Difluoromethylornithine Induced Changes in Polyamine Concentrations in Humans: Implications for Primary Prevention of Colorectal Carcinoma

Background: Colorectal carcinoma is a common and potentially fatal neoplasm, but effective preventive therapies are currently lacking. Difluoromethylornithine (DFMO) is an experimental agent that has shown potential to prevent colorectal cancer through inhibition of polyamine synthesis, but its long-term efficacy in human subjects has not been tested.

Objective: The purpose of this Phase IIb clinical investigation is to evaluate the long-term efficacy of DFMO in suppressing colonic polyamine concentrations using a randomized, double-blind, placebo-controlled trial of subjects with a history of colonic polyps.

Methods: Volunteer subjects (17F, 97M) with known colonic polyps were randomized to receive placebo or one of three doses of DFMO (0.075, 0.2 or 0.4 g/m^2/d) over a 12 month treatment period. Polyamine concentrations were measured from colonic biopsy samples obtained 0, 6, 12 and 15 months (3 months post-therapy). The efficacy of DFMO was assessed using a pre-specified primary endpoint of the spermidine to spermine ratio (Spd/Spm), paired t-test comparisons of means and intention-to-treat analysis.

Results: Following 6 months of DFMO therapy, there was a statistically significant -0.106 decrease in Spd/Spm (95% upper bound = -0.029, p=0.013) only in the 0.075 g/m^2/d dose group. Following 12 months of DFMO therapy, there was a statistically significant -0.143 decrease in Spd/Spm (95% upper bound = -0.023, p=0.026) only in the 0.4 g/m^2/d dose group. There was no evidence that suppression of Spd/Spm increased from the 6 to 12 month time points, and following cessation of DFMO therapy, Spd/Spm ratios returned to baseline limits in all dose groups.

Conclusions: At a dose of 0.4 g/m^2/d, DFMO therapy resulted in a significant and sustained reduction of colonic Spd/Spd, although the high attrition rate (36%) raises questions about the tolerability or safety of this dose. Further investigation of the safety and utility of this agent for the primary prevention of colorectal carcinoma is warranted.

Colorectal cancer is the third most common malignant neoplasm worldwide; in 1988, it was estimated to make up 9% of all diagnosed cancers, with approximately equal distribution among males and females.[1] While there is growing evidence that adenomatous polyps represent pre-cancerous lesions,[2, 3] routine screening strategies for detection of colonic polyps are currently debated.[4, 5] Epidemiologic investigations have identified overnutrition and an excess of dietary fat as key etiologic variables affecting the incidence of colorectal cancer.[6] This suggests that cancer risk is modifiable, but effective preventive strategies among high risk populations are currently lacking.

To date, approaches for preventing colorectal cancer have been concerned with modifying diet, with existing pharmaceutical agents like non-steroidal anti-inflammatory drugs, and with experimental chemicals. One novel approach uses difluoromethylornithine (DFMO), an experimental irreversible inhibitor of ornithine decarboxylase, the rate-limiting step in polyamine synthesis. Polyamines are important for cell replication in normal tissues and have a role in the transcription of specific growth-related genes, which may be a mechanism through which they modulate cell growth.[7] Depletion of polyamines has been shown to inhibit neoplastic growth in the laboratory and in animal models,[8] and DFMO has been shown to inhibit the production of polyamines and thereby modify carcinogenesis in murine tumor models and cancer cell lines.[9, 10] The suggestion, then, is that DFMO may prevent or reverse the development of colorectal cancer through inhibition of polyamine synthesis.

The safety and short-term efficacy of DFMO in humans has been recently established in phase I and IIa dose-ranging investigations.[11] In these studies, 28-day administration of DFMO was generally well-tolerated, although the highest doses were associated with ototoxicity and non-specific gastro-enterologic symptoms. Whether lower, safe doses of DFMO are effective at suppressing colonic polyamine concentrations over a longer treatment period is the subject of the present investigation.

QUESTIONS OF INTEREST

This investigation has four scientific aims. The primary aim is to determine whether safe doses of DFMO, administered over 6 months, will result in suppression of colonic polyamine concentrations compared to baseline levels. The second aim is to determine if continued therapy, as measured at 12 months, results in sustained colonic polyamine suppression compared to baseline levels. The third aim is to determine if colonic polyamine suppression increases with duration of DFMO therapy, which may have negative safety implications. The final aim is to determine if colonic polyamine concentrations return to baseline following cessation of DFMO therapy.

Comment [A1]: Please don't use this format for the summary, because I cannot insert comments.

In your summary:

- you should mention which polyamines we are interested in.
- your ability to do an ITT analysis was hampered by study dropout
- you should mention the study dropout, especially because there was a trend toward greater dropout at the highest doses.
- I would not use a 95% upper bound. More standard to report the 95% CI, which would correspond to a 97.5% upper bound. Also, when you report only the upper bound, it then makes us wonder whether you are using one-sided or two-sided p values. You would need to make this clear.
- Obviously you have a multiple comparison problem: You refer to .075 group at 6 months and 0.4 groups at 12 months. It is VERY MUCH of interest to know what the point estimates are doing in the other groups. Your selected reporting seems to suggest that you think lack of statistical significance means lack of effect. This is not the case, and we would need to see whether trends were similar in the other groups.
- You are being too deterministic to say that the spd:spm limits returned to baseline, without providing estimates or CI. It is perhaps OK to limit your presentation in the Summary, but I would still be less deterministic: "…3 months following cessation of DFMO therapy, there was no statistically significant difference between placebo and any of the dose groups." (I would really want this better quantified—referring to the point estimates would be nicer.)
- The dropout mentioned in your conclusions is never mentioned in your results. This is a major result and the questions raised are not just of toxicity, but also a systematic bias in your results (if the toxicity was due to low polyamines).

Comment [A2]: Interesting wording. Is this to say that we are not interested in the effects if the dose is not safe, or are we putting a caveat in about potential dropout. Either way is OK, but to the extent you are trying to say the latter, even better. But this brings up issues of population effects, intent-to-treat, and whether we actually measured this in this trial.

METHODS

A. Study Design. This randomized, double-blind, placebo-controlled investigation of DFMO was performed at the University of California Irvine Medical Center. Subjects (n=114) were randomly assigned to placebo or one of three doses of DFMO (0.075, 0.2 and 0.4 g/m^2/day) for 12 months duration. Colonoscopy was performed at baseline to screen for colonic polyps and to obtain biopsy samples. Serial colonoscopies and biopsies were then performed at 6 and 12 months of treatment, and at 15 months (3 months post-cessation of therapy). Polyamine concentrations were measured from these biopsy samples, including putrescine, spermidine and spermine concentrations. The spermidine to spermine ratio, previously shown to be an effective marker of ornithine decarboxylase activity,[12] served as the primary efficacy endpoint. This study was approved by the Institutional Review Board and Scientific Review Committee of the University of California Irvine Medical Center. DFMO was administered under FDA-approved Investigational New Drug application #B5172007.

B. Study Population. Volunteer subjects with a history of colonic polyps were recruited from local databases and referrals. Subjects were then contacted by telephone and invited to participate. Subjects were excluded if they had a prior history of colon cancer, bleeding diatheses, a profound aversion to needles, or an inability to give informed consent. Compliance with the medication was self-reported and subjects could voluntarily withdraw from this study for any reason. Informed written consent was obtained from all subjects prior to their enrollment.

C. Data Sources. Baseline characteristics of the participants were obtained using self-reported age and gender. Putrescine, spermidine and spermine concentrations were measured from biopsy samples using previously described methodology. As several biopsy samples were obtained at each time point, the mean polyamine concentration from these samples was used for analysis.

D. Statistical Methods. For the descriptive statistics, estimates of the mean, standard deviation (SD), minimal, median and maximal values are reported for continuous variables, while counts and proportions are provided for dichotomous variables. The spermidine to spermine ratio (Spd/Spm) was considered the primary outcome in the statistical analysis. To compute ratios for data points where spermine concentrations were zero (due to left censoring of concentration measurements), these zero values were replaced by one-half the smallest positive value in the total sample. Screening for outliers was performed with the understanding that subjects with elevated polyamine levels may be of particular scientific interest. Stratified counts are shown to highlight the progression of dropouts over the study period.

The methodologies for statistical inference were determined *a priori* after careful consideration of the scientific aims. Since the first and second scientific questions address the effect of the drug over time rather than the need to determine the "best" dose, longitudinal data was used and the relevant paired t-tests were performed to compare the difference of the means at the 6 and 12 month time points to their baseline levels, stratified by dose groups. Because the aim of the study is specifically to determine if Spd/Spm is suppressed by DFMO therapy, one-sided analyses were performed. Spd/Spm in the placebo group was also analyzed over time in order to determine the natural trend of this ratio over the course of the treatment period. To assess sustainability of dose-effect, one-sided paired t-tests were used to compare the difference of the means at the 12 and 6 months measurement points. To assess if Spd/Spm returned to baseline following cessation of therapy, a two-sided paired t-test was used to compare mean Spd/Spm at 15 months and baseline. Two-sided analyses were performed because there are potential clinical implications if the Spd/Spm returned either above or below original baseline values. Exploratory analyses utilized unpaired, two-sample t-test comparisons of means, allowing for unequal variances.

As data regarding medical compliance were unavailable, intention-to-treat analysis was performed. All available data were used for descriptive analysis; baseline data from participants who failed to complete the trial were thus included. All computations were anachronistically performed using STATA 10.0 for Windows (StataCorp LP, College Station TX). One-sided analyses report the 95% upper confidence bound (UCB); two sided analyses report 95% confidence intervals (CI). A p-value threshold of <0.05 was used to determine statistical significance for all inferences; significant p-values are listed in text, non-significant p-values are reported or detailed in Table 3.

The authors had full access to the data and take responsibility for the integrity of this analysis. Portions of the study design and subject recruitment have been fictionalized for completeness and entertainment.

Comment [A3]: Good to note and this reference to prior literature protects you from having to explain the rationale (even though I might explain it here as well)

Comment [A4]: You might say what that turned out to be (admittedly a result, but saying it here would dispense with having to mention it further)

Comment [A5]: This could be left out—it would be presumed.

Comment [A6]: NO. This is only of very limited interest. We are interested in how DFMO affects the levels. We randomized in order to isolate that effect from effects due to aging, calendar time, secular trends in diet or other aspects of the environment, and laboratory drift. Hence the major comparison should be across dose groups.

Comment [A7]: This is a good thing to do. A one-sided analysis should be reporting one-sided p values compared to 0.025. We generally still report two-sided 95% CI, though you could report the 97.5% one-sided confidence bound.

Comment [A8]: This approach ruined a perfectly good experiment: We had randomized to remove confounding, and you ignored it.

Comment [A9]: Good observation. But using one-sided sometimes and two-sided other times makes the reporting more complicated. Because of this point, I would have just reported two-sided p values at all times, and then when presenting the results point out the times that we were only interested in one direction.

Comment [A10]: Yes, particularly the access to the full data aspect. We did in fact have data on adverse events.

RESULTS

A. Study Population. A total of 114 subjects (17 females, 97 males) were enrolled. The baseline characteristics of the study population and the baseline polyamine concentrations are shown in Table 1. Subjects ranged in age from 45 to 81 years, with a mean ± SD of 63.9 ± 8.2 years. The estimated mean ± SD baseline putrescine, spermidine and spermine concentrations were 0.648 ± 0.486, 3.410 ± 1.553, and 8.415 ± 5.897 µmol/mg respectively. Although the distribution of polyamine concentrations appeared heavy-tailed, no spurious values were detected or excluded.

The randomization of subjects to treatment groups is within expected norms. There are no significant between-group differences in age or baseline polyamine concentrations, supporting successful randomization. While no females were randomized to the 0.2 g/m^2/d dose group, the overall number of females in this study is low (17/114).

There was substantial attrition in all treatment groups. Of the 114 subject initially enrolled, 22 subjects were lost to follow up, including 5 (16%), 3 (10%), 4 (16%) and 10 (36%) in the placebo and 0.075, 0.2 and 0.4 g/m^2/d treated groups respectively. However, at each measurement point, there were no statistically significant estimated differences in means of baselines Spd/Spm concentrations for those that completed the study compared to those who were lost to follow up (p-values ranged 0.26-0.92).

B. Change in Spd/Spm in Response to Therapy. The spermidine to spermine ratio (Spd/Spm) at baseline and follow up are detailed in Table 2. For the full population, baseline Spd/Spm ranged from 0.118 to 1.729, with a mean ± SD of 0.464 ± 0.247. Mean baseline ratios and standard deviations appear similar across all dose groups. Figure 1 demonstrates temporal trends in point estimates and 95% confidence intervals for each of the four groups.

Table 3.1 summarizes the means of the differences in Spd/Spm between 6 month and baseline, stratified by dose level, with relevant 95% UCB and one-sided p-values. In the placebo treated group, there was an estimated increase in the mean Spd/Spm of 0.041 compared to the baseline mean, however this increase did not reach statistical significance. Conversely, there were estimated decreases in each of the three actively treated dose groups. While the estimated decrease of 0.106 (95% UCB of a decrease $\geq$ 0.029) in the 0.075 g/m^2/d dose group reached the level of statistical significance (p=0.013), the estimated decreases in the 0.2 and 0.4 g/m^2/d dose groups were not statistically significant compared to their baseline levels.

Table 3.2 summarizes the means of the differences in Spd/Spm between 12 months and baseline, stratified by dose level, with relevant 95% UCB and one-sided p-values. In the placebo treated group, there was an estimated increase in Spd/Spm of 0.180 compared to the baseline mean, however this increase again did not reach the level of statistical significance. Again, conversely, there were estimated decreases in each of the three actively treated dose groups. While the estimated decrease in Spd/Spm for the 0.4 g/m^2/d dose group of 0.142 (95% UCB of a decrease $\geq$ 0.023) reached statistical significance (p=0.026), the estimated decreases in the 0.075 and 0.2 g/m^2/d dose groups were not statistically significant compared to their baseline levels.

C. Change in Polyamine Ratios in Response to Duration and Cessation of Therapy. Table 3.3 summarizes the means of the differences in Spd/Spm between 12 months and 6 months, stratified by dose level, with relevant 95% UCB and one-sided p-values. There were no statistically significant differences in the estimated mean Spd/Spm ratios between 6 months and 12 months for any of the four dose groups.

Table 3.4 summarizes the means of the differences in Spd/Spm between 15 months and baseline, stratified by dose level, with relevant 95% CI and two-sided p-values. There were no statistically significant differences in the estimated mean Spd/Spm ratios between baseline and 15 months for any of the four dose groups.

D. Effect of Sex on Polyamine Ratios. The 0.2 g/m^2/day dose group showed no significant decrease in Spd/Spm at any measurement point but was also the only dose group without female subjects. To assess for effect modification by sex, stratified analysis was performed on all subjects taking active DFMO therapy. Sex-stratified point-estimates and 95% confidence intervals for Spd/Spm are shown in Figure 1 (Panel B). While females (n=10) had an estimated 0.055 (95% CI: -0.296 to 0.186) greater decrease in Spd/Spm than males (n=66) at the 12 month measurement point, this difference did not reach statistical significance (p=0.629). Similar findings were observed at the 6 month measurement point. Thus sex does not appear to significantly alter the observed association between DFMO therapy and Spd/Spm levels (although the counts used for this analysis were small).

Comment [A11]: A little bit of statistical jargon. Maybe "a tendency toward some extreme values"

Comment [A12]: If by this you mean not statistically significant, this was a waste of time and ink. If you mean no important differences in the point estimates, this is a very appropriate observation. For that reason, I would use something other than "significant", because readers might fear you performed statistical tests, and were using the p values to tell you whether you needed to be worried. In that latter case, I would worry that your use of the wrong criteria meant that I had to be on the lookout for important differences that you did not detect. One cannot use statistical hypothesis testing to evaluate confounding. Lack of statistical significance does not prove equality ever, much less in the sample.

Comment [A13]: good to note

Comment [A14]: You seem again to be placing too much emphasis on statistical significance. I am worried about bias long before I would expect to have statistical significance. I note that this loss to follow-up may or may not bias our results—we have no way of knowing. But it is suggestive of possible toxicity. We would want to know the stated reasons for drop-out.

Comment [A15]: again, nonstandard

Comment [A16]: You never answered the scientific question, because you never compared the dose groups. This is a VERY serious problem.

Comment [A17]: And again, you needed to compare across dose groups and not regard that lack of statistical significance proved equality. Commenting on the precision of your CI is very important.

Comment [A18]: A good observation, and good to motivate the exploratory analysis. Of course, you would not really have adequate precision. This analysis combining all dose groups is ill-advised to address this question.

DISCUSSION

This study is the first phase IIb investigation of the efficacy of DFMO in suppressing colonic polyamine concentrations. The data from this randomized, double-blind, placebo controlled trial show that after 6 months of therapy, there was a statistically significant 0.106 estimated decrease (95% UCB of at least 0.029) in Spd/Spm only in the 0.075 g/m^2/day dose group, but this decrease was no longer significant at 12 month of therapy. These data further show that after 12 months of therapy, there was a statistically significant 0.142 estimated decrease (95% UCB of at least 0.023) in Spd/Spm only in the 0.4 g/m^2/day dose group. Thus only the highest DFMO dose appears effective at sustained suppression of polyamine concentrations over 12 months of therapy.

Additionally, these data showed no convincing evidence that Spd/Spm were significantly lower at 12 months as compared to 6 months in any of the treatment groups. Nor was there evidence that Spd/Spm at 15 months were significantly different (either higher or lower) than their respective baseline levels. Together, these data suggest that prolonged DFMO therapy does not cause continued Spd/Spm suppression, nor does one year of DFMO therapy result in sustained effects on mean Spd/Spm after drug discontinuation.

Choice of Statistical Methodology. This study sought to determine if DFMO therapy resulted in suppression in polyamine concentrations. Spd/Spm was chosen as the primary endpoint for two main reasons. First, previous data has suggested that this is an effective measure of ornithine decarboxylase activity,[12] the main target of DFMO therapy. Second, there was concern about the statistical implications of multiple testing, and a single measure of efficacy was preferred. Also for this reason, no between-dose group comparisons were performed.

Although strongly considered, log transformation of the polyamine concentration data was not performed. Although baseline polyamine concentrations appeared somewhat heavy-tailed, these were not felt to result in significant skew of the data. Moreover, it was felt that log transformation could result in significant loss of precision.

Finally, polyamine concentrations were compared with their baseline levels rather than with the placebo group because this was felt to best answer the scientific questions posed. The overarching purpose of this investigation is to test and develop an agent that can be used to reduce the risk of colorectal cancer in a high risk population. Spd/Spm serves as a surrogate measure of colon cancer risk. Thus for this analysis, a reduction in risk was defined as an absolute reduction in Spd/Spm rather than merely a decrease compared with placebo. It was felt that if the experimental preventive agent was unable to reduce Spd/Spm compared to baseline levels, then it would not be significantly reducing the absolute risk of colorectal cancer in this high-risk population.

Placebo Group Performance. The above choice of analyses is important in light of the unusual trend seen in the placebo treated group. Rather than remaining constant over the course of the treatment period, placebo group Spd/Spm increased throughout the course of the trial, and then decreased following cessation of therapy. Although these changes did not reach the level of statistical significance (p values reported in Table 2), the estimated magnitude of increase in Spd/Spm ratio in the placebo group (+33% at 12 months) was greater than the estimated magnitude of decrease in any of the DFMO treated groups.

One hypothesis that might explain this trend is that blinded subjects may have felt a psychological protective benefit of taking a study drug and liberalized their diets in response; they then resumed stricter diets at study completion. Fatty diets are a risk factor for the development of colorectal cancer, and may also increase Spd/Spm ratios. Other possibilities include changes in NSAID use, however, these explanations are speculative and the observed changes may be solely due to random chance. But if patients taking active drug therapy had a similar changes in medication or lifestyle, these trends may weaken the observed Spd/Spm-lowering effects of DFMO when compared to baseline. Thus it is possible that an analysis of the data using a comparison to the placebo group could reach additional conclusions about the efficacy of DFMO. Such an analysis was not performed due to concerns about its *post hoc* origin and the assumption that in order to justify further drug development (and placing subjects at additional risk), the drug should be more effective than lifestyle modifications or other contemporaneous preventive strategies.

Subject Dropout. There was substantial attrition over the course of this study, with ~19% of initially enrolled patients lost to follow up by the final 15 month sample point. This has both statistical and safety implications. There were no statistically significant differences in baseline Spd/Spm ratios between those who completed the study and those that did not, suggesting that dropout was unrelated to Spd/Spm. However, it remains possible that this dropout is due to atypical Spd/Spm responses to DFMO therapy, and is thus skewing the results.

Comment [A19]: Way, way, WAY overinterpreted. I would instead look at similarity of trends, carefully considering the precision with which I could detect differences. The question we have to address is why a low dose would have a more rapid effect that waned (if we are to take your stance of living and dying by statistical significance), while the higher dose had an effect that appeared later. You need to make it all fit together.

Comment [A20]: How do you reconcile these results with the first paragraph in which you seemed to suggest that in the 0.4 group there was no difference at 6 mos but there was at 12 mos. You need to consider trends and precision.

Comment [A21]: You completely missed the boat on this one. The between dose comparisons were the most important ones to do.

Comment [A22]: Actually, with analyses of biomarkers, I find that log transformation is most often easily justified biochemically (Micheealis-Menten kinetics), and statistically greater precision is most often obtained. In my experience, it is very rare that inference about the geometric mean is less precise than inference about the mean, when both are equally scientifically relevant.

Comment [A23]: Nope

Comment [A24]: No, we want to know whether DFMO would reduce levels from what they might otherwise be. As noted above, aging, seasonal effects, laboratory drift and many other things might cause the placebo group to differ over time.

Comment [A25]: And the possibility of this is what you were trying to protect yourself against when you included a placebo group.

Comment [A26]: But NSAID use would be expected to 1) lead to lower proliferation and 2) be similar across the groups (unless DFMO gives you headaches or aches and pains)

Comment [A27]: I would lead off the discussion with this point. In addition to its possible relevance to the toxicity of the drug, it present a possible bias in our results.

Comment [A28]: Nope. First, the sample sizes are small so we lack precision—lack of stat signif does not prove equality. Second, we want to knw about Spd:Spm at the time of dropping out. The subjects with the lowest levels of spd:spm at follow-up may not be the ones with the lowest baseline values.

As the dropout rate in the 0.075 and 0.2 g/m^2/day groups are similar to the placebo group (10% and 16% vs. 16% respectively), it seems likely that dropout in these groups is due to chance alone. However, the highest-dose group had substantially more attrition (36% vs. 16% for placebo group), raising concerns about the tolerability of the medication at this dose. Analysis of the safety data and reasons for discontinuation may be informative.

Comment [A29]: Yes.

Study Limitations. This study and analysis has several limitations. First, data regarding medical compliance was not available, and thus an intention-to-treat analysis was performed. This may serve to reduce the estimated differences between on-therapy Spd/Spm levels and baseline. Second, as discussed above, there was substantial attrition in this study, and data regarding safety and reasons for discontinuation are unavailable. Third, as exemplified by the performance of the placebo group, there is substantial individual variation in Spd/Spm over time, perhaps related to changes in diet or other factors. This may serve to minimize apparent differences between on-therapy Spd/Spm ratios and baseline, thus making a Type II statistical error more likely. Finally, there is an overrepresentation of males in this study compared to disease prevalence. As there are no known biological effect differences in Spd/Spm or DFMO metabolism between males and females, and no effect modification was detected, this is not felt to significantly alter the validity of these results.

Comment [A30]: No. Hopefully you did an ITT analysis because that is THE MOST appropriate analysis. A per-protocol analysis is very problematic. While we do sometimes do that in an exploratory way, we would never do that preferentially

CONCLUSIONS.

In this phase IIb study, 6 months of DFMO was effective at reducing colonic Spd/Spm ratios compared to baseline only in the 0.075 g/m^2/day dose group, but this benefit was no longer apparent at the 12 month follow up point. The highest, 0.4 g/m^2/day dose group showed significantly reduced colonic Spd/Spm ratios at 12 months compared to baseline, but had substantial subject attrition. Thus DFMO at a dose of 0.4 g/m^2/day appears effective at suppressing polyamine levels over a sustained time period, and further investigation of the potential safety and utility of this agent for primary prevention of colorectal carcinoma is warranted.

Comment [A31]: Does all of this (as written) make any scientific sense at all? Why would a lower dose cause a more rapid effect? Why would we trust the high dose effect, if we observed only a transient, faster effect at the lower dose? Wouldn't we worry that the effect at the higher dose would also be transient?

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Table 1: Characteristics of study population, including serial polyamine concentrations, stratified by DFMO dose group.

	Dose = 0 g/m2/day						Dose = 0.075 g/m2/day						Dose = 0.2 g/m2/day						Dose = 0.4 g/m2/day					
	N	**Mean**	**SD**	**Min**	**p50**	**Max**	**N**	**Mean**	**SD**	**Min**	**p50**	**Max**	**N**	**Mean**	**SD**	**Min**	**p50**	**Max**	**N**	**Mean**	**SD**	**Min**	**p50**	**Max**
Randomized, n	32						29						25						28					
Females, n (%)	6	(19%)					5	(17%)					0	(0%)					6	(21%)				
Age, years	32	65.9	8.5	45.5	66.4	77.2	28	61.3	7.7	47.8	61.4	76.9	25	62.8	8.3	45.4	63.7	77.6	28	63.9	7.8	48.5	65.0	81.0
Putrescine Concentrations																								
Baseline	32	0.665	0.441	0.061	0.569	1.975	29	0.655	0.522	0.009	0.537	2.588	25	0.611	0.418	0.000	0.595	1.964	28	0.654	0.570	0.000	0.596	2.300
6 Months	30	1.055	1.593	0.050	0.719	9.135	28	0.470	0.267	0.000	0.487	1.053	23	0.453	0.542	0.000	0.273	2.432	25	0.333	0.432	0.000	0.194	1.726
12 Months	28	1.158	0.835	0.000	0.904	3.180	26	1.085	1.027	0.036	0.693	4.285	21	0.796	0.789	0.000	0.510	3.212	20	0.882	1.424	0.000	0.401	5.484
15 Months	27	1.541	1.773	0.303	0.800	6.511	26	1.193	0.889	0.000	0.911	3.526	21	1.003	0.714	0.000	0.868	2.585	18	1.177	0.832	0.227	0.849	3.197
Spermidine Concentrations																								
Baseline	32	3.262	1.451	1.398	2.934	7.054	29	3.474	1.551	1.509	2.911	7.017	25	3.350	1.329	1.700	2.924	6.218	28	3.565	1.885	0.660	3.084	7.600
6 Months	30	3.369	1.532	1.505	3.025	6.912	28	2.639	0.892	1.388	2.456	5.120	23	2.583	1.643	1.068	1.847	7.844	25	2.677	1.428	1.059	2.069	6.344
12 Months	28	3.256	1.314	1.013	2.816	5.910	26	2.920	0.994	1.352	2.859	4.923	21	2.712	1.395	0.293	2.509	6.454	20	1.950	0.799	0.000	1.929	3.417
15 Months	27	2.688	0.933	1.249	2.445	4.616	26	2.951	0.989	0.000	2.984	4.832	21	2.979	0.903	1.806	2.807	4.805	18	2.704	0.866	1.289	2.687	4.465
Spermine Concentrations																								
Baseline	32	8.215	5.536	1.455	7.516	35.550	29	8.429	5.860	4.130	7.318	37.667	25	9.026	7.038	2.537	7.527	41.684	28	8.083	5.496	2.277	6.807	34.044
6 Months	30	7.341	2.707	3.308	6.507	14.389	28	8.075	2.487	4.595	8.010	15.674	23	7.177	2.485	2.345	7.366	12.129	25	8.044	3.949	2.760	8.015	17.199
12 Months	28	6.550	3.591	2.324	5.240	14.553	26	7.751	3.116	3.145	8.346	14.128	21	7.155	3.151	2.956	6.629	13.826	20	5.926	2.582	0.000	5.973	10.664
15 Months	27	6.395	2.448	2.831	5.785	12.051	26	6.691	3.322	0.000	6.295	12.381	21	6.078	3.555	1.930	4.611	12.376	18	6.425	2.533	2.525	6.000	11.518

Abbreviations**:** SD=standard deviation, Min=minimum, p50=median, Max=maximum.
Polyamine concentration units in μmol/mg

Table 2: Primary endpoint: spermidine to spermine ratios, stratified by DFMO dose group.

Dose Group	N	Missing	Mean	SD	Min	Median	Max
Placebo Group							
Baseline	32	0	0.464	0.228	0.118	0.367	1.157
6 Months	30	2	0.505	0.270	0.144	0.406	1.101
12 Months	28	4	0.619	0.432	0.207	0.491	2.068
15 Months	27	5	0.452	0.162	0.245	0.461	0.967
Dose = 0.075 g/m^2/d							
Baseline	29	0	0.459	0.217	0.130	0.432	1.112
6 Months	28	1	0.343	0.123	0.197	0.309	0.637
12 Months	26	3	0.410	0.140	0.192	0.392	0.726
15 Months	26	3	0.508	0.281	0.000	0.362	1.214
Dose = 0.2 g/m^2/d							
Baseline	25	0	0.456	0.315	0.120	0.339	1.729
6 Months	23	2	0.400	0.333	0.167	0.278	1.725
12 Months	21	4	0.447	0.277	0.027	0.375	1.027
15 Months	21	4	0.677	0.502	0.247	0.537	2.185
Dose = 0.4 g/m^2/d							
Baseline	28	0	0.478	0.243	0.201	0.405	1.160
6 Months	25	3	0.446	0.440	0.157	0.322	2.202
12 Months	20	8	0.339	0.158	0.000	0.342	0.757
15 Months	18	10	0.456	0.155	0.223	0.407	0.748

Abbreviations: N= number of subjects, SD=standard deviation, Min=minimum, Max=maximum.

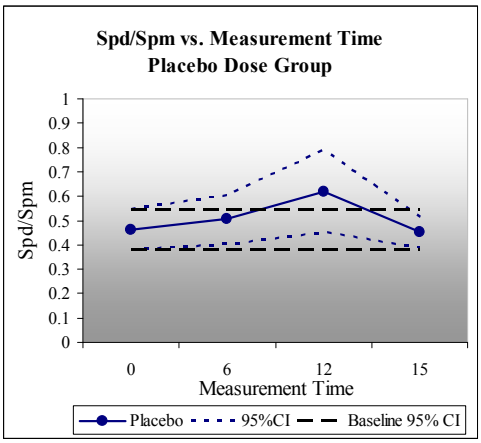
Table 3: Summary of inferential statistical analyses, stratified by scientific aim, with corresponding point estimates of mean differences in spermidine to spermine ratios, 95% confidence intervals and respective p-values.

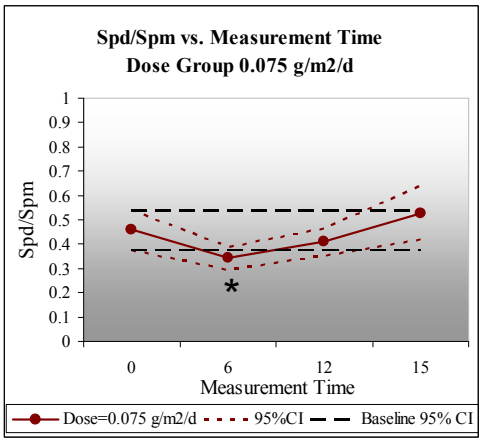
Scientific Aim	Null Hypothesis	Dose Group	Mean Difference	95% CI		p-value
1) Suppression of polyamines?	*H_0: mean of 6Mo - BL >= 0, 1-sample t-test, 1-sided p-value*	Placebo	0.041	- Inf	0.143	0.749
		0.075	-0.106	- Inf	-0.029	0.013*
		0.2	-0.053	- Inf	0.108	0.289
		0.4	-0.043	- Inf	0.150	0.352
2) Sustainability of suppression?	*H_0: mean of 12Mo - BL >= 0, 1-sample t-test, 1-sided p-value*	Placebo	0.180	- Inf	0.335	0.972
		0.075	-0.032	- Inf	0.059	0.278
		0.2	-0.005	- Inf	0.191	0.482
		0.4	-0.142	- Inf	-0.023	0.026*
3) Progression of suppression?	*H_0: mean of 12Mo - 6Mo >= 0, 1-sample t-test, 1-sided p-value*	Placebo	0.074	- Inf	0.222	0.801
		0.075	0.061	- Inf	0.124	0.944
		0.2	0.060	- Inf	0.220	0.739
		0.4	-0.115	- Inf	0.073	0.151
4) Return to normal?	*H_0: mean of 15Mo - BL = 0, 1-sample t-test, 2-sided p-value*	Placebo	0.092	-0.074	0.257	0.264
		0.075	0.016	-0.147	0.178	0.844
		0.2	0.078	-0.176	0.332	0.529
		0.4	0.109	-0.226	0.444	0.501

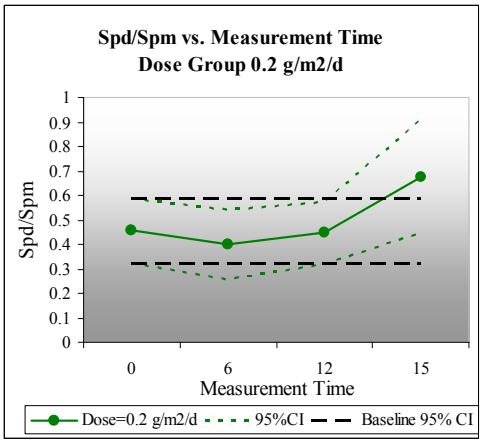
Abbreviations: CI = confidence intervals, BL = baseline measurement point, 6Mo = 6 month measurement point, 12Mo = 12 months measurement point, 15Mo = 15 month measurement point, - Inf = negative infinity.
Dose group units in g/m^2/day.
* labels statistical significance at $p<0.05$.

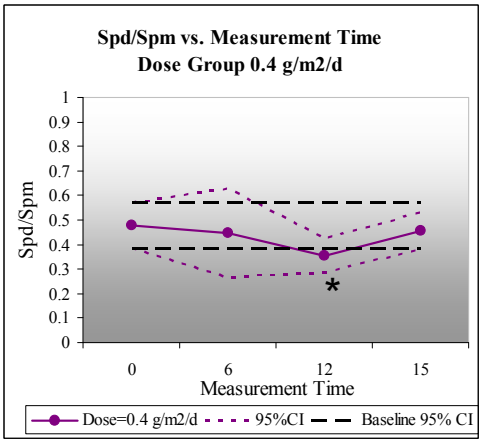
Figure 1: Panel A: Spermidine to spermine ratios versus measurement time, stratified by dose group. Panel B: Spermidine to spermine ratios versus measurement time, stratified by sex. (* labels significance at p<0.05)

PANEL A









PANEL B