

## Aspirin and Other NSAIDs as Chemoprevention Agents in Melanoma

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### Abstract

Melanoma incidence is increasing and, despite recent therapeutic advances, the prognosis for patients with metastatic disease remains poor. Thus, early detection and chemoprevention are promising strategies for improving patient outcomes. Aspirin (acetylsalicylic acid) and other nonsteroidal anti-inflammatory drugs (NSAID) have demonstrated chemoprotective activity in several other cancers, and have been proposed as chemopreventive agents for melanoma. Throughout the last decade, however, a number of case-control, prospective, and interventional studies of NSAIDs and melanoma risk have yielded conflicting results. These inconsistent findings have led to uncertainty about the clinical utility of NSAIDs for melanoma chemoprevention. This mini-review highlights current knowledge of NSAID mechanisms of action and rationale for use in melanoma, provides a comparative review of outcomes and limitations of prior studies, and discusses the future challenges in demonstrating that these drugs are effective agents for mitigating melanoma risk. *Cancer Prev Res*; 7(6); 557–64. ©2014 AACR.

### Introduction

Despite the recent advent of molecular targeted-based (1) and immunological-based (2, 3) therapeutics, most patients with metastatic melanoma ultimately succumb to their disease (4). It is clear that melanoma prevention (or early detection) is favorable to melanoma therapy for advanced disease. Skin screening (i.e., secondary prevention) has traditionally been targeted to patients at highest risk—namely those with personal or family history of melanoma, and those with numerous and/or atypical melanocytic nevi (moles; ref. 5). Population-based melanoma screening may also be an effective approach, as illustrated by recent efforts in Germany (6). Nevertheless, screening is not currently universally implemented and melanoma detection may be delayed even in patients under surveillance (7). Chemoprevention (i.e., primary prevention), in which a drug is administered chronically for the purpose of reducing melanoma risk, would be highly desirable if a safe and effective approach could be developed. Sunscreen may represent a viable chemopreventive agent for melanoma, as Green and colleagues (8) demonstrated melanoma development was reduced by half in sunscreen users in a pro-

spective randomized trial. Relying on sunscreen alone, however, may be inadequate as it is often not applied as recommended (9) and products designed to prevent sunburn may not block all potentially carcinogenic ultraviolet wavelengths or protect against other deleterious effects of sun exposure.

Several oral agents have been considered for melanoma chemoprevention (10). These include antioxidants such as epigallocatechin-3-gallate, found in green tea, which inhibited B16 melanoma metastasis in syngeneic mice (11); N-acetylcysteine, approved for patients with acetaminophen-induced oxidative liver damage, which delayed the onset of UV-induced melanoma in mice (12); and selenium, required for selenoprotein-containing antioxidants, which had chemoprotective effects against UV-induced melanoma in mice (13). Other proposed agents for melanoma chemoprevention include dietary supplements such as  $\beta$ -carotene, vitamin E, resveratrol, lycopene, flavonoids and grape seed extract, and various lipid-lowering drugs (14). None of these agents, however, have consistently demonstrated positive effects in human trials.

There is considerable rationale for use of anti-inflammatory drugs for cancer chemoprevention. Indeed, chronic administration of aspirin (acetylsalicylic acid, ASA) and/or other nonsteroidal anti-inflammatory drugs (NSAID) has been shown to reduce risk of gastric (15), colon (16), breast (17), and prostate cancer (18) in humans. With respect to melanoma, however, there have been conflicting results regarding NSAID use and melanoma risk. The recent report by Gamba and colleagues (19) from the Women's Health Initiative demonstrating a 20% reduction in melanoma incidence in women taking ASA has renewed interest in the potential chemopreventive benefit of ASA to reduce melanoma risk. Here, we review potential mechanisms of

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NSAID action and rationale for their use in melanoma, the outcomes and limitations of studies performed, and discuss the future challenges of demonstrating that these drugs are effective agents for melanoma chemoprevention.

### NSAID Mechanism of Action and Rationale for Use in Melanoma Prevention

There is considerable evidence that NSAIDs exert activity against multiple cancer cell types *in vitro*. As the specific activities of NSAIDs have been defined in greater detail, it is now clear that NSAIDs may function through several pathways, affecting both canonical and noncanonical targets. Here, we briefly review the major mechanisms and anti-cancer activities of NSAIDs (Fig. 1), and their potential relevance to melanoma.

#### COX-dependent mechanisms

Prostaglandin-endoperoxide synthase, or cyclooxygenase (COX) is an enzyme with multiple isoforms (COX-1, COX-2) that is responsible for catalyzing the conversion of arachidonic acid to prostaglandins. Although COX-1 expression tends to be constitutive, COX-2 is upregulated in inflammatory states and cancer (20). It is well known that NSAIDs inhibit the enzymatic activity of COX isozymes 1 and 2 by directly competing with arachidonic acid for the enzymes' active sites (21). ASA can also irreversibly inhibit COX activity by acetylating the N-terminal serine residue in

the domain of the enzymatic active site (22). This inhibition of COX enzymes decreases the catalytic production of prostaglandins, which are endogenous signaling molecules that play critical roles in pain, inflammation, hemostasis, protection of gastric mucosa, and other cellular and systemic processes. Selective COX-2 inhibitors (i.e., celecoxib) were developed to target inflammation and pain while not compromising COX-1-mediated activities such as protection of gastric mucosa. Prostaglandin E<sub>2</sub> (PGE<sub>2</sub>) synthesis is particularly relevant to cancer cell processes, as PGE<sub>2</sub> is involved in angiogenesis (23, 24), proliferation (25, 26), migration and invasion (27–29), and metastasis (30).

#### COX-independent mechanisms

Although early reports indicated that the anticancer effects of NSAIDs were related to COX inhibition, there is a growing body of evidence suggesting that NSAIDs also mediate anticancer activities independent of COX inhibition (31). For instance, some NSAIDs have been shown to inhibit activation of NF- $\kappa$ B (32, 33), a complex pathway implicated in apoptosis inhibition (34), cellular adhesion (35), and promotion of metastasis (36). Other evidence suggests that NSAIDs and their derivatives that lack the capacity to inhibit COX isozymes may abrogate cancer progression by downregulating  $\beta$ -catenin (37) or inhibiting its transcriptional activity (38). In addition, metabolites of some NSAIDs (such as sulindac) that lack COX-inhibitory

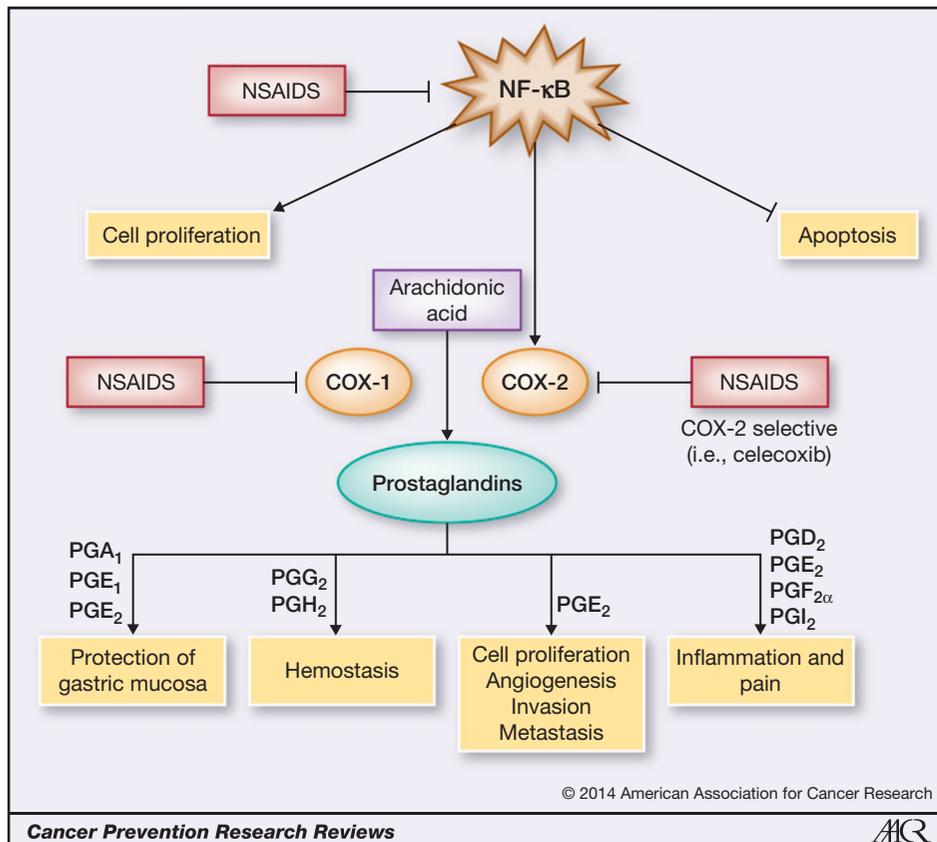


Figure 1. NSAIDs modulate COX-dependent and COX-independent cancer-related pathways. By directly inhibiting COX enzymes, NSAIDs block the catalytic conversion of arachidonic acid to prostaglandins, and decrease the presence of prostaglandin E<sub>2</sub>, which is implicated in various behaviors of cancer cells such as invasion, proliferation, and angiogenesis. NSAIDs may also have anticarcinogenic effects by blocking activation of NF- $\kappa$ B and by downregulating  $\beta$ -catenin. These NSAID-mediated activities result in apoptosis, diminish cell proliferation, and may block epithelial-to-mesenchymal transition.

activity downregulate EGF receptor signaling (39) and exert chemopreventive activity in animal models (40). These COX-independent activities may potentially allow development of agents with chemotherapeutic efficacy while circumventing the toxic effects of NSAIDs because of COX inhibition.

### Melanoma-specific effects of NSAIDs

COX-2 may be a reasonable target in melanoma, as it is generally not expressed in benign melanocytic nevi but is highly expressed in most melanomas (41, 42). Analysis of primary and metastatic melanoma lesions revealed increased COX-2 expression with melanoma progression (43), and that high COX-2 expression correlates inversely with patient survival (44). In addition, NF- $\kappa$ B, which is inhibited by NSAIDs as described above, may also be targeted because it is activated in melanoma compared with normal melanocytes (45). Vad and colleagues (46) observed toxicity of ASA in melanotic (but not amelanotic) melanoma cell lines, which was attributed to oxidation of ASA by tyrosinase and generation of reactive oxygen species. Similarly, Albano and colleagues (47) observed enhanced apoptosis in melanoma lines treated with diclofenac that increased intracellular reactive oxygen species and mitochondrial dysfunction, but found no significant effects on normal fibroblasts.

### Conflicting Results from Clinical Studies

Over the past decade, a number of studies examining the potential association between NSAID use and melanoma risk have yielded conflicting results. These various case-control, prospective, and interventional studies are summarized in Table 1.

#### Case-control studies

Among the case-control studies there is a wide range of findings, with some studies reporting low relative risk and others reporting high relative risk in NSAID users. For instance, Harris and colleagues (48) reviewed 110 women with melanoma and 609 female controls and determined the relative risk of melanoma for persons taking regularly taking nonselective NSAIDs to be 0.45. Similarly, Curiel-Lewandrowski and colleagues (49) reported a decreased risk of melanoma (OR, 0.73) associated with "ever use" of NSAIDs in a study of 400 melanoma and 600 control cases. Further corroborating these findings, Johannesdottir and colleagues (50) reported a risk reduction (incidence rate ratio 0.87) for "ever use" of NSAIDs in a study of 3,242 melanoma cases with 32,400 matched controls. Alternatively, Asgari and colleagues (51) and Vinogradova and colleagues (52) reported no association between melanoma development and NSAID use.

**Table 1.** Summary of studies on NSAID use and melanoma risk

Study	Cases/controls	Drug	Risk	Limitations
<b>Case-control</b>				
Johannesdottir et al. (50)	3,242/32,400	All NSAIDs, ever use	IRR = 0.87	Dispensed drugs only
Vinogradova et al. (52)	3,249/16,000	COX-2 inhibitors	OR = 1.05	Dispensed drugs only
Jeter et al. (54)	327/119	Current ASA, non-ASA NSAID use	OR = 1.45 (ASA), 0.71 (other NSAID)	Self-reported drug administration, small sizes
Curiel-Lewandrowski et al. (49)	400/600	All NSAIDs, ever use	OR = 0.73	Small sample sizes
Joosse et al. (53)	1,318/6,786	Daily ASA	OR = 0.54 (women only)	Small subgroup sizes
Asgari et al. (51)	349/63,809	All NSAIDs, ever use	HR = 0.99	Self-reported drug administration
Harris et al. (48)	110/609	Nonselective NSAIDs, regular use	RR = 0.45	Small sample sizes
<b>Prospective</b>				
Gamba et al. (19)	548/59,806	ASA, regular use	HR = 0.80	Self-reported drug administration, only postmenopausal Caucasian females
Jeter et al. (57)	658/92,125	Current ASA, non-ASA NSAID use	RR = 1.32 (ASA), 0.96 (other NSAID)	Only Caucasian females
Jacobs et al. (56)	190/146,113	325 mg ASA or no drug use	RR = 0.99	Self-reported drug administration, confounding variables
Sorenson et al. (55)	167/172,057	All NSAIDs	SIR = 1.0	Dispensed drugs only, confounding variables
<b>Interventional</b>				
Cook et al. (58)	138/39,876	100 mg ASA QOD vs. placebo	RR = 0.97	Confounding variables, likely insufficient dosing

Abbreviations: IRR, incidence rate ratio; QOD, every other day; RR, relative risk; SIR, standardized incidence ratio.

Others have reported significant findings within subgroups such as gender and drug type. For instance, Joosse and colleagues (53) concluded that continuous, low dose (30–100 mg/d) ASA was associated with significant reduction in melanoma risk for women but not for men (OR, 0.54 vs. OR, 1.01). In addition, Jeter and colleagues (54) found that relative risk was decreased in non-ASA NSAID users but increased in ASA users (OR, 0.71 vs. OR, 1.45) in a study of 327 melanoma cases and 119 controls.

### Prospective studies

Conflicting findings also exist among prospective studies. Sørensen and colleagues (55) found a significant protective effect of NSAID use for colon, rectal, stomach, and ovarian cancer, but not for melanoma in a large population-based study. These findings were supported by a prospective study by Jacobs and colleagues (56) that reviewed cancer incidence among 69,810 men and 76,303 women in the Cancer Prevention Study II Nutrition Cohort over a 10-year period. Participants self-reported daily administration of  $\geq 325$  mg ASA and were compared with those with no reported use. Among the ASA users there was no association with melanoma incidence (relative risk 1.15), but associations were found for overall cancer in men, colorectal cancer, and prostate cancer (relative risk 0.84, 0.68, and 0.81, respectively). In reviewing 92,125 Caucasian women in the Nurse's Health Study, Jeter and colleagues (57) did not find an association between current use of non-ASA NSAIDs and decreased incidence of melanoma as they did for "ever use" of non-ASA NSAIDs in their previous study (54); they did, however, report an increased risk of melanoma (relative risk 1.32) in ASA users, in agreement with their previous report.

Most recently, Gamba and colleagues (19) studied 59,806 postmenopausal women enrolled in the Women's Health Initiative. Participants were grouped into ASA users, non-ASA NSAID users, and NSAID nonusers according to self-report, and were followed for a median of 12 years. Although there was no statistically significant association between non-ASA NSAID users and melanoma risk (HR, 0.94), regular ASA use for 5 or more years was significantly associated with reduced melanoma incidence (HR, 0.70). Another interesting result was the effect of drug duration on melanoma incidence. In participants using ASA less than 1 year, the HR was only 0.89, which decreased to 0.79 after 1 to 4 years, and finally to 0.70 after 5 years of ASA use.

### Interventional studies

To our knowledge, there is only one randomized controlled clinical trial that examined ASA use and melanoma, which supports the notion that regular use of ASA is not associated with decreased risk. Cook and colleagues (58) conducted a trial of 39,876 women randomized to 100 mg of ASA or placebo every other day for an average of 10 years in the Women's Health Study. There was no significant risk reduction for melanoma (relative risk 0.97) or other cancers, although lack of effect on colon polyps suggested that the dosage of 100 mg every other day may not have been sufficient to observe potential chemoprotective effects.

### Limitations of Prior Clinical Studies

Although some studies have particular advantages over others, each also has distinct limitations that are important to consider when evaluating their conclusions regarding NSAID use and melanoma risk. Understanding these limitations, which are summarized in Table 1, may help us account for some of the variability in the reported results and construct a unified plan for future studies.

One major limitation of many studies is that limited sample size necessarily constricts statistical power in the NSAID-user subgroups, which may, in some cases, have been combined to improve statistical power. Loss of potential subgroups may result in forfeiting resolution among the subjects in terms of drug dosage and duration. For instance, some studies categorize NSAID users by number of pills taken per week or number of patient prescriptions, but not by absolute dosage of the drug (19, 48, 49, 52–55, 57), whereas others (50, 51, 56, 58) were able to retain these dosage subgroups. This lack of uniformity in considering individual NSAID drugs and dosages might be a critical contributing factor in the variation observed in reported results. Moreover, some studies relied on patient-reported drug use, which may not be accurate. Another major limitation in some studies is the selection of study subjects. Although some studies were population-based (48–50, 52, 55), others were restricted to specific patient populations enrolled in larger studies (19, 51, 53, 54, 56–58). For example, the recent study by Gamba and colleagues (19) was restricted to postmenopausal Caucasian women. A final limitation to consider is the potential for residual confounding factors. For example, although some studies controlled for sun exposure history (19, 48, 49, 51, 54, 57), others did not (50, 52, 53, 55, 56, 58). Similar discrepancies are found among these studies in controlling for other important confounding variables like smoking, body mass index, number of nevi and atypical nevi, history of melanoma, and other potential melanoma risk factors. These variations within study design are often unavoidable, but may contribute substantially to the variety of clinical results that have been reported.

### Toxicities Associated with Chronic NSAID Use

In considering any chemopreventive agent, one must weigh potential benefits against potential risks or toxicities. Although NSAIDs are generally safe when taken for short periods of time and allergic reactions are uncommon (59), chronic ingestion of any drug can be associated with some rate of toxicity or unanticipated side effects. The most serious long-term risks with NSAID use are gastrointestinal bleeding and hemorrhagic stroke. A more common side effect is peptic ulcer disease. A population-based study from the United Kingdom involving more than 450,000 persons found relative risk of peptic ulcer disease to be 2.9 with ASA and 4.0 with other NSAIDs (60). Rarely, NSAIDs are associated with nephrotoxicity and hypertension, particularly when combined with angiotensin-I converting enzyme inhibitors. One study examined 2,278 patients treated with NSAIDs, 328 with angiotensin-I converting enzyme

inhibitors, and 162 with both. No nephrotoxicity was found in conjunction with monotherapy, but 3 cases of reversible renal failure were found in conjunction with combination therapy (61).

Of the NSAIDs currently available, the most studied is ASA. A meta-analysis of 24 randomized trials in 66,000 subjects found a rate of gastrointestinal bleeding in subjects taking ASA of 2.4% (vs. 1.42% for placebo), although there seemed to be no correlation with dose (62). The average risk of NSAID-associated gastrointestinal bleeding increases from 1% to 3% to more than 5% in subjects older than age 70 (without prior history of bleeding and not taking corticosteroids or anticoagulants; refs. 63 and 64). In terms of hemorrhagic stroke risk, a meta-analysis of 16 trials involving more than 55,000 patients taking ASA (average duration 37 months) found that although risk of myocardial infarction and ischemic stroke were reduced, risk of hemorrhagic stroke was increased by about 0.1% (65).

Finally, many NSAIDs may increase the risk of adverse cardiovascular events. A meta-analysis of 280 randomized trials of NSAIDs versus placebo and 474 trials of one NSAID versus another NSAID found that major coronary events were increased by a coxib (relative risk 1.76), diclofenac (relative risk 1.70), or ibuprofen (relative risk 2.22; ref. 66). Compared with placebo, of 1,000 patients taking a coxib or diclofenac for a year, 3 more had major cardiovascular events, 1 of which was fatal (66). Naproxen did not significantly increase major vascular events (relative risk 0.93), but heart failure risk was roughly doubled by all NSAIDs (66). Thus, some NSAIDs may be safer to take chronically than others, and selecting the optimal drug for melanoma chemoprevention will require careful consideration of these drug-specific effects to minimize the adverse effects of chronic NSAID use.

Interestingly, specific genetic polymorphisms in several genes have been associated with increased risk of side effects in patients taking ASA (67). Two single nucleotide polymorphisms in COX-1 (A842G and C50T) confer increased sensitivity to ASA (68). Genetic variants in several cytochrome p450 genes (CYP<sub>4</sub>F<sub>11</sub>, CYP<sub>2</sub>C<sub>9</sub>, CYP<sub>2</sub>D<sub>6</sub>; ref. 69) and the eNOS gene (70) were significantly associated with increased risk of ASA-associated gastrointestinal bleeding. With respect to NSAID-associated peptic ulcer disease, increased risk has been associated with genetic polymorphisms in genes encoding interleukin-1 $\beta$  and interleukin-1 receptor antagonist (71, 72), and TNF- $\alpha$  (73, 74). In this era of personalized medicine, genetic testing may allow us to predict which patients are less likely to have side effects with chronic NSAID use and therefore, most suitable candidates for a chemoprevention regimen involving NSAIDs.

### Unanswered Questions and Future Directions

Many questions remain regarding the potential utility of chronic ASA or other NSAID administration for melanoma chemoprevention. Just as particular individuals will be genetically predisposed or resistant to side effects, we expect variability in the antineoplastic efficacy of NSAIDs among individuals. Unfortunately, we are not aware of any genetic

biomarkers to predict who is most likely to benefit from chronic ASA use. Presumably, using the lowest effective dose would minimize the side effects described above, but the precise dosage and optimal frequency of administration have not yet been defined. Furthermore, it cannot be assumed that the optimal dose for ASA-mediated chemoprevention of melanoma will be the same as for other cancers. Because the studies reviewed above involve different subject populations, it is also unclear who the ideal subjects are. Another remaining question is the optimal age at which melanoma chemoprevention should be initiated. The recent study by Gamba and colleagues (19) showed that melanoma risk reduction increased with duration of chemoprevention up to 5 years, yet it remains unknown if greater duration translates into greater risk reduction. Ideally, one would begin a melanoma chemoprevention regimen for the optimal duration before the age of peak onset (age range 50–70; ref. 75), although melanoma incidence is also increasing in children and adolescents (76).

Presumably the greatest benefit to risk ratio will be for those patients with highest likelihood of developing melanoma—namely those with prior personal history or significant family history of the disease, and those having numerous or atypical melanocytic nevi (5). Such individuals are likely to have an inherent genetic susceptibility, although in most cases it is undefined. By comparison, chronic ASA usage for colon cancer chemoprevention has been recommended for predisposed patients with Lynch syndrome, but not the general population (77). Interestingly, this clinical recommendation is not without contention in the literature, as several studies have published conflicting results regarding the efficacy of ASA in preventing colorectal cancers in both general subjects and those with Lynch syndrome (78, 79).

Given the number of points of uncertainty, it is not feasible to expect all of these questions to be answered in randomized controlled trials, although the number of subjects could be reduced by enrolling patients with melanoma risk factors. Nevertheless, large numbers of patients will be required in any trial where melanoma diagnosis is the endpoint. Unlike the case of colon cancer, where colon polyps which are bona fide cancer precursors that can serve as intermediate endpoints, it is unclear what (if any) markers would be similarly suitable for melanoma. Although nevi are associated with melanoma risk and ~20% of melanomas arise from nevi (5), most nevi never progress to melanoma (5) and thus changes in numbers of nevi during a proposed study period may not reflect changes in melanoma risk. Short of a randomized controlled trial, we would advocate defining a disease-related mechanism or target in an animal model that is modified by ASA or another NSAID, which results in prevention or delay of melanoma development. A subsequent study showing modulation of that target or mechanism by the drug in a group of human subjects would then be appropriate before recommending its use for melanoma chemoprevention. Although it is not feasible to screen for chemopreventive activity in animals or humans, screening libraries of

compounds could be a powerful unbiased *in vitro* approach to define potential targets/mechanisms before testing candidate agents could be tested in animal models prior to human studies. It will always be more expeditious, however, to begin with drugs that already have a demonstrated safety record in humans.

## Conclusions

Given the conflicting results of clinical trials and the number of uncertainties discussed above, chronic administration of ASA or other NSAIDs cannot be recommended for melanoma chemoprevention in the general population at this time. Similarly, for patients at increased risk (personal history of melanoma, 10-fold; numerous/atypical nevi, 4-fold; ref. 5) who would be most likely to benefit, there is insufficient evidence of efficacy for any particular drug or dosing regimen. Although a prospective random-

ized controlled trial in such high-risk patients offers the best hope of minimizing confounding variables and determining whether chronic administration of a particular NSAID can reduce melanoma risk, this would likely require a multi-institutional effort. Demonstration of drug targeting in an animal model of melanoma, with subsequent validation in human studies, may be a more reasonable approach.

## Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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