Long-Term Use of Nonsteroidal Anti-inflammatory Drugs Decreases the Risk of Cutaneous Melanoma: Results of a United States Case–Control Study

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Experimental and observational studies continue to demonstrate conflicting results regarding the role of several commonly used drugs as melanoma chemopreventive agents. This case–control study was designed to assess the associations between cutaneous melanoma (CM) and exposure to nonsteroidal anti-inflammatory drugs (NSAIDs) and statins in current users. A total of 400 CM and 600 eligible age- and gender-matched community-based controls were prospectively recruited and interviewed. We assessed participants' demographic characteristics, CM risk factors, and current and previous use of medications. Multivariable conditional logistic regression models were used to estimate adjusted odds ratios (ORs) and 95% confidence intervals (CIs) for associations between NSAIDs and/or aspirin (ASA), statin exposure, and CM risk. Half of the subjects were men (mean age 60 years). After adjusting for confounders, use of any type of NSAIDs for more than 5 years significantly reduced the risk of melanoma development compared with the low-exposure group (adjusted OR = 0.57; 95% CI = 0.43–0.77). Subgroup analyses showed that the observed risk reduction was primarily driven by continuous ASA use (>5 years adjusted OR = 0.51, 95% CI = 0.35–0.75). No significant protective effect was observed with statin exposure (OR = 0.97, 95% CI = 0.73–1.29). Long-term use of NSAIDs, especially ASA, is associated with a significantly decreased risk of CM development. Clinical intervention studies are warranted to further investigate the potential role of ASA and other NSAIDs as chemopreventive agents for CM.

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INTRODUCTION

With the incidence and mortality of malignant cutaneous melanoma (CM) increasing more rapidly than other common cancers in the US (Jemal *et al.*, 2009), prevention efforts, beyond reduction in sun exposure, merit additional consideration (Bordeaux *et al.*, 2007; Tucker, 2009).

Chemoprevention is a relatively unexplored strategy to reduce the incidence of CM (Vogel, 2007). Commonly used drugs such as

nonsteroidal anti-inflammatory drugs (NSAIDs), including acetyl-salicylic acid (ASA), and statins with acceptable safety and other potential health benefits are appealing candidates for chemoprevention. The protective effect of NSAIDs has been confirmed by multiple studies for colorectal cancer (González-Pérez *et al.*, 2003; Dubè *et al.*, 2007) with findings suggesting the need for extended and frequent NSAID exposure, usually beyond 5 years (González-Pérez *et al.*, 2003). Less definitive data exist in the setting of breast, esophagus, and stomach cancers (Zerbini *et al.*, 2006).

Several *in vitro* and observational studies have suggested that regular use of NSAIDs, and statins may reduce the likelihood of CM development and progression, but not all studies have been supportive of this association resulting in overall conflicting results (Downs *et al.*, 1998; Jeter *et al.*, 2005; Ramirez *et al.*, 2005; Koomen *et al.*, 2007; Asgari *et al.*, 2008; Kuoppala *et al.*, 2008; Joosse *et al.*, 2009; Bonovas *et al.*, 2010). Therefore, population-based epidemiological studies specifically designed to evaluate the role of these commonly prescribed drugs, as potential chemopreventive agents for CM are needed.

The objective of this case–control study was to assess the association of long-term use of lipid-lowering agents (LLAs) and NSAIDs use with CM risk.

Table 1. Distribution of selected demographics, lifestyle factors, and health-care utilization between cases and controls, and univariate logistic regression analysis

Characteristic	No. of cases (%) (*n*=400)	No. of controls (%) (*n*=600)	Crude OR (95% CI)
Age (years)			
40–44	48 (12)	61 (10)	1.00 (Reference)
45–54	116 (29)	194 (32)	0.79 (0.52–1.21)
55–64	117 (29)	174 (29)	0.88 (0.58–1.36)
65–74	77 (19)	105 (18)	0.97 (0.61–1.54)
>75	42 (11)	66 (11)	0.86 (0.49–1.48)
Gender			
Female	193 (48)	301 (50)	1.00 (Reference)
Male	207 (52)	299 (50)	1.08 (0.84–1.34)
Ethnicity			
Non-white	3 (1)	15 (3)	1.00 (Reference)
White	397 (99)	585 (97)	4.75 (1.41–16.03)
Marital status			
Married	304 (76)	464 (77)	1.00 (Reference)
Single	39 (10)	51 (9)	1.17 (0.75–1.81)
Other	57 (15)	85 (14)	1.02 (0.71–1.47)
Education			
High school graduate or less	74 (19)	121 (20)	1.00 (Reference)
College graduate or less	184 (46)	264 (44)	1.14 (0.81–1.61)
Graduate school	138 (35)	208 (35)	1.09 (0.76–1.56)
Working status			
Full time	202 (51)	272 (45)	NA
Retired	116 (30)	181 (30)	
Other	82 (21)	147 (25)	
Annual household income ($)			
⩽30,000	36 (9)	55 (9)	1.00 (Reference)
>30,000–75,000	112 (28)	178 (30)	0.96 (0.58–1.60)
>75,000	208 (47)	246 (41)	1.30 (0.80–2.11)
Not provided	44 (11)	121 (20)	0.56 (0.31–0.99)
Time in present residence			
>10 years	239 (60)	434 (72)	1.00 (Reference)
6–10 years	79 (20)	106 (18)	1.35 (0.96–1.91)
<5 years	82 (21)	57 (10)	2.61 (1.77–3.86)

Table 1. Continued

Characteristic	No. of cases (%) (*n*=400)	No. of controls (%) (*n*=600)	Crude OR (95% CI)
Smoking status			
Never smoked	164 (41)	266 (44)	1.00 (Reference)
Former smoker	196 (49)	260 (43)	1.22 (0.93–1.61)
Current smokers	31 (8)	60 (10)	0.84 (0.51–1.38)
Visits to primary care physician per year			
0–2	262 (66)	430 (72)	1.00 (Reference)
3–5	106 (27)	141 (24)	1.23 (0.92–1.66)
⩾6	31 (8)	29 (5)	1.75 (1.03–2.98)
Visits to a medical specialist per year			
0	85 (21)	170 (28)	1.00 (Reference)
1–2	169 (42)	242 (40)	1.40 (1.01–1.94)
3–5	100 (25)	128 (21)	1.56 (1.08–2.26)
⩾6	46 (12)	60 (10)	1.53 (0.96–2.44)
Skin checks: times in lifetime			
0	117 (29)	191 (32)	1.00 (Reference)
1–2	100 (25)	188 (31)	0.87 (0.62–1.21)
3–5	56 (14)	81 (14)	1.23 (0.75–1.70)
⩾6	125 (31)	138 (23)	1.48 (1.06–2.07)
Number of prescription medications used in the last year			
None	123 (31)	196 (33)	1.00 (Reference)
1–2	132 (33)	214 (36)	0.98 (0.72–1.34)
3–5	109 (27)	146 (24)	1.19 (0.85–1.66)
⩾6	36 (9)	42 (7)	1.37 (0.83–2.25)

Abbreviation: NA, not applicable.

RESULTS

Sample

About half the cases and controls were female with a mean age of 58 years (Table 1). More than 96% of cases and controls were Caucasian. No statistically significant differences in the distribution of marital status, education level, working status, annual income, and smoking status were observed between cases and community-matched controls. The utilization of health care was comparable between cases and controls, except that CM patients reported more skin checks during their life before diagnosis than did controls (⩾6; odds ratio (OR) = 1.48 (95% confidence interval (CI) 1.06–2.07) compared with no skin checks; Table 1).

Several phenotypic characteristics including light hair and eye color, and increased number of moles and freckles were associated with a 2- to 3-fold-increased risk of CM. Skin response to sun, weeks at the beach, and number of sunburns were significantly higher among cases than

Table 2. Distribution of possible melanoma risk factors between melanoma cases and controls

Possible risk factor	No. of cases (*n*=400)(%)	No. of controls (*n*=600) No. (%)	OR (95% CI)
Hair color			
Dark brown to black	76 (19)	177 (30)	1.00 (Reference)
Medium brown	124 (31)	187 (31)	1.54 (1.09–2.20)
Blond to light brown	164 (41)	210 (35)	1.82 (1.30–2.55)
Red	36 (9)	26 (4)	3.23 (1.82–5.71)
Eye color			
Brown	108 (27)	211 (35)	1.00 (Reference)
Green or hazel	135 (34)	169 (28)	1.56 (1.13–2.16)
Blue or gray	157 (39)	220 (37)	1.39 (1.023–1.90)
Previous Hx of NMSC			
No	326 (82)	523 (87)	1.00 (Reference)
Yes	74 (19)	76 (13)	1.56 (1.10–2.21)
Family Hx of melanoma			
No	335 (84)	511 (85)	1.00 (Reference)
Yes	59 (15)	64 (11)	1.41 (0.96–2.06)
Number of moles as a teenager			
I had no moles	83 (21)	193 (32)	1.00 (Reference)
I had a few moles	271 (68)	372 (62)	1.69 (1.25–2.29)
I had many moles	44 (11)	30 (5)	3.41 (2.01–5.80)
Number of freckles			
Face/teen			
I had no freckles	125 (31)	290 (48)	1.00 (Reference)
I had a few freckles	194 (49)	221 (37)	2.04 (1.53–2.71)
I had many freckles	81 (20)	84 (14)	2.24 (1.55–3.24)
Arms/teen			
I had no freckles	87 (22)	245 (41)	1.00 (Reference)
I had a few freckles	222 (56)	264 (44)	2.37 (1.75–3.21)
I had many freckles	91 (23)	87 (15)	2.95 (2.01–4.32)
Skin response to strong sunlight			
At the beginning of summer			
No burn	37 (9)	102 (17)	1.00 (Reference)
Mild burn	225 (56)	346 (58)	1.79 (1.19–2.71)
Severe or painful burn	135 (34)	150 (25)	2.48 (1.59–3.86)

Table 2. Continued

Possible risk factor	No. of cases (*n*=400)(%)	No. of controls (*n*=600) No. (%)	OR (95% CI)
Skin response at the end of summer			
Deep tan	64 (16)	162 (27)	1.00 (Reference)
Mild or moderate tan	290 (73)	384 (64)	1.91 (1.38–2.65)
No tan	38 (10)	50 (8)	1.92 (1.15–3.21)
No. of weeks/year at the beach			
As an adolescent			
0	66 (17)	141 (24)	1.00 (Reference)
1	74 (19)	119 (20)	1.33 (0.88–2.01)
2–3	112 (28)	142 (24)	1.69 (1.15–2.47)
⩾4	146 (37)	198 (33)	1.58 (1.10–2.26)
In the past 5 years			
0	124 (31)	219 (37)	1.00 (Reference)
1	91 (23)	125 (21)	1.29 (0.91–1.82)
2–3	104 (26)	130 (22)	1.41 (1.01–1.98)
⩾4	80 (20)	126 (21)	1.12 (0.79–1.60)
Number of sunburns			
As a child			
0	136 (34)	294 (49)	1.00 (Reference)
1–4	131 (33)	175 (29)	1.61 (1.19–2.19)
>4	108 (27)	100 (17)	2.34 (1.66–3.28)
As an adolescent			
0	133 (33)	252 (42)	1.00 (Reference)
1	140 (35)	194 (32)	1.37 (1.01–1.85)
⩾2	127 (32)	154 (26)	1.56 (1.14–2.14)
Sun bed use (times/lifetime)			
0	311 (78)	482 (80)	1.00 (Reference)
<10	37 (9)	61 (10)	0.94 (0.60–1.48)
⩾10	52 (13)	57 (10)	1.41 (0.93–2.15)

Abbreviations: CI, confidence interval; Hx, history; NMSC, non-melanoma skin cancer; OR, odds ratio.

controls (Table 2). Most cases had a superficial spreading histological subtype of melanoma (46%) and almost half (48%) had a Breslow thickness <1 mm, and a quarter were thicker than 2 mm.

Statins and CM

Of the 280 users of LLAs, 276 (98.6%) reported taking statins in the last 12 months. All of those users reported a frequency of exposure >4 days per week. Participants reported using atorvastatin (69.9%), simvastatin (17.8%), pravastatin (5.1%), rosuvastatin (2.9%), lovastatin (2.1%), and fluvastatin (2.1%). Cases and controls had similar use of statins (27.3 vs. 27.8%; OR = 0.97, 95% CI = 0.73–1.29; Table 3). The proportion of

Table 3. Comparison of statin and NSAID exposure measures between melanoma cases and controls

Drug exposure	No. of cases (*n*=400) (%)	No. of controls (*n*=600) (%)	(Adjusted) OR (95% CI)	Multivariate OR (95% CI)
Statin use				
No use	291 (73)	433 (72)	1.00 (Reference)	NA
Any use	109 (27)	167 (28)	0.97 (0.73–1.29)	
Duration of any statin use (years)[1]				
<2 or no use	317 (79)	479 (80)	1.00 (Reference)	NA
2–5	63 (16)	85 (14)	1.12 (0.79–1.60)	
>5	20 (5)	36 (6)	0.84 (0.48–1.48)	
Any NSAID use[1]				
No use	138 (35)	167 (28)	1.00 (Reference)	NA
Any use	262 (66)	433 (72)	0.73 (0.56–0.96)	
Duration of any NSAID use (years)[1]				
<2 or no use	179 (45)	226 (38)	1.00 (Reference)	NA
2–5	110 (28)	129 (22)	1.08 (0.78–1.48)	
>5	111 (28)	245 (41)	0.57 (0.43–0.77)	
Any ASA use (± NSAID)[2]				
No use	256 (64)	342 (57)	NA	1.00 (Reference)
Any use	144 (36)	258 (43)		0.72 (0.55–0.94)
Duration of use of ASA (years)[2]				
<2 or no use	279 (70)	381 (64)	NA	1.00 (Reference)
2–5	68 (17)	92 (15)		1.03 (0.72–1.49)
>5	53 (13)	127 (21)		0.51 (0.35–0.75)
Any use of a non-ASA NSAID (± ASA)[3]				
No use	237 (59)	349 (58)	NA	1.00 (Reference)
Any use	163 (41)	251 (42)		0.92 (0.70–1.19)
Duration of use of a non-ASA NSAID (years)[3]				
<2 or no use	268 (67)	382 (64)	NA	1.00 (Reference)
2–5	60 (15)	73 (12)		1.25 (0.85–1.83)
>5	72 (18)	145 (24)		0.64 (0.46–0.89)

Abbreviations: ASA, acetyl salicylic acid; CI, confidence interval; NA, not applicable; NSAID, nonsteroidal anti-inflammatory drug; OR, odds ratio.
[1]Not adjusted in multivariate model, except age-, gender-, and town-matched.
[2]Adjusted for number of sunburns during childhood.
[3]Adjusted for extent of freckling on arms.

cases and controls that had used statins for >5 years was comparable (5.0 and 6.0%, respectively) and prolonged exposure to statins was not associated with a decreased risk of CM (OR = 0.84, 95% CI = 0.48–1.48 compared with <2 years; Table 3). Men demonstrated a higher prevalence of statin use than women (36 vs. 19%), but the findings were comparable after stratification for gender (Table 4).

NSAIDs and melanoma

Of the 1,000 cases and controls, 695 (69.5%) reported exposure to NSAIDs within the year before the interview. Of these 695 participants, 402 (57.8%) had used ASA (Table 3). Among the non-ASA NSAID users, the most commonly used drugs were ibuprofen (45.3%) and naproxen (10.6%). Only 6.6% of participants reported exposure to Cox-2 inhibitors.

Table 4. Comparison of statin and NSAID exposure measures between melanoma cases and controls stratified for gender

Drug exposure	Males				Females			
	No. of cases (*n*=207) (%)	No. of controls (*n*=299) (%)	(Adjusted) OR (95% CI)	Multivariate OR (95% CI)	No. of cases (*n*=193) (%)	No. of controls (*n*=301) (%)	(Adjusted) OR (95% CI)	Multivariate OR (95% CI)
Statin use								
No use	134 (65)	191 (64)	1.00 (Reference)	NA	157 (81)	242 (80)	1.00 (Reference)	NA
Any use	73 (35)	108 (36)	0.96 (0.67–1.40)		36 (19)	59 (20)	0.94 (0.59–1.49)	
Duration of any statin use (years)[1]								
<2 or no use	149 (72)	219 (73)	1.00 (Reference)	NA	168 (87)	260 (86)	1.00 (Reference)	NA
2–5	43 (21)	51 (17)	1.24 (0.79–1.96)		20 (10)	34 (11)	0.91 (0.51–1.64)	
>5	15 (7)	29 (10)	0.76 (0.39–1.47)		5 (3)	7 (2)	1.11 (0.35–3.54)	
Any NSAID use[1]								
No use	69 (33)	89 (30)	1.00 (Reference)	NA	69 (36)	78 (26)	1.00 (Reference)	NA
Any use	138 (67)	210 (70)	0.85 (0.58–1.24)		124 (64)	223 (74)	0.63 (0.43–0.93)	
Duration of any NSAID use (years)[1]								
<2 or no use	90 (44)	116 (39)	1.00 (Reference)	NA	89 (46)	110 (37)	1.00 (Reference)	NA
2–5	65 (31)	70 (23)	1.20 (0.77–1.85)		45 (23)	59 (20)	0.94 (0.58–1.52)	
>5	52 (25)	113 (38)	0.59 (0.39–0.91)		59 (31)	132 (44)	0.55 (0.37–0.84)	
Any ASA use (± NSAID)[2]								
No use	115 (56)	146 (49)	NA	1.00 (Reference)	141 (73)	196 (65)	NA	1.00 (Reference)
Any use	92 (44)	153 (51)		0.73 (0.50–1.08)	52 (27)	105 (35)		0.67 (0.45–1.02)
Duration of use of ASA (years)[2]								
<2 or no use	127 (61)	169 (57)	NA	1.00 (Reference)	152 (79)	212 (71)	NA	1.00 (Reference)
2–5	46 (22)	56 (19)		1.03 (0.66–1.71)	22 (11)	36 (12)		0.98 (0.55–1.77)
>5	34 (16)	74 (25)		0.56 (0.34–0.92)	19 (10)	53 (18)		0.44 (0.24–0.81)
Any use of a non-ASA NSAID (± ASA)[3]								
No use	138 (67)	199 (67)	NA	1.00 (Reference)	99 (51)	150 (50)	NA	1.00 (Reference)
Any use	69 (33)	100 (33)		0.95 (0.65–1.40)	94 (49)	151 (50)		0.92 (0.64–1.34)
Duration of use of a non-ASA NSAID (years)[3]								
<2 or non-exposed	154 (74)	209 (70)	NA	1.00 (Reference)	114 (59)	173 (58)	NA	1.00 (Reference)
2–5	28 (14)	38 (13)		1.03 (0.60–1.78)	32 (17)	35 (12)		1.53 (0.88–2.65)
>5	25 (12)	52 (17)		0.61 (0.36–1.04)	47 (24)	93 (31)		0.69 (0.45–1.08)

Abbreviations: ASA, acetyl salicylic acid; CI, confidence interval; NA, not applicable; NSAID, nonsteroidal anti-inflammatory drug; OR, odds ratio.
[1]Not adjusted in multivariate model, except age-, gender-, and town-matched.
[2]Adjusted for number of sunburns during childhood.
[3]Adjusted for extent of freckling on arms.

Overall, controls were more likely than cases to have used NSAIDs 4–5 times weekly (4.2 vs. 1.8%).

The distribution of several NSAID exposure measures among CM cases and controls are presented in Table 3. Age- and gender-matched controls were significantly more likely to have longer NSAID exposure (Tables 3 and 4). After adjusting for potential confounders in a multivariate model, the use of at least one NSAID for >5 years decreased the likelihood of developing CM by almost half (adjusted OR = 0.57; 95% CI = 0.43–0.77 compared with <2 years and no-use).

Comparing with cases, a significantly higher proportion of controls reported ASA use in the past year (overall exposure: 43 vs. 36%). After adjusting for sunburns during childhood (see Tables 3 and 4 for adjusted variables), people who used ASA were still about 25% less likely to have a CM diagnosis (adjusted OR = 0.72; 95% CI = 0.55–0.94). Controls were also more likely to have used ASA for >5 years than cases (21 vs. 13%, *P* = 0.002). For long-term ASA users, CM risk was about half that of never-users of ASA or of those who had used ASA for <2 years, after adjusting for sunburns during childhood (adjusted OR = 0.51; 95% CI = 0.35–0.75)

The use of non-ASA NSAIDs (with or without prior and/or concomitant exposure to ASA) did not differ between cases and controls (41 vs. 42%, adjusted OR = 0.92; 95% CI = 0.70–1.19). However, controls were slightly more likely than cases to have used non-ASA NSAIDs for >5 years (24 vs.18%, *P* = 0.072). Participants who used non-ASA NSAID for >5 years were significantly less likely to develop CM compared with participants who used it for 2 years or less (adjusted OR = 0.64; 95% CI = 0.46–0.89).

Sensitivity analyses

Restricting the univariate analyses to participants who used ASA without previous and/or concomitant exposure to other NSAIDs did not change the findings substantially (>5 years: OR = 0.48; 95% CI = 0.31–0.74 compared with <2 years-of-use). Also, the NSAID analysis that excluded participants who used ASA was comparable to the presented results (>5 years: OR = 0.63; 95% CI = 0.43–0.93 compared with <2 years-of-use).

Including all variables that were significantly associated with CM in the univariate analysis into a full multivariate model showed similar findings for long-term use of all NSAIDs (>5 years; adjusted OR = 0.57, 95% CI = 0.42–0.77, compared with <2 years-of-use), and ASA use (>5 years; adjusted OR = 0.50, 95% CI = 0.30–0.82, compared with <2 years-of-use). In contrast to the multivariate analysis that included variables based on the bivariate approach, non-ASA NSAID use (±ASA exposure) for >5 years was associated with a significantly reduced melanoma risk (>5 years; adjusted OR = 0.64, 95% CI = 0.46–0.89, compared with <2 years-of-use).

In the multivariate model that included variables based on the level of significance in the univariate analysis, participants who used non-ASA NSAID without concomitant ASA exposure remained significantly less likely to develop CM (>5 years; adjusted OR = 0.61; 95% CI = 0.40–0.93 compared with <2 years-of-use). After stratification for gender, the protective effect of NSAID and ASA exposure for >5 years was similar to those of the entire study population, with a trend toward a more pronounced effect in women (Table 4).

DISCUSSION
NSAIDs and melanoma
The findings of this case–control study indicate that continuous use of ASA for 5 years or more reduces the CM risk by almost half. This suggests the need of longer exposure for potential chemopreventive agents such as ASA to exert a protective biological effect (Elwood *et al.*, 2009). Regular, long-term use of non-ASA NSAIDs may also reduce CM risk, but because only 25% of non-ASA NSAID users were taking the medications ⩾4 times weekly the power to detect a significant association is limited. In contrast to a recent Dutch pharmaco-epidemiological study, we observed a protective effect of ASA on CM development in both men and women (Joosse *et al.*, 2009). In addition to a different dosing regimen, ASA may have a different effect on CM development than other NSAIDs because of its anti-oxidant properties, inhibition of activation of NF-κβ, upregulation of tumor suppressor genes such as *TP53*, *CDKN1A*, and *BAX*, and the downregulation of anti-apoptotic genes such as *BCL2* (Elwood *et al.*, 2009).

It is difficult to compare our findings with other studies because of the difference in study design and exposure definition. Two cohort studies have demonstrated a potential protective effect by NSAIDs in women exposed to continues use of low-dose ASA and in the setting of Cox-2 inhibitor exposure when assessing for incidence of second primaries, metastatic disease, and recurrences (Ramirez *et al.*, 2005; Joosse *et al.*, 2009). The Vitamins and Lifestyle cohort study did not detect a protective effect of NSAIDs on CM development, but this study had the following limitations: (1) NSAID exposure was defined as at least once weekly NSAID use for 1 year in the last 10 years without other temporal association other than ''preceding'' the date of diagnosis of melanoma; and (2) the magnitude of effect for several known melanoma risk factors, such as family history, hair color, and skin reaction to sunlight, were not confirmed in this study (Asgari *et al.*, 2008).

Statins and melanoma
Our initial statistical consideration assumed a 20% prevalence of statin use. We observed an overall 28% exposure rate, which could be explained by the increasing rate of statin use observed in the last decade. The prevalence of statin use in our case–control study, including gender distribution, is in range with recently reported studies (Ma *et al.*, 2005; Buettner *et al.*, 2008). A similar rate of statin exposure was observed between cases and controls irrespective of potential relevant factors such as duration of use and gender effect (Tables 3 and 4). In addition, all users related a frequency of exposure >4 times per week further supporting the negative association between statin exposure and melanoma risk reduction.

This neutral effect of statins in melanoma incidence concurs with two recent meta-analyses studies demonstrating ORs ranging from 0.87 to 0.92 (Freeman *et al.*, 2006; Bonovas *et al.*, 2010). In addition, studies evaluating the chemopreventive effect of statins in multiple neoplasias have

failed to demonstrate a reduction in short-term cancer risk (Browning and Martin, 2007). Of interest, a positive effect of statins in melanoma progression has been suggested by one recent study (Koomen *et al.*, 2007). The authors identified that statin use was associated with a reduced Breslow thickness (−19%, 95% CI = −33, −2.3, P = 0.03) without significant impact on overall melanoma incidence.

Strengths and limitations

This large case–control study was specifically designed to test the associations between drug exposure and CM development. In total, 400 melanoma patients participated, but a limited sample size (i.e., type II error) may have affected our findings, especially for the chemopreventive effects of non-ASA NSAIDs. As the initial sample size calculations were based on the association between statin exposure and CM risk, the statistical power was calculated *post hoc* for all NSAIDS and ASA based on the proportions of cases and controls who were exposed to these drugs for >5 years (28 vs. 41% and 13 vs. 21%, respectively) and was high at an alpha level of 0.05 (99.6 and 90.9%, respectively). The controls were population based and matched for age, gender, and community. Although it could be argued that this approach introduced overmatching, which would tend to underestimate associations, this procedure was chosen to minimize biases due to differences in sociodemographic factors, health care access, ethnicity, and possibly several lifestyle exposures (Le Marchand *et al.*, 2006; Whiteman *et al.*, 2006). Our confirmation of known melanoma risk factors such as demographics, phenotype, sun exposure, and lifestyle supports the accuracy of our data (Le Marchand *et al.*, 2006; Whiteman *et al.*, 2006; Tucker, 2009).

In the Materials and Methods section, we indicate that the selection of participants >40 years of age was based on general population exposure to statins. Several case–control studies evaluating the relationship of statins and NSAID exposure on cancer incidence have used a similar age cutoff (Shadman *et al.*, 2009; Yu *et al.*, 2009). This approach raises the possibility of selection bias; however, SEER data suggest that the probability of developing melanoma within birth to age 39 by gender between 2002 and 2005 was significantly lower when compared with those >40 years of age (Jemal *et al.*, 2009); therefore our results are likely relevant to the majority of the population at risk for melanoma. An inherent potential limitation in the recruitment of community controls relates to the risk of selection bias toward individuals with greater awareness toward health-related inquiries; however, cases and controls responded similarly to the indication of use for ASA (71 vs. 73% respectively), and both groups demonstrating similar level of access to medical care (Table 1).

The specific focus of the overall study was exposure to medications and we sought to limit recall bias. We used structured questions to elicit information on NSAID and statin exposure and read a list of all generic and brand names of the most commonly used drugs to participants. The doses of drugs used and their precise indication were not assessed because of their complexity (especially for NSAIDs). Although we did not verify the data from the standardized retrospective drug history by any other means (e.g., contacting primary care physicians, pharmacy, or hospital records), types of NSAIDs and statins used were similar between cases and controls and the most frequently used NSAID (70% was accounted for by ASA and ibuprofen) and statins (70% accounted for atorvastatin) was in accordance with previously reported data. (Ma *et al.*, 2005). This suggests that recall bias was low and most likely to be non-differential, which would then bias the risk estimates toward the null hypothesis.

Typically non-ASA NSAIDs are commonly used short-term for acute inflammatory processes and ASA more often long-term for cardiovascular disease prophylaxis (e.g., daily low-dose ASA). Although the specific ASA dose was not ascertained, 70% of people who indicated exposure to ASA used it ⩾4 times a week suggesting possible use for cardiovascular prophylaxis. In contrast to previous studies, we were able to evaluate the temporal association of long-term NSAID and melanoma detection in more detail.

Future melanoma chemoprevention strategies will likely be subjected to the current notion that multiple pathways of melanoma pathogenesis exist. This theory asserts that certain types of melanoma (i.e., head and neck) may be more prevalent in individuals with higher cumulative doses of sun exposure and low nevus counts, whereas intermittent UV exposure may be more closely linked to truncal melanoma and high nevus counts (Whiteman *et al.*, 2003). Also recent data have shown that differences in frequency of BRAF or NRAS mutations are also related to patterns of sun exposure (Curtin *et al.*, 2005).

In summary, our findings suggest that long-term use of NSAIDs, particularly ASA, decreases the risk of CM by half, with statin exposure demonstrating a neutral effect. The chemopreventive property of ASA in cancer development may be an additional argument to strongly encourage ASA adherence (Elwood *et al.*, 2009). Considering the risk to benefit ratio of low-dose ASA including the risk for bleeding and its cardiovascular effects, ASA would be an ideal candidate for clinical chemoprevention studies in melanoma in very high-risk populations (e.g., familial melanoma syndrome, atypical mole syndrome, and previous melanoma) or in populations using ASA for other health endpoints. In addition, the potential effect of NSAIDs in melanoma progression should be further explored.

MATERIALS AND METHODS
Sample

CM cases were recruited from the melanoma clinics and dermatology practices at affiliated Dana Farber Harvard Cancer Center institutions and Dermatology Associates of Concord, Boston (USA) from 15 March 2004 to 18 June 2007 (Figure 1). The Dana Farber Harvard Cancer Center and the New England Institutional Review Board approved the study and all procedures. Written informed patient consent was waived to minimize ascertainment bias between cases and controls. A verbal consent was obtained for all participating subjects and the protocol was compliant with the Helsinki Guidelines. Cases were eligible if they were at least 40 years of age, spoke English, were able to complete a telephone interview, and had received a diagnosis of primary CM within 90 days from the recruitment date. The rationale for recruiting individuals at least 40 years of age was based on the low prevalence of drug exposure, particularly for statins, in younger cohorts (see statistical analysis section). We contacted cases after their physician had obtained verbal consent. Of 483 cases that were invited
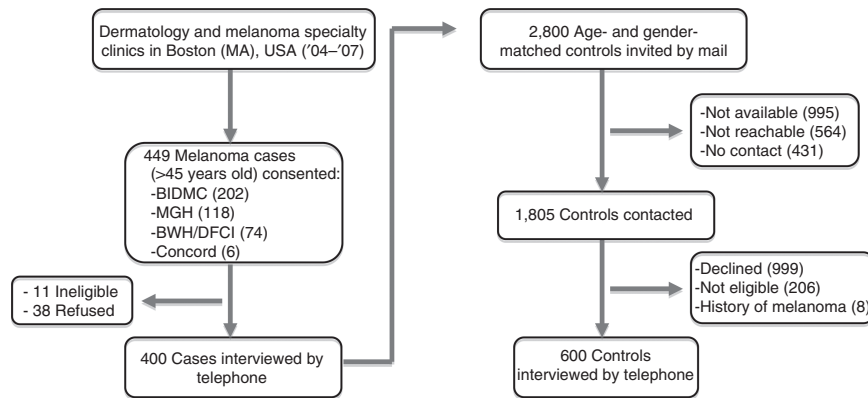
Figure 1. **Ascertainment of Cases and Controls.** BIDMC, Beth Israel Deaconess Medical Center; MGH, Massachusetts General Hospital; BWH/DFCI, Brigham and Women's Hospital/Dana Farber Cancer Institute.

to participate 449 verbally consented, and a total of 400 completed the interview (Figure 1). After completing the interview with a case, we matched each case to seven potential controls of the same sex, 5-year age-group, and home precinct obtained from city/town registry of adult residents (Sakamoto *et al.*, 2006; Zhang *et al.*, 2009). To minimize variability in the interview process (see data collection section), we approached cases and controls similarly by mailing an invitation letter explaining the telephone interview process without revealing our key study objective. All participants were asked to list current and former exposure to prescription and over the counter medications targeted in the study. Of the 2,800 potential-matched controls who were sent an invitation letter, we successfully contacted 1,805 after one to three telephone calls, 999 declined participation and 206 were not eligible. Every case had at least one interviewed age- and gender-matched control, and half of the CM cases were successfully matched with two controls (on average a 1:1.5 ratio), ultimately resulting in 608 consented and interviewed age- and gender-matched controls, representing 21.4% out of 57.1% of contacted and eligible controls (Figure 1). Of these, eight with a history of melanoma were excluded.

Data collection

Four trained interviewers telephoned all participants, verbally consented the controls, and administered a 42-item questionnaire that elicited information on demographic characteristics, medical history, and lifestyle characteristics, including utilization of medical care, known CM risk factors, and medication use (Rosso *et al.*, 1996, 2002; Zanetti *et al.*, 1996). (See Tables 1 and 2 for selected variables). The questionnaire took an average of 25 minutes to complete and participants were not compensated for their time. Data entry was checked for consistency of data entry by re-entering 50 interviews into a separate database. This procedure detected errors in only 0.5% of entries.

Assessment of medication use

We defined current NSAID, ASA, LLA, and statin users as persons taking the drug at least once weekly within a year preceding the interview. The interviewers ascertained drug exposure history in three ways by asking participants to (1) list the number of current prescription and over the counter medications taken at least once weekly in the last year; (2) identify medical conditions that might be associated with use of the drug classes of interest (for NSAIDs, any use within the last year of a prescription or over the counter drug for pain,

headache, arthritis, cardiovascular prophylaxis, or for other typical indications. For LLAs, any history of hyperlipidemia and treatment); (3) verify names of drugs in response to the interviewer's listing of the most commonly used NSAIDs (that reflected more than 90% of NSAIDs marketed in the US), and a complete listing of LLAs (including fibrates and statins) using generic and brand names (Intercontinental Marketing Services website: http://www.imshealth.com/portal/site/imshealth, accessed 27 January 2004). The interviewer recorded the drug name, frequency, and duration of use given by the respondent.

For NSAIDs and LLAs users, we assessed the drug with longest exposure duration. Among individual NSAIDs, the pattern of use could vary considerably, so we also assessed the following NSAIDs exposures: (1) all NSAIDs (ASA and non-ASA NSAIDs); (2) ASA with or without concomitant NSAIDs exposure, and (3) non-ASA NSAIDs with or without ASA exposure.

Statistical analysis

The analyses of the data reported on this paper were on the study hypotheses that focused on the association between NSAID and statin use and CM development. Sample size calculations were based on the prevalence of drug use and risk reduction assumption. In the case of statins, which have lower prevalence or use, it was assumed that 20% of the general population age 45 years and older used statins (personal communication by Dr D. Kaufman) and that the expected CM risk reduction would be 30%, as suggested in other studies of chemopreventive agents. With an $\alpha = 0.05$, $\beta = 0.80$, and a 1:2 ratio for cases and controls, about 500 cases and 1,000 controls needed to be enrolled. However, following interim analysis the studied was halted at 400 cases when no association between statins and CM risk reduction was observed.

Continuous variables were presented as means, and standard deviations. Mean differences between cases and controls were assessed with the Student's t-test. Differences between groups in the distribution of categorical variables were tested using the χ^2 test. We used univariate conditional logistic regression models to analyze the association between NSAIDs, ASA, statins, and CM by calculating ORs with 95% CI. Potential confounding factors were evaluated based on whether inclusion of the factor into the regression model changed the OR of NSAID, ASA, and CM risk by 10% or more (''bivariate approach''; Vandenbroucke *et al.*, 2007). Modeling was conducted for statins, and each of the three definitions of NSAIDs. We categorized duration of exposure for NSAID, ASA, or statins into

<2 years, 2–5 years, and >5 years (Dubè *et al.*, 2007). In our final analysis, we combined the ''never exposed'' and ''exposed for less than 2 years'' categories because exposure of <2 years before index date of CM is likely to have had no effect on CM carcinogenesis and in preliminary analyses, and no significant differences in risk estimates were identified between no-use and <2 years-of-exposure for both study drugs (Dubè *et al.*, 2007).

In sensitivity analyses, ASA exposure without previous or concomitant NSAID use, and NSAID exposure without ASA use were analyzed as dependent outcomes to assess possible differences between the effects of these two drug classes. In addition to the bivariate approach, we performed a multivariate analysis including all variables that differed significantly between cases and controls in the univariate analyses to test the impact of selecting confounders using the bivariate approach.

All tests of statistical significance were two-sided and *P*-values <0.05 were significant. Analyses were performed using STATA statistical software, version 10 (College Station, TX) and SPSS statistical software, version 17.0 (SPSS , Chicago, IL).

CONFLICT OF INTEREST
The authors state no conflict of interest.

REFERENCES

Asgari MM, Maruti SS, White E (2008) A large cohort study of nonsteroidal anti-inflammatory drug use and melanoma incidence. *J Natl Cancer Inst* 100:S967–71

Bonovas S, Nikolopoulos G, Filioussi K *et al.* (2010) Can statin therapy reduce the risk of melanoma? A meta-analysis of randomized controlled trials. *Eur J Epidemiol* 25:29–35

Bordeaux JS, Lu KQ, Cooper KD (2007) Melanoma: prevention and early detection. *Semin Oncol* 34:460–6

Browning DR, Martin RM (2007) Statins and risk of cancer: a systematic review and metanalysis. *Int J Cancer* 120:833–43

Buettner C, Davis RB, Leveille SG *et al.* (2008) Prevalence of musculoskeletal pain and statin use. *J Gen Intern Med* 23:1182–6

Curtin JA, Fridlyand J, Kageshita T *et al.* (2005) Distinct sets of genetic alterations in melanoma. *N Engl J Med* 353:2135–47

Downs JR, Clearfield M, Weis S *et al.* (1998) Primary prevention of acute coronary events in men and women with average cholesterol levels: results of AFCAPS/TexCAPS. Air Force/Texas Coronary Atherosclerosis Prevention Study. *JAMA* 279:1615–22

Dubè C, Rostom A, Lewin G *et al.* (2007) The use of aspirin for primary prevention of colorectal cancer: a systematic review prepared for the U.S. Preventive Services Task Force. *Ann Intern Med* 146:365–75

Elwood PC, Gallagher AM, Duthie GG *et al.* (2009) Aspirin, salicylates, and cancer. *Lancet* 373:1301–9

Freeman SR, Drake AL, Heilig LF *et al.* (2006) Statins, fibrates, and melanoma risk: a systematic review and meta-analysis. *J Natl Cancer Inst* 98:1538–46

González-Pérez A, García Rodríguez LA, López-Ridaura R (2003) Effects of non-steroidal anti-inflammatory drugs on cancer sites other than the colon and rectum: a meta-analysis. *BMC Cancer* 3:28

Jemal A, Siegel R, Ward E *et al.* (2009) Cancer statistics, 2009. *CA Cancer J Clin* 59:225–49

Jeter JM, Bonner JD, Johnson T *et al.* (2005) Non-steroidal anti-inflammatory drugs and the risk of melanoma. *J Clin Oncol* 23:1027 (abstr.)

Joosse A, Koomen ER, Herings R *et al.* (2009) Non steroidal anti-inflammatory drugs and melanoma risk: Large Dutch population based case-control study. *J Invest Dermatol* 129:2620–7

Koomen ER, Joosse A, Herings RM *et al.* (2007) Is statin use associated with a reduced incidence, a reduced Breslow thickness or delayed metastasis of melanoma of the skin? *Eur J Cancer* 43:2580–9

Kuoppala J, Lamminpää A, Pukkala E (2008) Statins and cancer: a systematic review and meta-analysis. *Eur J Cancer* 44:2122–32

Le Marchand L, Saltzman BS, Hankin JH *et al.* (2006) Anatomic site, sun exposure, and risk of cutaneous melanoma. Sun exposure, diet, and melanoma in Hawaii Caucasians. *Am J Epidemiol* 164: 232–45

Ma J, Sehgal NL, Ayanian JZ *et al.* (2005) National trends in statin use by coronary heart disease risk category. *PLoS Med* 2:e123

Ramirez CC, Ma F, Federman DG *et al.* (2005) Use of cyclooxygenase inhibitors and risk of melanoma in high-risk patients. *Dermatol Surg* 31:748

Rosso S, Miñarro R, Schraub S *et al.* (2002) Reproducibility of skin characteristic measurements and reported sun exposure history. *Int J Epidemiol* 31:439–46

Rosso S, Zanetti R, Martinez C *et al.* (1996) The multicentre south European study 'Helios' II: different sun exposure patterns in the etiology of basal cell and squamous cell carcinomas of the skin. *Br J Cancer* 73:1447–54

Sakamoto C, Sugano K, Ota S *et al.* (2006) Case control study on the association of upper gastrointestinal bleeding and nonsteroidal anti-inflammatory drugs in Japan. *Eur J Clin Pharmacol* 62:765–72

Shadman M, Newcomb PA, Hampton JM *et al.* (2009) Non-steroidal anti-inflammatory drugs and statins in relation to colorectal cancer risk. *World J Gastroenterol* 15:2336–9

Tucker MA (2009) Melanoma epidemiology. *Hematol Oncol Clin North Am* 23:383–95

Vandenbroucke JP, von Elm E, Altman DG *et al.* (2007) STROBE Initiative. Strengthening the Reporting of Observational Studies in Epidemiology (STROBE): explanation and elaboration. *Epidemiology* 18:805–35

Vogel VG (2007) Chemoprevention strategies 2006. *Curr Treat Options Oncol* 8:74–88

Whiteman DC, Stickley M, Watt P *et al.* (2006) Anatomic site, sun exposure, and risk of cutaneous melanoma. *J Clin Oncol* 24:3172–7

Whiteman DC, Watt P, Purdie DM *et al.* (2003) Melanocytic nevi, solar keratoses, and divergent pathways to cutaneous melanoma. *J Natl Cancer Inst* 95:806–12

Yu O, Boudreau DM, Buist DS *et al.* (2009) Statin use and female reproductive organ cancer risk in a large population-based setting. *Cancer Causes Control* 20:609–16

Zanetti R, Rosso S, Martinez C *et al.* (1996) The multicentre south European study 'Helios'. I: skin characteristics and sunburns in basal cell and squamous cell carcinomas of the skin. *Br J Cancer* 73:1440–6

Zerbini LF, Czibere A, Wang Y *et al.* (2006) A novel pathway involving melanoma differentiation associated gene-7/interleukin-24 mediates nonsteroidal anti-inflammatory drug-induced apoptosis and growth arrest of cancer cells. *Cancer Res* 66:11922–31

Zhang CX, Ho SC, Chen YM *et al.* (2009) Greater vegetable and fruit intake is associated with a lower risk of breast cancer among Chinese women. *Int J Cancer* 125:181–8