7.6% of the dose in rats, less than 30% of the dose in dogs, and about 40% of the dose in rhesus monkeys [21].

The pharmacokinetics of piroxicam may alter with age in rats. After a single dose of 1 mg/kg-bw iv, plasma $t_{½}$ increased from 5.9 hrs in 5 month old animals to 30.6 hrs in 2 year old animals [26]. Concomitantly, clearance decreased from 0.048 L/hr·kg in young rats to 0.021 L/hr·kg in old rats.

The prodrug ampiroxicam is metabolized in the intestine of rats, dogs and monkeys, so that only piroxicam is detected in the plasma [27]. Bioavailability studies show that conversion is 90%, 70%, and 50% in these species, respectively. In the rat and monkey, equivalent doses of prodrug and drug resulted in t_{max} of 2 hrs, but C_{max} was higher for piroxicam (14 *vs* 12 μg/ml in the rat; 36 *vs* 16 in the monkey).

CLINICAL SAFETY: PHASE I STUDIES

A CB-sponsored Phase I study of the combination of piroxicam and DFMO is being carried out in patients previously treated for early skin cancer (Dr. P. Carbone, University of Wisconsin). The first step of the study evaluating the drug effect and safety of doses of piroxicam less than the standard therapeutic dose of 20 mg qd (Table I) has been completed. Twelve patients were randomized to receive piroxicam at 10 mg qd or qod for six months. The qod dosage regimen takes advantage of the relatively long plasma $t_{½}$ of piroxicam (14–158 hrs, see ADME below) to reduce the dosage and still maintain blood levels of the drug. Three out of six patients treated with 10 mg qd completed the study; 2/3 patients who withdrew experienced grade 2 tinnitus, however, no hearing loss was noted. Grade I GI symptoms were also observed at both doses. All six patients completed the study at the low dose, and 10 mg qod was selected for the combination arm with 0.5 g DFMO/m^2, qd.

Literature reports of previous safety and ADME studies of piroxicam are summarized below, and compared with the results available from the Phase I study.

Drug Effect Measurement Serum PG levels, primarily of PGE_2 and PGF, are generally used and well-documented as drug effect measurements for NSAIDs. It is critical that procedures for PG measurements are standardized and validated for specific tissues studied in chemoprevention trials, such as colon mucosa. In the piroxicam arm of the Phase I study described above, a decrease in serum TxB_2 activity was not observed. The investigators suggested that this may have been due to the large coefficient of variance inherent in the RIA methodology used and the relatively small doses of piroxicam administered. These effects will be investigated further during the combination arm of the study. Also, no significant changes in urinary polyamine synthesis or TPA-induced ODC activity in skin punch biopsies were observed; these measurements would be expected to respond to DFMO treatment.

Safety Data compiled from several clinical trials indicated that GI side effects occurred in about 13% of more than 73,000 patients studied (many of whom were receiving dosages of greater than 20 mg qd) [18–20]. Less than 5% of the patients experienced side effects involving the CNS, skin, or cardiovascular systems. At daily doses of 10, 20, 30, and 40 mg piroxicam (duration unreported), the percentages of GI side effects observed were 9.6, 18.4, 22.3 and 29.9, respectively. Primary symptoms included epigastric distress, nausea, stomatitis, anorexia, and dizziness. The incidences of patients experiencing peptic ulceration were 0.5%, 0.9%, 2.6% and 6.9%, respectively. These symptoms necessitated termination of piroxicam dosing in about 4% of the patients. A lower overall incidence of GI effects was reported when doses of 20 mg qd or less were used.

Side effects other than those affecting the GI tract have been infrequent. Generally, less than 1% of the treated population have experienced dermatological effects such as skin rash and pruritus [20], phototoxicity [20,28,29], and erythema multiforme [20]. However, in one study of 31 case reports [30], 47% of patients had skin reactions, with 5 of these being photosensitivities. Piroxicam has also been reported to cause edema, hair loss, paresthesia and, rarely, aplastic anemia [31]. Case reports suggest that piroxicam, like some other NSAIDs, is associated with pancreatitis [*e.g.*, 32].

ADME The kinetics and metabolism of piroxi-

cam in humans have been extensively reviewed [18–21,24]. Results from studies in humans ingesting 10–100 mg piroxicam show that it is rapidly and fully absorbed. Piroxicam dissolves slowly in the stomach as the nonionized form, passes readily through the cell membranes of the gastric mucosa, and assumes an ionized form upon entering the bloodstream. In this state, it becomes more hydrophilic, binds to plasma proteins, and does not penetrate tissues [21]. However, at a site of inflammation, equilibration of the nonacidic drug with its nonionized lipophilic form is facilitated and piroxicam penetrates into the site [21].

In volunteers given 40 mg orally or rectally, absorption by rectal administration was more gradual [33]. No data are available on the degree to which piroxicam is absorbed topically, but a study on the antiinflammatory effects of piroxicam administered by this route in rats provides indirect evidence that dermal absorption also occurs at levels comparable to oral and rectal exposure [34].

Following administration of single and repeated doses, the pharmacokinetics of piroxicam are linear, with dose-related plasma C_{max} and AUC values [20]. The t_{max} was 1–6 hrs after oral and rectal dosing [20]. It has been proposed that the ingestion of food slows absorption [35], but some observers have shown otherwise [23,32]. For example, steady-state plasma concentrations of piroxicam were observed after administration of 20 mg qd for 7 days [33], and were unaffected by food intake [20]. In male volunteers, C_{max} was roughly related to dosage levels; values of 0.85 μg/ml and 13.5 μg/ml were reported after a single 10 or 100 mg dose, respectively [23]. Preliminary results from the Phase I study show C_{max} values of 1.1 and 2.1 μg/ml after three months of 10 mg piroxicam on qod and qd dosing schedules, respectively. Multiple peaks in the plasma concentration of piroxicam are frequently observed after ingestion and may be indicative of enterohepatic circulation or tubular reabsorption [23,36].

The elimination $t_{½}$ was 14.1 to 158 hrs in humans (average, 38–45 hrs). Its long plasma disappearance time in humans has been attributed to its strong binding with plasma proteins (approximately 99%) [20,22,23] and its low clearance rate (0.13–0.15 L/hr) [35] and V_d [24]. No bioaccumulation of piroxicam has been observed either in laboratory rats or humans.

Piroxicam undergoes extensive hepatic biotransformation [24]. Hydroxylation of the pyridyl ring has been observed as the major metabolic pathway of piroxicam in rats, dogs, monkeys and man [25,36]. Although cyclodehydration and amide hydrolysis leading to decarboxylation, ring contraction and *N*-dealkylation have also been observed in experimental animals, none of these metabolites represent more than 5% of the dose in humans [25]. The principal metabolite identified in animals and humans is formed by hydroxylation of the pyridyl ring at the 5′ position and this metabolite is excreted via urine as the free compound or as the glucuronic acid conjugate [20,23].

Due to extensive metabolism, only 5–10% of a piroxicam dose is excreted unchanged in urine [24]. An early study following a single 20 mg oral dose of piroxicam in humans found that 10% of the dose was excreted unchanged in the urine and 32% was excreted in the feces over a period of 8 days [35]. More recent data indicate that only 2–5% of a 20 mg dose is excreted unchanged in humans. No specific information was found on the relative drug effects of the metabolites.

Prodrugs ampiroxicam and droxicam, synthesized to reduce piroxicam's gastrointestinal toxicity, appear to be hydrolysed to the NSAID during absorption through the intestinal wall [reviewed in 24]. Ampiroxicam has lower aqueous solubility, and is absorbed more slowly after oral administration than piroxicam. The t_{max} values were 4.4 hrs and 7 hrs for ampiroxicam and droxicam, respectively, compared with 2.2 hrs for the parent after equivalent doses; however, the AUC, Cl, and V_d values were similar.

CLINICAL EFFICACY: PHASE II STUDIES

One Phase II study sponsored by NCI, DCPC is in progress in patients with previous colonic adenoma (Dr. D. Earnest, University of Arizona; see Table I). A preliminary dose-finding step (IIa) in 40 subjects identified 7.5 mg piroxicam qd as the lowest dose that significantly (≥20%) reduced rectal mucosa PGE_2 levels; the usual antiarthritic dose is 20 mg qd [37,38]. The second step (IIb) is comparing the effects of the same dose of piroxicam with placebo on proliferation in colorectal mucosa (BrdU labeling index). Based on preclinical efficacy studies, colon and bladder cancers are the primary targets for chemoprevention by piroxicam. The CB is considering additional Phase II studies in these tissues for piroxicam alone and in combination with DFMO.

PHARMACODYNAMICS

In preclinical studies, the lowest effective dose of piroxicam in rat colon (25 ppm in diet,

ca. 0.004 mmol/kg-bw/day) caused gastric ulceration and altered hematological parameters in the CB-funded 90-day rat toxicity study. The daily anti-inflammatory dose in humans (20–40 mg, or 0.0009–0.0017 mmol/kg-bw) is already below the effective preclinical dose. However, since 25 ppm was the lowest dose tested in rats, even lower doses may inhibit colon carcinogenesis. Thus, a dose of 7.5 mg qd (*ca.* 0.0003 mmol/kg-bw qd) is being evaluated against a proliferative biomarker in a Phase II trial of colonic polyp patients. It may be possible to decrease the dose even further in combination with DFMO.

PROPOSED STRATEGY FOR CLINICAL DEVELOPMENT

Drug Effect Measurement Issues

PG synthesis, primarily of PGE_2 and PGF, is generally used and well-documented as a drug effect measurement for NSAIDs. It is critical that procedures for PG measurements are standardized and validated for specific tissues studied in chemoprevention trials, such as colon mucosa. The apparent lack of effect on serum TxB_2 levels observed after low piroxicam doses in the Phase I trial will be investigated further in the combination arm.

Safety Issues

No additional specific toxicology studies will be required to develop piroxicam as a chemopreventive agent. However, gastric ulceration and bleeding, induced most probably by inhibition of PGs and TxA_2 synthesis (via inhibition of cyclooxygenase), is a significant side effect of long-term NSAID therapy and of piroxicam specifically. It will be important to develop information that delineates dosage regimens resulting in chemopreventive efficacy and minimal toxicity. Such dose evaluation is part of the current Phase I trial (Table I, Dr. P. Carbone).

A strategy to optimize results with potentially toxic drugs is to use them in combination with other drugs having complementary or supplementary chemopreventive activity. The combination should allow maintenance of efficacy with lower and less toxic doses of both drugs. This strategy will be pursued with piroxicam. Synergistic chemopreventive activity of piroxicam and DFMO has been observed in the AOM-induced rat colon cancer model. The combination is being compared to piroxicam alone in the Phase I clinical study in progress (Dr. P. Carbone). Phase II trials of the combination in colon and bladder are under consideration and depend on a favorable outcome in the Phase I trial.

Pharmacodynamics Issues

The ADME of piroxicam suggests that the highest levels of exposure occur in the colon and bladder. The demonstrated chemopreventive efficacy of piroxicam in these tissues suggests that regimens can be designed to minimize gastric ulceration and bleeding while maintaining chemopreventive efficacy. The safety and drug effect of lower doses of piroxicam which take advantage of the long plasma $t_{1/2}$ are being investigated in the ongoing Phase I trial. The issue is whether these doses are also effective as a cancer chemopreventive regimen. The combination with DFMO is an alternate strategy to increase efficacy.

Regulatory Issues

No specific regulatory issues exist for piroxicam, which is already an approved drug for chronic use. A Phase I clinical trial of the combination of piroxicam with DFMO has started based on the 90-day toxicity studies in rats and dogs. Chronic toxicity studies will be necessary for long-term administration in future clinical trials.

Supply and Formulation Issues

Chas. Pfizer and Co., Inc., currently holds a patent for the use of piroxicam in the treatment of cancer. The current Phase I study uses a 10 mg capsule formulation of piroxicam commercially available from this company as well as other suppliers. Any dosages of <10 mg or that are not multiples of 10 mg to be used in chemoprevention studies will require purchase of the bulk drug and reformulation.

The availability of DFMO solely as an oral solution and piroxicam as a capsule will complicate the dosing in any blinded combination study. To avoid this problem, formulation of DFMO in capsule form, either as the sole ingredient or with piroxicam, will be required.

Intermediate Biomarker Issues

Several types of intermediate biomarkers are being evaluated in preclinical studies with pirox-

icam, including indicators of proliferation (*e.g.*, PCNA), oncogene expression, and precancerous lesions.

Evidence from preclinical studies suggests that other types of intermediate biomarkers should be carefully chosen when assessing the effect of NSAIDs on colon carcinogenesis. For example, changes in proliferation biomarkers do not always correlate with decreases in colon tumor incidence or local PG synthesis. Oral aspirin treatment of either control or DMH-exposed rats decreased colon PGE_2 production by *ca.* 96% [39]. In contrast, the NSAID has no effect on mucosal proliferation (measured as [^{3}H]-thymidine incorporation) in the DMH-induced group even though colon adenocarcinoma incidence significantly decreased. Furthermore, aspirin enhanced colon proliferation in the absence of carcinogen. In a related example, indomethacin had no effect on colon PGE_2 synthesis at a dose which reportedly inhibited colon tumor formation [40]; administration of a stable PGE analog did not neutralize the chemopreventive efficacy of indomethacin [reviewed in 39]. Conversely, numerous reports have demonstrated that prostaglandins can inhibit proliferation of animal and human tumor cells *in vitro* and *in vivo* and rat colon mucosa *in vitro* [reviewed in 41]. Thus, the influence of NSAIDs on colon carcinogenesis is complex. The response may depend on the identity of the NSAID or carcinogen, or the dose employed. Differences in the cell populations sampled (*e.g.*, scraping of the entire mucosa) may also be a confounding factor; it has been suggested that host cells rather than tumor cells are the major sources of prostaglandins that contribute to colon carcinogenesis [41]. Finally, the carcinogenic mechanism related to cyclooxygenase activity in the colon may not be related to a direct effect of the PG end-products. For example, generation of mutagens could be decreased by inhibition of PG synthase-related production of reactive species or co-oxidation of carcinogens. Other possible mechanisms include altered signal transduction or immune response, or induction of apoptosis. Thus, genetic or differentiation biomarkers should be investigated along with proliferation biomarkers as potential surrogate endpoints for clinical trials of piroxicam as a colon chemopreventive agent.

In the Phase I trial of the combination of piroxicam and DFMO, standardization of the ODC assay protocol is of high importance. The type of buffer, the protein content of the reaction mixture, and the choice of negative control can affect the result by ≥20% [42].

Clinical Studies Issues

Based on preclinical efficacy, colon and bladder are primary targets for chemopreventive intervention by piroxicam. One Phase II study in colon is now in progress. Additional Phase II trials of the combination of piroxicam and DFMO are being considered for the colon and bladder. Further development of the combination depends on the favorable outcome of the Phase I trial; the combination arm is in progress.

Alternatives to the development of piroxicam are the prodrugs droxicam and ampiroxicam, which circumvent the gastric lesions produced by the parent NSAID due to slower hydrolysis at stomach pH, and delayed conversion to piroxicam in the small intestine mucosa [27,43,44]. The anti-inflammatory activities of both prodrugs appear to be identical to piroxicam.

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Table I. Clinical Trials of Piroxicam Sponsored/Funded by NCI, DCPC

Study No. Title (PI) Period of Performance IND No.	Cancer Target	Study Population No. of Subjects	Dose(s) Study Duration	Endpoints	Remarks
Phase I (Safety, ADME)					
NO1-CN-85109-02 Phase I and Pharmacokinetic Studies of Difluoromethylornithine (DFMO) Plus Piroxicam (Dr. Paul P. Carbone, University of Wisconsin) 6/91–10/94 IND 39,462 (NCI)	---	Previously treated early stage basal cell or squamous cell skin cancer Piroxicam Arm 12 patients (6/dose)	Piroxicam Arm 10 mg qd or 10 mg qod for 6 months Piroxicam+DFMO Arm 10 mg piroxicam qod + 0.5g DFMO/m^2 qd	Piroxicam Arm Drug effect measurement: TxB_2 in platelets, ODC in skin punch biopsies, polyamines in urine Piroxicam+DFMO Arm Drug effect measurement: Urinary polyamines, 2, 3-dinor-TXB_2	Piroxicam Arm Two patients (10 mg qd) removed due to adverse effects: chronic tinnitus, mild nausea and gastritis. Other minor effects: shortness of breath, joint pain, body pain, diarrhea, chest pain, headaches and migraines Piroxicam+DFMO Arm Study in progress
Phase II (Dose titration, efficacy, intermediate biomarkers)					
Planned Study Phase IIa/IIb Chemoprevention Trials of DFMO + Piroxicam in Patients Previously Treated with BCG for Superficial Bladder Cancer 1995 IND 39,462	Bladder	Previous superficial bladder cancer resected and treated intravesically with BCG	Oral (doses to be determined) 1 month (Phase IIa) 1 year (Phase IIb)	Drug effect measurement: (to be determined) Efficacy: Tumor recurrence, histopathology, intermediate biomarkers (to be determined)	Efficacy and evaluation of intermediate biomarkers as surrogate endpoints

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Study No. Title (PI) Period of Performance IND No.	Cancer Target	Study Population No. of Subjects	Dose(s) Study Duration	Endpoints	Remarks
Phase II (Dose titration, efficacy, intermediate biomarkers) ***(continued)***					
PO1-CA-41108 Phase IIa Randomized Cancer Prevention Study of the Effects of Piroxicam on Colon Mucosa (Dr. David Earnest, University of Arizona) 9/86–4/95 (Program Project) IND 29,294	Colon	Patients aged 40–80 years with previous polyp removed within 10 years prior to entry	Oral 5, 7.5, 10, 20 mg qd for 3 months	Pharmacokinetics Drug effect measurement: Rectal mucosa PGE_2 Efficacy: [^{3}H]-Thymidine labelling	Study complete Plasma drug levels for 3 lowest doses were 2–4 μg/ml; the plasma level was 3-fold higher at 20 mg qd, which suggests nonlinear kinetics and accumulation Significant reductions in mucosal PGE_2 at doses of 7.5–20 mg qd; however, no effect on proliferation was observed. The 7.5 mg qd dose selected for Phase IIb Published report: [45]
PO1-CA-41108 Phase IIb Randomized Cancer Prevention Study of the Effects of Piroxicam on Colon Mucosa (Dr. David Earnest, University of Arizona) 9/86–4/95 (Program Project) IND 29,294	Colon	Patients aged 40–80 with previous 3 mm polyp removed within 6 months prior to entry 100 Patients	Oral 7.5 mg qd for 3 years	Drug effect measurement: PGE_2 concentration in rectal mucosa Efficacy: BrdU labeling index in rectal mucosa	Study in progress

Study No. Title (PI) Period of Performance IND No.	Cancer Target	Study Population No. of Subjects	Dose(s) Study Duration	Endpoints	Remarks
Phase II (Dose titration, efficacy, intermediate biomarkers) ***(continued)***					
Planned Study Phase II Chemoprevention Trials of DFMO + Piroxicam in Colon Cancer 1995 IND 39,462	Colon	Patients with previously resected colorectal adenomatous polyps	Oral 3 years	Efficacy: Intermediate biomarkers, histopathology	Efficacy (polyp reduction) and evaluation of intermediate biomarkers

PIROXICAM DEVELOPMENT STATUS