Summary

Colorectal cancer is the second leading cause of cancer death in the United States. Difluoromethylornithine (DFMO) irreversibly inhibits ornithine decarboxylase (ODC), which catalyzes the synthesis of polyamines thought to be necessary for colon cancer cell growth. This study investigates whether DFMO suppresses polyamine concentrations in human colon tissue, whether or not such suppression is prolonged with extended treatment, and whether polyamine concentrations return to normal after treatment is ended.

A randomized, double-blind, placebo controlled, Phase IIb trial was conducted at the University of California, Irvine, with 114 patients who had colon polyps in the past. They were randomized to receive placebo or DFMO at 0.075, 0.2 or 0.4 g/m²/day for 12 months. Polyamine concentrations were measured from colonoscopy biopsy samples at 0, 6, 12, and 15 months. Primary outcomes were putrescine concentration and the ratio of spermidine to spermine concentrations.

At 6 months, geometric mean putrescine was decreased by 91% with 0.4 g/m²/day DFMO vs. placebo (geometric mean ratio = 0.09; 95% CI: 0.03, 0.29; p = 0.0002) and was also decreased with lower DFMO doses. The geometric mean spermidine to spermine ratio was decreased by 25% with 0.4 g/m²/day DFMO vs. placebo (geometric mean ratio = 0.75; 95% CI: 0.54, 1.05; p = 0.0972) and was also decreased with lower DFMO doses. By 12 months, putrescine was suppressed by 88% with 0.4 g/m²/day DFMO vs. placebo (geometric mean ratio = 0.12; 95% CI: 0.02, 0.67; p= 0.0173), but such a strong effect was not seen at lower DFMO doses. The spermidine to spermine ratio was 39% lower with 0.4 g/m²/day DFMO vs. placebo (geometric mean ratio = 0.61; 95% CI; 0.47, 0.79; p=0.0005) and similar reductions were seen with lower DFMO doses. At 15 months (3 months after the end of treatment), putrescine was not decreased with 0.4 g/m²/day DFMO vs. placebo (geometric mean ratio = 0.98; 95% CI: 0.61, 1.56; p = 0.9206) or at any lower DFMO doses. The spermidine to spermine ratio was not decreased with 0.4 g/m²/day DFMO vs. placebo (geometric mean ratio = 1.01; 95% CI: 0.82, 1.25; p = 0.9057) or at any lower DFMO doses.

These results give evidence that DFMO effectively suppresses putrescine and the spermidine to spermine ratio at 6 months. Suppression continues for the duration of treatment in the highest dose group. Regardless of dose, the concentrations return to normal levels by three months after stopping treatment.

Comment [A1]: Overall, a very nice paper.

Comment [A2]: You could mention ages, sex here.

Comment [A3]: Somewhere in here I would comment on the missing data, as there is a trend toward greater missing data in the highest dose group.

Comment [A4]: We would likely be interested in seeing a dose-response (one of Koch's postulates). Hence giving some estimates for the ower dose would be nice.

Background

Colon cancer is one of the most common cancers and has a high fatality rate.(1, 2) Precancerous lesions in the colon called polyps may develop into colon cancer.(3) Adenomatous polyps, the type most likely to develop into cancer, are present in approximately 30% of middle-aged or elderly people.(4) Patients with colon polyps present an important opportunity for cancer prevention.

Difluoromethylornithine (DFMO) is being investigated as a colon cancer preventive agent because of its ability to block one biological pathway involved in cell proliferation. Several authors have reviewed the relevant biological mechanisms and their implications for colon cancer prevention.(5-9) Here we summarize the key concepts.

Cell proliferation involves increased synthesis of the polyamines putrescine, spermidine (thought to be essential for DNA replication), and spermine. The first step in polyamine synthesis is the conversion of ornithine to putrescine by the enzyme ornithine decarboxylase (ODC). DFMO blocks the enzymatic action of ODC. In studies of DFMO administration in experimental tumors, putrescine levels consistently show marked decrease, and spermidine levels often decrease moderately, but spermine levels remain stable or even increase slightly.(10,11) Moderate decreases in spermidine coupled with slight increases in spermine may produce substantial decreases in the spermidine to spermine ratio, even when changes in spermidine and spermine themselves are not remarkable.(10,11) The spermidine to spermine ratio is thought to be important for cell proliferation,(10) and proliferating tumors are found to have higher spermidine to spermine ratios than regressing tumors or non-cancerous tissue.(12,13) In cell cultures, DFMO effectively inhibits cell proliferation, and in animal studies it inhibits tumor growth.(5-9)

DFMO is appealing as a possible colon cancer prevention agent in patients with a history of colon polyps, because it is well-tolerated by patients and has been shown to have low, reversible toxicity at doses below 1 g/m²/day.(14) ODC and polyamine levels are elevated in colon polyps to 3-4 times the levels found in normal colon cells.(5-9) Inhibiting polyamine synthesis in the colon tissue might prevent the progression of polyps to colon cancer. Thus the present trial seeks to determine whether DFMO treatment will effectively inhibit polyamine synthesis in colon tissue in patients with a history of colon polyps.

Questions of Interest

Client Questions:

1) In humans, is DFMO administration at 0.075, 0.2, or 0.4g/m2/day associated with a change in colonic mucosal polyamine concentrations at 6 months when compared with placebo? If so, which dose and which polyamine?
2) If a change is seen at 6 months with a particular dose of DFMO, is that effect persistent at 12 months of therapy when compared with placebo?
3) If an change is seen at 12 months of therapy, does the effect persist at 15 months, with 3 months off of therapy when compared with placebo?

Our Questions:

Is there an association between DFMO dose, when compared with placebo, and colonic mucosal polyamine concentrations at 6, 12 and 15 months in this dataset?

Our outcome variables for polyamine concentration include putrescine and the spermidine/spermine ratio and our primary outcome measure was the ratio of geometric means comparing each dosage group to placebo.

Sources of Data

This study was a randomized, double-blind, placebo-controlled trial conducted at the University of California, Irvine. 114 subjects with a history of colonic polyps were randomly assigned to one of three doses of DFMO (0.075, 0.2, or 0.4 g/sq m/day) or placebo. We do not have information about randomization procedures. The only demographic information we have available for each subject is their age and sex. Men are overrepresented in all dose groups (88.6-100% of subjects in the four groups). The 0.2g/m2/day dose group had no women. This gender balance is not representative of the current population with colon cancer (RR 1.34 for men vs. women in 2004).(15)

Comment [A5]: Indeed. About half the patients came from a VA hospital. In this age range, such patients are overwhelmingly male.

At 0, 6, 12 and 15 months, participants underwent a colonoscopy with a mucosal tissue biopsy that was analyzed for polyamine concentrations (putrescine, spermine, and spermidine micromole/mg protein). Over the course of the study, 5 (15.6%) participants on placebo, 3 (10.3%) on dose 0.075, 4 (16.0%) on dose 0.2, and 10 (35.7%) on dose 0.4 were lost to follow-up and failed to provide biopsies for some time points after baseline. Participants with missing biopsies were similar to other participants at baseline with respect to age, sex, and polyamine levels (analyses not shown).

Comment [A6]: Good to note. I probably would have put this in the Results for this particular analysis, while other times I would put it here in Methods as you did. What tips the balance this time is the fact that there was a suggestion of increased trend in the highest dose group. This suggests the possibility of toxicities. Furthermore, if these are toxicities, it could be related to polyamine levels and thus be biasing.

We considered age and sex as possible confounders. Age was well-balanced across dose groups and thus would not be considered a confounder in our analyses even if it predicted polyamine concentrations. Sex would not be considered a confounder in our analyses because there is no evidence from the literature that sex would influence polyamine levels,(5-9) and polyamine levels in our sample were similar for men and women throughout the course of the study.

Statistical Methods

Baseline patient characteristics were tabulated descriptively by treatment group to assess for potential effect modification. Arithmetic means are displayed in this case (Table 1). For all descriptive and inferential analyses, polyamine levels of 0 micromol/mg protein were replaced with 50% of the next lowest value of

that polyamine. These included 26 putrescine measurements, 2 spermidine measurements and 2 spermine measurements. We considered this a reasonable approximation given the potential limits of laboratory detection and the possibility of technician error in processing samples. The outcome variable of the ratio of spermidine to spermine was created including these adjusted values. The distributions of polyamine concentrations and spermidine to spermine ratio were right-skewed, the variance was substantially different across dose groups, and the standard deviations were proportional to the means. Log-transformation of the polyamine concentrations and the spermidine to spermine ratio improved the skewness and made the variances more similar across dose groups (plot not shown). Therefore, we compared geometric means between dose groups instead of arithmetic means. The graphically displayed descriptive statistics reflect geometric means. Associations between dose group and placebo were assessed using geometric means and two-sided two-sample t-tests, assuming unequal variance. Statistical testing was performed using Intercooled STATA™, College Station, TX.

Comment [A7]: This could go in the Methods instead. And even though this describes your methods perfectly, I would have included the "imputed" value (which value is of course a "result".

Comment [A8]: If only you had not included the "therefore". You should make decisions like this before looking at the data.

Our summary measures (arithmetic or geometric means) can be interpreted as our best estimates of a particular polyamine at a particular time point in a particular dose group. The confidence intervals (in the inferential analysis) can be interpreted as follows: our observed results would not be unusual if the true values in the population were within the CI. The p-value represents the probability that we would have observed by chance the experimental result, or a more extreme result, if the null hypothesis were true. Although a multiple regression analysis would be a more rigorous way to analyze these data, that tool was not at our disposal. Although we descriptively plotted the summary measures for each dose group for each polyamine over time, we chose to perform inferential analysis on the dose group summary measures compared with placebo rather than a paired analysis within each patient. This was a reasonable choice given the randomized study design and the relative similarity of the patients at baseline. There was also a significant change from baseline in the placebo group, which may have complicated the interpretation of within-patient comparisons. We thereby avoided time-varying covariates and the necessity to account for correlated observations.

Comment [A9]: Not only reasonable, but really the only correct choice. Comparisons over time confound the effect of DFMO with aging, secular trends in the environment, seasonality, laboratory drift, etc.

Comment [A10]: We included a randomized placebo group to protect us agains this

Descriptive Results

The study sample consisted of 114 patients (97 men, 17 women) ranging in age from 45 to 81 years at baseline. Baseline patient characteristics by dose group are shown in Table 1. Mean age at baseline was similar across dose groups, varying from 61.3 to 65.9 years. Sex, however, was not well-balanced by randomization. Although dose groups 0, 0.075, and 0.4 had 17-21% women, dose group 0.2 had no women. Arithmetic mean polyamine concentrations in biopsied colon tissue were similar across all treatment groups at baseline. The distributions of polyamine concentrations tended to be right-skewed, with some outliers in the upper tails.

Geometric mean polyamine concentrations by dose group over the duration of follow-up are shown in Table 2 and Figure 1. In the placebo group, putrescine levels increased steadily throughout follow-up; spermidine levels remained stable through 12 months, then declined; spermine levels tended to decline throughout follow-up. The non-placebo groups had similar patterns of polyamine concentrations. Putrescine levels were lower at 6 months than at baseline, but subsequently rose again; spermidine levels were also lower at 6 months than at baseline, and remained relatively stable through month 15; spermine levels declined steadily throughout follow-up, as in the placebo group. Differences from the placebo group tended to be dose-related, particularly for putrescine and spermine at six and 12 months.

The ratio of spermidine to spermine (Figure 1, panel D) in the placebo group increased through month 12 but returned to the baseline level by month 15. In the three non-placebo groups, the ratio was lower at six months than at baseline, tended to remain low through 12 months, but returned to or exceeded the baseline value at 15 months.

Results of inferential statistics

All inferential tests are based on the ratio of geometric means and the null hypotheses are that there are no differences in putrescine concentration or the spermidine to spermine ratio between the dosage groups at any given time point. P-values are based on a two-sided t-test with unequal variances. Results for

putrescine and spermidine/spermine ratio at 6, 12, and 15 months are shown in Table 3, and the results significant at the 0.05 level are summarized below.

Comment [A11]: In this analysis involving multiple dose groups and multiple times, the small sample sizes would mean that it is quite likely that we would not always have statistical significance. But the estimates would still be of interest. So I would not necessarily restrict attention to only the stat signif results. Instead provide the overall picture.

Putrescine concentrations were significantly reduced at 6 months for all dosage groups and at 12 months for the highest dose. At 6 months, geometric mean putrescine concentration for the 0.075 g/m²/day dose group was 65% lower than for the placebo group (95% CI: 21% to 85% lower; p = 0.0136); for the 0.2 g/m²/day dose group it was 67% lower than for the placebo group (95% CI: 31% to 84% lower; p = 0.0049); and for the 0.4 g/m²/day dose group it was 93% lower than for the placebo group (95% CI: 71% to 97% lower; p = 0.0002). At 12 months, geometric mean putrescine concentration for the 0.4 g/m²/day dose group was 88% lower than for the placebo group (95% CI: 33% to 99% lower; p = 0.0173). Thus we would reject the null hypotheses of no difference in putrescine concentration compared to placebo for 0.075, 0.2 or 0.4 g/m²/day DFMO at 6 months, and for 0.4 g/m²/day DFMO at 12 months.

Spermidine/spermine ratios were significantly reduced at 6 and 12 months for the lowest dose, and at 12 months for the highest dose. The geometric mean spermidine/spermine ratio for the 0.075 g/m²/day dose group was 28% lower than for the placebo group (95% CI: 9% to 42% lower; p = 0.0055) at 6 months and 27% lower than for the placebo group (95% CI: 7% to 43% lower; p = 0.0119) at 12 months. The geometric mean spermidine/spermine ratio for the 0.4 g/m²/day dose group was 39% lower than for the placebo group (95% CI: 21% to 53% lower; p = 0.0005) at 12 months. Thus we would reject the null hypotheses of no difference in spermidine/spermine ratio compared to placebo for 0.075 g/m²/day at 6 and 12 months and for 0.4 g/m²/day at 12 months.

Discussion

We found evidence of an association between DFMO administration at any dose between 0.075 and 0.4 g/m2/day and a decline in putrescine levels at 6 months, but this effect persisted at 12 months only in the 0.4 dose group and was absent by 15 months (3 months off of study drug). We found a trend toward a decrease in spermidine/spermine ratio at 6 months in all dose groups, but this was only statistically significant in the 0.075 dose group. At 12 months, this effect was seen only in the 0.4 dose group. At 15 months, no difference from placebo could be determined.

Based on this trial, DFMO would be recommended as an agent of suppression of polyamine concentrations at any of these doses for six months. At 0.4g/m2/day, it is possible that the effect might persist for 12 months or more. Regardless of dose, the effect of DFMO on polyamine concentrations did not persist for 3 months after the study drug was stopped.

One limitation of our analysis was multiple testing. We reported results of 18 separate t-tests and did not perform any statistical adjustment for multiple comparisons. However, simply by chance, 18 tests would be expected to yield less than one p-value < 0.05, and we observed 7 p-values < 0.05. Therefore it would be unlikely that our significant results arose because of multiple testing.

A second limitation was missing biopsy data at time points after baseline, particularly among patients in the dose 0.4 group, in which the percentage missing at each time point was much higher than for other dose groups or placebo. Other studies have shown only low and reversible toxicity by DFMO at doses up to 1 g/m2/day.(14) However, the pattern of missing data in our study raises concerns for dose-related toxicity even at 0.4 g/m2/day that might be severe enough to discontinue treatment. The higher drop-out rate in dose 0.4 could have caused DFMO to appear spuriously favorable if DFMO would have had less impact on polyamines in participants who dropped out compared to participants who remained in the study. Unfortunately, we do not have variables describing adherence to the treatment regimen or the reasons for failure to provide biopsy. Thus we cannot verify whether missing biopsies were due to toxicity-related drop-out or to other factors.

Comment [A12]: Personally, I would lead off with this paragraph. It limits the interpretability of all other results, if the loss of follow-up was due to toxically low polyamine levels.

Gender would be an effect modifier in this relationship only if polyps are more likely to be found in men than women. When plotted descriptively (not shown), there does not appear to be evidence of effect modification by gender. We cannot predict whether DFMO would be effective in patients with colon cancer, as the subjects in this study only had a history of colonic polyps. Polyps are quite common in the

Comment [A13]: No. Sex is an effect modifier if the effect of DFMO on geom. mean polyamines is different across sexes.

population and colon cancer relatively rare, so the study population is likely more representative of the overall population than the patients who might be likely to receive DFMO if it were shown to be effective against colon cancer. We do not know from the information available whether the patients in this study were more likely to have familial polyposis syndromes than the overall population. These patients might benefit more given their higher risk of colon cancer.

References

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Table 1: Patient characteristics at baseline by DFMO treatment group.

	Mean (SD)	Min	Median	Max
	Dose: 0 g/m^2/d (N = 32)			
Female (%)	18.8			
Age in years	65.9 (8.5)	45.5	66.4	77.2
Putrescine	0.66 (0.44)	0.06	0.57	1.98
Spermidine	3.26 (1.45)	1.40	2.93	7.05
Spermine	8.22 (5.54)	1.46	7.52	35.55
	Dose: 0.075 g/m^2/d (N = 29)			
Female (%)	17.2			
Age in years	61.3 (7.7)	47.8	61.4	76.9
Putrescine	0.65 (0.52)	0.01	0.54	2.59
Spermidine	3.47 (1.55)	1.51	2.91	7.02
Spermine	8.43 (5.86)	4.13	7.32	37.67
	Dose: 0.2 g/m^2/d (N = 25)			
Female (%)	0			
Age in years	62.8 (8.3)	45.4	63.7	77.6
Putrescine	0.61 (0.42)	0.001	0.60	1.96
Spermidine	3.35 (1.33)	1.70	2.92	6.22
Spermine	9.03 (7.04)	2.54	7.53	41.68
	Dose: 0.4 g/m^2/d (N = 28)			
Female (%)	21.4			
Age in years	63.9 (7.8)	48.5	65.0	81.0
Putrescine	0.65 (0.57)	0.001	0.60	2.30
Spermidine	3.56 (1.88)	0.66	3.08	7.60
Spermine	8.08 (5.50)	2.28	6.81	34.04

Age was missing for one participant in the 0.075 g/m^2/d dose group.
Polyamine concentrations were measured as micromoles/mg protein.
Values of zero for polyamines were replaced with half the next lowest value.

Table 2. Geometric mean (range) polyamine concentrations and spermidine to spermine ratio at baseline, 6, 12, and 15 months by DFMO dose.

DFMO dose ($g/m^2/d$)	N (% missing)	Putrescine	Spermidine	Spermine	Spermidine to spermine ratio
Baseline					
0 (placebo)	32 (0)	0.52 (.061-1.98)	2.97 (1.40-7.05)	7.17 (1.46-35.55)	0.41 (.12-1.16)
0.075	29 (0)	0.46 (.01-2.59)	3.17 (1.51-7.02)	7.61 (4.13-37.67)	0.42 (.13-1.11)
0.2	25 (0)	0.35 (.001-1.96)	3.13 (1.70-6.22)	7.94 (2.54-41.68)	0.39 (.12-1.73)
0.4	28 (0)	0.27 (.001-2.3)	3.11 (.66-7.6)	7.19 (2.28-34.04)	0.43 (.20-1.16)
6 months					
0 (placebo)	30 (6.3)	0.71 (.05-9.14)	3.07 (1.51-6.91)	6.88 (3.31-14.39)	0.45 (.14-1.10)
0.075	28 (3.4)	0.28 (.001-1.05)	2.51 (1.39-5.12)	7.75 (4.60-15.67)	0.32 (.20-.64)
0.2	23 (8.0)	0.25 (.001-2.43)	2.23 (1.07-7.84)	6.71 (2.35-12.13)	0.33 (.17-1.72)
0.4	25 (10.7)	0.09 (.001-1.73)	2.39 (1.06-6.34)	7.11 (2.76-17.20)	0.34 (.16-2.20)
12 months					
0 (placebo)	28 (12.5)	0.75 (.001-3.18)	3.01 (1.01-5.91)	5.67 (2.32-14.55)	0.53 (.21-2.07)
0.075	26 (10.3)	0.71 (.04-4.29)	2.75 (1.35-4.92)	7.12 (3.15-14.13)	0.39 (.19-.73)
0.2	21 (16.0)	0.48 (.001-3.21)	2.32 (.29-6.45)	6.52 (2.96-13.83)	0.36 (.03-1.03)
0.4	20 (28.9)	0.20 (.001-5.48)	1.71 (.15-3.42)	5.40 (.73-10.66)	0.32 (.18-.76)
15 months					
0 (placebo)	27 (15.6)	0.98 (.30-6.51)	2.54 (1.25-4.62)	5.95 (2.83-12.05)	0.43 (.24-.97)
0.075	26 (10.3)	0.94 (.001-3.53)	2.79 (0.15-4.83)	5.85 (.73-12.38)	0.48 (.27-1.21)
0.2	21 (16.0)	0.79 (.001-2.59)	2.85 (1.81-4.81)	5.11 (1.93-12.38)	0.56 (.25-2.18)
0.4	18 (35.7)	0.96 (.23-3.20)	2.57 (1.29-4.47)	5.96 (2.53-11.52)	0.43 (.22-.75)

Polyamine concentrations were measured as micromoles/mg protein.
Values of zero for polyamines were replaced with half the next lowest value.

Figure 1. Geometric mean polyamine concentrations and spermidine to spermine ratio at baseline, 6, 12, and 15 months by DFMO dose.

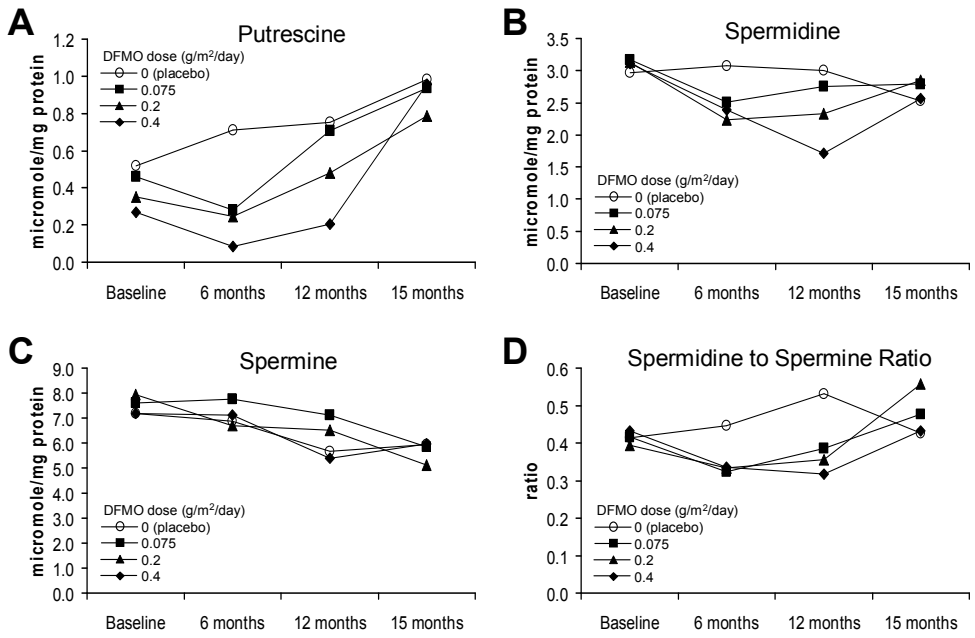

Table 3. Results of t-tests comparing the ratio of geometric means of the given dose group to placebo.

			95% Confidence Interval			
DFMO Dose	Time (Months)	Ratio of Geom Means	Lower Bound	Upper Bound	2-sided p-value	
			Putrescine			
0.075	6	0.35	0.15	0.79	0.0136	*
	12	1.15	0.50	2.70	0.7368	
	15	0.79	0.40	1.59	0.5029	
0.2	6	0.33	0.16	0.69	0.0049	*
	12	0.68	0.24	1.92	0.4675	
	15	0.64	0.29	1.43	0.2631	
0.4	6	0.09	0.03	0.29	0.0002	*
	12	0.12	0.02	0.67	0.0173	*
	15	0.98	0.61	1.56	0.9206	
			Spermidine/spermine ratio			
0.075	6	0.72	0.58	0.91	0.0055	*
	12	0.73	0.57	0.93	0.0119	*
	15	1.08	0.85	1.35	0.5646	
0.2	6	0.75	0.55	1.00	0.0515	
	12	0.67	0.45	1.01	0.0554	
	15	1.32	0.97	1.75	0.076	
0.4	6	0.75	0.54	1.05	0.0972	
	12	0.61	0.47	0.79	0.0005	*
	15	1.01	0.82	1.25	0.9057	

Polyamine concentrations were measured as micromoles/mg protein.
Asterisk (*) denotes p-values < 0.05.