Levels of Rectal Mucosal Polyamines and Prostaglandin E2 Predict Ability of DFMO and Sulindac to Prevent Colorectal Adenoma

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BACKGROUND & AIMS: Combination of polyamine and prostaglandin E2 (PGE2)–synthesis inhibitors reduced the risk of colorectal adenoma (CRA) by 70% in patients who received polypectomies. We studied effects of the combination of difluoromethylornithine (DFMO) and sulindac on biomarkers and investigated factors that modify their efficacy.

METHODS: We analyzed rectal mucosal levels of polyamines (spermidine, spermine, and putrescine) and PGE2, treatment regimens, and risk of CRA in 267 participants of a phase IIb/III chemoprevention trial of DFMO/sulindac.

RESULTS: In the group that received DFMO/sulindac, spermidine-to-spermine ratio (Spd:Spm) in rectal mucosa decreased between baseline and 12- and 36-month follow-up examinations (0.30, 0.23, and 0.24, respectively; P < .001 for both comparisons to baseline). Putrescine levels decreased between baseline and 12 months (0.46 vs 0.15 nmol/mg protein; P < .001) but rebounded between 12 and 36 months (0.15 vs 0.36 nmol/mg protein; P = .001). PGE2 levels did not change, although aspirin use was significantly associated with lower baseline levels of PGE2. No significant associations were observed between changes in biomarker levels and efficacy. However, drug efficacy was greatest in subjects with low Spd:Spm and high PGE2 at baseline; none of these subjects, versus 39% of those given placebo, developed CRA (P < .001). Efficacy was lowest in subjects with high Spd:Spm and low PGE2 at baseline; 28% developed CRA, compared with 36% of patients given placebo (P = .563).

CONCLUSIONS: 

A combination of DFMO and sulindac significantly suppressed production of rectal mucosal polyamines but not PGE2. No relationship was found between changes in biomarker levels and response. However, baseline biomarker levels modified the effect of DFMO/sulindac for CRA prevention.

Keywords: Biomarkers; Chemoprevention; Colorectal Cancer.

We recently reported on the positive results of a randomized trial of difluoromethylornithine (DFMO) plus sulindac (a nonsteroidal anti-inflammatory drug, or NSAID) in subjects with a history of colorectal adenoma (CRA). In the postpolypectomy setting, combination DFMO/sulindac was associated with a significant 70% risk reduction in the development of metachronous CRA and >90% risk reduction in advanced or multiple lesions, compared with double placebo. DFMO is a suicide inhibitor of ornithine decarboxylase (ODC) that has been shown to decrease colorectal tissue polyamine levels and tumorigenesis. Polyamines are produced as a critical component of cellular metabolism, acting as signaling molecules for cell growth. The pharmacologic inhibition of polyamine synthesis results in growth arrest and prevention of tumor development in animal models. Sulindac acts on both prostaglandin E2 (PGE2) and polyamine metabolism through inhibition of cyclooxygenase-2 (COX-2)–derived prostaglandins and by increased cellular polyamine catabolism and export. The role of prostaglandins in tumorigenesis is well documented. Modulation of these pathways and their mediators, polyamines and PGE2, is strongly suspected to explain the anticancer activity of DFMO and NSAIDs.

In the present analysis, we examined the relationships between polyamine and PGE2 levels in normal rectal mucosa, DFMO/sulindac treatment, and development of metachronous CRA among subjects treated after polypectomy. We hypothesized that tissue polyamine and PGE2 levels would decrease in response to DFMO/sulindac treatment as biomarkers of drug response. Secondarily, we investigated the relationships between these biomarker levels and DFMO/sulindac drug efficacy. We also explored the potential role of low-dose aspirin use in these associations.

Abbreviations used in this paper: CI, confidence interval; COX, cyclooxygenase; CRA, colorectal adenoma; CV, coefficient of variation; DFMO, difluoromethylornithine; NSAID, nonsteroidal anti-inflammatory drug; ODC, ornithine decarboxylase; PGE2, prostaglandin E2; RR, relative risk; Spd:Spm, spermidine-to-spermine ratio.
Subjects and Methods

Study Population

The details of our phase IIb/III clinical trial (NCT00005882, NCT00118365) of combination DFMO/sulindac have been reported. Briefly, this double-blinded, placebo-controlled, multicenter randomized trial tested the efficacy of combination DFMO/sulindac on preventing metachronous CRA. A total of 375 participants with prior history of CRA were randomly assigned to receive daily DFMO (500 mg) plus sulindac (150 mg) or double placebo for 3 years (Supplementary Figure 1). Data on self-reported regular low-dose aspirin use (≤81 mg daily or ≤325 mg twice weekly) were collected at baseline; patients using full-dose aspirin (325 mg/d) were excluded from the study. The trial was terminated early by the Data and Safety Monitoring Board because efficacy endpoints had been achieved; thus, 267 participants had an end-of-study colonoscopy. Of these 267 participants, 178 were from the phase IIb portion of the study and 89 from the phase III. In the phase IIb protocol, a 12-month rectal biopsy was required for eligibility but involved a less-invasive endoscopy procedure and was not a colonoscopy. This eligibility requirement was dropped for the phase III component. Thus, subjects in the phase IIb protocol had a 12-month endoscopy procedure specifically for rectal biopsy (n = 177 performed). The mean time to the end-of-study colonoscopy for the 267 subjects was 36.0 ± 4.3 months and is designated as “36 months.”

Colon Biopsy Sample Collection

Normal (tumor-free) rectal mucosal biopsies were obtained during 3 separate endoscopy procedures: at baseline and after approximately 12 (phase IIb participants only) and 36 (end-of-study) months. Three of 8 cores were placed in separate standard MiniFuge tubes for polyamine analyses, and the remaining 5 were placed in similar tubes containing indomethacin in buffer to inhibit in vitro formation of PGE2; all 8 were immediately snap-frozen in liquid nitrogen.

PGE2 Content

PGE2 analysis was performed as described previously. Briefly, tissue cores that were frozen in indomethacin were thawed, homogenized in an indomethacin buffer, and clarified at low speed. PGE2 content was measured in the supernatant with the use of the Prostaglandin E2 Biotrak enzyme immunoassay system (GE Healthcare Life Sciences, Piscataway, NJ). Samples were assayed in duplicate at 2 dilutions that were 10-fold different in concentration. Nine samples were excluded from the data set because of vial mishandling. Values that fell outside the linear portion of the curve were also excluded: 41 participants (19 placebo, 22 treatment) at baseline, 19 (11 placebo, 8 treatment) at 12 months, and 51 (27 placebo, 24 baseline) at 36 months. Furthermore, values for which the coefficient of variation (CV) between duplicates was >15% were excluded: 0 participants at baseline, 1 (placebo) at 12 months, and 1 (placebo) at 36 months. Finally, values whose associated standard curves were of low quality were excluded: 37 participants (18 placebo, 19 treatment) at baseline, 46 (24 placebo, 22 treatment) at 12 months, and 50 (31 placebo, 19 treatment) at 36 months. Among those who finished the trial, 192 participants (95 placebo, 97 treatment) had valid PGE2 measurements at baseline, along with 108 (43 placebo, 65 treatment) at 12 months and 140 (70 placebo, 70 treatment) at 36 months. A sensitivity analysis was performed by conducting analyses with and without the excluded values. Including these values introduced noise, as would be expected; the results did not substantially differ, but generally they were less precise, and the significance of the associations was diminished. The mean CV for all samples falling within the linear range (excluding all nonlinear samples and respective duplicates) was 11.3%; after excluding measurements with CV >15%, the mean CV was 5.7%. Measures below the limit of detection were set to the lower-limit value of 0.04 ng/mg protein.

Polyamine Content

Polyamine analysis was performed as described previously. Briefly, frozen tissue cores were homogenized and extracted. Polyamine (spermidine, spermine, and putrescine) content was measured with the use of reverse-phase, ion-paired high-performance liquid chromatography. Protein contents were determined with the use of the bicinchoninic acid protein assay (Thermo Fisher Scientific, Rockford, IL). The same 9 samples as for PGE2 were excluded from the data set because of vial mishandling. We used the spermidine-to-spermine ratio (Spd:Spm) in our analyses to minimize the influence of assay variability. The determinations of day-to-day precision and accuracy of measurements for the polyamines were carried out on the basis of the performance of the standards, which were separately injected at intervals of every 5 study specimens. Every study specimen was spiked with an internal standard to estimate recovery and to assess performance of the chromatography. The CV for the measurement of the internal spiked sample 1,6-diaminohexane was <10% in all batches conducted in the study and averaged 5.45%. The CV for the measurement of spermine, spermidine, and putrescine in the standard in all runs was below 15% and averaged 5.06%, 2.82%, and 3.07%, respectively. Among those who finished the trial, 265 participants (129 treatment, 136 placebo) had valid polyamine measurements at baseline, along with 172 (76 treatment, 96 placebo) at 12 months and 241 (118 treatment, 123 placebo) at 36 months. Measures below the limit of detection were set to the lower-limit value of 0.01 nmol/mg protein.
Table 1. Baseline Characteristics of Clinical Trial Participants, by Treatment Group

|                          | All randomized participants (n = 375) | Subjects who completed the trial (n = 267) |
|--------------------------|--------------------------------------|-------------------------------------------|
|                          | Placebo (n = 184)                    | DFMO/sulindac (n = 191)                   |
|                          | Placebo (n = 129)                    | DFMO/sulindac (n = 138)                   |
| Male sex, n (%)          | 138 (75.0)                           | 147 (77.0)                                | 94 (72.9)                                | 108 (78.3) |
| Age (y), mean ± SD       | 61.4 ± 8.2                           | 60.6 ± 8.6                                | 61.4 ± 8.0                               | 60.2 ± 8.5 |
| White ethnicity/race, n (%) | 157 (85.3)                           | 155 (81.2)                                | 109 (84.5)                               | 115 (83.3) |
| Aspirin use, n (%)a      | 69 (37.5)                            | 77 (40.3)                                 | 50 (38.8)                                | 53 (38.4)  |
| PGE2 (ng/mg protein), mean ± SDb | 0.67 ± 0.94                         | 0.81 ± 1.32                               | 0.53 ± 0.59                              | 0.66 ± 0.99 |
| Spd:Spm, medianb         | 0.38                                 | 0.33                                      | 0.38                                     | 0.27       |
| Putrescine (nmol/mg protein), mean ± SDc | 0.63 ± 0.55                         | 0.70 ± 0.82                               | 0.64 ± 0.54                              | 0.69 ± 0.83 |
| Putrescine (nmol/mg protein), medianc | 0.50                                | 0.46                                      | 0.50                                     | 0.46       |

DFMO, difluoromethylornithine; PGE2, prostaglandin E2; SD, standard deviation; Spd:Spm, spermidine-to-spermine ratio.
aSelf-reported regular aspirin use at baseline.
bSample size for PGE2 was 134 (placebo) and 135 (treatment) among all participants randomly assigned; 95 (placebo) and 97 (treatment) among those who finished the trial.
cSample size for polyamines was 184 (placebo) and 188 (treatment) among all participants randomly assigned; 129 (placebo) and 136 (treatment) among those who finished the trial.

Assessment of Metachronous CRA

Endpoint colonoscopies were performed approximately 36 months (the mean time was 36.0 ± 4.3 months) after baseline colonoscopy for 267 participants. The reports for all resected polyps were submitted for central pathology review. A person was classified as having a metachronous CRA if ≥1 adenomatous polyp or adenocarcinoma was diagnosed during the trial.

Statistical Methods

Individuals’ tissue biomarker (PGE2, Spd:Spm, and putrescine) levels were tested for change between each pair of time points (baseline and 12 months, baseline and 36 months, 12 months and 36 months) with the use of nonparametric pairwise sign-rank tests. Relative risks (RRs) and 95% confidence intervals (CIs) were calculated for the association between DFMO/sulindac treatment and CRA, stratified by baseline biomarker levels and by biomarker response. For baseline biomarker levels, we divided the population into “low” versus “high” groups according to the median of the study population. We defined biomarker response with a cut point of ≥30% decrease in biomarker levels between baseline and 36 months, to be consistent with standard clinical trial assessments for solid tumors. Logistic regression was used to model the presence of metachronous CRA as the outcome variable and predictors that included treatment, baseline biomarker group (or biomarker response), and interaction between treatment and baseline biomarker group (or biomarker response). Similarly, logistic regression was used to model the presence of metachronous CRA as the outcome variable and predictors that included treatment, aspirin use, and interaction between treatment and aspirin use. Likelihood ratio tests were applied to test for interactions. Adjustment for age and sex did not substantially change the models, so we present unadjusted models only. Fisher’s exact tests were used to assess differential drug efficacy when considering different biomarkers simultaneously. All statistical analyses were performed with Stata 11.0 (StataCorp, College Station, TX), all reported P values are 2-sided, and no adjustments were made for multiple comparisons.

Results

Baseline Characteristics

The treatment arms were well balanced on sex (76% male), age (mean, 61 years), ethnicity (83% white), and self-reported regular low-dose aspirin use (39%; Table 1). No significant differences were observed in any baseline characteristics between the participants who did and did not complete the trial. In addition, no substantial differences were observed in baseline measures of PGE2, Spd:Spm, or putrescine by treatment group. Aspirin users had significantly lower median PGE2 levels at baseline than nonaspirin users (0.21 vs 0.42 ng/mg protein; P < .001; Table 2). We detected no significant differences in baseline Spd:Spm or putrescine levels according to aspirin use.

Effect of DFMO/Sulindac on Biomarker Levels

The mean (± SD) time between baseline and the 12-month rectal biopsy procedure was 13.0 ± 1.6 months, and the mean (± SD) time to the 36-month procedure was 36.0 ± 4.3 months. The mean time to the 12-month procedure in the placebo and treatment arms was 12.8 and 13.2 months, respectively (P = .254), and the mean
time to the 36-month procedure in the 2 groups was 35.7 and 36.3 months, respectively ($P = .304$). We detected no change in median PGE2 levels between any pair of time points in either group (all $P > .05$; Figure 1A).

Median Spd:Spm levels decreased significantly in the DFMO/sulindac group between baseline and 12 months (0.30 vs 0.23; $P < .001$) and between baseline and 36 months (0.30 vs 0.24; $P < .001$), but no significant change was observed between 12 and 36 months (0.23 vs 0.24; $P = .638$; Figure 1B). In the placebo group, Spd:Spm levels increased slightly between baseline and 12 months (0.30 vs 0.31; $P = .012$), but no significant change was observed between baseline and 36 months (0.30 – 0.29; $P = .459$) or between 12 and 36 months (0.31– 0.29; $P = .368$).

Median putrescine levels decreased significantly in the DFMO/sulindac group between baseline and 12 months (0.46 vs 0.15; $P < .001$), but they increased between 12 and 36 months (0.15 vs 0.36; $P = .001$; Figure 1C). The apparent rebound did not fully restore putrescine levels to baseline concentrations, but no significant difference was observed in putrescine levels between baseline and 36 months (0.46 vs 0.36; $P = .108$). In the placebo group, putrescine levels increased slightly between baseline and 12 months (0.50 vs 0.61; $P = .041$), but no significant change was observed between baseline and 36 months (0.50 vs 0.55; $P = .359$) or between 12 and 36 months (0.61 vs 0.55; $P = .634$).

**Effect of Baseline Biomarker Levels on the Association Between Treatment and Metachronous CRA**

We stratified the analysis of the association between DFMO/sulindac treatment and metachronous CRA by baseline biomarker levels. We found no signifi-
cant differences in the effect of treatment on metachro-
nous CRA according to individual baseline biomarker 
levels (all \(P > .05\); Table 3). However, our results suggest 
possible effect modification by baseline Spd:Spm ratio 
(\(P = .087\)), whereby participants with low baseline Spd: 
Spm achieved a nonsignificant 2.5-fold greater reduction 
in risk with DFMO/sulindac (RR = 0.17; 95% CI, 0.07–
0.41) than those with high baseline Spd:Spm (RR = 0.42; 
95% CI, 0.23–0.77). Participants with high baseline PGE2 
had a similar, nonsignificant 2.5-fold greater benefit from 
treatment (RR = 0.17; 95% CI, 0.05–0.54) than those 
with low baseline PGE2 (RR = 0.50; 95% CI, 0.28–0.91; \(P 
= .132\)). When we restricted these analyses to subjects 
who received their follow-up colonoscopy between 33 
and 39 months, our findings did not substantially 
change, although the interaction between treatment and 
baseline Spd:Spm levels on CRA became more significant 
(\(P = .040\)). Baseline polyamine and PGE2 levels were not 
correlated (\(P = .034, P = .639\)), suggesting that any 
effect modification of treatment response by each of 
these biomarkers probably act independently of each 
other.

When considering these 2 biomarkers simultaneously, 
we observed an important combined effect of baseline 
PGE2 and Spd:Spm on treatment efficacy. Among par-
ticipants with both low Spd:Spm and high PGE2, 0 of 24 
(0%) participants developed CRA on treatment, compared 
with 11 of 28 (39%) in the placebo group (\(P < .001\; 
\text{Figure 2})). Among participants with low PGE2 and low 
Spd:Spm, 3 of 21 (14%) treated persons developed CRA, 
compared with 11 of 20 (55%) in the placebo group (\(P 
= .009\)). Among participants with high PGE2 and high

### Table 3. Treatment Efficacy in Preventing Metachronous Colorectal Adenoma, Stratified by Biomarker Level at Baseline

| Biomarker level at baseline\(^a\) | Low                  | High                 |
|----------------------------------|----------------------|----------------------|
| **PGE2**                        |                      |                      |
| Placebo, events/total (%)        | 19/42 (45.2)         | 21/53 (39.6)         |
| DFMO/sulindac, events/total (%)  | 12/53 (22.6)         | 3/44 (6.82)          |
| RR (95% CI)                      | 0.50 (0.28–0.91)     | 0.17 (0.05–0.54)     |
| **Spd:Spm**                      |                      |                      |
| Placebo, events/total (%)        | 31/68 (45.6)         | 24/61 (39.3)         |
| DFMO/sulindac, events/total (%)  | 5/64 (7.81)          | 12/72 (16.7)         |
| RR (95% CI)                      | 0.17 (0.07–0.41)     | 0.42 (0.23–0.77)     |
| **Putrescine**                   |                      |                      |
| Placebo, events/total (%)        | 24/62 (38.7)         | 31/67 (46.3)         |
| DFMO/sulindac, events/total (%)  | 7/70 (10.0)          | 10/66 (15.2)         |
| RR (95% CI)                      | 0.26 (0.12–0.56)     | 0.33 (0.18–0.61)     |

Cl, confidence interval; DFMO, difluoromethylomithine; PGE2, prostaglandin E2; RR, relative risk; Spd:Spm, spermidine-to-spermine ratio.
\(^a\)Low versus high determined by the median biomarker level: low is below the median; high is above the median.
\(^b\)\(P\) value for the likelihood ratio test of interaction between treatment group and low versus high biomarker level at baseline.

**Figure 2.** DFMO/sulindac efficacy, by biomarker levels. Participants were categorized according to their baseline levels of prostaglandin E2 (PGE2) and the spermidine-to-spermine ratio (Spd:Spm). Low versus high cut points were defined by the median biomarker levels in the study population. The table below the figure includes the number (and percentage) of participants with metachronous adenoma in each stratum. A Fisher’s exact test was conducted to test for a significant difference between the 2 groups in the proportion of persons with metachronous adenoma.
Putrescine Spd:Spm, 3 of 20 (15%) treated persons developed CRA, compared with 10 of 25 (40%) in the placebo group \( (P = .100) \). Finally, among participants with low PGE2 and high Spd:Spm, 9 of 32 (28%) treated persons developed CRA, compared with 8 of 22 (36%) in the placebo group \( (P = .563) \). Although limited by small numbers, these results suggest that baseline biomarker levels, in combination, are important determinants of DFMO/sulindac efficacy.

**Effect of Changing Biomarker Levels on the Association Between Treatment and Metachronous Adenoma**

We also stratified the analysis of the association between DFMO/sulindac treatment and metachronous CRA by biomarker response. We divided the study population into 2 groups according to the level of biomarker response, which was defined as a decrease in biomarker level \( \geq 30\% \) between baseline and 36 months. For all 3 biomarkers (PGE2, Spd:Spm, and putrescine), we detected no significant interactions between biomarker response and DFMO/sulindac treatment on metachronous CRA \( (P > .1; \text{Table 4}) \). Participants with \( \geq 30\% \) decrease in Spd:Spm may have achieved a greater benefit from DFMO/sulindac treatment \( (RR = 0.23; 95\% \text{ CI}, 0.11–0.49) \) than participants lacking Spd:Spm response \( (RR = 0.49; 95\% \text{ CI}, 0.25–0.96) \). However, tests for interaction between Spd:Spm response and treatment on CRA were not significant \( (P = .202) \), even after restricting the analysis to subjects with a final colonoscopy between 33 and 39 months \( (P = .482) \).

**Potential Effect Modification by Aspirin**

Given the recognized inhibitory action of aspirin on rectal mucosal PGE2 levels (see Table 2 and Krishnan et al\(^1\)) and suspected role in polyamine catabolism,\(^2\) we investigated whether potential effect modification by self-reported low-dose aspirin use at baseline could account for differences in treatment response by subgroups in Figure 2. We detected no interaction between aspirin and DFMO/sulindac treatment on metachronous CRA \( (P = .443) \). Furthermore, we detected no significant interactions between aspirin and DFMO/sulindac on biomarker response (defined as \( \geq 30\% \) decrease in biomarker level between baseline and 36 months) for PGE2 \( (P = .582) \), Spd:Spm \( (P = .140) \), or putrescine \( (P = .868) \). To explore this question further, we conducted a multivariate analysis by adjusting for aspirin use. We found no evidence that aspirin use confounds the relationships between biomarker levels and drug efficacy. Thus, despite the significant association observed between aspirin and baseline PGE2 levels, aspirin use at baseline does not appear to explain the differential drug efficacy by biomarker levels shown in Figure 2. However, this analysis is limited by its small sample size, as well as imprecise measures of aspirin exposure, and we cannot rule out the potential for effect modification of DFMO/sulindac by aspirin use.

**Discussion**

In this study, we examined the effect of combination DFMO/sulindac treatment on rectal mucosal concentrations of polyamines and PGE2 as putative drug response biomarkers. Sulindac, an NSAID and nonselective COX inhibitor, has known inhibitory action on PGE2 as well as effects on polyamine transport.\(^5\) DFMO inhibits ODC with demonstrated effects on tissue polyamine levels, particularly putrescine and spermidine.\(^13\) We also explored the potential effect of self-reported low-dose aspirin use on biomarker levels, because aspirin is a commonly used NSAID with similar properties to sulindac.

Consistent with previous studies showing that DFMO treatment depletes putrescine and lowers Spd:Spm in rectal mucosa,\(^3\),\(^13\) we found a significant effect of DFMO/
sulindac on rectal mucosal polyamines. In the treatment group, the decline in Spd:Spm was achieved by 12 months, with no evidence of additional change between 12 and 36 months. Putrescine levels showed a similar behavior to Spd:Spm, with change achieved by 12 months. However, putrescine subsequently increased between 12 and 36 months; consequently, putrescine levels at 36 months were no longer significantly different from those at baseline. A similar pattern was observed in a prior phase IIb biomarker trial investigating tissue polyamine responses in patients with CRA after random assignment to 0.075 g/m² DFMO, 0.2 g/m² DFMO, 0.4 g/m² DFMO, or placebo. In the 2 lower-dose DFMO groups (approximately 150 mg/d and 400 mg/d; ie, lower than the 500 mg/d dose used in the present study), putrescine inhibition was observed at the earlier time point (6 months) but not the later time point (12 months). In contrast, putrescine levels remained suppressed at both 6 and 12 months in the highest DFMO group (approximately 800 mg/d). Effects of DFMO on putrescine levels at 36 months were not previously assessed. Our findings here in the 3-year trial validate the results of the prior dose de-escalation study and support the selection of 500 mg/d as the lowest possible dose to achieve an effect on polyamine endpoints at the tissue level. Taken together, these findings support the potential for an adaptive tissue response to prolonged ODC suppression, resulting in increased cellular uptake of diet- or bacteria-derived putrescine from the colonic lumen,13,14 Putrescine is an important factor in colonic mucosal renewal15; thus, prolonged inhibition of ODC with DFMO and increased export by sulindac may lead to compensatory uptake of luminal polyamines in the normal rectal mucosa.

In contrast with the polyamines, we found no measurable effect of treatment on PGE2 concentrations in rectal mucosa. Although we observed a clear difference in PGE2 levels at baseline between aspirin users and nonusers that persisted at both 6 and 12 months in the highest DFMO group (approximately 800 mg/d). Effects of DFMO on PGE2 at 12 or 36 months. The reason for the lack of treatment effect on tissue PGE2 levels is unknown and should be interpreted with caution, given the sample size. The lack of evidence for a strong effect of treatment on PGE2 could alternatively suggest that treatment-associated reductions in colorectal tissue polyamine contents, as shown in this report, might be more important than previously appreciated for CRA prevention with combination DFMO/sulindac, as reported earlier.1 As noted, sulindac has been shown to activate polyamine catabolism and export,7 possibly acting to complement the effects of DFMO to reduce tissue polyamine pools. This hypothesis needs to be tested in future clinical trials.

When we evaluated the role of biomarker change and response to intervention, we found no effect of biomarker response (ie, percentage of change over time) on DFMO/sulindac efficacy for any of the 3 biomarkers investigated. We had hypothesized that persons who achieved benefit from treatment would show greater reductions in their polyamine or PGE2 levels. Note that we observed a nonsignificant greater benefit for participants who exhibited a ≥30% decrease in Spd:Spm (ie, responders) than did nonresponders.

To further explore a potential mechanistic role for PGE2 and polyamines in DFMO/sulindac treatment for CRA prevention, we stratified the association between treatment and metachronous CRA by baseline biomarker levels. We investigated whether baseline status of the biomarker could act as an indicator of drug response. Although we lacked sufficient power to detect statistically significant differences, we found evidence suggesting that baseline PGE2 and Spd:Spm levels may modify responsiveness to DFMO/sulindac for CRA prevention. Our findings suggest that subjects with low baseline Spd:Spm were more responsive to the chemopreventive effects of DFMO/sulindac than were persons with high baseline Spd:Spm. Interestingly, the most responsive subgroup to treatment were subjects who entered the study with both high PGE2 and low Spd:Spm in their rectal mucosal biopsy. This subgroup experienced zero recurrences, compared with 39% observed in the corresponding placebo subgroup. Strikingly, the subgroup with the exact opposite characteristics, low PGE2 and high Spd:Spm, showed no significant treatment benefit. These findings suggest that high Spd:Spm levels in tissues, which may differ among persons as a consequence of genetic variability (eg, ODC polymorphisms) and/or exposures to dietary or gut flora polyamines, render subjects less pharmacologically responsive to DFMO/sulindac for CRA prevention. Further, the magnitude of risk reduction for CRA appears to be reduced in persons who do not express a COX-dependent, pro-PGE2 background in the target tissue. Importantly, among persons with low baseline Spd:Spm levels, we observed significant risk reduction with DFMO/sulindac even in the absence of high baseline PGE2, although this subgroup experienced a few “breakthrough” CRAs. Possible COX2 negativity of these few CRAs should be considered. Although highly speculative, we believe that these results show an important role for polyamine metabolism in CRA risk and suggest that the combination of DFMO/sulindac is most effective when the NSAID target (COX2) is present and the polyamine levels are in a treatable range (low). In a separate analysis, we found significant metachronous CRA risk reduction ascribed to DFMO/sulindac in patients consuming low-to-moderate levels of dietary polyamines, but there was no benefit among persons in the highest quartile of polyamine intake (Raj KP et al, unpublished results; Abstract 279; Gastrointestinal Cancers Symposium; Orlando, FL; January 22–24, 2010). Taken together, these data also suggest that the ability to overcome high polyamine background levels (eg, dietary
modification to reduce polyamine intakes or other drug targets in the pathway) may lead to even greater efficacy of drug combinations directed at the polyamine/COX2 or polyamine/“other” pathways. Although these results derive from subgroup analyses, they strongly suggest a role for baseline PGE2 and polyamine status as potential drug-response predictors and warrant further investigation.

A number of limitations are noted in the current study. A strong overall effect of DFMO/sulindac treatment on the development of CRA and resulting small sample size from the early termination of the trial yielded inadequate statistical power. Thus, we were limited in our ability to fully study the relationships between biomarker levels and CRA development as originally planned. Additional limitations are inherent to the measurement of rectal mucosal levels of PGE2 and polyamines, which are prone to measurement error, and, as intermediate markers, they may not accurately reflect biological effects at the CRA level. This latter issue may be particularly true for normal rectal colonocytes, which, unlike colonocytes taken from neoplastic tissues, express low levels of the DFMO and sulindac drug targets, ODC\textsuperscript{16} and COX2,\textsuperscript{17} respectively. Further, greater dependence of normal rectal mucosa on exogenously derived luminal polyamines\textsuperscript{18} may explain the significant increase in putrescine between 12 and 36 months and our inability to associate biomarker change in the surrogate tissue with response to intervention.

In conclusion, future studies should consider the measurement of surrogate tissue sources of Spd:Spm and PGE2 as potential baseline predictors of drug responsiveness. Investigation of urinary measures, as suggested by Kawakita and Hiramatsu,\textsuperscript{19} may prove more feasible in monitoring response to DFMO/NSAIDs in planned future trials than the collection of rectal mucosal biopsy specimens. Finally, although the number of metachronous CRAs in the DFMO/sulindac treatment group was low, additional study of the factors contributing to these “breakthrough” adenomas is needed.

**Supplementary Material**

Note: To access the supplementary material accompanying this article, visit the online version of *Gastroenterology* at www.gastrojournal.org, and at doi: 10.1053/j.gastro.2010.06.005.

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Conflicts of interest
The authors disclose the following: F.L.M. and E.W.G. have ownership interest in Cancer Prevention Pharmaceuticals, LLC. The remaining authors disclose no conflicts.

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Supplementary Figure 1. Study schema.

502 Patients assessed for eligibility

375 Randomized

184 Placebo

55 Lack follow-up colonoscopy

129 Completed trial

Data used in current analysis:
- 95 Baseline PGE2
- 129 Baseline polyamines
- 43 12-month PGE2
- 76 12-month polyamines
- 70 36-month PGE2
- 118 36-month polyamines

191 500 mg DFMO/150 mg sulindac

53 Lack follow-up colonoscopy

138 Completed trial

Data used in current analysis:
- 97 Baseline PGE2
- 136 Baseline polyamines
- 65 12-month PGE2
- 96 12-month polyamines
- 70 36-month PGE2
- 123 36-month polyamines