Research Article

Cancer Prevention Research

Efficacy of Difluoromethylornithine and Aspirin for Treatment of Adenomas and Aberrant Crypt Foci in Patients with Prior Advanced Colorectal Neoplasms

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Abstract

Difluoromethylornithine (DFMO), an inhibitor of polyamine synthesis, was shown to act synergistically with a NSAID for chemoprevention of colorectal neoplasia. We determined the efficacy and safety of DFMO plus aspirin for prevention of colorectal adenomas and regression of rectal aberrant crypt foci (ACF) in patients with prior advanced adenomas or cancer. A double-blinded, placebo-controlled trial was performed in 104 subjects (age 46–83) randomized (1:1) to receive daily DFMO (500 mg orally) plus aspirin (325 mg) or matched placebos for one year. All polyps were removed at baseline. Adenoma number (primary endpoint) and rectal ACF (index cluster and total) were evaluated at a one year colonoscopy. ACF were identified by chromoendoscopy. Toxicity was monitored, including audiometry. Eighty-seven subjects were evaluable for adenomas or ACF modulation ($n = 62$). At one year of treatment, adenomas were detected in 16 (38.1%) subjects in the DFMO plus aspirin arm ($n = 42$) versus 18 (40.9%) in the placebo arm ($n = 44$; $P = 0.790$); advanced adenomas were similar ($n = 3$/arm). DFMO plus aspirin was associated with a statistically significant reduction in the median number of rectal ACF compared with placebo ($P = 0.036$). Total rectal ACF burden was also reduced in the treatment versus the placebo arm relative to baseline (74% vs. 45%, $P = 0.020$). No increase in adverse events, including ototoxicity, was observed in the treatment versus placebo arms. While adenoma recurrence was not significantly reduced by one year of DFMO plus aspirin, the drug combination significantly reduced rectal ACF number consistent with a chemopreventive effect.

Introduction

Colorectal cancer is the fourth most common cancer and second leading cause of cancer-related mortality in the United States (1). Colonoscopy has been shown to effectively reduce colorectal cancer and disease-related mortality (2), yet it is an invasive test that must be repeated at regular intervals and is limited by a significant miss rate for colorectal adenomas estimated to be 26% in a meta-analysis of 43 publications involving approximately 15,000 tandem colonoscopies (3, 4) Chemoprevention is an attractive strategy, especially in high risk patients, but requires agents that are effective, safe, and well tolerated for long-term administration. The combination of difluoromethylornithine (DFMO), an inhibitor of polyamine biosynthesis, and sulindac was associated with marked reduction (approximately 70%) in colorectal adenoma recurrence at 3 years relative to matching placebo in a randomized trial (5). A synergistic interaction between DFMO and sulindac or aspirin was observed in animal models of colon cancer (6–10) that was related to a reduction in polyamine synthesis by DFMO and a concurrent increase in cellular export of polyamines due to activation of spermidine/spermine acetyl transferase (SAT1) by an NSAID (11, 12). Aspirin is an attractive drug

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for chemoprevention given abundant evidence from observational studies and interventional trials supporting its efficacy for the prevention of colorectal adenomas (13–17). Furthermore, the preventive effects of aspirin on adenomas may differ by polyp location (18). Of note, however, is that higher all-cause mortality was observed among apparently healthy older adults (≥70 years for whites) who received daily aspirin than among those who received placebo that was attributed primarily to cancer-related death that included colorectal cancer (19). Results are inconsistent with prior observational studies, and longer term follow-up data from this study are awaited. Aspirin demonstrates cardiovascular safety for long-term use in contrast to other NSAIDs (20). Further advances in the area of chemoprevention are needed that include maintaining drug efficacy while reducing treatment duration to enhance patient acceptance and compliance, and a surrogate endpoint biomarker (SEB) of efficacy that has remained elusive.

DFMO and aspirin have each been shown to significantly reduce the incidence and multiplicity of aberrant crypt foci (ACF) in rodent models of colon carcinogenesis (9), yet data in humans for this combination are lacking. ACF are clusters of abnormal-appearing crypts in the colorectal mucosa that are believed to be precursors of adenomas (21–23). ACF are a potential surrogate endpoint biomarker, and studies in a Japanese cohort found that ACF can be eradicated in only a few months by certain chemopreventive agents (24–26) compared with prevention of adenomas that took at least one or two years. ACF can be visualized at colonoscopy by application of a mucosal dye, that is, chromoendoscopy (27). Prior studies have shown a stepwise increase in the number of rectal ACF from normal subjects to those with adenoma and colorectal cancer (27), and multiple studies demonstrate a positive correlation between endoscopic ACF counts and synchronous or prior colorectal adenomas (24, 28–33). Human ACF are located predominantly within the distal colorectum (27, 34, 35) and are mostly hyperplastic (34), although a subset show dysplasia (36, 37). To date, chemoprevention studies in humans have shown conflicting and mostly negative data for the targeting of ACF (27–29, 38), although studies in patients with prior advanced adenomas or colorectal cancer have not been performed. Given the strong association of ACF with colonic neoplastic development and progression and their potential utility as a surrogate endpoint biomarker that could lead to shorter studies to evaluate chemopreventive efficacy, further study of ACF is warranted.

We conducted a randomized, placebo-controlled trial of DFMO plus aspirin for the prevention of adenoma recurrence and regression of rectal ACF number. Adenoma recurrence rates among patients with prior sporadic adenomas or colon cancer range from 27% to 47% in the placebo arms of randomized chemoprevention trials (13–15). The aspirin dose selected (325 mg/day) was associated with a significant reduction in colorectal adenoma recurrence in a placebo-controlled trial in patients with prior colon cancer (14). In contrast to aspirin, other NSAIDs have been associated with cardiovascular toxicity (20, 39) making them less than ideal for chemoprevention. We chose a one year intervention based on the potent efficacy for the combination of DFMO (500 mg/day) plus sulindac seen in patients with sporadic adenomas (5). We selected patients with a history of prior advanced adenomas or colon cancer since chemoprevention strategies are a major unmet need in this high-risk population.

Materials and Methods

Study population and design

We conducted a randomized, double-blinded, and placebo-controlled trial. Participants ($N = 104$) ages 46 to 83 years were randomized to receive DFMO (500 mg once daily) plus aspirin (325 mg once daily) or matching placebo that were taken continuously for 1 year. This aspirin dose was chosen based on a study showing a statistically significant chemopreventive effect in patients with prior colon cancer (14). All patients were required to have a surveillance colonoscopy to the cecum within 45 days prior to randomization that included removal of all polyps. In addition, all patients received an end-of-study colonoscopy performed at 1 year postrandomization. Those undergoing baseline post enrollment colonoscopy had chromoendoscopy of the distal sigmoid and rectum. Subjects who had a prestudy colonoscopy within 45 days of study enrollment underwent flexible sigmoidoscopy with chromoendoscopy for identification and quantitation of rectal ACF. Evaluation of rectal ACF is described in detail below. We recorded data for total adenoma number, number of adenomas with size ≥ 5 mm, and advanced adenomas, defined as size of ≥ 10 mm by endoscopic assessment, villous or tubulovillous histology, or high-grade dysplasia. All polypectomy tissue underwent histopathologic review at Mayo Clinic (Rochester, MN). All study-related colonoscopies (Olympus Corp., 190-series) were performed by endoscopists at Mayo Clinic who were associated with the study protocol. A specific bowel preparation was not specified per protocol, however, Movi-Prep (Salix Pharmaceuticals) is generally used for patients undergoing colonoscopy at our institution.

Eligibility criteria were as follows: at least 40 years of age with a history of histologically confirmed, advanced colorectal adenomas or cancer; ECOG (Eastern Cooperative Oncology Group) performance status < 2, and a negative pregnancy test prior to enrollment among women of childbearing potential. A preregistration component was utilized to identify patients with five or more rectal ACF by chromoendoscopy who were eligible to participate. An

index cluster of five or more rectal ACF was chosen to enable an assessment of a quantifiable change after treatment. Those with five or more rectal ACF at baseline had chromoendoscopy as a component of their end-of-study colonoscopy. To enhance study accrual, the pre-registration component requiring a minimum number of 5 rectal ACF at baseline was dropped mid study because approximately 40% of subjects failed to meet such criteria and were excluded. Given this modification to the study design, the primary endpoint became metachronous adenomas (see Statistical analysis). Exclusion criteria were as follows: ileostomy/colostomy, rectal surgery, rectal cancer or prior pelvic radiotherapy. Regular use of NSAIDs, including aspirin, $\leq$ 6 weeks prior to randomization was not permitted and such patients had to undergo a "washout." Other exclusions include statins, corticosteroids, anticoagulant drugs, history of invasive malignancy (other than colon cancer) within the preceding 5 years; heritable cancer syndrome, inflammatory bowel disease, confirmed peptic ulcer disease. All participants were recruited and enrolled at Mayo Clinic, Rochester, and provided written informed consent. The study protocol was approved by the Institutional Review Board (IRB) at Mayo Clinic (Rochester, MN).

Safety and evaluation

At study enrollment, baseline history and physical examination, ECOG performance status, and laboratory testing were performed. All subjects underwent baseline and 1-year audiograms per protocol; in the event of symptoms, earlier evaluation was performed. Postrandomization evaluations were performed every month via telephone interview to monitor patient safety and to identify and record adverse events, adherence with the study medications, and use of concomitant medications. A follow-up clinic visit was conducted at month 6 that included a physical examination and laboratory testing. Safety and tolerability of the study drugs was evaluated by reviewing patterns of adverse events within and across the intervention and placebo groups. The NCI CTC Version 3.0 was used to grade all adverse events.

Chromoendoscopy for evaluation of rectal ACF

The rectum was defined as extending from the anal verge to the middle rectal fold ($\sim$15 cm). After removal of any identified polyps, the rectum was prepared for chromoendoscopy by application of 60 mL of 10% Mucomyst solution applied with a spray catheter to coat the mucosa with a dwell time of 1–2 minutes, after which washing with 60 mL of water was performed to remove residual mucus. Methylene blue dye (0.2%) was then applied to the rectal mucosa using a spray catheter (dwell time of 2 minutes; refs. 27, 40), and assessment of rectal ACF was performed by slow, deliberate pull-back of the endoscope. Still photography of regions of interest was performed, and the procedure was videotaped to enable future review. ACF number, region and distance from the anal verge were recorded.

In subjects with at least five rectal ACF at baseline, a repeat chromoendoscopy with ACF quantitation at study completion (1 year) was performed. An index cluster of 5 or more rectal ACF were identified at baseline and a mucosal tattoo (SPOT) was placed at the site to enable repeat evaluation of the same cluster/region. In patients with 4 or fewer rectal ACF, all identified ACF were biopsied at baseline and the tissue stored for further biomarker studies. All patients had biopsies of normal appearing mucosa of the sigmoid colon at baseline and at 1 year for future biomarker studies.

Statistical analysis

The primary study endpoint was adenoma number in the DFMO + aspirin study arm compared with the double placebo arm at the 1-year follow-up colonoscopy exam. All randomized participants who received at least one follow-up colonoscopy were considered evaluable for the primary endpoint per the intent-to-treat principle. We regarded a 50% reduction in adenoma number at year 1 to be clinically significant and would justify a phase III trial. On the basis of published data for adenoma recurrence rates ranging from 27% to 47% in the placebo arms of randomized chemoprevention trials (13–15), we estimated a recurrence rate of 40% for the double placebo arm. Assuming 47 evaluable participants per arm, we had 80% power to detect a decrease from 40% to 20% in the 1-year adenoma recurrence rate for the active treatment arm versus the double placebo arm (1-sided level of significance of 0.10). A comparison of the data was performed in nonaspirin users with those from the full study cohort.

A secondary endpoint was change in ACF number in an index cluster at one year when compared with baseline between the treatment arms. ACF were categorized as reduced or nonreduced based upon change in total ACF number quantified in the entire rectum. We also correlated the total ACF number with adenoma number at the one year end-of-study colonoscopy. χ^2 or Fisher Exact tests were used to test for the association between categorical variables, and Wilcoxon rank-sum P values were used for the association between continuous variables and 2-level categorical variables. Safety and tolerability of the study drugs was evaluated by reviewing patterns of adverse events, within and across the intervention and placebo group, via frequency tables and univariate statistics.

The studies were conducted in accordance with guidelines outlined in the Declaration of Helsinki. All subjects provided written informed consent and the study was approved by the Mayo Clinic IRB.

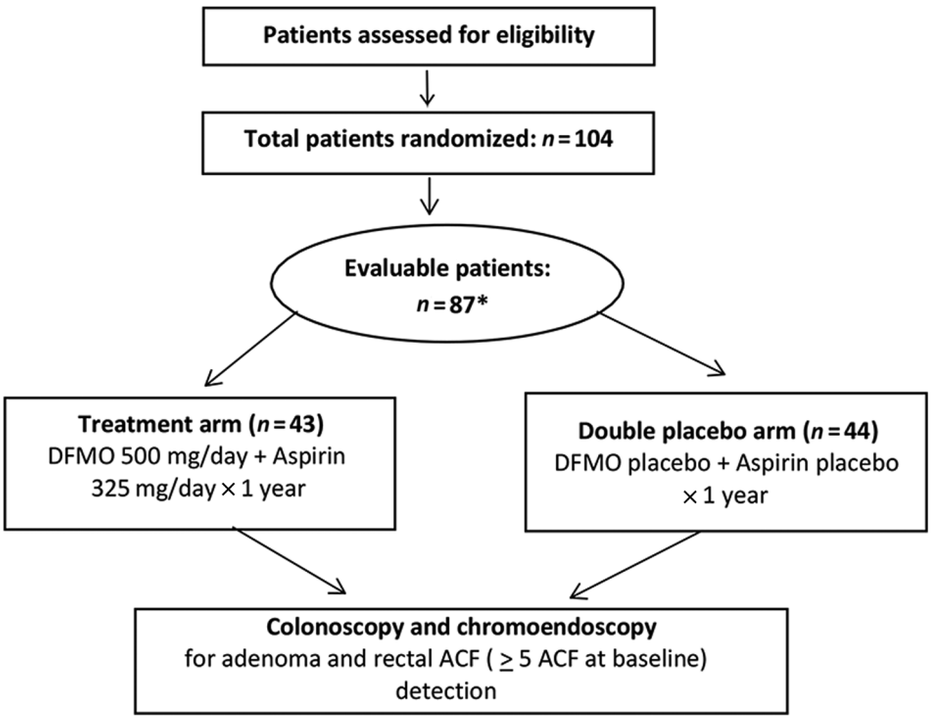

Figure 1.
Study schema.

Results

Baseline characteristics

All study participants had a history of prior advanced colorectal adenomas ($n = 102$) or colon cancer ($n = 2$). The study schema in shown in Figure 1. Patient characteristics at baseline, including age, sex, race, body mass index (BMI), and prior low-dose aspirin use (81 mg daily) were balanced between the study arms (Table 1). Among the 104 randomized patients, 87 were evaluable for the study endpoints including 86 for the 1-year adenoma recurrence rate (primary endpoint) and 62 for ACF modulation (secondary endpoint; Fig. 1); one patient was evaluable for ACF but not adenoma recurrence due to poor bowel preparation. There were 43 patients in the treatment arm and 44 in the placebo arm. Data were available on 104 patients for baseline characteristics and adverse events (Table 1). At least 80% adherence with the study medications based on pill counts was required. Reasons for patient non evaluable status included drop out or withdrawal with failure to complete the year 1 colonoscopy exam ($n = 12$) or due to tinnitus (placebo arm; $n = 1$). Other reasons for nonevaluable status included nonadherence with study drugs ($n = 2$), inadequate bowel prep at year 1 colonoscopy ($n = 1$), and ineligibility ($n = 1$). Demographics of nonevaluable patients were similar to those in the evaluable study population. Patient-reported data on concomitant medications taken during the study

Table 1. Characteristics of the study population

	Study arm			
	DFMO + aspirin (*N* = 52)	**Double placebo (*N* = 52)**	**Total (*N* = 104)**	***P***
Age				0.7424[a]
N				
Mean (SD)	62.8 (9.43)	62.3 (8.83)	62.6 (9.09)	
Median	63.0	62.0	62.5	
Range	46.0–83.0	50.0–79.0	46.0–83.0	
Sex, *n* (%)				0.1678[b]
Female	27 (51.9%)	20 (38.5%)	47 (45.2%)	
Male	25 (48.1%)	32 (61.5%)	57 (54.8%)	
Race, *n* (%)				0.3607[b]
White	50 (96.2%)	52 (100.0%)	102 (98.1%)	
African American	1 (1.9%)	0 (0.0%)	1 (1.0%)	
Other	1 (1.9%)	0 (0.0%)	1 (1.0%)	
Prior aspirin use, *n* (%)				0.8356[b]
Yes	18 (34.6%)	17 (32.7%)	35 (33.7%)	
No	34 (65.4%)	35 (67.3%)	69 (66.3%)	
Prior cancer, *n* (%)				1.0000[b]
Yes	1 (1.9%)	1 (1.9%)	2 (1.9%)	
No	51 (98.1%)	51 (98.1%)	102 (98.1%)	
Prior advanced adenomas, *n* (%)				0.3150[b]
Yes	52 (100.0%)	51 (98.1%)	103 (99.0%)	
No	0 (0.0%)	1 (1.9%)	1 (1.0%)	
BMI				0.0682[a]
Mean (SD)	31.3 (7.66)	28.7 (5.67)	30.0 (6.83)	
Median	30.3	27.4	28.3	
Range	19.4–54.2	20.6–52.9	19.4–54.2	

[a]Wilcoxon rank-sum *P* value.
[b]χ^2 *P* value. Missing data: DFMO + aspirin ($n = 2$), Double placebo ($n = 1$).

Table 2. Adenoma detection by study arm

	Colonoscopy at 1 year (all patients)				Colonoscopy at 1 year (nonaspirin users)				First poststudy colonoscopy (all patients)[d]			
	DFMO + aspirin ($n = 42$)	Double placebo ($n = 44$)	Total ($n = 86$)	P[a]	DFMO + aspirin ($n = 28$)	Double placebo ($n = 29$)	Total ($n = 57$)	P	DFMO + aspirin ($n = 20$)	Double placebo ($n = 18$)	Total ($n = 38$)	P
Any adenoma *n* (%)	16 (38.1)	18 (40.9)	34 (39.5)	0.7896[a]	8 (28.6)	13 (44.8)	21 (36.8)	0.2034[a]	10 (50)	10 (55.6)	20 (52.6)	0.7568[b]
Adenoma $\geq$ 5 mm, *n* (%)	5 (11.9)	5 (11.4)	10 (11.6)	1.0000[b]	3 (10.3)	3 (10.0)	6 (10.2)	1.0000[b]	5 (25)	7 (38.9)	12 (31.6)	0.4889[b]
Advanced adenomas, *n* (%)	3 (7.1)	3 (6.8)	6 (7)	1.0000[b]	3 (10.3)	3 (10.0)	6 (10.2)	1.0000[b]	2 (10)	2 (11.1)	4 (10.5)	1.0000[b]
Multiple adenomas, *n* (%)	7 (16.7)	12 (27.9)[4]	19 (22.4)	0.2136[a]	5 (17.9)	10 (35.7)[c]	15 (26.8)	0.1314[a]	0	0	0	0

[a]χ^2 *P* value.
[b]Fisher exact *P* value.
[c]Missing data; double placebo arm ($n = 1$).
[d]Median interval of 35 months post end-of-study colonoscopy.

indicated single use of NSAIDs in 7 patients of whom 5 were in the placebo arm.

Adenomas

At the 1-year end-of-study colonoscopy, 86 patients were evaluable for adenoma recurrence. One or more adenomas were detected in 16 of 42 (38.1%) and 18 of 44 (40.9%) subjects from the DFMO plus aspirin arm versus double placebo arm, respectively ($P = 0.790$; Table 2). Of note, the adenoma recurrence rate in the placebo arm of our study was similar to the 41.1% rate (after 2–39 months of treatment) found in the study of DFMO plus sulindac that evaluated a lower risk population (5). Among patients in the treatment arm, 7 (16.7%) patients had more than one adenoma removed compared with 12 patients (27.9%) in the placebo arm ($P = 0.214$; Table 2). When patients with adenomas of at least 5 mm in size were analyzed, an equal number were found in the treatment and placebo arms at year 1 (Table 2). Three patients in each of the treatment and placebo arms developed advanced adenomas at the 1-year colonoscopy. One-third of the study population were users of low dose aspirin prior to study enrollment (Table 1). To determine whether preenrollment aspirin use could influence the study results, we performed an exploratory analysis that was restricted to nonusers of low-dose aspirin. Eight of 28 (28.6%) subjects were found to have one or more adenomas in the treatment arm compared with 13 of 29 (44.8%) in placebo arm ($P = 0.203$). Furthermore and among nonusers of prior aspirin, more than one adenoma was found in 5 (17.9 %) patients in the treatment arm compared with 10 patients (35.7 %) in the placebo arm ($P = 0.131$).

ACF

Rectal ACF were visualized using chromoendoscopy (Fig. 2). Sixty-two of 87 (71.3%) subjects had at least 5 rectal ACF in an index cluster (see Methods) at the baseline chromoendoscopy exam and were, therefore, eligible for repeat ACF evaluation at the 1-year end-of-study colonoscopy. The combination of DFMO plus aspirin was associated with a statistically significant reduction in rectal ACF number compared with subjects in the placebo arm ($P = 0.036$; Table 3; Fig. 3). Specifically, the drug combination reduced rectal ACF number in an index cluster by a median of 5 ACF compared with a median decrease of 3 ACF for the placebo arm. We also determined the total ACF number in the rectum by chromoendoscopy and categorized subjects as having reduced versus nonreduced ACF after 1 year of continuous drug or placebo treatment. Among patients treated with the drug combination and compared with baseline, 74.2% showed improvement in global rectal ACF at 1 year versus 44.8% with improvement in the double placebo arm ($P = 0.020$; Table 3; Fig. 4). We also examined the correlation between the total rectal ACF number with adenoma number at the one year end-of-study colonoscopy, but the relationship was not statistically significant ($r = 0.23$; $P = 0.083$).

In an exploratory analysis, we examined data among preenrollment nonaspirin users and found that the drug combination reduced rectal ACF number in the index cluster

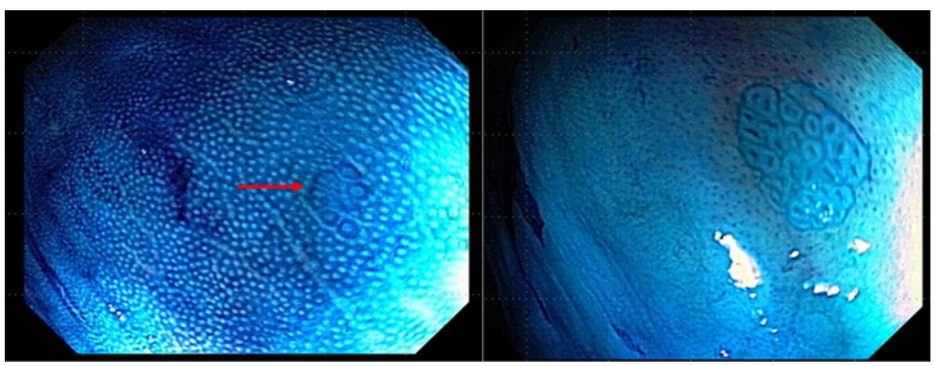

Figure 2.
Rectal ACF consist of clusters of abnormal appearing and enlarged colonic crypts that can be visualized at chromoendoscopy.

Table 3. Detection of rectal ACF by study arm

	Study arm				Nonaspirin users			
	DFMO + aspirin (*n* = 32)	**Double placebo (*n* = 30)**	**Total (*n* = 62)**	***P***	**DFMO + aspirin (*n* = 24)**	**Double placebo (*n* = 19)**	**Total (*n* = 43)**	***P***
Baseline ACF				0.1801[a]				0.1175[a]
Mean (SD)	10.0 (10.34)	6.9 (3.15)	8.5 (7.84)		11.0 (11.74)	6.6 (2.85)	9.0 (9.15)	
Median	6.0	5.0	6.0		6.0	5.0	5.0	
Range	5.0–56.0	5.0–17.0	5.0–56.0		5.0–56.0	5.0–14.0	5.0–56.0	
12 Month ACF				0.3631[a]				0.4501[a]
Mean (SD)	3.4 (2.91)	4.6 (3.99)	4.0 (3.49)		3.4 (2.92)	4.5 (3.81)	3.9 (3.34)	
Median	3.0	4.5	4.0		2.5	5.0	4.0	
Range	0.0–10.0	0.0–14.0	0.0–14.0		0.0–9.0	0.0–11.0	0.0–11.0	
ACF absolute change				0.0355[a]				0.0227[a]
Mean (SD)	−6.6 (10.04)	−2.4 (3.42)	−4.5 (7.82)		−7.6 (11.15)	−2.1 (2.88)	−5.2 (8.90)	
Median	−5.0	−3.0	−4.0		−5.0	−2.0	−4.0	
Range	−47.0–5.0	−10.0–7.0	−47.0–7.0		−47.0–1.0	−6.0–3.0	−47.0–3.0	
ACF global assessment				0.0203[b]				0.0549[b]
Reduced	23 (74.2%)	13 (44.8%)	36 (60.0%)		17 (73.9%)	8 (44.4%)	25 (61.0%)	
Not reduced	8 (25.8%)	16 (55.2%)	24 (40.0%)		6 (26.1%)	10 (55.6%)	16 (39.0%)	
Missing	1	1	2		1	1	2	

[a]Wilcoxon rank-sum *P* value.
[b]χ^2 *P* value.

by a median of 5 ACF compared with a median of 2 ACF in the placebo arm ($P = 0.023$) [Table 3]. We then categorized preenrollment nonaspirin users as having reduced or non-reduced global rectal ACF and found that 73.9% showed a reduction in the treatment arm compared with baseline versus 44.4% with reduction in the placebo arm ($P = 0.055$) [Table 3].

Safety and tolerability

The study population of 104 patients was evaluable for toxicity that included 53 in the treatment arm and 51 in the placebo arm. Given the reported potential for DFMO to produce ototoxicity (41), all subjects underwent baseline and one year audiograms per protocol. Adverse events (AE) recorded during the study period included those that were grade 1 ($n = 4$; 3.8%), grade 2 (11; 10.5%), and grade 3 ($n = 4$; 3.8%); there were no grade 4 events (Table 4A). Importantly, no statistically significant differences in the rate of AEs were found by study treatment arm (Table 4B). Two patients from the treatment arm (3.8%) and 3 patients from the placebo arm (5.9%) had grade 2 tinnitus (Table 4B). Of the four grade 3 AEs, all occurred in the placebo arm and were nonhematologic toxicities, consisting of abdominal pain, diarrhea, headache and tinnitus (one patient had grade 2 and 3 and withdrew; Table 4A). The pure tone audiometry thresholds did not reveal significant differences by study arm. Furthermore, repeat audiograms obtained during the study for patients who reported tinnitus did not differ significantly from their baseline studies.

Poststudy adenoma recurrence data at colonoscopy

We examined data from colonoscopic exams performed on participants following completion of their study

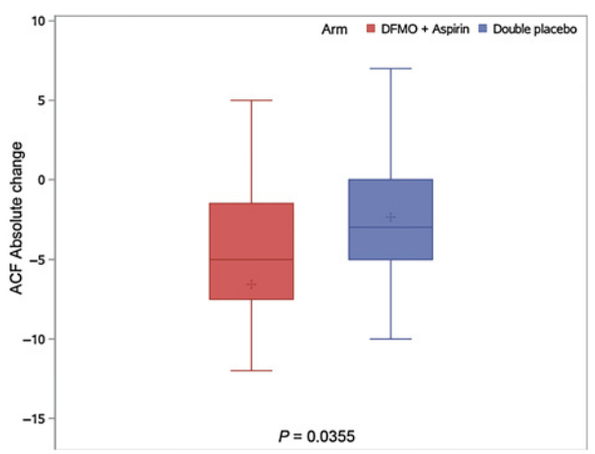

Figure 3.
Effect of treatment with DFMO plus aspirin versus double placebo on the change in rectal ACF number in an index cluster evaluated by chromoendoscopy after one year of treatment compared with baseline. Box plots show median values and interquartile ranges.

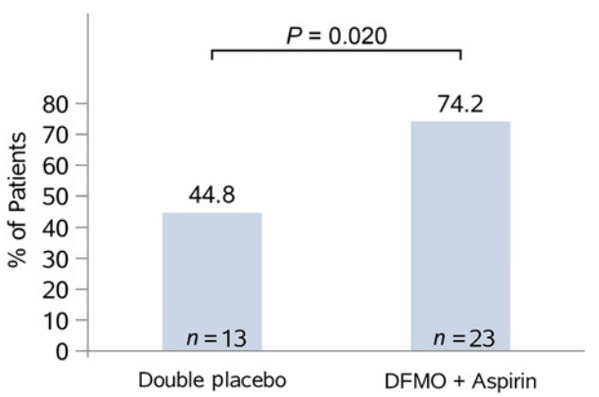

Figure 4.
Percentage of patients who had a reduction in the total number of rectal ACF after treatment with DFMO + aspirin or double placebo given continuously for 1 year.

Table 4A. Adverse event grade by study arm in the overall population

Adverse Event		Grade			
Type	**Arm**	**1**	**2**	**3**	**4**
		n (%)	*n* (%)	*n* (%)	*n* (%)
Tinnitus	Placebo		3 (5.9)	1 (2.0)	
	DFMO + ASA		2 (3.8)		
Headache	Placebo			1 (2.0)	
	DFMO + ASA		1 (1.9)		
Abnormal audiogram	Placebo	1 (2.0)			
	DFMO + ASA		1 (1.9)		
Abdominal pain	Placebo			1 (2.0)	
Diarrhea	Placebo			1 (2.0)	
Dizziness	DFMO + ASA	1 (1.9)			
Gastrointestinal disorder	DFMO + ASA		1 (1.9)		
Musculoskeletal disorder	DFMO + ASA	1 (1.9)			
Nausea	Placebo	1 (2.0)			
Pain	DFMO + ASA		1 (1.9)		
Rectal hemorrhage	DFMO + ASA		1 (1.9)		
Rectal pain	DFMO + ASA		1 (1.9)		

NOTE: Total $N = 104$; DFMO + aspirin, $n = 53$; double placebo, $n = 51$.

participation. By medical record review, we recorded adenoma number found at the first poststudy surveillance colonoscopy that was performed at a median of 35 months following study completion (median time interval for DFMO + aspirin group was 37.3 months and for double placebo group was 33 months). Recurrence of adenomas occurred in 10 of 20 (50.0%) versus 10 of 18 (55.6%) patients previously enrolled in the treatment and placebo arms, respectively ($P = 0.757$; Table 2). In prior participants in the treatment arm, 5 (25%) patients had adenomas $\geq$ 5 mm compared with 7 (38.9%) patients in the placebo arm ($P = 0.489$). Advanced adenomas were found in 2 patients from each of the study arms (Table 2).

Discussion

The combination of low-dose DFMO plus aspirin given continuously for 1 year did not produce a statistically significant reduction in colorectal adenoma recurrence in our high-risk patient population. This was true for any adenoma, multiple adenomas, those at least 5 mm in size, as well as advanced adenomas. Of note, there was a suggestion of greater benefit from the drug combination among nonusers of low-dose aspirin although the difference in adenoma number was not statistically significant likely due to the small sample size. The possibility exists that a longer treatment duration for DFMO plus aspirin may have been more efficacious. In this regard, a larger randomized trial found that DFMO (at the same dose used here) plus sulindac significantly reduced adenoma recurrence relative to placebo at 3 years (5). While DFMO plus sulindac was evaluated in lower risk subjects with sporadic adenomas (5), our study was restricted to subjects with prior advanced adenomas or cancer. NSAIDs including aspirin and sulindac were shown to activate polyamine catabolism by increasing the expression and activity of the *SAT1* promoter via an NFκB-dependent mechanism (12) or via a PPARγ element, respectively (42). Support for the dosage of DFMO used in our study was shown by its ability to significantly suppress polyamine levels in human colorectal mucosa (43).

Table 4B. Frequency of adverse events by patient study arm

Adverse events	**DFMO + aspirin ($n = 53$)**	**Placebo ($n = 51$)**
All grade $\geq$ 3	0	4 (7.8%)
Nonhematologic, grade $\geq$ 3	0	2 (3.9%)
Any AE	10 (18.8%)	8 (15.6%)
Cardiovascular	0	0
Gastrointestinal	1 (1.9%)	0
Abdominal pain	0	1 (2%)
Diarrhea	0	1 (2%)
Tinnitus	2 (3.8%)	4 (7.8%)
Abnormal audiogram	1 (1.9%)	1 (2%)
Headache	1 (1.9%)	1 (2%)
Dizziness	1 (1.9%)	0
Nausea	0	1 (2%)
Musculoskeletal disorder	1 (1.9%)	0
Pain	1 (1.9%)	0
Rectal hemorrhage	1 (1.9%)	0
Rectal pain	1 (1.9%)	0

In contrast to adenomas, we found that DFMO plus aspirin produced a statistically significant reduction in rectal ACF number at 1 year compared with placebo suggesting a chemopreventive effect. We quantified ACF in an index cluster of at least five rectal ACF with the region marked with a mucosal tatoo to enable a quantitative evaluation at end of treatment compared with baseline. We also observed a statistically significant reduction in total rectal ACF number for the combination versus double placebo. Of note, a reduction in ACF was seen in the placebo arm and collected data on concomitant medications revealed single use of NSAIDs in 7 patients of whom 5 were in the placebo arm. While the impact of such intake is unclear, we also cannot exclude the potential for changes in diet or lifestyle to influence our results. While the study had a focus on ACF modulation, only about 60% of screened patients met the minimum ACF requirement at baseline that was dropped during the study to facilitate accrual since the primary endpoint variable was adenoma occurence. Our findings for ACF regression are consistent with preclinical data for the combination of DFMO and aspirin that synergistically reduced ACF and colon tumors in the azoxymethane rodent model of colon carcinogenesis (9). Furthermore, provision of dietary polyamines was shown to reverse the suppression of ACF produced by DFMO (44). DFMO plus aspirin was shown to decrease the mitotic index in ACF while apoptosis was increased only by DFMO in a rodent model (9). Human ACF were shown to be hyperproliferative relative to normal colonic epithelium (28), and express genes or their alterations that are important in MAPK signaling including *BRAF*, *KRAS*, *NRAS*, and *ERBB2* (36, 45–47). Recent data indicate that 75% of the loci harboring DNA methylation changes in ACF were also altered in human colorectal cancer samples (48).

Prior studies in humans targeting ACF have produced conflicting results. Compared with other studies, we evaluated a higher risk population of patients with prior advanced adenomas or cancer as dysplastic ACF were more frequently observed in patients with colorectal cancer or adenoma (34). In addition, we examined the potential for ACF modulation given that DFMO plus aspirin were given continuously for only 1 year. A randomized trial in 189 subjects found that rectal ACF number at 2 months was significantly suppressed by sulindac, but not by the selective COX-2 inhibitor etodolac (24). In that study, suppression of total polyps and adenomas was significantly greater among ACF responders versus nonresponders to sulindac (24). In a nonrandomized and open-label trial of curcumin treatment in 41 subjects, a significant reduction (40%) in rectal ACF number was observed with the 4 gm ($P < 0.005$), but not the 2 gm curcumin dose (25). In another study, treatment of 26 nondiabetic patients with metformin (250 mg/day) for 1 month was associated with a significant decrease in the mean number of rectal ACF, along with a significant reduction in proliferating cell nuclear antigen (PCNA) expression in normal rectal epithelium in the treatment but not the control arm (26). However, the selective COX-2 inhibitor celecoxib (vs. placebo) significantly reduced adenomas but not rectal ACF after 8 to 12 months of treatment in participants in the Adenoma Prevention with Celecoxib (APC) trial ($P = 0.77$; ref. 28). Furthermore, 6-month interventions with atorvastatin, sulindac, oligofructose-enriched inulin or maltodextrin did not significantly reduce rectal ACF number among 88 randomized subjects [49].

We found that the combination of DFMO and aspirin was well tolerated with no statistically significant increase in toxicities relative to matching placebo. Furthermore, there was no increase in ototoxicity from low-dose DFMO administered in this study, as was reported for DFMO plus sulindac (50). Strengths of our study include the randomized, double-blinded and placebo-controlled design, and performance of colonoscopy and chromoendoscopy by a limited number of experienced endoscopists. Weaknesses include the relatively short treatment duration of one year, modest sample size, absence of family history information, and lack of data on ACF histology, polyamines, or other mucosal biomarkers although biospecimens were collected and banked to enable future biomarker studies. Interestingly, endocytoscopy has been shown to provides real-time histologic images *in vivo*, with clear visualization of cellular details and features of dysplasia in colorectal ACF (51).

In summary, DFMO plus aspirin did not significantly reduce colorectal adenoma recurrence at 1 year in a high-risk population compared with placebo, although we cannot exclude the possibility that a longer treatment duration may have been more effective in reducing metachronous adenomas. In contrast to adenomas, the drug combination was found to significantly reduce the number of rectal ACF relative to placebo consistent with a chemopreventive effect. Toxicities were not increased in the treatment versus placebo arm and specifically, no ototoxicity occurred. These results require validation to support ACF modulation as a potential biomarker of drug efficacy in a high risk cohort. Such a study could be embedded into a randomized, controlled trial that enables ACF modulation to be correlated with effects on occurrence of adenomas. Barriers exist to evaluating ACF in clinical trials that include the need to screen patients to identify those with sufficient numbers at baseline to enable their quantitative assessment using rigorous methodology to enable reproducible assessment.

Disclosure of Potential Conflicts of Interest

C. Gostout is a medical officer (paid consulting) at Apollo Endosurgery, is a consultant at Olympus Medical Systems, AdaptivEndo, and has ownership interest (including patents) in Apollo Endosurgery. J.B Kisiel reports receiving a commercial research grant and has ownership interest (including patents) from Exact Sciences. No potential conflicts of interest were disclosed.

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