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Randomized, Double-blind, Placebo Controlled Trial of Sulindac in Individuals at Risk for Melanoma: Evaluation of Potential Chemopreventive Activity

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Abstract

Background—Reduced melanoma risk has been reported with regular use of non-steroidal anti-inflammatory drugs (NSAIDs). However, NSAIDs ability to reach melanocytes in vivo and modulate key biomarkers in pre-neoplastic lesions such as atypical nevi has not been evaluated.

Methods—Randomized, double-blind, placebo controlled trial of sulindac conducted in individuals with atypical nevi (AN) to determine bioavailability of sulindac and metabolites in nevi and effect on apoptosis and vascular endothelial growth factor A (VEGFA) expression in AN. Fifty subjects with 4mm AN and one benign nevus (BN) were randomized to sulindac (150 mg BID) or placebo for 8 weeks. Two AN were randomized for baseline excision, and 2 AN and BN were excised post-intervention.

Results—Post-intervention sulindac, sulindac sulfone, and sulindac sulfide concentrations were 0.31 ± 0.36 , 1.56 ± 1.35 , 2.25 ± 2.24 $\mu\text{g/ml}$ in plasma, and 0.51 ± 1.05 , 1.38 ± 2.86 , 0.12 ± 0.12 $\mu\text{g/g}$ in BN, respectively. Sulindac intervention did not significantly change VEGFA expression but did increase expression of the apoptotic marker cleaved caspase 3 in AN (increase of 3 ± 33 in sulindac vs. decrease of 25 ± 45 in placebo arm, $p=0.0056$), although significance was attenuated ($p=0.1103$) after adjusting for baseline expression.

Conclusion—Eight weeks of sulindac intervention resulted in high concentrations of sulindac sulfone, a pro-apoptotic metabolite, in BN but did not effectively modulate VEGFA and cleaved caspase-3 expression. Study limitations included limited exposure time to sulindac and the need to optimize a panel of biomarkers for NSAIDs intervention studies.

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Keywords

melanoma; prevention and control; chemoprevention; nevus; anti-inflammatory agents; non-steroidal; atypical nevi

Introduction

Melanoma is the fifth most common cancer in men and the seventh in women in the US, with 70,230 newly diagnosed cases and 8,790 deaths anticipated in 2011 [ACS Cancer Facts & Figures 2011]. Solar UVR exposure remains the major environmental risk factor, and incidence continues to increase despite public health initiatives promoting sun protection. The increasing incidence of melanoma and its poor prognosis in advanced stages mandate the investigation of novel primary prevention approaches such as chemoprevention. To date, limited intervention trials have been conducted to evaluate potential chemopreventive agents for melanoma prevention.¹⁻³ Individuals with atypical or dysplastic nevi have been targeted for chemoprevention efforts because dysplastic nevi are the most important clinical marker of increased melanoma risk and can also serve as potential precursor lesions.⁴⁻⁷

Preclinical studies suggest that certain non-steroidal anti-inflammatory drugs (NSAIDs) inhibit cell proliferation and induce growth inhibition in melanoma cell lines.^{8,9} NSAIDs have also been shown to suppress tumor growth of melanoma cells implanted in surgical wounds¹⁰ and augment the antitumor effect of interleukin-1 alpha or interferon-gamma on mouse models of melanoma.^{11,12} Recent epidemiological studies suggest that regular use of NSAIDs reduces the risk of melanoma, although there are some inconsistencies in the data between studies.¹³⁻¹⁷ Controlled intervention trials are clearly warranted to define the role of NSAIDs for melanoma prevention.

We conducted a randomized, double-blind, placebo controlled trial of sulindac to evaluate its potential activity for melanoma prevention in individuals with multiple atypical nevi (AN). We prioritized sulindac over other NSAIDs for evaluation in the melanoma prevention setting because it demonstrates a broad spectrum of anti-cancer activity and possesses pharmacokinetic characteristics that are likely to have favorable drug distribution to the skin after oral administration. In addition, it has demonstrated bioactivity in the skin in rodent models following oral administration.¹⁸⁻²⁰ We hypothesized that sulindac intervention can result in distribution of sulindac and related metabolites to the target tissue and favorable changes in surrogate endpoint biomarkers in AN.

Patients and Methods

Study Design and Subjects

A randomized, double-blind, placebo controlled intervention trial was conducted in healthy men and women at risk for melanoma, who were randomly assigned to receive either sulindac (150 mg BID) or placebo for eight weeks. Adults between 18 and 65 years of age with at least 4 clinical AN and one BN suitable for biopsy and measuring at least 5mm in size were eligible to participate. Subjects were recruited from the Pigmented Lesion Clinics at the Arizona Cancer Center/University of Arizona and Stanford University Medical Center and Cancer Institute. Individuals were considered ineligible if the following exclusion criteria were identified: contraindication to prolonged NSAIDs intake, more than one prior invasive cutaneous melanoma (one prior stage I, IIa or IIb melanoma permissible if off treatment for >3 months); family history of ≥ 2 first-degree relatives with melanoma; modified dermoscopy score of >4.8 for the selected AN; histological diagnosis of melanoma on baseline biopsy; pregnant or nursing women; use of tanning beds within 6 months of

study entry; and unwilling to minimize exposure to sunlight while enrolled. Sulindac and placebo tablets were supplied by the National Cancer Institute/Division of Cancer Prevention. The study was approved by the Institutional Review Boards at University of Arizona and Stanford University. Informed consent was obtained from all participants.

Study Procedures

During the initial visit, participants underwent eligibility evaluation, including an interview and physical exam to obtain medical history, performance status, height, weight, blood pressure, pulse, and temperature. A blood sample was collected for complete blood count (CBC) with differentials and comprehensive metabolic panel (CMP). A dermatologic exam was performed to determine whether the participants had more than 4 large atypical nevi and one benign nevus.

Study AN were selected by clinical evaluation using unaided visual inspection following the definition proposed by the WHO in 1990: 5mm, must include a macular component in at least one area and at least two of the following features: ill defined borders, color variegation, and uneven contour. The study AN were required to measure 5mm and < 15 mm. Epiluminescent microscopy (ELM) examination was performed using the pattern analysis and modified ABCD rule algorithms to obtain a modified dermoscopy score.²¹ These criteria were used to select 4 clinically AN, 1 BN (consistent with a compound or intradermal common nevus), and to exclude lesions suspicious for melanoma or severe dysplasia.

The selected 4 study AN were numbered according to a fixed schema and photographed (standard and ELM photos), with 2 lesions randomized for baseline evaluation and 2 for post-intervention evaluation. The 2 lesions assigned to baseline evaluation were removed by excisional biopsy for histological and biomarker evaluation. Each AN was bisected, with one half oriented, embedded in OCT, and frozen to preserve histological features and then stored at -80°C. The second half was immediately fixed in 10% neutral buffered formalin for 24 hrs, and then transferred to 70% ethanol prior to routine processing and paraffin embedding.

A board-certified dermatopathologist immediately processed and provided the initial histological report on the baseline AN. Once any concerning melanocytic proliferations were excluded pathologically, study subjects were randomized (1:1, 25 per arm) to receive sulindac (150 mg BID) or placebo (BID) intervention for 8 weeks.

Participants returned to the clinic after 4 weeks of agent intervention for adherence and safety evaluation. During this interim visit, the subjects returned the remaining study agent, intake calendar, and adverse event (AE) diary. After review of the documents, the subjects were given a new supply of study agent, intake calendar, and AE diary.

Upon completion of the intervention at 8 weeks, the remaining 2 study AN were similarly evaluated for clinical changes by ELM using the pattern analysis and modified ABCD rule algorithms and then photographed. The AN were surgically excised for post-intervention evaluation. The BN was also removed for sulindac and sulindac metabolite concentration determination. Post-intervention AN were processed as described above. The BN was excised and snap frozen in liquid nitrogen and then stored at -80°C. A blood sample was collected for determination of plasma sulindac and sulindac metabolite concentrations. A blood sample was also collected for CBC with differentials and CMP.

Quantification of sulindac and its metabolites in plasma and skin biopsies

Plasma concentrations of sulindac and its metabolites were quantified using a high-performance liquid chromatography tandem mass spectrometry (LC-MS/MS) method developed in our laboratory.²² For the skin biopsies, the sample was weighed and homogenized in 0.2 M perchloric acid. An aliquot of the supernatant was mixed with the internal standard solution and then extracted with dichloromethane. The organic layer was collected, processed and analyzed by LC-MS/MS similar to the plasma samples.²²

Determination of VEGF-A Expression and Cleaved Caspase 3 in AN

The expression of VEGF-A and cleaved caspase 3 was determined by immunohistochemistry (IHC) assays. Tissue blocks were sectioned at 4 μ m followed by deparaffinization and rehydration. For VEGF-A, tissue slides were stained with anti-VEGF-A (Santa Cruz #7269, 1:50 dilution) using an overnight incubation and antigen retrieval was performed with Tris/Borate/EDTA (TBE(Biocare Medical)) buffer (pH 9). For cleaved caspase 3, tissue slides were stained with anti-cleaved caspase- 3 (Cell Signaling Technologies #9661) at a 1:800 dilution overnight while antigen retrieval was performed with DIVA buffer (Biocare Medical). Positive controls included UVB irradiated HaCat cells and angiosarcoma or a brain melanoma for VEGF-A. UVB irradiated HaCat cells that were formalin fixed and paraffin-embedded, UVB irradiated abdominal skin, and tonsil tissue were used for cleaved caspase 3.

For VEGF-A and cleaved caspase 3, IHC was performed using a red alkaline phosphatase indirect biotin streptavidin system (Vector Labs) and a hematoxylin counterstain (Surgipath), and hand stained. Melanocytes were detected through Melan-A stain.

IHC staining was evaluated by an expert dermatopathologist to determine the intensity of the stain (defined as the percent of cells positive multiplied by intensity). Staining intensity was scored on a scale of 0–3, where 0 was no staining, 1 was weak, 2 was moderate, and 3 was strong. Individual scores were generated for both cytoplasmic and nuclear components of keratinocytes, junctional melanocytes, and dermal melanocytes.

Statistical Analysis

The primary endpoint was to determine the bioavailability of sulindac and sulindac metabolite in the target tissue, measured by post-intervention nevus sulindac and sulindac metabolite concentrations. The secondary endpoints included assessment of cleaved caspase 3, a protein marker of apoptosis, and VEGF expression in AN and determination of plasma sulindac and metabolite levels. VEGF and cleaved caspase 3 expression levels for each lesion were derived by multiplying the intensity score (0-3) with the associated percentage (0-100). For each participant, the average expression over all baseline lesions and all post-intervention lesions, respectively, was calculated to perform comparisons between the intervention groups. Descriptive statistics were performed on each of the endpoints within each intervention group. The distributions for some of the endpoints were not symmetrical. Therefore, a two-sided Wilcoxon Rank-Sum test was performed to test if the changes from baseline to post-intervention in each of the endpoints, including VEGF and cleaved caspase 3 expression levels and the dermoscopy score, differed by the intervention groups. Fisher's exact tests were performed to compare the histological diagnosis of the study nevi between the intervention groups and between baseline and post-intervention.

Results

Fifty-one subjects were consented between February 2009 and July 2010, with one who did meet inclusion criteria. Study subject characteristics are summarized in Table 1. As

expected, the majority of the subjects were Caucasian. The mean age of the subjects was 46.4 years in the sulindac group and 45.9 years in the placebo group, with a male:female distribution of 13/12 and 15/10 in the sulindac and placebo group, respectively. The majority of the enrolled subjects in the sulindac arm had atypical mole syndrome following the definition by Slade et al.²³ while all subjects in the placebo group demonstrated this phenotype (23/25). In addition, 14 subjects in the sulindac arm had a prior history of one primary invasive melanoma compared to 8 subjects in the placebo group.

Tolerability, Adherence, and Safety of Oral Sulindac Administration in Atypical Nevus Patients

Study subjects were closely monitored for potential AEs for the duration of the study. For those AEs recorded as “possibly or probably related” to study agents, Grade 1 heartburn was the most common symptom with 16% (n=4) of subjects in the sulindac group and 8% (n=2) on subjects in the placebo group affected (Table 2). One subject in the sulindac study arm experienced Grade 1 constipation, liver function test (LFT) elevation, as well as Grade 2 diarrhea, flatulence, and abdominal pain. The study agent administration was discontinued after the dose of sulindac was reduced by 50%, and symptoms persisted. Ultimately, the subject’s symptoms and LFT abnormalities resolved spontaneously. An additional subject experienced Grade 1 nausea, and 3 subjects reported individual Grade 2 tinnitus, fatigue, and hypertension. In this case the diagnosis of mild hypertension was established at the time of the annual physical examination, which took place while the subject was in the study. Since the subject’s baseline blood pressure was within normal limits at the initial study visit, the event was considered as possibly related to the intervention. A 100% compliance was achieved in the study since all subjects reported >than 80% study agent intake throughout the course of the study.

Nevi and Plasma Concentrations of Sulindac and Sulindac Metabolites

Baseline plasma samples and post-intervention nevi and plasma samples were obtained for measurements of sulindac and sulindac metabolite concentrations. Post-intervention nevi and plasma samples were obtained within 24 hrs from the last study agent intake dose. The post-intervention BN was not collected from one of the subjects in the sulindac group because the post-intervention visit occurred more than one week after the subject was taken off agent due to an adverse event. Sulindac and sulindac metabolites were not detected in any of the baseline plasma samples and the post-intervention nevi and plasma samples from the placebo group. Sulindac and sulindac metabolites were present in all the post-intervention plasma samples following the sulindac intervention. Sulindac sulfone was present in all the post-intervention nevi samples, while sulindac and sulindac sulfide was present in 75% and 83% of the BN, respectively. Figure 1 shows the sulindac and sulindac metabolite concentrations in the post-intervention nevi and plasma samples. Sulindac, sulindac sulfone, sulindac sulfide concentrations were 0.33 ± 0.36 , 1.63 ± 1.34 , 2.35 ± 2.24 $\mu\text{g/ml}$, respectively, in post-intervention plasma samples. Sulindac, sulindac sulfone, sulindac sulfide concentrations were 0.51 ± 1.05 , 1.38 ± 2.86 , 0.12 ± 0.12 $\mu\text{g/g}$ tissue, respectively, in post-intervention BN. The nevi-to-plasma concentration ratio was 1.68 ± 1.54 , 0.88 ± 0.79 , 0.09 ± 0.11 for sulindac, sulindac sulfone, sulindac sulfide, respectively.

Clinical and Histological Characteristics of Study Nevi

A total of 200 clinically AN were selected corresponding to 4 lesions per study subject. Following consensus review of the study nevi by 3 board-certified dermatopathologists, a total of 73.4% were diagnosed as dysplastic nevi (DN) and 26.6% as benign nevi (BN). Table 3 summarizes the histological diagnoses of the study nevi by treatment group. There was a non-statistically significant higher prevalence of DN diagnosis in the baseline (pre-intervention) nevi in the sulindac group than the placebo group (78% vs. 66%, $p=0.27$). The

prevalence of histological DN diagnosis decreased in the post-intervention nevi in the sulindac group (from 78% to 72%) while the prevalence of DN diagnosis increased in the placebo group (from 66% to 77.5%), although this differential change in prevalence was not significant when analyzed by a generalized linear mixed effects model ($p=0.14$).

The modified ABCD dermoscopy score was obtained from each of the study AN. Table 4 summarizes the dermoscopy score for study nevi randomized for post-intervention excision where a dermoscopy score was available at baseline and post-intervention. Eight weeks of agent intervention did not result in changes in dermoscopy score.

Cleaved Caspase 3 and VEGF Expression in Atypical Nevi

The expression of VEGF-A and cleaved caspase 3 was assessed according to the cell of origin and architectural location of the melanocytic cells (Figure 2). Table 5 summarizes the expression of these biomarkers for all study nevi that were deemed clinically atypical. The baseline expression of VEGF-A and cleaved caspase 3 was similar between the two study groups. VEGF-A expression was high in the melanocytic junctional component followed by the melanocytic dermal component and the keratinocytes. Cleaved caspase 3 was primarily expressed in the melanocytic junctional component. Eight weeks of agent intervention did not result in significant changes in VEGF-A and cleaved caspase 3 expression when analyzed for all study nevi (Table 5).

Similarly, when the melanocytic nevi were classified as dysplastic histologically, the baseline expression of VEGF-A was not different between the two study groups, and eight weeks of agent intervention did not result in significant changes in VEGF-A expression (Table 6). When evaluating the baseline expression of cleaved caspase 3 in junctional melanocytes in the sulindac arm corresponding to dysplastic nevi, a significantly lower level of expression was identified when compared to the placebo group (100 ± 24 vs. 150 ± 60 , $p=0.0029$). There was a statistically significant intervention effect on the cleaved caspase 3 expression in the melanocytic junctional component (an increase of 3 ± 33 in the sulindac arm vs. a decrease of 25 ± 45 in the placebo arm, $p=0.0056$). However, after adjusting for baseline values, the significance of this finding was attenuated ($p=0.1103$) based on a linear regression model.

Discussion

Atypical nevi are the most important clinical risk factor for melanoma and may serve as non-obligate biologic intermediates in melanoma tumorigenesis, making them useful for early-phase candidate chemoprevention trials. Our phase IIa randomized, placebo controlled trial demonstrated that sulindac and sulindac metabolites were bioavailable in the skin, specifically in BN following oral administration. Sulindac sulfone, a pro-apoptotic metabolite, reached the target tissue at concentrations similar to the respective plasma concentrations. The nevus distribution of sulindac sulfide, the metabolite responsible for cyclooxygenase inhibition, was limited. Eight weeks of sulindac intervention did not result in significant changes in VEGF expression, but it resulted in a marginal modulation of a marker of apoptosis, cleaved caspase 3, in the melanocytic junctional component in dysplastic nevi. This borderline effect could be due to small sample size (with only 20 subjects in the sulindac arm and 21 in the placebo arm with evaluable dysplastic nevi data) or relatively short duration of the intervention. However, this finding should be explored further since possible induction of apoptosis with sulindac intervention would be consistent with the observed identification of high concentrations of sulindac sulfone in the nevi.

Prior studies have evaluated the effect of topical tretinoin (retinoic acid analog) on AN as a surrogate marker for the chemoprevention of melanoma.²⁴⁻²⁷ Although some of these

studies demonstrated significant histologic change toward benignity, the endpoint of evaluating degree of histological dysplasia was complicated by the resulting inflammatory response from topical application of retinoid to a large segment of the back where the study nevi were located. In addition, a small pilot study examined the effect of topical imiquimod on AN.²⁸ There were no obvious clinical changes in the size and morphology of the study nevi after 16 weeks of imiquimod 5% cream applied 3 times per week. Histologically, 4 of 14 treated nevi showed a significant reduction of junctional and intraepidermal melanocytes suggestive of partial regression, compared with untreated nevi.

Despite potential activity, topical application of melanoma preventive agents imposes a number of limitations. Most importantly, while there are certain anatomical sites that are known to be at higher risk of developing melanoma according to gender and sub-type of melanoma, most melanomas arise de novo and not from precursor nevi (common, congenital, or dysplastic), and so targeting selected nevi on the skin may prove to be less effective for melanoma prevention compared with a systemic approach.

Prior studies have evaluated oral isotretinoin²⁵ and beta-carotene²⁹ in patients with dysplastic nevi but failed to demonstrate activity. We conducted the current study to evaluate the potential activity of NSAIDs for melanoma prevention because recent epidemiological studies suggest that regular use of NSAIDs, particularly aspirin reduces the risk of melanoma.¹³⁻¹⁷ We elected to study sulindac because it demonstrates a broad spectrum of anti-cancer activity and well-documented safety. Given the long prescription history of this agent, adverse reactions can be minimized with knowledge of the established predisposing risk factors, including but not limited to history of adverse reaction to any NSAID and active GI disease.

The anti-inflammatory activity of sulindac is believed to be mostly through inhibition of prostaglandin synthesis by the sulfide metabolite.^{30, 31} Sulindac sulfone possesses minimal activity toward cyclooxygenase; however, it has been shown to be a pro-apoptotic compound. In melanoma cell lines, both sulindac metabolites are effective in reducing the number of viable cells.³² The decrease in cell viability is accompanied by an induction of apoptosis, measured by morphological changes and fragmented DNA in cell lysates.³²

Because of their long half-lives and high lipophilicity,³² sulindac metabolites may distribute to the skin to a greater extent than other NSAIDs and as so may be better candidates for skin cancer prevention. In preclinical studies, oral administration of sulindac at physiological concentrations has shown biological activities in the skin.¹⁸⁻²⁰ As we demonstrated, sulindac metabolites are bioavailable in the skin after oral sulindac administration, with sulindac sulfone showing favorable nevi distribution which correlated with the observed changes in the apoptotic marker.

Reduced VEGF production and gene activation are among the proposed mechanisms of NSAID-induced inhibition of angiogenesis.^{34,35} Melanoma cells have been shown to strongly express VEGF (or VEGF-A), whereas benign melanocytic nevi and melanocytes are largely thought not to express VEGF.³⁶⁻⁴⁰ Expression of other VEGF family members has also been described in melanoma.^{41,42} Our research group previously showed that VEGF expression in melanocytic cells was low or absent in BN, increased significantly in dysplastic nevi, and was further increased in primary melanoma.⁴³ Collectively, these studies suggest that angiogenesis may be an early event of melanocytic lesion progression and could be a useful target for melanoma prevention. In our study, VEGF expression was not modulated with eight weeks of sulindac intervention. Further studies are needed to determine whether a longer duration of NSAIDs intervention would affect these findings.

An important outcome of our study relates to the feasibility of conducting this type of intervention in individuals at risk for melanoma. We were able to recruit the target cohort of 50 subjects within 16 months from 2 geographically distinct study sites. In addition, the introduction of dermoscopy-based algorithms for selection of study AN appears to effectively improve the clinical-pathological correlation. A pattern analysis-based algorithm was utilized to enhance the sensitivity of selecting AN while the ABCDE clinical algorithm was implemented with the objective of excluding concerning melanocytic lesions. Overall, 73.4% of AN were histologically classified as dysplastic, which demonstrates high clinical-pathological correlation when compared to other studies.⁴⁴

Potential limitations of the study are the small sample size and short intervention duration for assessing tissue biomarker and pathological changes, as the study was designed and powered for the primary endpoint. The identification of optimal and reproducible biomarkers to assess the effect on sulindac and other NSAIDs in melanocytic nevi requires further evaluation. An important consideration for future studies will be to clinically target AN that represent a compound melanocytic process in order to increase the likelihood of identifying a junctional and dermal component for the evaluation of biomarkers. In addition, more subjects in the sulindac arm had a prior history of melanoma which may affect the tissue biomarker expression. While we did not find a significant difference in baseline tissue biomarker expression in subjects with or without prior melanoma (data not shown), stratification based on prior melanoma history may still be an important consideration for future clinical trial design.

We conclude that sulindac and sulindac metabolites can reach measurable levels in melanocytic nevi after oral sulindac administration, with sulindac sulfone, a pro-apoptotic compound, showing favorable nevi distribution. Eight weeks of sulindac intervention demonstrated a marginal effect in the expression of a marker of apoptosis in dysplastic nevi and did not result in significant changes in VEGF expression. The study findings support the further evaluation of sulindac as a chemopreventive agent for melanoma.

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Figure 1.

Nevi and Plasma Concentrations of Sulindac and Sulindac Metabolites

The graph summarizes the sulindac and sulindac metabolite (sulfone and sulfide) concentrations in the post-intervention benign nevi and corresponding plasma samples obtained within 24hrs from the last study agent intake dose.

Sulindac, sulindac sulfone, sulindac sulfide concentrations were 0.33 ± 0.36 , 1.63 ± 1.34 , 2.35 ± 2.24 $\mu\text{g/ml}$, respectively, in post-intervention plasma samples.

Sulindac, sulindac sulfone, sulindac sulfide concentrations were 0.51 ± 1.05 , 1.38 ± 2.86 , 0.12 ± 0.12 $\mu\text{g/g}$ tissue, respectively, in post-intervention BN.

Figure 2.

Dermoscopy and histological example of the AN included in the study Representative images of study nevi showing a dermoscopy image (A); Hematoxyline/Eosin (20X) (B); immunohistochemistry stain for VEGF (20X) (C); and cleaved caspase-3 (20X) (D). The expression of VEGFA was observed on both, the junctional and dermal component of the nevi, while the expression of cleaved caspase 3 was predominantly observed at the junctional level.

Table 1

Study subjects characteristics.

Variable	Sulindac (n= 25)	Placebo (n= 25)	p-value ^a
Age (mean±SD)	46.4 ± 9.76	45.9 ± 8.59	0.85
Weight/ Lbs (mean±SD)	175.6 ± 36.47	172.2 ± 29.40	0.72
Height/ Inches (mean±SD)	68.9 ± 3.95	68.7 ± 3.20	0.84
Gender: Male/Female	13 M/12 F	15 M/10 F	0.78
Race			
White	25	24	1.00
Unknown	0	1	
Atypical Mole Syndrome	23/2	25/0	0.49
Yes/No			
History of Melanoma	14/11	8/17	0.15
Yes/No			

^a derived from two-sample t test with unequal variances for continuous variables and Fisher's exact test for categorical variables.

Table 2

Summary of possibly and probably related adverse events based on the number of subjects experiencing the event and the highest grade experienced.

	Sulindac (N = 25)		Placebo (N = 25)		p-value ^a
	Grade 1 N (%)	Grade 2 N (%)	Grade 1 N (%)	Grade 2 N (%)	
Gastrointestinal					
Constipation	1 (4%)	0	0	0	1.00
Diarrhea	0	1 (4%)	0	0	1.00
Flatulence	0	1 (4%)	0	0	1.00
Heartburn	4 (16%)	0	2 (8%)	0	0.67
Nausea	1 (4%)	0	0	0	1.00
Other (increased appetite)	1 (4%)	0	0	0	1.00
Metabolic/Laboratory					
ALT	1 (4%)	0	0	0	1.00
AST	1 (4%)	0	0	0	1.00
Pain					
Abdomen	0	1 (4%)	0	0	1.00
Stomach	0	1 (4%)	1 (4%)	0	1.00
Auditory/Ear					
Tinnitus	0	1 (4%)	0	0	1.00
Cardiac					
Hypertension	0	1 (4%)	0	0	1.00
Constitutional symptoms					
Fatigue	0	1 (4%)	0	0	1.00

^a derived from Fisher's exact test

Table 3

Histological diagnosis of the study nevi.

	Sulindac	Placebo	p-value^a
Baseline	78% DN/22% BN	66% DN/34% BN	0.27
Post-intervention	72% DN/28% BN	77.5% DN/ 22.5% BN	0.64
p-value ^a	0.65	0.27	

^a based on a Fisher's exact test.

DN=Dysplastic nevi; BN= Benign nevi

Table 4

Dermoscopy score for study nevi excised post-intervention.

	Sulindac	Placebo	p-value^b
Baseline	2.20 ± 1.00 ^a	2.20 ± 1.00	<u>0.25</u>
Post-intervention	2.20 ± 1.50	1.70 ± 1.00	<u>0.30</u>
Change	0.00 ± 0.00	0.50 ± 0.00	<u>0.61</u>

^a median ± interquartile range

^b derived from a Wilcoxon rank-sum test

Table 5

VEGF-A and Cleaved Caspase 3 expression in all study nevi.

Variable	Sulindac	Placebo	p-value ^b
VEGF-A			
Melanocytic Junctional Component	N=25	N=25	
Baseline	200 ± 110 ^a	200 ± 75	0.75
Change	0 ± 70	0 ± 85	0.66
Melanocytic Dermal Component	N=20	N=20	
Baseline	123 ± 95	130 ± 60	0.83
Change	0 ± 53	15 ± 85	0.48
Keratinocytic Component	N=24	N=25	
Baseline	48 ± 50	30 ± 35	0.09
Change	- 1 ± 33	10 ± 30	0.20
Cleaved Caspase 3			
Melanocytic Junctional Component	N=25	N=25	
Baseline	100 ± 25	125 ± 55	0.08
Change	5 ± 45	-15 ± 40	0.09 ^c
Melanocytic Dermal Component	N=15	N=20	
Baseline	0 ± 0	0 ± 0	0.62
Change	0 ± 0	0 ± 0	0.40

^a median ± interquartile range (IQR)^b derived from a Wilcoxon rank-sum test^c after adjusting for baseline value, p-value becomes 0.3344 based on a linear regression model.

Table 6

VEGF-A and Cleaved Caspase 3 expression in dysplastic nevi.

Variable	Sulindac	Placebo	p-value ^b
VEGF-A			
Melanocytic Junctional Component	N=20	N=21	
Baseline	200 ± 98 ^a	200 ± 100	0.90
Change	23 ± 63	0 ± 85	0.39
Melanocytic Dermal Component	N=15	N=12	
Baseline	120 ± 60	130 ± 58	0.98
Change	0 ± 60	15 ± 55	0.73
Keratinocytic Component	N=20	N=21	
Baseline	48 ± 50	40 ± 40	0.19
Change	- 6 ± 38	10 ± 23	0.21
Cleaved Caspase 3			
Melanocytic Junctional Component	N=20	N=21	
Baseline	100 ± 24	150 ± 60	0.00
Change	3 ± 33	- 25 ± 45	0.01 ^c
Melanocytic Dermal Component	N=10	N=12	
Baseline	0 ± 0	0 ± 0	0.92
Change	0 ± 0	0 ± 0	0.79

^a median ± interquartile range (IQR)^b derived from a Wilcoxon rank-sum test^c after adjusting for baseline, p-value becomes 0.1103 based on a linear regression model.