

## Randomized Phase II Trial of Sulindac, Atorvastatin, and Prebiotic Dietary Fiber for Colorectal Cancer Chemoprevention

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### Abstract

Sulindac, atorvastatin, or prebiotic dietary fiber may reduce colorectal cancer (CRC) risk. However, clinical trial data are currently limited. We conducted a randomized, phase II chemoprevention trial involving subjects 40 years or older, with previously resected colon cancer or multiple/advanced colorectal adenomas. Magnification chromoendoscopy (MCE) was performed to identify and characterize rectal aberrant crypt foci (ACF); eligibility criteria required five or more rectal ACFs at baseline. Intervention assignments were as follows: (a) atorvastatin 20 mg qd; (b) sulindac 150 mg bid; (c) oligofructose-enriched inulin (as ORAFIT<sup>®</sup>Synergy1) 6 gm bid; or (d) control (maltodextrin) 6 gm bid, for 6 months. Percent change in rectal ACF number (% $\Delta$ ACF) within arm was the primary endpoint. Secondary endpoints included changes in proliferation (Ki67) and apoptosis (caspase-3), as measured from normal mucosa biopsy samples. Among 85 eligible randomized subjects, 76 (86%) completed the trial per protocol. The median (range) of rectal ACF was 9 (5–34) and 8 (0–37) at baseline and postintervention, respectively. The median (SD) for % $\Delta$ ACF was 5.6 (–69% to 143%), –18.6 (–83% to 160%), –3.6 (–88% to 83%), and –10.0 (–100% to 117%) in the atorvastatin, sulindac, ORAFIT<sup>®</sup>Synergy1 and control arms, respectively. Neither within-arm ( $P = 0.12$ – $0.59$ ) nor between-arm ( $P = 0.30$ – $0.92$ ) comparisons of % $\Delta$ ACF were statistically significant. The active and control interventions also seemed to have similar effects on mucosal proliferation and apoptosis ( $P > 0.05$  for each comparison). Data from this multicenter, phase II trial do not provide convincing evidence of CRC risk reduction from 6-month interventions with atorvastatin, sulindac, or ORAFIT<sup>®</sup>Synergy1, although statistical power was limited by the relatively small sample size. *Cancer Prev Res*; 4(2); 259–69. ©2011 AACR.

### Introduction

Colorectal cancer (CRC) has the third highest incidence rate of all cancers in the United States, with more than 145,000 new cases diagnosed each year (1). Early detection (and removal) of premalignant neoplasia remains the cornerstone of CRC prevention. Chemoprevention may provide adjunctive benefits in high-risk patient popula-

tions. Despite strong potential, chemoprevention has achieved relatively limited clinical impact to date, in large part because of reportedly unfavorable and/or incompletely defined risk:benefit ratios for many candidate agents (2, 3).

Existing data support further evaluation of nonsteroidal anti-inflammatory drugs (NSAID), statin drugs (also 3-hydroxy-3-methylglutaryl coenzyme A reductase inhibitors), and prebiotic dietary fiber in early-phase chemoprevention trials (4). Sulindac (a nonselective NSAID) has been shown to regress polyps among individuals genetically predisposed to developing CRC (5), although results from subsequent clinical trials of sulindac (alone or in combination with other candidate chemoprevention agents) involving subjects with sporadic colorectal neoplasia remain inconclusive (6–9). Atorvastatin, the most widely prescribed drug in the United States ([www.imshealth.com](http://www.imshealth.com); accessed April 13, 2010), is a lipophilic statin and may have anti-inflammatory, proapoptotic or other growth inhibitory properties based on preclinical data (10). Some, but not all, prospective observational studies have reported an inverse association between statin

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use and CRC risk (11–14). However, to date, no statin-based CRC chemoprevention trials have been reported. ORAFTI<sup>®</sup>Synergy1 is a prebiotic fiber composed of oligofructose and polyfructose chains and has been shown to inhibit intestinal tumors in rodents (15). Favorable effects on several stool- and blood-based biomarkers were recently observed when ORAFTI<sup>®</sup>Synergy1 was administered in combination with two probiotic agents to subjects with a history of resected colorectal neoplasia (16).

To maximize efficiency, early-phase chemoprevention trials should apply validated, modifiable, easy-to-measure surrogate endpoint biomarkers as the primary endpoint (17). Aberrant crypt foci (ACF), which are characterized by aggregated clusters of thickened and enlarged mucosal crypts, respond to chemopreventive interventions in animal models and have been correlated with synchronous colorectal neoplasia in some, but not all, observational studies as recently reviewed (18, 19). Rectal ACF can be readily quantified by magnification chromoendoscopy (MCE) and have been analyzed as primary endpoints in a limited number of previous CRC chemoprevention trials (17, 20). Using the infrastructure of the multicenter Cancer Prevention Network (CPN), we conducted a randomized, phase II clinical trial of 6-month interventions with sulindac, atorvastatin, ORAFTI<sup>®</sup>Synergy1, or control (maltodextrin) to assess the effects of these candidate agents on percentage change in rectal ACF number (primary endpoint) and other tissue-based biomarkers (secondary endpoints) among subjects at increased risk for sporadic CRC.

## Materials and Methods

All aspects of the study protocol were reviewed and approved by the appropriate Institutional Review Board for human research at each participating site. The Data and Safety Monitoring Board of the Mayo Clinic Cancer Center reviewed safety data every 6 months and efficacy data within 6 months of the planned interim analysis.

### Baseline evaluation

Subjects were enrolled through 10 CPN member organizations (Brigham and Women's Hospital, Boston, MA; Fox Chase Cancer Center, Philadelphia, PA; Hines Veterans Administration, Hines, IL; Indiana University, Indianapolis, IN; Kaiser Permanente Medical Group, Sacramento, CA; Mayo Clinic Arizona, Scottsdale, AZ; Mayo Clinic Rochester, Rochester, MN; University of Connecticut, Farmington, CT; University of Illinois at Chicago, Chicago, IL; and University of Pittsburgh, Pittsburgh, PA), beginning in April 2006 and ending in August 2008. The target population was defined as patients 40 years or older with a history of previously resected colon cancer (excluding subjects with stage IV disease) or "advanced" colorectal adenomas ( $\geq 1$  cm in maximal diameter,  $\geq 3$  in total number, villous morphology, or high-grade dysplasia). After informed consent was obtained, willing participants completed a focused interview, brief CRC risk factor survey, limited physical examination, and peripheral blood draw. General

eligibility criteria were as follows: no current use of NSAIDs (excluding low-dose aspirin, defined as  $\leq 162.5$  mg per day or  $\leq 325$  mg every other day), corticosteroids, statin drugs, anticoagulant/antiplatelet drugs, investigational drugs, or other compounds likely to interfere with the planned study interventions; and ECOG (Eastern Cooperative Oncology Group) performance status 2 or less. Women of child-bearing potential were also required to document a negative pregnancy test prior to enrollment. Exclusion criteria were as follows: history of invasive malignancy (other than colon cancer) within the preceding 5 years; rectal surgery; heritable cancer syndrome, inflammatory bowel disease, pelvic radiation therapy; endoscopically confirmed peptic ulcer disease; currently breast-feeding; allergy to compounds of similar chemical structure or biological composition as the study agents; or uncontrolled intercurrent illness that, in the investigator's opinion, might limit compliance with the study requirements.

Trial enrollment further required 5 or more rectal ACFs at the baseline MCE examination. Study endoscopists were provided with a reference set of endoscopic images (depicting rectal ACF and/or other mucosal abnormalities) and were asked to complete a short knowledge assessment test based on these images before conducting their first study-related procedure. MCE examinations were performed using Olympus close focus prototype colonoscopes (model CF-Q160ALE). Preprocedure bowel cleansing was achieved using oral purgatives (i.e., polyethylene glycol preparation) or rectal enemas for subjects who were unwilling or unable to comply with oral purgation. Following routine mucosal inspection, the distance from the anal verge to the middle valve of Houston was recorded to define the rectal segment ( $\sim 15$  cm in length). Mucosal washings were performed until more than 90% of the rectal mucosa was well visualized. All polyps 2 mm or more in size were removed from the rectal segment; adequate hemostasis was achieved and additional mucosal washings were performed, as needed, before proceeding. Using a spray catheter, the rectal mucosa was coated with at least 60 mL of 10% Mucomyst solution (left in place for 2 minutes), washed with 60 mL of water to clear any residual mucus, and dyed with at least 60 mL of 0.2% methylene blue (21) solution (left in place for 2 minutes). Adequate dye-spray application was defined as 90% or more of the rectal mucosa interpreted as well stained by the study endoscopist. Rectal ACFs were identified by a minimum of 5 aggregated crypts in a single grouping (maximum spacing between crypts no more than 2 times the average crypt diameter) and a crypt diameter at least 1.5 times the diameter of surrounding normal crypts (Fig. 1). Recorded ACF characteristics included total count, tissue plane (flat, elevated, depressed), staining intensity (equal, lighter, darker), predominant luminal shape (irregular, mixed, normal, oval/round, slit-like), crypt number (5–9, 10–19,  $\geq 20$ ) and distance from the anal verge (to allow comparison between pre- and postintervention examinations).

For subjects with 5 or more rectal ACFs at the baseline MCE examination, up to 8 biopsy samples were obtained

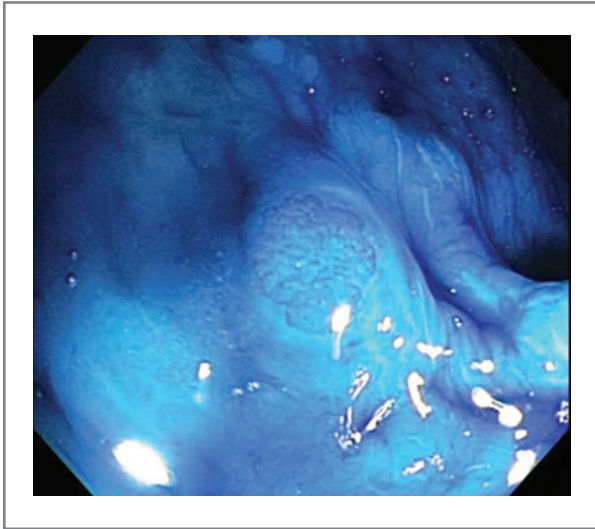


Figure 1. Representative image of rectal aberrant crypt foci (ACF) detected by magnification chromoendoscopy.

from normal appearing rectal mucosa; no biopsy was carried at baseline out for rectal ACFs for these subjects. Subjects with fewer than 5 rectal ACFs at the baseline MCE examination were not eligible for randomization, but up to 8 biopsy samples were obtained from normal appearing rectal mucosa and up to 4 biopsy samples were obtained from rectal ACF for histologic assessment and other correlative studies. Biopsy samples were divided and fixed in 10% neutral buffered formalin, followed by paraffin-embedding (odd numbered samples), or immediately frozen in liquid nitrogen (even numbered samples) for future biomarker analyses.

#### Intervention assignments and on-study monitoring

Subjects were randomly assigned to 1 of 4 intervention arms for 6 months: (a) atorvastatin 20 mg tablet once per day; (b) sulindac 150 mg tablet twice per day; (c) ORAFIT<sup>®</sup>Synergy1 6 g powder twice per day; or (d) control (maltodextrin powder twice per day; formulated to permit blinded comparison to the ORAFIT<sup>®</sup>Synergy1 intervention arm). Randomization was performed by CPN personnel by a dynamic allocation procedure (22) that balanced the intervention arms based on the following stratification factors: history of colon resection (yes vs. no), baseline ACF number (5–9 vs.  $\geq 10$ ), and participating site. Telephone interviews were conducted at months 1, 2, 4, and 5 postrandomization and an interim office visit was performed at month 3 ( $90 \pm 10$  days) postrandomization (including a limited physical examination and a peripheral blood draw). Agent compliance and concomitant medication use were recorded. Adverse events were classified and graded using NCI Common Toxicity Criteria, version 3.0 (available at [www.ctep.cancer.gov](http://www.ctep.cancer.gov)). Attribution of agent-related adverse events was performed by the designated medical monitor (GDZ).

#### Postintervention evaluation

Subjects returned at 6 months ( $180 \pm 10$  days) post-randomization to assess compliance, concomitant medication use, and adverse events. A limited physical examination and peripheral blood draw were also performed. MCE examinations were performed using the same standardized protocol applied at the baseline. Rectal ACFs were counted and characterized, including classification of incident (newly identified) versus prevalent (present at the baseline exam) lesions. For all subjects, up to 8 biopsy samples were obtained from normal appearing rectal mucosa and up to 8 biopsy samples were obtained from rectal ACF (if  $>8$  rectal ACFs were identified, the largest lesions were targeted for biopsy). Biopsy samples were divided and fixed in 10% neutral buffered formalin, followed by paraffin-embedding (odd numbered samples), or immediately frozen in liquid nitrogen (even numbered samples) for future biomarker analyses.

#### Tissue biomarker assays

Formalin-fixed, paraffin-embedded rectal biopsy samples were processed on site and shipped to Mayo Clinic Rochester for further analyses. Results were reported by an experienced gastrointestinal pathologist (T.C.S.) without prior knowledge of the intervention assignment or endoscopic findings. General histology was classified as normal, hyperplastic, dysplastic, or other. When present, dysplasia was graded as low-grade, high-grade, invasive carcinoma, or indeterminate. Mucosal proliferation and apoptosis were measured from normal appearing rectal biopsy samples by a dual immunohistochemistry (IHC) staining protocol to label Ki67 and cleaved caspase-3, respectively. IHC results were recorded as the percent of positively stained cells per visualized field.

#### Statistical analyses

The primary trial endpoint was defined as the percent change in number of rectal ACF ( $\% \Delta \text{ACF} = [\text{postintervention ACF number} - \text{baseline ACF number}] / \text{baseline ACF number}$ ). Trial participants were considered evaluable for the primary endpoint if ACF data were available from both the baseline and postintervention MCE examinations, applying the intent-to-treat principle. The original sample size was powered to detect a difference of at least 1SD in the mean  $\% \Delta \text{ACF}$ , based on 3 pairwise comparisons [i.e., each active intervention arm vs. the control (maltodextrin) arm], with a planned variability assessment (without halting accrual) of the  $\% \Delta \text{ACF}$  to be conducted after data from 40 subjects were available ( $n = 10$  per intervention arm). The SD in the mean  $\% \Delta \text{ACF}$  was conservatively estimated as the range of possible values (from 0 to 1, divided by 4;  $\text{SD} = 25\%$ ); 25 subjects per intervention arm yielded 92% power using the 2-sample  $t$  test, with a 2-sided  $\alpha = 0.016$  (adjusted for multiple comparisons).

Because of bidirectionality in the observed  $\% \Delta \text{ACF}$  data at the planned variability assessment, the estimated SD was revised to include a wider range of possible values (from  $-1$

to 1, divided by 4; SD = 50%). Under these considerations, 17 subjects in each of the sulindac and control arms yielded 76% power to detect a difference of at least 1 SD in the mean  $\% \Delta \text{ACF}$ , with a 2-sided  $\alpha = 0.05$  (1-sample Wilcoxon test, unadjusted for multiple comparisons). Given emerging controversy regarding the application of rectal ACF as an intermediate endpoint biomarker for CRC chemoprevention trials (23, 24), as well as slower than anticipated accrual, a decision was made to proceed with this revised, albeit moderately powered, sample size estimate.

Summary statistics and frequency tables were used to describe baseline patient characteristics and adverse event rates and compared between intervention arms using a chi-square (or Fisher's exact) test or the Kruskal-Wallis test for categorical and continuous variables, respectively. Descriptive statistics were used to summarize the baseline and postintervention levels of the proliferation (Ki67) and apoptosis (caspase-3) biomarkers.  $\% \Delta \text{ACF}$  and percentage change in the biomarker levels were compared within each arm by the Wilcoxon signed rank procedure. The between-arm comparisons (both between the active intervention arms and each active intervention arm and control) were carried out using the Wilcoxon rank-sum test. Adverse events were reported as maximum severity per subject and type, across the duration of intervention. All attributions collected for adverse events were reported and all statistical tests are 2-sided, unless otherwise noted. Data analyses were conducted using SAS version 9.1.3 (SAS Institute, Inc.).

## Results

### Cohort description

A CONSORT overview of subject recruitment is shown in Figure 2. One hundred forty-two unique subjects provided informed consent and were preregistered for the baseline evaluation. Baseline MCE examinations were completed for 129 subjects, of whom 33 had fewer than 5 rectal ACFs.

Eight additional subjects were excluded on the basis of failure to meet other eligibility criteria. Randomization was performed for 88 subjects. Three subjects were subsequently withdrawn postrandomization after further review of complete baseline evaluation data. Therefore, the intervention cohort included 85 subjects. Baseline demographics are provided in Table 1 by trial participation status. In general, subjects in the intervention cohort ( $n = 85$ ) had similar baseline characteristics as ineligible/withdrawn subjects (except with respect to characteristics that directly influenced trial participation status).

### Baseline ACF

Mucosal dye spray allowed for adequate inspection in 127 of 129 (99%) subjects, including 85 of 85 (100%) subjects in the intervention cohort. Nine subjects had no rectal ACF identified. In the remaining 120 subjects, a total of 998 rectal ACFs were found (Table 2). The median number (range) of rectal ACF was 2 (0–12) and 9 (5–34) for ineligible/withdrawn and intervention cohort subjects, respectively ( $P < 0.001$ ). Other baseline ACF characteristics that differed by trial participation status included lumen shape ( $P = 0.005$ ), tissue plane ( $P < 0.001$ ), and staining intensity ( $P < 0.001$ ).

### Intervention arms, agent compliance, and adverse events

By design, the intervention arms were evenly balanced (Table 3) with respect to history of colon resection (yes vs. no;  $P = 1.00$ ), baseline ACF number (5–9 vs.  $\geq 10$ ;  $P = 0.98$ ), and participating site ( $P = 0.97$ ). Baseline age, gender, body mass index (BMI), cigarette smoking status, daily alcohol consumption, family history of CRC, and history of chronic NSAID/analgesic use were similarly distributed across intervention arms ( $P > 0.05$  for each variable). Agent compliance for at least 3 months was reported by 77 of 85 (91%) subjects; 74 of 85 (87%)

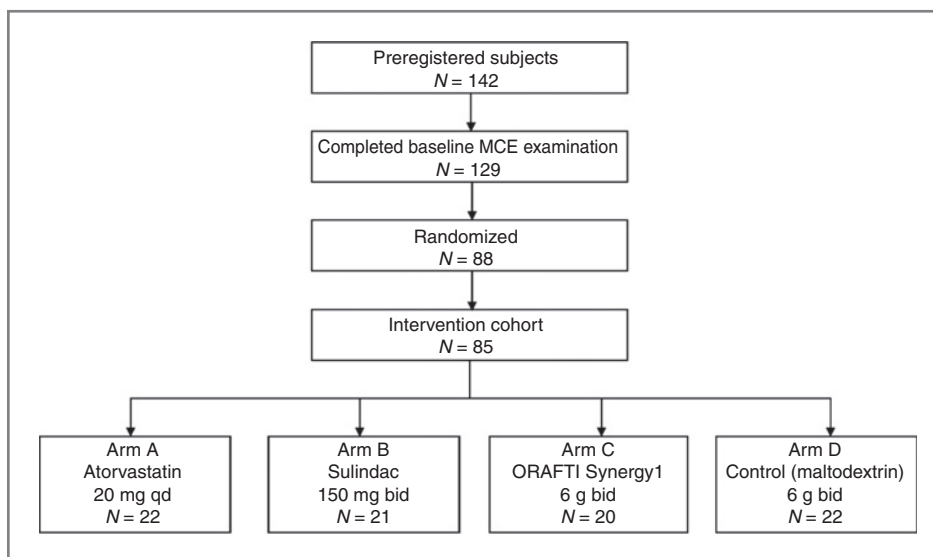


Figure 2. CONSORT flow diagram.



**Table 1.** Baseline demographics by trial participation status

Characteristic <sup>a</sup>	All registered (n = 142)	Ineligible or withdrawn (n = 57)	Intervention cohort (n = 85)	P <sup>b</sup>
Age, y				0.49
Median	58	59	58	
Range	41–78	41–76	41–78	
Gender, n (%)				0.22
Women	55 (38.7)	26 (45.6)	29 (34.1)	
Men	87 (61.3)	31 (54.4)	56 (65.9)	
BMI, kg/m <sup>2</sup>				0.81
Median	28.3	28.2	28.3	
Range	15.5–51.3	22.5–47.7	15.5–51.3	
Cigarette smoking status, n (%)				0.82
Never	56 (42.4)	21 (44.7)	35 (41.2)	
Current	18 (13.6)	5 (10.6)	13 (15.3)	
Former	58 (43.9)	21 (44.7)	37 (43.5)	
Alcohol, drinks per day, n (%)				0.85
None	50 (37.9)	17 (36.2)	33 (38.8)	
<1	69 (52.3)	24 (51.1)	45 (52.9)	
2–3	10 (7.6)	5 (10.6)	5 (5.9)	
≥4	3 (2.3)	1 (2.1)	2 (2.4)	
Family history of CRC, n (%) <sup>c</sup>				0.12
Yes	27 (20.4)	6 (12.8)	21 (24.7)	
No	105 (79.6)	41 (87.2)	64 (75.3)	

<sup>a</sup>Data for 1 or more characteristics were missing for n = 10 ineligible/withdrawn subjects.

<sup>b</sup>P value for comparison between ineligible or withdrawn subjects versus intervention cohort subjects using Fisher's exact test for categorical responses and rank-sum test for continuous responses.

<sup>c</sup>Defined as 1 or more first-degree, biological (nonadopted) relative(s) with colorectal cancer.

subjects were compliant with the full 6-month intervention. The median (range) of planned agent doses received was 96.7% (1.4; 108.1%) overall. Reported compliance was lowest in the control arm [median (range) of 86.4% (1.4, 103.9);  $P = 0.02$  for comparison across intervention arms]. At least 1 adverse event, regardless of attribution, was reported by 60 of 85 (71%) subjects (Table 3). The large majority of these adverse events were classified as grade 1 (56%) or grade 2 (31%), with fewer grade 3 (6%) and no grade 4 adverse events reported. On the basis of medical monitor's adjudication, none of the grade 3 adverse events were related to the study agent.

#### Postintervention ACF

Complete postintervention MCE data were available for 77 of 85 (91%) subjects. Reasons for incomplete data on the remaining 8 subjects were as follows: lost to follow-up ( $n = 3$ ), consent withdrawn ( $n = 2$ ), medical contraindication to the study-related procedures ( $n = 1$ ), or other ( $n = 2$ ). Among the 77 subjects who completed the postintervention MCE examination, rectal ACFs were identified in 76 subjects ( $n = 766$  rectal ACFs total), with a median (range) of 8 (0–37; Table 4). Per the examining endoscopist, 518 ACFs (68%) were categorized as incident

(i.e., newly identified) and 248 ACFs (32%) were categorized as prevalent (i.e., present at the baseline evaluation). Incident ACFs had fewer crypts ( $P < 0.001$ ), were more likely to be elevated ( $P = 0.002$ ), and stained more intensely relative to the surrounding rectal mucosa ( $p < 0.001$ ) than prevalent ACFs.

Data regarding % $\Delta$ ACF, overall and by intervention arm, are shown in Table 4. Subjects assigned to the control arm exhibited no change in rectal ACF number [median value (range) of % $\Delta$ ACF =  $-10.0\%$  ( $-100\%$  to  $117\%$ )] between the postintervention and baseline MCE examinations. For subjects in the atorvastatin, sulindac, and ORAFTI<sup>®</sup>Synergy1 active arms, the median value (range) of % $\Delta$ ACF was  $5.6\%$  ( $-69\%$  to  $143\%$ ),  $-18.6\%$  ( $-83\%$  to  $160\%$ ), and  $-3.6\%$  ( $-88\%$  to  $83\%$ ), respectively. The observed % $\Delta$ ACF values were not statistically significant when each active intervention arm was independently compared with the control arm ( $P = 0.30, 0.60,$  and  $0.92$ ) or when the postintervention versus baseline ACF counts were compared within each active intervention arm ( $P = 0.59, 0.12,$  and  $0.54$ ).

#### Proliferation and apoptosis

Ki67 and caspase-3 assay data were measured from baseline and postintervention biopsy samples of normal

**Table 2.** Baseline rectal ACFs by trial participation status

	All registered (n = 129)	Ineligible or withdrawn (n = 44)	Intervention cohort (n = 85)	P <sup>a</sup>
ACF number, total	998	127	871	–
ACF number, per subject <sup>b</sup>				<0.001
Median (range)	7 (0–34)	2 (0–12)	9 (5–34)	
Crypts per ACF, n (%)				0.97
5–9	178 (17.8)	22 (17.3)	156 (17.9)	
10–19	345 (34.6)	43 (33.9)	302 (34.7)	
≥20	475 (47.6)	62 (48.8)	413 (47.4)	
Lumen shape, n (%)				0.005
Irregular	138 (13.8)	15 (11.8)	123 (14.1)	
Mixed	101 (10.1)	26 (20.5)	75 (8.6)	
Normal	15 (1.5)	3 (2.4)	12 (1.4)	
Oval/round	609 (61.0)	70 (55.1)	539 (61.9)	
Slit-like	132 (13.2)	13 (10.2)	119 (13.7)	
Other <sup>c</sup>	3 (0.3)	0 (0.0)	3 (0.3)	
Maximum diameter, mm <sup>2</sup>				0.09
Median (range)	1.0 (0.1–10.0)	1.0 (0.3–4.0)	1.0 (0.1–10.0)	
Tissue plane, n (%) <sup>d</sup>				<0.001
Depressed	2 (0.2)	1 (0.8)	1 (0.1)	
Flat	575 (57.6)	53 (41.7)	522 (59.9)	
Elevated	421 (42.2)	73 (57.5)	348 (40.0)	
Staining intensity, n (%) <sup>d</sup>				<0.001
Equal	336 (33.7)	50 (39.4)	286 (32.8)	
Lighter	122 (12.2)	27 (21.3)	95 (10.9)	
Darker	540 (54.1)	50 (39.4)	490 (56.3)	

<sup>a</sup>P value for comparison between ineligible or withdrawn subjects versus intervention cohort subjects using Fisher's exact test for categorical responses and rank-sum test for continuous responses.

<sup>b</sup>Median (range).

<sup>c</sup>Indistinct (n = 1) or unknown (n = 2).

<sup>d</sup>Relative to the surrounding rectal mucosa.

appearing rectal mucosa as secondary endpoints. Paired results were available for 61 or 85 (72%) and 55 of 85 (65%) subjects, respectively. For Ki67, the mean (SD) percentage change was 13.6 (44.1) for subjects assigned to the control arm (Fig. 3). The corresponding values were 6.5 (62.3), 12.2 (52.8), and 34.7 (70.6) for subjects assigned to receive atorvastatin, sulindac, or ORAF-TI<sup>®</sup>Synergy1, respectively. None of the within-arm or between-arm comparisons were statistically significant ( $P > 0.05$  for each comparison). For caspase-3, the mean (SD) percentage change was 130.6 (81.8) for subjects assigned to the control arm (Fig. 4). The corresponding values were 106.0 (140.3), 131.9 (154.1), and 120.5 (195.6) for subjects assigned to receive atorvastatin, sulindac, or ORAF-TI<sup>®</sup>Synergy1, respectively. Caspase-3 levels increased significantly when compared within each intervention arm (atorvastatin,  $P = 0.008$ ; sulindac,  $P = 0.005$ ; ORAF-TI<sup>®</sup>Synergy1,  $P = 0.05$ ; control,  $P = 0.001$ ). However, comparisons of change in caspase-3 level between each

active arm and the control arm were not statistically significant ( $P > 0.05$  for each comparison).

### Histologic assessment

Histologic findings were compared with MCE findings in secondary data analyses. Normal mucosa biopsy samples were available for review from 98 (34 ineligible/withdrawn, 64 randomized) subjects [median (range) = 2 (1–8) biopsies per subject], including 144 biopsies from the baseline evaluation and 56 biopsies from the postintervention evaluation. Histologic findings from the endoscopically normal appearing biopsy samples included normal mucosa ( $n = 180$ ; 90%), hyperplasia ( $n = 5$ ; 3%), low-grade dysplasia ( $n = 2$ ; 1%), and other ( $n = 13$ ; 7%). Rectal ACF biopsy samples were available for review from 68 subjects [median (range) = 3 (1–8) biopsies per subject], including 28 biopsies from the baseline evaluation and 164 biopsies from the postintervention evaluation. Histologic findings from the rectal ACF biopsy

**Table 3.** Baseline demographics, intervention compliance, and adverse events by randomization arm

Characteristic	Overall (n = 85)	Atorvastatin (n = 22)	Sulindac (n = 21)	ORAFIT <sup>®</sup> Synergy1 (n = 20)	Control (n = 22)	P <sup>a</sup>
Age, y						0.07
Median	58	61	55	64	57	
Range	41–78	44–78	41–74	48–78	44–72	
Gender, n (%)						0.51
Women	29 (34.1)	5 (22.7)	9 (42.9)	8 (40.0)	7 (31.8)	
Men	56 (65.9)	17 (77.3)	12 (57.1)	12 (60.0)	15 (68.2)	
BMI, kg/m <sup>2</sup>						0.78
Median	28.3	27.2	29.0	27.9	29.4	
Range	15.5–51.3	15.5–42.0	18.4–51.0	19.8–39.6	22.5–51.3	
Cigarette smoking status, n (%)						0.42
Never	35 (41.2)	9 (40.9)	9 (42.9)	11 (55.0)	6 (27.3)	
Current	13 (15.3)	5 (22.7)	4 (19.1)	1 (5.0)	3 (13.6)	
Former	37 (43.5)	8 (36.4)	8 (38.1)	8 (40.0)	13 (59.1)	
Alcohol, drinks per day, n (%)						0.16
None	33 (38.8)	9 (40.9)	6 (28.6)	12 (60.0)	6 (27.3)	
≤1	45 (52.9)	10 (45.5)	14 (66.7)	7 (35.0)	14 (63.6)	
2–3	5 (5.6)	3 (13.6)	0 (0.0)	1 (5.0)	1 (4.6)	
≥4	2 (2.4)	0 (0.0)	1 (4.8)	0 (0.0)	1 (4.6)	
Family history of CRC, n (%)						0.42
Yes	21 (24.7)	5 (22.7)	6 (28.6)	7 (35.0)	3 (13.6)	
No	64 (75.3)	17 (77.3)	15 (71.4)	13 (65.0)	19 (86.4)	
History of surgical resection, n (%)						1.00
Yes	25 (29.4)	7 (31.8)	6 (28.6)	6 (30.0)	6 (27.3)	
No	60 (70.6)	15 (68.2)	15 (71.4)	14 (70.0)	16 (72.7)	
Intervention compliance:						0.02
planned doses received, %						
Median	96.7	99.3	96.4	97.4	86.4	
Range	1.4–108.1	28.9–101.7	5.6–108.1	50.0–103.1	1.4–103.9	
Adverse events, n (%) <sup>b</sup>						
Any grade	60 (70.6)	15 (68.2)	14 (66.7)	12 (60.0)	19 (86.4)	0.25
Grade 1	48 (56.5)	11 (50.0)	12 (57.1)	10 (50.0)	15 (68.2)	0.60
Grade 2	26 (30.6)	5 (22.7)	4 (19.0)	4 (20.0)	13 (59.1)	0.02
Grade 3	5 (5.9)	2 (9.1)	2 (9.5)	0 (0.0)	1 (4.5)	0.71

<sup>a</sup>P value for comparison across randomization arms, using Fisher's exact test.

<sup>b</sup>Maximum severity adverse event per participant.

samples included normal mucosa ( $n = 113$ ; 59%), hyperplasia ( $n = 72$ ; 38%), low-grade dysplasia ( $n = 4$ ; 2%), and other ( $n = 3$ ; 2%).

## Discussion

In this prospective, randomized clinical trial, 6-month interventions with sulindac, atorvastatin, and ORAFIT<sup>®</sup>Synergy1 did not yield significant reductions in rectal ACF number, as compared with control (maltodextrin), among subjects at increased risk for sporadic CRC. To our knowledge, this study represents the largest ACF-based CRC chemoprevention trial reported to date and the first such trial to be conducted in a stand-alone fashion rather

than ancillary to or embedded within a larger parent study. Furthermore, secondary analyses of tissue-based biomarkers (assessed from normal appearing rectal mucosa) in our study revealed that none of the interventions had a statistically significant effect on cellular proliferation whereas all of interventions (including the maltodextrin control) were associated with increased apoptosis. Yet, because the observed proapoptotic effects did not differ appreciably between the active and control interventions, the relevance of these latter data to defining the CRC chemopreventive potential of sulindac, atorvastatin, and ORAFIT<sup>®</sup>Synergy1 remains indeterminate.

Only two other prospective CRC chemoprevention trials have been reported with change in rectal ACF number as

**Table 4.** Change in rectal ACF number by randomization arm

	Overall (n = 77)	Atorvastatin (n = 20)	Sulindac (n = 18)	ORAFIT <sup>®</sup> Synergy1 (n = 20)	Control (n = 19)
Baseline					
Median	9.0	9.0	9.5	8.5	9.0
Range	5–34	5–24	5–34	5–28	6–17
Mean	10.3	10.1	11.1	10.2	9.7
SD	5.2	5.0	6.9	5.5	3.1
Postintervention					
Median	8.0	8.0	7.5	8.5	8.0
Range	0–41	3–41	1–27	1–34	0–20
Mean	9.9	11.0	9.9	10.3	8.5
SD	7.6	8.6	8.0	8.7	4.9
% Change <sup>a</sup>					
Median	–7.1	5.6	–18.6	–3.6	–10.0
Range	–100 to 160	–69 to 143	–83 to 160	–88 to 83	–100 to 117
Mean	–5.3	8.4	–13.3	–8.8	–8.6
SD	50.3	49.1	55.4	49.2	48.8
P <sup>b</sup>	n/a	0.30	0.60	0.92	n/a
P <sup>c</sup>	0.20	0.59	0.12	0.54	0.41

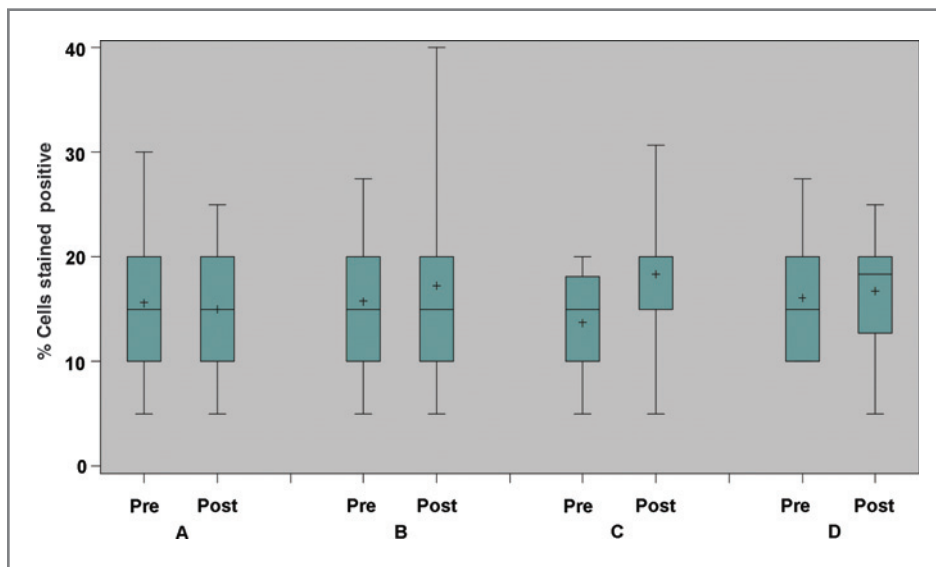
<sup>a</sup>(Postintervention ACF number – baseline ACF number)/Baseline ACF number.

<sup>b</sup>Wilcoxon rank-sum P value for comparison of % change in ACF between each active intervention (atorvastatin, sulindac, or ORAFIT<sup>®</sup>Synergy1) versus control.

<sup>c</sup>Signed rank P value for comparison of percentage change in ACF within each randomization arm.

the primary endpoint (17, 20). Takayama and colleagues initially described the results of an open-label trial of sulindac 100 mg tid conducted among a subset of subjects (n = 20) enrolled in an MCE study designed to evaluate ACF prevalence across CRC risk groups (20). Eleven subjects who received sulindac for 8 to 12 months had sig-

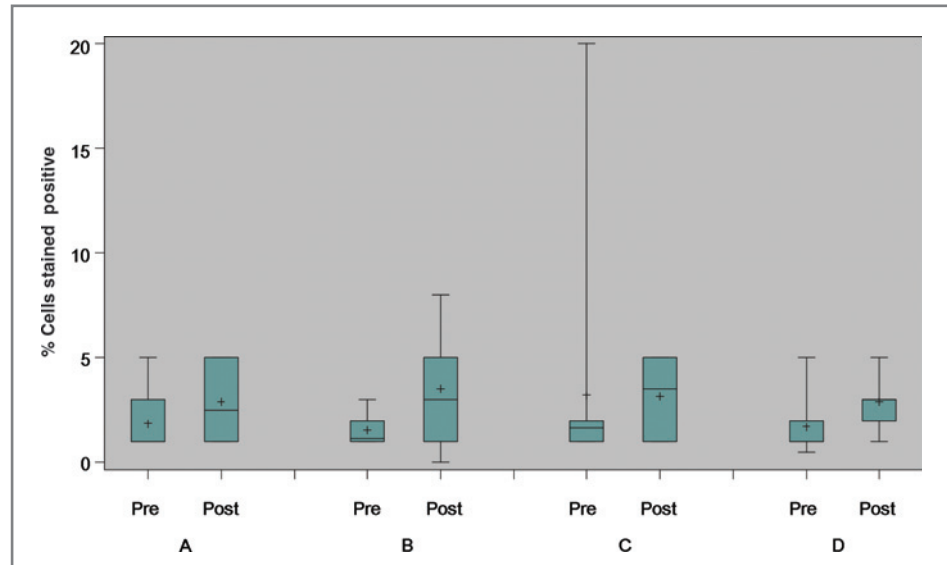
nificantly fewer rectal ACFs at the posttreatment MCE examination than 9 untreated subjects (P < 0.001 for difference between groups). More recently, Cho and colleagues (17) used a substudy design to assess change in ACF number among 45 participants enrolled in the Adenoma Prevention with Celecoxib (APC) trial. Following an 8- to



**Figure 3.** Change in proliferation (Ki67), by intervention arm. Based on biopsy samples from normal-appearing rectal mucosa obtained during the preintervention evaluations. Box and whisker plot, demonstrating median, interquartile range, and extreme values; mean value also represented (+). Arm A: atorvastatin 20 mg tablet once per day; Arm B: sulindac 150 mg tablet twice per day; Arm C: ORAFIT<sup>®</sup>Synergy1 6 gm powder twice per day; Arm D: control (maltodextrin powder) twice per day.



**Figure 4.** Change in apoptosis (caspase-3), by intervention arm. Based on biopsy samples from normal-appearing rectal mucosa obtained during the preintervention (baseline) and postintervention evaluations. Box and whisker plot, demonstrating median, interquartile range, and extreme values; mean value also represented (+). Arm A: atorvastatin 20 mg tablet once per day; Arm B: sulindac 150 mg tablet twice per day; Arm C: ORAFTI<sup>®</sup>Synergy1 6 gm powder twice per day; Arm D: control (maltodextrin powder) twice per day.



12-month intervention period, celecoxib (200 mg or 400 mg bid) did not seem to beneficially modulate rectal ACF number, with average  $\pm$  SE values for pre- to postintervention change in ACF number of  $1.8 \pm 1.1$  and  $2.3 \pm 1.7$  among subjects assigned to the active versus placebo arms, respectively ( $P = 0.77$ ). Biopsy of up to 5 rectal ACFs per subject was carried out at baseline; however, accounting for baseline ACF removal did not meaningfully alter the primary endpoint comparison ( $P = 0.60$ ).

Although rectal ACFs have been widely discussed as a putative precursor to colorectal adenoma and cancer (20, 25), concerns have been recently raised regarding measurement of these lesions as an intermediate endpoint for CRC chemoprevention trials (23), due in large part to the low prevalence of histologically confirmed dysplasia. Indeed, in the aforementioned studies by Takayama and colleagues (20) and Cho and colleagues (17), dysplastic ACFs comprised only 5% (161/3,155) and 0% (0/70), respectively, of the microscopically analyzed lesions. In our trial, dysplastic ACFs were similarly infrequent (2%; based on histologic review of postintervention ACF biopsy samples). Previous observational studies have reported a somewhat wider range of dysplastic ACFs (0%–23%), albeit from variably defined subject populations (26–30). Nonetheless, further investigation seems prudent to clarify the validity of rectal ACF as a surrogate marker for CRC risk before incorporating these lesions as intermediate endpoints in future prevention studies.

Emerging data suggest that the natural history of rectal ACF could also influence the results of short-term CRC chemoprevention trials. In an ancillary study to the Prostate, Lung, Colorectal and Ovarian (PLCO) cancer screening trial, Schoen and colleagues performed follow-up MCE examinations at 1 year on 64 subjects with rectal ACF identified (mean = 2.1 per subject), but not removed, at

the baseline evaluation (31). Forty-three percent of the baseline ACFs were reidentified at follow-up. Moreover, 56% of the subjects had newly identified rectal ACFs. A subsequent analysis of subjects who underwent rectal ACF removal at baseline and returned for follow-up MCE examination 1 year later showed relatively little change in the mean number of lesions per subject (2.25 vs. 1.93; ref. 32), indicating a dynamic change in ACF progression, regression, and/or detection rates over the lifespan of a typical phase II CRC chemoprevention trial. To explore this possibility in our intervention cohort, we conducted sensitivity analyses on the basis of incident versus prevalent ACFs and observed no appreciable differences in the agent-specific effects (data not shown).

Potential challenges related to the MCE examination should also be considered when interpreting the results of our phase II CRC chemoprevention trial. Most notably, based again on data from the PLCO ancillary ACF study, the interrater agreement for most endoscopic criteria used to identify rectal ACFs seems to be low (33). In addition, up to 55% of endoscopically identified ACFs have not been confirmed by histology in other North American studies (27), which is consistent with the "false-positive" rate observed in our trial. Thus, variability in the endpoint assessment might have contributed to our generally null results.

Major strengths of our study include the prospective, multicenter trial design that afforded successful achievement of our revised accrual target. We also utilized a standardized MCE examination protocol, which incorporated pretrial ACF knowledge assessment and uniform, prototype endoscopy equipment across study sites to minimize potential influence from technical challenges on the primary endpoint evaluation. Yet, even with this rigorous approach, striking SDs were observed in the  $\% \Delta$ ACF data.

Several additional factors might have affected our ability to detect a significant benefit from sulindac, atorvastatin, or ORAFTI® Synergy1. First, polymorphisms in flavin monooxygenase 3 have been shown to influence sulindac efficacy in other high-risk populations (34) but were not measured in our study cohort. Second, on the basis of a recently published meta-analysis of data from 18 studies and involving more than 1.5 million subjects (35), the magnitude of CRC risk reduction associated with statin use may be much lower (8%) than anticipated during the design phase of our trial. In fact, observational data from the APC trial cohort imply that statin drug use may be associated with increased adenoma risk among subjects with previously resected colorectal neoplasia (36). Third, while prebiotic dietary fiber alone might be anticipated to provide a somewhat lesser degree of CRC risk reduction than candidate pharmaceutical agents, limited available data suggest that ORAFTI® Synergy1 could have greater anticancer potential if given in combination with probiotic agents (16, 37). Fourth, despite being larger than any previously reported CRC chemoprevention trial with an ACF endpoint (17, 20), our study was only moderately powered to detect the defined intervention effect. Finally, because the assessed biomarkers (endoscopic and tissue-based) were measured from the rectum only, it remains conceivable that chemopreventive effects confined to the colon could have gone undetected.

In summary, data from this phase II randomized, partially blinded chemoprevention trial do not provide convincing evidence of CRC risk reduction from atorvastatin, sulindac, or ORAFTI® Synergy1. Larger sample size, longer intervention period, and/or alternate endpoints should be

considered if further evaluation of these candidate agents is pursued. Ongoing investigation of the endoscopic, histologic, and molecular characteristics of rectal ACFs that accurately reflect CRC risk may also serve to clarify if, or how, these lesions can be effectively applied as surrogate markers in future prevention studies.

### Disclosure of Potential Conflicts of Interest

P.J. Limburg served as a consultant for Genomic Health, Inc., from August 12, 2008 to April 19, 2010. Mayo Clinic has licensed P.J. Limburg's intellectual property to Exact Sciences and he and Mayo Clinic have contractual rights to receive royalties through this agreement.

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