available at www.sciencedirect.com

ScienceDirect

journal homepage: www.ejconline.com

Is statin use associated with a reduced incidence, a reduced Breslow thickness or delayed metastasis of melanoma of the skin?

E.R. Koomen[a,*], *A. Joosse*[a], *R.M.C. Herings*[b], *M.K. Casparie*[c], *W. Bergman*[d], *T. Nijsten*[e], *H.J. Guchelaar*[a,*]

[a]*Department of Clinical Pharmacy & Toxicology, Leiden University Medical Centre, P.O. Box 9600, 2300 RC Leiden, The Netherlands*
[b]*PHARMO, Institute for Drug Outcome Research, Utrecht, The Netherlands*
[c]*Foundation PALGA, Utrecht, The Netherlands*
[d]*Department of Dermatology, Leiden University Medical Centre, Leiden, The Netherlands*
[e]*Department of Dermatology, Erasmus Medical Centre Rotterdam, Rotterdam, The Netherlands*

ARTICLE INFO

Article history:
Received 27 July 2007
Received in revised form 3 September 2007
Accepted 5 September 2007
Available online 22 October 2007

Keywords:
Melanoma
Metastasis
Chemoprevention
HMG-CoA reductase inhibitors

ABSTRACT

Background: Statins show anticancer activity in melanoma cells. We investigated the association between statins and incidence and Breslow thickness of cutaneous melanoma (CM).
Methods: Data were used from PHARMO, a pharmacy database, and PALGA, a pathological database, in the Netherlands. Cases had a primary CM diagnosis between January 1st 1991 and December 14th 2004, were $\geqslant$18 years and had $\geqslant$3 years of follow up in PHARMO before CM diagnosis. Controls were matched for gender, date of birth and geographic region. Analyses were adjusted for age, gender, year of diagnosis, number of medical diagnoses and the use of NSAIDs and oestrogens.
Findings: Finally, 1318 cases and 6786 controls were selected. CM risk was not associated with statin use ($\geqslant$0.5 years) (adjusted odds ratio (OR) = 0.98, 95% confidence interval (CI) = 0.78–1.2). However, statin use was associated with a reduced Breslow thickness (−19%, 95% CI = −33, −2.3, $p = 0.03$).
Conclusion: Our study suggests protective effects of statins on melanoma progression.

1. Introduction

Cutaneous melanoma (CM) accounts for 77% of all deaths due to skin cancer. The incidence of CM is increasing considerably, about 3% each year.[1]

Until now, treatment of advanced CM has been disappointing.[2] Preventive public health measures aiming at early diagnosis have therefore received much attention. Chemoprevention would be another approach to inhibit the development or progression of CM. *In vitro* studies have shown that several agents including 3-hydroxy-3-methylglutaryl-coenzyme A reductase inhibitors (statins) have the potential to alter CM behaviour.[3] Statins are interesting candidates for chemoprevention because they are widely used and have an excellent long term safety.[4]

Statins inhibit the cholesterol biosynthesis through inhibition of the enzyme HMG-Co-A reductase and subsequently cause depletion of mevalonate, a precursor of cholesterol and farnesyl- and geranylgeranyl-moieties essential for post-translational activation of several intracellular proteins

* *Corresponding authors:* Tel.: +31 71 526 2790; fax: +31 71 526 6980.
E-mail addresses: e.r.koomen@lumc.nl (E.R. Koomen), h.j.guchelaar@lumc.nl (H.J. Guchelaar).
0959-8049/$ - see front matter
doi:10.1016/j.ejca.2007.09.004

through prenylation. By inhibiting prenylation, statins may affect several proteins such as the Rho family involved in signalling and regulation of cell differentiation and proliferation.[5,6] High-throughput screens for transcriptionally regulated targets in the metastatic process have shown that RhoC overexpression dramatically increases the metastatic potential of inoculated melanoma in mice.[7]

Therefore, statins may potentially affect incidence and metastasic spreading of CM. Indeed, in severely combined immunodeficient (SCID) mice atorvastatin prevented RhoC isoprenylation, invasion and metastasis of A375M melanocytes.[8]

Epidemiological studies and meta-analyses have suggested that use of statins is associated with a lower risk of developing cancer in general .[9–14] However, most studies do not have sufficient sample size to study site-specific cancers.[11] For colorectal cancer, a case-control study with 1809 cases and 1809 controls was published by Coogan and colleagues,[15] but for CM no studies with sufficient sample size have been published.

In an earlier nested case-control observational study we confirmed a significant risk reduction of cancer of 20% in statin users compared to non-users. For incident skin cancers, the risk reduction was 36% but statistically not significant (adjusted odds ration (OR) = 0.63; 95% confidence interval (CI), 0.22 to 1.8).[9] Although a Cochrane Review demonstrated no significant association between statin use and CM incidence (OR = 0.90, 95% CI 0.56–1.4), the authors concluded further exploration of the use of statins in melanoma prevention is warranted.[16,17]

The primary objective of this study is to investigate the effect of statins on the incidence and the Breslow thickness of CM. Also, a pilot study was performed to study the effects of statins on the time to metastasis.

2. Methods

2.1. Setting

Data were used from the PHARMO database, containing drug dispensing records of a defined population of over 2 million Dutch residents, thus representing more than 12% of the Dutch population. Residents are included regardless of type of insurance.[18]

Participants of PHARMO enter the database with the first prescription filled in a PHARMO pharmacy and are observed until the last prescription. Since, in the Netherlands, most individuals visit a single pharmacy, dispensing histories are virtually complete.[19] The computerised drug dispensing histories contain all dispensed prescriptions and include information on type, quantity, dosage form, strength, dispensing date and prescribed daily dose of the dispensed drug. PHARMO was linked to PALGA, the Dutch nationwide registry of histopathology and cytopathology, using a variation of a reliable probabilistic algorithm.[20] PALGA contains abstracts of all pathology reports with encrypted patient identification and diagnostic terms which are in scope with SNOMED classification. Since 1990 the registration reached 100% participation and, in 2004, data on over 9 million patients had been archived.[21] Therefore PALGA represents all Dutch patients and is the basis for the Dutch Cancer Registry.

2.2. Study population

Cases had a primary CM diagnosis in PALGA between January 1st 1991 and December 14th 2004 and were also registered in PHARMO in this period. End of follow up was defined as the date of CM diagnosis (index date). For the pilot study, 90 days (i.e. the usual prescription duration) after the last date in PHARMO or date of metastasis, whichever occurred first, was used as end of follow up.

For each case, all records in PALGA were interpreted by one of the two investigators (AJ, ERK). From these records the researchers extracted and recorded diagnosis and date of primary CM, Breslow depth (mm), CM subtype according to WHO classification[22] and body location (head-neck, trunk or extremities) as well as occurrence and date of pathologically confirmed metastasis of the lymph node (LN), skin and/or internal organs between Jan 1st 1991 and March 14th 2005 (90 days after end of study period). To assess inter-observer variation, 300 cases were randomly selected and scored by both researchers.

Potential cases were excluded if, in PHARMO, the date of entry was unknown, gender was unknown, follow up in the 3 years before CM diagnosis was incomplete, or, in PALGA, if the date of CM diagnosis was before the age of 18 or before January 1, 1991, the melanoma was not pathologically confirmed, or if the primary melanoma was not on the skin (e.g. in the eye) or if the melanoma was *in situ* (Fig. 1).

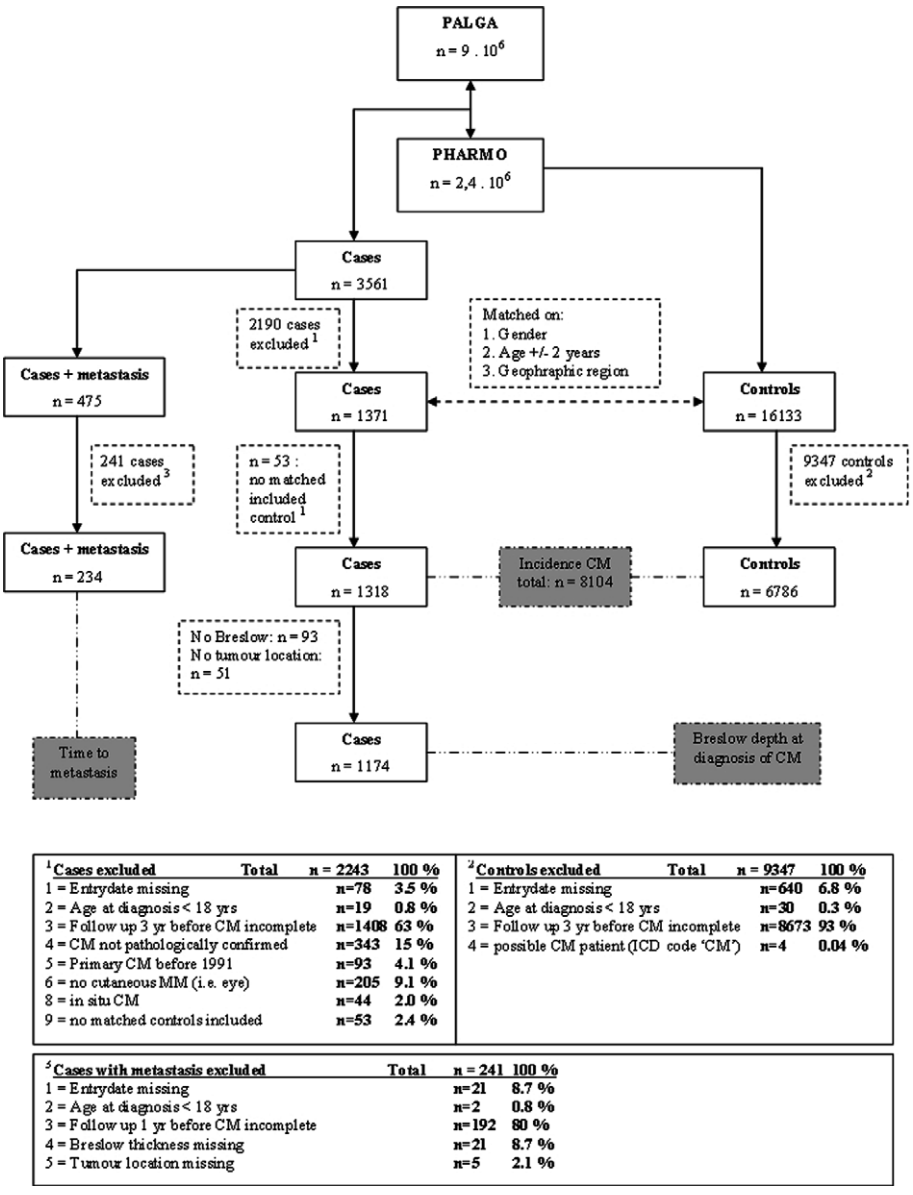
For every eligible case, an average of five controls was sampled from the population available in PHARMO, matched for gender, date of birth (+/− 2 years) and geographic region. Potential cases could not be selected as controls. To calculate follow up for controls, controls were assigned the index date of the matched case.

Controls were excluded if, in PHARMO, the date of entry was unknown, if they were younger than 18 years at the index date, if the follow up in the 3 years before index date was incomplete, or if they were diagnosed in PHARMO with previous melanoma according to the International Classification of Disease (Fig. 1).

2.3. Drug exposure

Statin exposure was defined as the use of one or more statins for at least 6 months of cumulative prescription duration in the 3 years before CM (i.e. we assumed this minimal exposure to be required for the hypothesised biological mechanism). All statins commercially available in the Netherlands within the study period were included: pravastatin, simvastatin, cerivastatin (since withdrawn), atorvastatin, rosuvastatin and fluvastatin (ATC codes: C10AAXX).

To further detail statin use, several variables related to statin exposure were created (Fig. 2), all with the 6 month threshold. The cumulative number of dispenses, cumulative dispensed dose and the cumulative prescribed duration were calculated. The average day dose was defined as the cumulative dose divided by the cumulative duration. Lag time was



[1] Cases excluded	Total	n = 2243	100 %
1 = Entrydate missing		n=78	3.5 %
2 = Age at diagnosis < 18 yrs		n=19	0.8 %
3 = Follow up 3 yr before CM incomplete		n=1408	63 %
4 = CM not pathologically confirmed		n=343	15 %
5 = Primary CM before 1991		n=93	4.1 %
6 = no cutaneous MM (i.e. eye)		n=205	9.1 %
8 = in situ CM		n=44	2.0 %
9 = no matched controls included		n=53	2.4 %

[2] Controls excluded	Total	n = 9347	100 %
1 = Entrydate missing		n=640	6.8 %
2 = Age at diagnosis < 18 yrs		n=30	0.3 %
3 = Follow up 3 yr before CM incomplete		n=8673	93 %
4 = possible CM patient (ICD code 'CM')		n=4	0.04 %

[3] Cases with metastasis excluded	Total	n = 241	100 %
1 = Entrydate missing		n=21	8.7 %
2 = Age at diagnosis < 18 yrs		n=2	0.8 %
3 = Follow up 1 yr before CM incomplete		n=192	80 %
4 = Breslow thickness missing		n=21	8.7 %
5 = Tumour location missing		n=5	2.1 %

Fig. 1 – Flow chart study population.

defined as the difference between the index date and the last day of statin use as calculated from the last dispense.

2.4. *Potential confounders*

Ever use of drugs possibly related to progression and development of CM was investigated, such as Non Steroidal Anti-Inflammatory Drugs (NSAIDs including COXibs) and anticonception and hormonal substitution oestrogens (OAC and HRT, ATC codes: G03AXXX & G03CXXX). Use of fibrates, heparins and lipid lowering drugs other than fibrates or statins was recorded, but the number of cases and controls using these drugs were too small (<1.0%) to be used for further analysis. Ever use of oestrogens was studied among female cases and controls.

In order to estimate health care consumption, which may be a confounder, a variable was created counting the total number of unique (i.e. singular) medical diagnoses (International Classification of Disease 9th revision, clinical modification; ICD9-CM) in PHARMO in the 3 years before CM.

In a pilot study, we investigated the association between statin use and time to metastasis among cases with pathologically confirmed metastasis (LN, skin and/or systemic). These cases were categorised in ever statin users and non statin users in the period between 1 year before CM diagnosis and metastasis. For this pilot, statin use was not detailed any further because of the limited sample size and the presence of metastasis risk prior to diagnosis.

2.5. *Statistical analysis*

Because CM may behave differently across gender, we analysed the total study population, but also men and women separately. To test for statistical differences, χ^2 and Student's

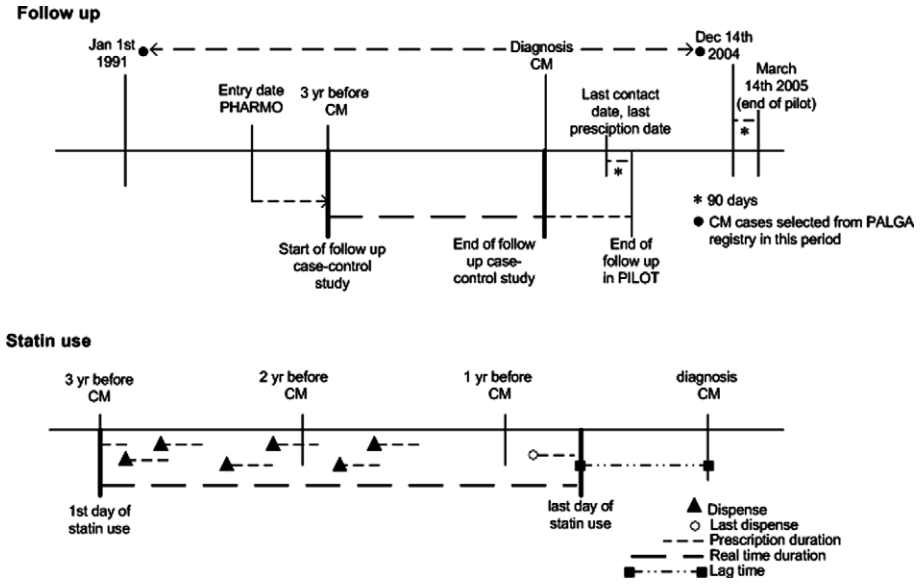


Fig. 2 – Schematic representation of follow up and statin use.

t-tests were used for categorical and continuous variables respectively. Non-normal distributions (tested using the Kolomogorov–Smirnov test) were log-transformed. All statistical tests were two sided, with a rejection of the null hypothesis at $p < 0.05$.

A multivariate logistic regression model was used to calculate adjusted OR and 95% CI for the association between CM incidence and statin use. The different statin variables were categorised based on quartiles among all users. Multiple linear regression, which used log transformed Breslow thickness as a dependent variable, was used to estimate the effect of statin use on local CM progression (adjusted coefficients and 95% CI). In this analysis, the statin variables were divided in categories of equal distances to facilitate the interpretation of the findings.

In the pilot study, a Kaplan–Meier curve and Cox proportional hazard model were used to estimate the hazard ratio between statin use and time to metastasis among cases with pathologically confirmed metastasis.

All statistical analyses were performed using SPSS 14.0 (.2) (SPSS Inc., Chicago, IL).

3. Results

3.1. Study population

Figure 1 demonstrates the ascertainment of cases and controls. In total 3561 subjects who were registered in PHARMO had a SNOMED code 'Melanoma' in PALGA. Of these cases, 1318 (37.0%) met the inclusion criteria. The main reason for not meeting inclusion criteria was registration in different time periods in PALGA and PHARMO or an incomplete follow up in PHARMO in the 3 years before CM diagnosis. Accordance between the two authors in a random sample of 300 cases was high (Kappa values >0.85), suggesting small inter-observer variation. Of the 16,133 controls matched on gender, age (+/− 2 years) and geographical region, 6786 (42.1%) could be included in the study (Fig. 1).

3.2. Risk of CM development and statin use

Mean age of cases and controls was 55.3 and 55.9 years ($p > 0.05$; Table 1A). Fifty-nine of the cases versus 60% of controls were female ($p > 0.05$). Male cases had significantly more unique diagnoses than male controls (0.84 versus 0.66, $p = 0.02$; Table 1B). Among females there was no significant difference. Statins were used for more than half a year in the study period by 7.3% of the cases and 7.4% of the controls ($p > 0.05$). Of the statins used, 62.4% was simvastatin, 14.2% pravastatin, 4.7% fluvastatin, 16.9% atorvastatin, 1.3% rosuvastatin and 0.5% cerivastatin. None of the statin related variables were significantly different between cases and controls. Women with CM were less likely to have used statins for more than 3 years (1.2% versus 2.4%, $p = 0.04$) and to have a cumulative dose between 1001–1500 DDD (0.6% versus 1.8%, $p = 0.02$). In men, cases using statins were more likely to have a lag time of 0.5 years or longer than controls who used statins ($p = 0.03$).

The average statin day dose prescribed to patients was 1.4 DDD per day (SD 0.82 DDD per day). Comparing prior drug use demonstrated significantly more use of NSAIDs and oestrogens in the 3 years prior to diagnosis among CM patients (Tables 1A and 1B).

After adjusting for confounding factors in a multivariate model, none of the statin related variables were significantly associated with CM incidence in the total study population (Table 2A). Although not statistically significant, a higher average daily statin dose was associated with a lower relative risk of CM, especially among women and to a lesser extent in men (Table 2B). The differences in the distribution of several characteristics of statin use observed in Tables 1A and 1B remained significant after adjusting for confounding variables. Compared to female non statin users, women who had 3 or

Table 1A – Characteristics of all cases and controls

		Cases $n = 1318$	Controls $n = 6786$	p-value
Gender[a]	male	540 (41.0%)	2714 (40.0%)	
	female	778 (59.0%)	4072 (60.0%)	0.51
Age at diagnosis[b]	yrs	55.3 (±15.9)	55.9 (±15.5)	0.18
Total unique diagnoses[b]	number	0.71 (±1.5)	0.61 (±1.55)	0.04
NSAIDs[a]	Yes	627 (47.6%)	2942 (43.4%)	
	No	691 (52.4%)	3844 (56.6%)	0.01
Oestrogens[a]	Yes	264 (20.0%)	1117 (16.5%)	
	No	1054 (80.0%)	5669 (83.5%)	<0.01
Statin use[a]	Non-exposed	1222 (92.7%)	6283 (92.6%)	
	Exposure >0.5 yr	96 (7.3%)	503 (7.4%)	0.87
Number of dispenses[a]	0	1222 (92.7%)	6283 (92.6%)	
	1–8	27 (2.0%)	131 (1.9%)	0.79
	9–11	17 (1.3%)	118 (1.7%)	0.25
	12	24 (1.8%)	111 (1.6%)	0.64
	>12	28 (2.1%)	143 (2.1%)	0.97
Cumulative prescription duration[a,c]	0 yrs	1222 (92.7%)	6283 (92.6%)	
	0.5–1.0 yrs	17 (1.3%)	53 (0.8%)	0.07
	1.0–2.0 yrs	18 (1.4%)	115 (1.7%)	0.40
	2.0–3.0 yrs	25 (1.9%)	140 (2.1%)	0.70
	>3.0 yrs	36 (2.7%)	195 (2.9%)	0.78
Cumulative dose[a]	0 DDD	1222 (92.7%)	6283 (92.6%)	
	1–600 DDD	32 (2.4%)	125 (1.8%)	0.17
	601–1000 DDD	24 (1.8%)	110 (1.6%)	0.61
	1001–1500 DDD	21 (1.6%)	145 (2.1%)	0.21
	>=1501 DDD	19 (1.4%)	123 (1.8%)	0.35
Average day dose[a]	0 DDD	1222 (92.7%)	6283 (92.6%)	
	0.01–0.99 DDD	29 (2.2%)	127 (1.9%)	0.44
	1.00–1.32 DDD	23 (1.7%)	94 (1.4%)	0.33
	1.33–1.99 DDD	27 (2.0%)	153 (2.3%)	0.65
	>= 2.00 DDD	17 (1.3%)	129 (1.9%)	0.13
Lag time[a,d]	Non-exposed	1222 (92.7%)	6283 (92.6%)	
	<0.5 yrs	87 (6.6%)	481 (7.1%)	0.55
	>=0.5 yrs	9 (0.7%)	22 (0.3%)	0.06

a Number of cases and controls presented, tested for statistical difference with χ^2-test.
b Mean value presented, tested for statistical difference with t-test.
c Time interval between first prescription and estimated last day of use based on last dispense and amount dispensed in the 3 years before diagnosis of cutaneous melanoma.
d Time interval between estimated last day of use based on last dispense and amount dispensed and date of diagnosis of cutaneous melanoma.

more years of statin use were about half as likely to have developed CM (adjusted OR = 0.49, 95% CI = 0.25–0.99). Female CM patients were also significantly less likely to have used a substantial cumulative dose than those without CM (for 1001–1500 DDD, adjusted OR = 0.35, 95% CI = 0.14–0.88, compared to 0 DDD). Men with CM were more than twice as likely to have used statins for less than a year and have a lag time of 0.5 years or more after adjusting for confounding variables.

3.3. *Breslow thickness of CM and statin use*

Cases with unknown Breslow depth or location of the CM were excluded (93 versus 51). Of the residual 1174 CM cases, 51.4% had a Breslow thickness <1.0 mm, 66.8% was of the superficial spreading type and 93.2% showed no regression (Table 3). Eighty-six percent were located on the trunk or extremities. Tumour characteristics such as Breslow depth, CM subtype and body location differed significantly between males and females. Tumour regression, however, did not differ significantly between male and female cases.

In our multivariate linear regression model, each of the associations between Breslow thickness and the statin variables in the 3 years prior to CM diagnosis were negative with p-values close to statistical significance ($p < 0.10$) (Table 4). Using statins for 6 months or longer significantly reduced the average Breslow thickness with 19.2% when compared to non users (95% CI = −33.2%, −2.3%, $p = 0.03$). After adjustment for gender, these findings were confirmed in men but not in women. In men, every increase of four dispenses or 0.66 DDD in average day dose was associated with a significantly reduced Breslow thickness (−10.7%, 95% CI = −18.5%,

Table 1B – Characteristics of male and female cases and controls

		Males			Females		
		Cases $n = 540$	Controls $n = 2714$)	p-value	Cases $n = 778$	Controls $n = 4072$	p-value
Age at diagnosis[a]	yrs	57.7 (±14.6)	58.0 (±14.2)	0.72	53.6 (±16.5)	54.6 (±16.1)	0.14
Total unique diagnoses[a]	number	0.84 (±1.76)	0.66 (±1.61)	0.02	0.62 (±1.33)	0.59 (±1.50)	0.55
NSAIDs[b]	Yes	239 (44.3%)	1125 (41.5%)		388 (50.1%)	1817 (44.6%)	
	No	301 (55.7%)	1589 (58.5%)	0.23	390 (49.9%)	2255 (55.4%)	0.01
Oestrogens[b]	Yes	–	–		264 (33.9%)	1117 (27.4%)	
	No	–	–	–	514 (66.1%)	2955 (72.6%)	<0.001
Statin use[b]	Non-exposed	477 (88.3%)	2446 (90.1%)		745 (95.8%)	3837 (94.2%)	
	Exposure >0.5 yr	63 (11.7%)	268 (9.9%)	0.21	33 (4.2%)	235 (5.8%)	0.72
Number of Dispenses[b]	0	477 (88.3%)	2446 (90.1%)		745 (95.8%)	3837 (94.2%)	
	1–8	17 (3.1%)	68 (2.5%)	0.37	10 (1.3%)	63 (1.5%)	0.56
	9–11	11 (2.0%)	66 (2.4%)	0.63	6 (0.8%)	52 (1.3%)	0.23
	12	15 (2.8%)	61 (2.2%)	0.43	9 (1.2%)	50 (1.2%)	0.84
	>12	20 (3.7%)	73 (2.7%)	0.19	8 (1.0%)	70 (1.7%)	0.16
Cumulative prescription duration[b,c]	0 yrs	477 (88.3%)	2446 (90.1%)		745 (95.8%)	3837 (94.2%)	
	0.5–1.0 yrs	12 (2.2%)	28 (1.0%)	0.02	5 (0.6%)	25 (0.6%)	0.95
	1.0–2.0 yrs	11 (2.0%)	61 (2.2%)	0.81	7 (0.9%)	54 (1.3%)	0.32
	2.0–3.0 yrs	13 (2.4%)	80 (2.9%)	0.55	12 (1.5%)	60 (1.5%)	0.93
	>3.0 yrs	27 (5.0%)	99 (3.6%)	0.13	9 (1.2%)	96 (2.4%)	0.04
Cumulative dose[b]	0 DDD	477 (88.3%)	2446 (90.1%)		745 (95.8%)	3837 (94.2%)	
	1–600 DDD	21 (3.9%)	66 (2.4%)	0.06	11 (1.4%)	59 (1.4%)	0.90
	601–1000 DDD	14 (2.6%)	60 (2.2%)	0.55	10 (1.3%)	50 (1.2%)	0.93
	1001–1500 DDD	16 (3.0%)	71 (2.6%)	0.61	5 (0.6%)	74 (1.8%)	0.02
	>= 1501 DDD	12 (2.2%)	71 (2.6%)	0.65	7 (0.9%)	52 (1.3%)	0.37
Average day dose[b]	0 DDD	477 (88.3%)	2446 (90.1%)		745 (95.8%)	3837 (94.2%)	
	0.01–0.99 DDD	17 (3.1%)	63 (2.3%)	0.24	12 (1.5%)	64 (1.6%)	0.91
	1.00–1.32 DDD	17 (3.1%)	56 (2.1%)	0.12	6 (0.8%)	38 (0.9%)	0.64
	1.33–1.99 DDD	17 (3.1%)	71 (2.6%)	0.46	10 (1.3%)	82 (2.0%)	0.17
	>= 2.00 DDD	12 (2.2%)	78 (2.9%)	0.45	5 (0.6%)	51 (1.3%)	0.15
Lag time[b,d]	Non-exposed	477 (88.3%)	2446 (90.1%)		745 (95.8%)	3837 (94.2%)	
	<0.5 yrs	57 (10.6%)	258 (9.5%)	0.42	30 (3.9%)	223 (5.5%)	0.07
	>=0.5 yrs	6 (1.1%)	10 (0.4%)	0.03	3 (0.4%)	12 (0.3%)	0.70

a Mean value presented, tested for statistical difference with t-test.
b Number of cases and controls presented, tested for statistical difference with χ^2-test.
c Time interval between first prescription and estimated last day of use based on last dispense and amount dispensed in the three years before diagnosis of cutaneous melanoma.
d Time interval between estimated last day of use based on last dispense and date of diagnosis of cutaneous melanoma.

Table 2A – Multivariate analysis of risk factors 3 years before diagnosis of CM

		Adjusted OR[a]	95% CI
Statin use	Non-exposed	1.0	Referent
	>0.5 yr	0.98	0.78–1.2
No. of dispenses	0	1.0	Referent
	1–8	1.1	0.70–1.6
	9–11	0.73	0.44–1.2
	12	1.1	0.71–1.7
	>12	1.0	0.67–1.5
Cumulative prescription duration	0 yrs	1.00	Referent
	0.5–1.0 yrs	1.7	0.97–2.9
	1.0–2.0 yrs	0.80	0.48–1.3
	2.0–3.0 yrs	0.91	0.59–1.4
	>3.0 yrs	0.96	0.66–1.3
Cumulative dose	0 DDD	1.00	Referent
	1–600 DDD	1.3	0.89–2.0
	601–1000 DDD	1.1	0.72–1.8
	1001–1500 DDD	0.74	0.47–1.2
	>= 1501 DDD	0.78	0.48–1.3
Average day dose	0 DDD	1.0	Referent
	0.01–0.99 DDD	1.2	0.79–1.8
	1.00–1.32 DDD	1.3	0.79–2.0
	1.33–1.99 DDD	0.91	0.60–1.4
	>= 2.00 DDD	0.67	0.40–1.1
Lag time[b]	Non-exposed	1.0	Referent
	<0.5 yrs	0.94	0.73–1.2
	>= 0.5 yrs	2.0	0.92–4.4

a Adjusted for age, gender, year of diagnosis, total number of unique ICD diagnoses, the use of NSAIDs and oestrogens.
b Time interval between estimated last day of use (based on last dispense and amount dispensed) and date of diagnosis of CM.

−2.2%, $p = 0.02$ and −11.0%, 95% CI = −19.7%, −1.2%, $p = 0.03$, respectively).

3.4. *Time to CM metastasis and statin use - pilot study*

Of all 3561 CM cases, 475 (13.3%) had pathologically confirmed metastasis (Fig. 1). Of these 475 cases with metastasis, 234 (49.3%) could be included in the analysis (average age was 54.7 years and 46.2% were females). The average number of months to metastasis was significantly higher for statin users than for non users (28.4 [SD 26.9] versus 16.5 [SD 22.7], $p = 0.03$) (Fig. 3).

After adjustment for gender, age, year of CM diagnosis, body site, Breslow thickness, histological subtype, presence of regression, use of NSAID and oestrogens in a Cox proportional hazard model, ever statin use between the year prior to CM diagnosis and date of metastasis reduced the likelihood of metastasis but was no longer significant (HR 0.69, 95% CI = 0.42–1.1). A survival analysis model that excluded Breslow thickness was performed as well. This model showed a significant effect of statin use on time to metastasis (HR = 0.58, 95% CI = 0.36–0.94).

4. Discussion

4.1. *Incidence cutaneous melanoma*

None of the statin related independent variables in our study consistently supports a risk reduction of statin use on the incidence of CM (Tables 2A and 2B). Possibly, the average daily doses in our population (median: 1.3 to 2.0 DDD) are not high enough to prove a chemopreventive effect. The follow up may be too short and persistence (i.e. compliance with statin intake) may be poor, a problem of statin therapy as described by Johnson and colleagues.[23] However, our findings are in concordance with the Cochrane Review.[16,17]

4.2. *Breslow thickness at diagnosis*

To our knowledge, this is the first study investigating an association between statin use and Breslow depth at diagnosis of CM. Our data suggest that statin use is associated with a significantly reduced Breslow thickness at diagnosis (−19.2%, 95% CI = −33.2, −2.3, $p = 0.03$). As non statin-users in our database had a mean Breslow thickness of 1.8 mm, this would indicate an average reduction in the depth of the lesion of 0.35 mm with statin use. This is an important finding since the Breslow thickness at diagnosis is one of the strongest determinants for prognosis.[24,25]

Among men this effect was even more pronounced with a reduction in Breslow thickness of −27.8% (95% CI = −43.7%, −7.4%, $p = 0.01$). Male non-statin users had a mean Breslow thickness of 2.1 mm; therefore, statin use for 0.5 years or more would result in a mean reduction of 0.58 mm. Because male cases, especially, had a significant higher number of unique ICD diagnoses compared to male controls (0.84 versus 0.66, $p = 0.02$), one could also argue that statin use among

Table 2B – Multivariate analysis of risk factors of men and women 3 years before diagnosis of CM

		Males		Females	
		Adjusted OR[a]	95% CI	Adjusted OR[b]	95% CI
Statin use	Non-exposed	1.0	Referent	1.0	Referent
	>0.5 yr	1.2	0.88–1.6	0.75	0.51–1.1
No. of dispenses	0	1.0	Referent	1.0	Referent
	1–8	1.3	0.73–2.2	0.86	0.44–1.7
	9–11	0.84	0.44–1.6	0.62	0.26–1.4
	12	1.3	0.72–2.3	0.93	0.45–1.9
	>12	1.4	0.82–2.3	0.61	0.29–1.3
Cumulative prescription duration	0 yrs	1.0	Referent	1.0	Referent
	0.5–1.0 yrs	**2.1**	**1.1–4.2**	1.1	0.43–3.0
	1.0–2.0 yrs	0.91	0.47–1.7	0.68	0.31–1.5
	2.0–3.0 yrs	0.82	0.45–1.5	1.1	0.57–2.0
	>3.0 yrs	1.4	0.90–2.2	**0.49**	**0.25–0.99**
Cumulative dose	0 DDD	1.0	Referent	1.0	Referent
	1–600 DDD	1.6	0.96–2.6	1.0	0.53–1.9
	601–1000 DDD	1.2	0.66–2.2	1.1	0.54–2.1
	1001–1500 DDD	1.2	0.67–2.0	**0.35**	**0.14–0.88**
	>= 1501 DDD	0.83	0.44–1.6	0.71	0.32–1.6
Average day dose	0 DDD	1.0	Referent	1.0	Referent
	0.01–0.99 DDD	1.4	0.81–2.4	0.99	0.53–1.9
	1.00–1.32 DDD	1.5	0.85–2.5	0.88	0.37–2.1
	1.33–1.99 DDD	1.3	0.73–2.2	0.63	0.33–1.2
	>= 2.00 DDD	0.75	0.40–1.4	0.53	0.21–1.3
Lag time[c]	Non-exposed	1.0	Referent	1.0	Referent
	<0.5 yrs	1.1	0.79–1.5	0.72	0.48–1.1
	>= 0.5 yrs	**2.9**	**1.0–8.1**	1.3	0.36–4.6

a Adjusted for age, year of diagnosis, total number of unique ICD diagnoses and the use of NSAIDs.
b Adjusted for age, year of diagnosis, total number of unique ICD diagnoses, the use of NSAIDs and oestrogens.
c Time interval between estimated last day of use (based on last dispense and amount dispensed) and date of diagnosis of CM.

Table 3 – Melanoma characteristics of the primary CM of the cases

	Total n = 1174	Male n=487	Female n = 687	p-value
Breslow				
mm	1.75	2.06	1.53	[a]<0.001
Breslow in AJCC categories				
0–1 mm	604 (51.4%)	223 (45.8%)	381 (55.5%)	[b]0.001
1.01–2 mm	284 (24.2%)	123 (25.3%)	161 (23.4%)	
2.01–4 mm	188 (16.0%)	85 (17.5%)	103 (15.0%)	
>4 mm	98 (8.3%)	56 (11.5%)	42 (6.1%)	
Type of melanoma				
Superficial spreading	784 (66.8%)	315 (64.7%)	469 (68.3%)	[b]0.02
Nodular	187 (15.9%)	96 (19.7%)	91 (13.2%)	
Lentigo maligna	153 (13.0%)	59 (12.1%)	94 (13.7%)	
Unknown/others	50 (4.3%)	17 (3.5%)	33 (4.8%)	
Regression of primary tumour				
Yes	80 (6.8%)	31 (6.4%)	49 (7.1%)	[b]0.61
No / Unknown	1094 (93.2%)	456 (93.6%)	638 (92.9%)	
Location of primary tumour				
Head/neck	160 (13.6%)	86 (17.7%)	74 (10.8%)	[b]<0.001
Trunk	490 (41.7%)	270 (55.4%)	220 (32.0%)	
Extremity	524 (44.6%)	131 (26.9%)	393 (57.2%)	

a Number of male versus female cases tested for statistical difference with t-test, equal variances not assumed.
b Number of male versus female cases tested for statistical difference with χ^2-test.

Table 4 – Multivariable linear regression analysis between Breslow thickness and statin use

Variables	Coefficient[a]	95% CI	*p*	Change in independent variable	Estimated % change in mean Breslow	95% CI
TOTAL (*n* = 1174)						
Statin use for at least 0.5 years	**−0.213**	**−0.40–0.02**	**0.03**	**Yes/No**	**−19.2**	**−33.2–2.3**
Nr. of dispenses of statin	−0.066	−0.14–0.004	0.06	4 dispenses	−6.4	−12.6–0.4
Cumulative duration of prescriptions	−0.052	−0.11–0.01	0.10	1 year	−5.1	−10.8–0.9
Cumulative dose	−0.058	−0.12–0.01	0.08	500 DDD	−5.6	−11.5–0.6
Average dose per day	−0.072	−0.15–0.01	0.10	0.66 DDD per day	−7.0	−13.8–0.6
MALE (*n* = 487)						
Statin use for at least 0.5 years	**−0.326**	**−0.57–0.08**	**0.01**	**Yes/No**	**−27.8**	**−43.7–−7.4**
Nr. of dispenses of statin	**−0.113**	**−0.20–0.02**	**0.02**	**4 dispenses**	**−10.7**	**−18.5–−2.2**
Cumulative duration of prescriptions	−0.073	−0.15–0.01	0.07	1 year	−7.0	−14.0–0.6
Cumulative dose	−0.077	−0.16–0.01	0.08	500 DDD	−7.4	−15.0–0.9
Average dose per day	**−0.116**	**−0.22–0.01**	**0.03**	**0.66 DDD per day**	**−11.0**	**−19.7–−1.2**
FEMALE (*n* = 687)						
Statin use for at least 0.5 years	−0.049	−0.35–0.25	0.75	Yes/No	−4.8	−29.6–28.8
Nr. of dispenses of statin	−0.006	−0.12–0.10	0.91	4 dispenses	−0.6	−11.0–11.0
Cumulative duration of prescriptions	−0.024	−0.13–0.08	0.65	1 year	−2.4	−11.8–8.2
Cumulative dose	−0.044	−0.14–0.06	0.39	500 DDD	−4.3	−13.4–5.8
Average dose per day	−0.010	−0.13–0.11	0.87	0.66 DDD per day	−1.0	−12.3–11.6

a Adjusted for age, gender (total group only), year of diagnosis, total number of unique ICD diagnoses, use of oestrogens (not in sub analysis males and use of NSAIDs).

men is simply associated with earlier diagnosis of a CM lesion and not with slower progression of the CM lesion.

4.3. *Strengths and limitations*

PALGA and PHARMO are general population based databases that closely reflect the Dutch population.[20,21] Moreover, pharmacy data are gathered prospectively. Therefore, recall bias is avoided.

Another strength of our study is that PHARMO enabled us to study dose-effect responses. For example, our data suggest thinner melanoma in patients who use higher doses of statins.

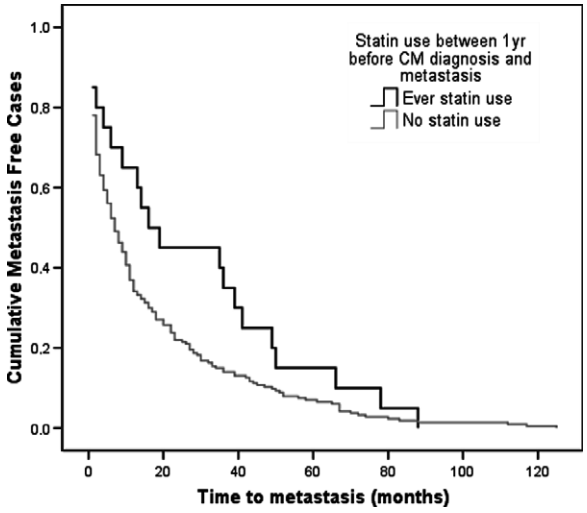


Fig. 3 – Statin use between 1 year before CM diagnosis and metastasis.

Since risk factors for melanoma do not play a role in the prescription of statins, confounding by indication seems unlikely. However, statin users are likely to have more health care contacts and therefore might be more likely to be diagnosed with melanoma. We included the number of unique medical diagnoses (ICD codes) in our study to adjust for this. Nevertheless, not all health consumption may be reflected in these diagnoses and ascertainment bias is still possible.

A limitation of our study is the relatively high frequency of simvastatin prescriptions; 63% of the prescriptions were simvastatin. Because the inhibitory effect of statins may not be equal for all statins,[26] the results of our study cannot be generalised to all statins.

We were not able to study the effects of statin use longer than 3 years before CM, but all patients included did have full follow up for the 3 years before diagnosis of CM. For some sub analyses the sample sizes may be too small. Most cases were excluded because they were registered in PHARMO in a different time period. Following this line of reasoning, with a required follow up of only 1 year the number of cases would increase from 1318 (37.0%) to 1697 (47.7%).

PHARMO does not provide information on lifestyle variables, such as sun exposure, a risk factor for the development of melanoma. It seems unlikely however that the use of statins is associated with sun exposure. However, it is possible that statin use is associated with the intake of certain foods and some authors have suggested that specific food items may influence the incidence of melanoma.[27]

Therefore, we cannot rule out residual biases or confounding as possible explanations for our findings. A possible causal relationship with regard to our findings should be studied in a prospective randomised trial.

4.4. *Time to metastasis*

In a small sample of about 250 patients, univariate analysis suggested that statin use may delay time to metastasis. After adjusting for Breslow thickness and other factors, this association was no longer significant (HR = 0.69, 95% CI = 0.42–1.1). To differentiate between the direct effects of statins on the process of metastasis and their effect on metastasis through Breslow thickness, we also performed an analysis excluding Breslow thickness. This model did show a significant effect of statin use on time to metastasis (HR = 0.58, 95% CI = 0.36–0.94), which suggests that the effect of statins on time to metastasis may not only be caused by the effect of statins on the Breslow thickness.

Unfortunately, we were not able to perform a sensitivity analysis, excluding cases with a positive sentinel node procedure ($N = 52$), since only one statin user had a positive sentinel node procedure. Therefore, bias due to early detection of metastasis in a sentinel node procedure is possible.

5. Conclusion

Our observational study suggests a protective effect of statins on the progression of melanoma. A validation of our findings is justified, preferably in a prospective randomised study. Also, linkage of datasets like ours to death registers may be helpful in the further exploration of the effect of statins on (progression of) melanoma.

Conflict of interest statement

None declared.

Acknowledgement

We thank Professor Jan Vandenbroucke for critical discussion of the design of the study and Dr. Mark Tinga for data selection in PHARMO.

REFERENCES

1. http://www.cancer.org/, Cancer facts and figures 2004, visited 7th Jan 2007.
2. Eigentler TK, Caroli UM, Radny P, Garbe C. Palliative therapy of disseminated malignant melanoma: a systematic review of 41 randomised clinical trials. *The Lancet Oncology* 2003;**4**:748–59.
3. Graaf MR, Richel DJ, van Noorden CJF, Guchelaar HJ. Effect of statins and farnesyltransferase inhibitors on the development and progression of cancer. *Cancer Treat Rev* 2004;**30**:609–41.
4. Demierre MF. What about chemoprevention for melanoma? *Curr Opin Oncol* 2006;**18**:180–4.
5. Goldstein JL, Brown MS. Regulation of the mevalonate pathway. *Nature* 1990;**343**:425–30.
6. Bos JL. Ras oncogenes in human cancer: A review. *Cancer Res* 1989;**49**:4682–9.
7. Clark EA, Golub TR, Lander ES, Hynes RO. Genomic analysis of metastasis reveals an essential role for RhoC. *Nature* 2000;**406**:532–5.
8. Collisson EA, Kleer C, Wu M, et al. Atorvastatin prevents RhoC isoprenylation, invasion, and metastasis in human melanoma cells. *Mol Cancer Ther* 2003;**2**:941–8.
9. Graaf MR, Beiderbeck AB, Egberts ACG, Richel DJ, Guchelaar HJ. The risk of cancer in users of statins. *J Clin Oncol* 2004;**22**:2388–94.
10. Dale KM, Coleman CI, Henyan NN, Kluger J, White CM. Statins and cancer. A meta-analysis. *J Am Med Assoc* 2006;**295**(1):74–80.
11. Bonovas S, Filioussi K, Tsavaris N, Sitaras NM. Statins and cancer risk: a literature-based meta-analysis and meta-regression analysis of 35 randomized controlled trials. *J Clin Oncol* 2006;**24**(30):4808–17.
12. Browning DRL, Martin RM. Statins and risk of cancer: a systematic review and meta-analysis. *Int J Cancer* 2006;**120**(4):833–43.
13. Cholesterol Treatment Trialists' (CTT) Collaborators. Efficacy and safety of cholesterol-lowering treatment: prospective meta-analysis of data from 90 056 participants in 14 randomized trials of statins. *Lancet* 2005; **366**: 1267–1278.
14. Bjerre LM, LeLorier J. Do statins cause cancer? A meta-analysis of large randomized clinical trials. *Am J Med* 2001;**110**:716–23.
15. Coogan PF, Smith J, Rosenberg L. Statin use and risk of colorectal cancer. *J Natl Cancer Inst* 2007;**99**(1):32–40.
16. Dellavalle RP, Drake A, Graber M, et al. *Cochrane Database Syst Rev* 2005;**19**(4):CD003697.
17. Freeman SR, Drake AL, Heilig LF, et al. Statins fibrates and melanoma risk: a systematic review and meta-analysis. *J Natl Cancer Inst* 2006;**98**(21):1538–46.
18. http://www.pharmo.nl/, PHARMO pharmacy database, visited Jan 8th 2007.
19. Lau HS, de Boer A, Beuning KS, et al. Validation of pharmacy records in drug exposure assessment. *J Clin Epidemiol* 1997;**50**:619–25.
20. Herings R. Pharmo, A record linkage system for postmarketing surveillance of prescription drugs in the Netherlands [thesis in pharmaco-epidemiology and pharmacotherapy]. The Netherlands, Utrecht University; 1993: 17–32.
21. Casparie MK, Tiebosch T, Burger G, et al. Pathology databanking and biobanking in The Netherlands, a central role for PALGA, the nationwide histopathology and cytopathology data network and archive. *Cellular Oncology* 2007;**29**(1):19–24.
22. LeBoit PE, Burg G, Weedon D, Sarasin A (editors). WHO classification of tumours Volume 6, IARC WHO classification of tumours No 6, Pathology and genetics of tumours of the skin; 2005.
23. Johnson ES. Pharmacy databases can identify waste. *Pharmacoepidemiol Drug Saf* 2006;**15**:207–9.
24. Gimotty PA, Botbyl J, Soong S, Guerry DP. A population based validation of the American Joint Committee on Cancer Melanoma Staging System. *J Clin Oncol* 2005;**23**:8065–75.
25. de Vries E, Houterman S, Janssen-Heijnen MLG, et al. Up-to-date survival estimates and historical trends of cutaneous malignant melanoma in the south-east of The Netherlands. *Ann Oncol* 2007;**18**:1110–6.
26. Negre-Aminou P, van Vliet AK, van Erck M, et al. Inhibition of proliferation of human smooth muscle cells by various HMG-CoA redyuctase inhibitors: Comparison with other human cell types. *Biochim Biochim Biophys Acta* 1997;**1345**:259–68.
27. Vinceti M, Bonvicini F, Pellacani G et al. Food intake and risk of cutaneous melanoma in an Italian population. *Eur J Clin Nutr* 2007 [epub ahead of printing].