Article type: Original article

Title: Statins may reduce disease recurrence in patients with ulcerated primary melanoma

Lena A von Schuckmann, MBBS, MPH[1,2], Kiarash Khosrotehrani, MD, PhD[3,4], Reza Ghiasvand, PhD[5] Maria Celia B Hughes, MMedSci[1], Jolieke C van der Pols, PhD[6], Maryrose Malt, B.Bus[1], B Mark Smithers MBBS[7], Adele C Green, MBBS, PhD[1,8]

1. *Population Health Department, QIMR Berghofer Medical Research Institute, Australia*
2. *School of Public Health, The University of Queensland, Australia*
3. *Experimental dermatology group, The University of Queensland Diamantina Institute, Translational research institute, Brisbane, Australia*
4. *Department of Dermatology, Princess Alexandra Hospital, Brisbane, Australia*
5. *Oslo Centre for Biostatistics and Epidemiology, Department of Biostatistics, Institute of Basic Medical Sciences, University of Oslo, Norway*
6. *School of Exercise and Nutrition Sciences, Queensland University of Technology, Australia*
7. *Queensland Melanoma Project, Princess Alexandra Hospital, The University of Queensland, Australia*
8. *CRUK Manchester and Faculty of Biology, Medicine and Health, University of Manchester, Manchester, UK*

Corresponding author:

Lena von Schuckmann
QIMR Berghofer Institute of Medical Research, 300 Herston Road, Herston (4006), Australia
Phone +61 7 3362 3226
Email: lena.vonschuckmann@uqconnect.edu.au

Acknowledgements:

The study is supported by NHMRC Program Grants 1073898 and 552429. LAvS is funded by NHMRC Postgraduate Scholarship 1133317, KK is funded by a NHMRC Career Development Fellowship and RGH is funded by the Norwegian Cancer Society (6823329). The funding body played no role in study design; in the collection, analysis and interpretation of data; in the writing of the report; or in the decision to submit the article for publication.

Running head: Statins and ulcerated primary melanoma

Presentation: None

Conflicts of interest: None

Word count: 2276

Figures & tables: 3 tables

Abstract: 241 words

Purpose

Statins may restrict cellular functions required for melanoma growth and metastasis. We examined whether long-term statin use commenced before diagnosis of the primary is associated with reduced risk of melanoma recurrence.

Patients and methods

We prospectively followed a cohort of patients newly diagnosed between 2010 and 2014 with localised tumour-stage T1b to T4b melanoma in Queensland, Australia. We used Cox-regression analyses to examine associations between long-term statin use and melanoma recurrence for the entire cohort, and then separately by sex and by presence of ulceration due to evidence of effect modification.

Results

Amongst 700 patients diagnosed with stage T1b to T4b primary melanoma (mean age 62, 59% male, 28% with ulcerated tumours), 94 patients (13%) developed melanoma recurrence within 2 years. Long-term statin users (n=204, 29%) had a significantly lower risk of disease recurrence compared to non-users (Adjusted hazard ratio (HR_{adj}) 0.55, 95% Confidence Interval (CI) 0.32-0.97) regardless of statin subtype or potency. Compared to non-statin users, risk of recurrence was significantly decreased in male statin-users (HR_{adj} 0.39, 95% CI 0.19-0.79) but not female statin users (HR_{adj} 0.82, 95% CI 0.29-2.27) and in statin-users with ulcerated (HR_{adj} 0.17, 95% CI 0.05-0.52) but not non-ulcerated (HR_{adj} 0.91, 95% CI 0.46-1.81) primary melanoma.

Conclusion

Statins commenced before melanoma diagnosis, may reduce the risk of melanoma recurrence, especially in males and those with ulcerated tumours. Clinical trial evaluation of the potential role of statins in improving the prognosis of high-risk melanoma is warranted.

Introduction

Melanoma is the most serious form of skin cancer and incidence rates are increasing in most Caucasian populations, with the greatest disease burden occurring in Australian, North American and European populations[1-3]. Thin (≤1mm) localised melanomas are usually curable with surgical resection, with long-term patient survival exceeding 98% in the population[4]. Thick tumours and ulcerated tumours are associated with high rates of disease recurrence, and despite novel treatments for metastatic disease, long-term prognosis remains relatively poor[5]. Therefore, an important approach to decreasing disease burden is identifying other ways to halt disease progression and metastasis. With emerging preclinical evidence for the anticancer effects of commonly used medications such as statins, it has become a research priority to evaluate their potential efficacy in preventing recurrence or as adjunct treatment for advanced disease [6].

Statins lower serum cholesterol by inhibiting the enzyme 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase[7] and have proven preventive and therapeutic effects in cardiovascular diseases[8]. Statins also reduce mevalonate production and its downstream products which play important roles in cancer biology by inhibiting pathways required for growth and metastasis[9,10]. We recently described the lower likelihood of statin users compared with non-users being diagnosed with ulcerated melanomas[11], hypothesising that statins inhibited the inflammatory pathways associated with tumour ulceration[12]. Based on in-vitro[13-15] and animal models[16,17], it is further possible that statins' anti-inflammatory properties inhibit growth and progression of melanoma cells. Epidemiological and clinical evidence regarding statins' ability to prevent melanoma recurrence in humans is inconclusive however, as most published studies are limited by their use of crude outcome measures such as overall survival, or of routine cancer registry data which lack clinical and histopathological details [18-20].

In order to justify expensive clinical trials, strong observational evidence for statins' possible role in reducing melanoma recurrence is required. We therefore investigated the association between statin

use and melanoma recurrence in a large prospective study of patients newly diagnosed with primary melanoma at high risk of spread.

Methods

Study population

Participants were recruited prospectively between 2010 and 2014, from various specialist public hospital clinics, private clinics of collaborating surgeons and pathology services in Queensland. Patients were invited to participate if they had a histologically-confirmed new diagnosis of tumour stage 1b to 4b cutaneous melanoma, were 16 years or older, and had capacity to complete the study questionnaire. Patients who were found to have macroscopic lymph node involvement or visceral metastatic disease within 30 days of the primary melanoma diagnosis were ineligible. Of 1,254 invited patients, 825 (66%) gave written consent to take part, of whom 125 (10%) later proved ineligible with additional clinical information (n=36) and the change from the 7th to the 8th AJCC melanoma staging criteria[5] (n=89), leaving 700 eligible patients. Details of the study have been described elsewhere[21]. The study was approved by the Human Research Ethics Committees of the Metro South Health Service and the QIMR Berghofer Medical Research Institute.

Data collection

All participants completed a self-administered questionnaire at baseline giving personal details, including sex, age, height and weight, smoking history (current, ex-smoker, never smoked) and personal history of melanoma (later confirmed by histology reports). Information about melanoma in a first degree relative (yes, no) was obtained and whether participants had ever been diagnosed with diabetes or cardiovascular disease (yes, no). Patients were asked to report use of statins in the preceeding 5 years, including drug class, dosage (mg), frequency (daily, weekly, etc.) and starting and stopping dates. History of statin use was updated by questionnaire for all active participants in April

2016. For patients who were passive participants (no longer actively completing questionnaires), information on statin use was obtained from clinical records.

Histological details of all primary melanomas were extracted from pathology reports including thickness (mm), presence of ulceration (yes, no), regression (yes, no) or mitosis (per mm^2 or per high power field) as well as site (head or neck, trunk, upper limb, lower limb) and melanoma subtype. Results from sentinel lymph node biopsies (SLNB) (if performed) were collected from clinical notes and pathology reports.

Patients completed 6-monthly follow-up questionnaires (repeat of baseline survey) in the first 2 years after diagnosis when melanoma recurrences were self-reported and then confirmed by histology, imaging and clinic reports. We defined a recurrence as histological or radiological evidence of a metastatic melanoma deposit (local, in-transit, lymph node or distant organ), diagnosed at least 1 month after diagnosis of the primary melanoma. Hospital records and the Queensland Cancer Registry were searched 6-monthly for information about recurrences or deaths in patients passively followed up.

Statistical analysis

Based on patients' responses to the baseline questionnaire or the follow-up statin survey, or information provided by clinic letters, patients were categorised as *long-term* statin users if they were taking 3 or more doses per week, every week, in the 2 years before until 1 year after primary melanoma diagnosis, and *ever-users* if they took statins for any continuous 3-month period. Based on biochemical properties, statins were classified as lipophilic (atorvastatin, simvastatin) or lipophobic subtypes (rosuvastatin, pravastatin); and low/moderate potency or high potency (high potency defined as atorvastatin ≥40mg or rosuvastatin ≥20mg)[22]. Body mass index (BMI) was calculated as weight (kg)/height $(m)^2$ and categorised as <25, 25-30, or >30.

Baseline characteristics were described according to recurrence status (yes/no) and statin use (ever/never) with frequencies and proportions, and differences between groups were assessed using Pearson's chi-squared test (significance level $p<0.05$). To determine whether statin use was associated with disease recurrence, we used unadjusted and adjusted proportional-hazard regression models with *long-term* statin use as the exposure variable and 2-year disease recurrence as the primary outcome variable. Those with no recurrence, and those who died within 2 years before a recurrence occurred (n=3, all melanoma-related deaths), were censored at 24 months or date of death respectively. We assessed confounding by patient and tumour characteristics and adjusted the final model for age, sex, diabetes, heart disease, smoking status and BMI. Proportionality was assessed using time-dependent covariates. Because of evidence of effect modification (identified with interaction terms; $p<0.05$), the final model was then also stratified by sex and ulceration. Subgroup analyses were conducted in patients with ulcerated tumours to examine the associations between statin potency and subtype and melanoma recurrence. We conducted several sensitivity analyses in the subgroup of patients with ulcerated tumours: excluding patients with a past history of melanoma; excluding patients with a positive SLNB; and including known prognostic factors as co-variates (thickness, mitotic rate, site of primary and subtype). Recurrence-free survival (RFS) proportions at 2 years after diagnosis were calculated whilst stratifying by sex and ulceration. All analyses were conducted using SAS Version 9.4 (SAS Institute, Cary, NC).

Results

Patient and tumour characteristics

Of the 700 participants (mean age 62 years, 59% male, 20% with a previous melanoma), 94 (13%) developed a confirmed recurrence within 2 years of diagnosis and during 15512.65 person-months of follow-up (recurrence rate: 6.1/1000 person-months)[23]. As expected, recurrence was more common

in patients with thick or ulcerated tumours, or with >3/mm^2 mitotic rate or located on the head or neck; recurrence was less common in superficial spreading melanomas compared to other subtypes (all p<0.05) (Table 1). One-third of patients (n=223, 32%) had taken statins for at least 3 continuous months in the time from 2 years before until 1 year after diagnosis, while two-thirds had never taken statins (n=474, 67%). Statin history was unknown for 3 patients. Most statin users were long-term users (n=204, 92%) with only 12 (5%) patients ceasing statins before diagnosis of the study melanoma and 7 (3%) commencing statins after diagnosis. The most frequently used long-term statin was atorvastatin (n=100, 49%), while 56 (27%) used rosuvastain, 36 (18%) simvastatin and 7 (4%) pravastatin (5 (2%) were missing). Statin users versus non-users were more commonly male, older than 65 years, BMI >30 and more commonly had cardiovascular disease or diabetes (Table 2).

Risk of recurrence

A post-hoc power analysis indicated that our study had 90.7% power to detect a significant difference in recurrence rates for statin users versus non-users, for a significance level of p<0.05. The risk of melanoma recurrence within 2 years of diagnosis was significantly lower in the 204 (29%) long-term statin users compared to non-users of statins (HR_{adj} 0.55, 95% CI 0.32-0.97) (Table 3). Patients who took statins for at least 3 months prior to melanoma diagnosis (n=216) also had a reduced risk of recurrence (HR 0.57, 95% CI 0.33-0.98), as did those who took statins for at least 3 months after their diagnosis (n=211) (HR 0.62, 95% CI 0.36-1.05). When conducting analyses by presence or absence of tumour ulceration, risk of recurrence was lower in statin users versus non-users with ulcerated tumours, but not in statin users with non-ulcerated tumours (HR 0.17, 95% CI 0.05-0.52; HR 0.91, 95% CI 0.46-1.81 respectively) (Table 3). Similarly, a lower risk of recurrence was seen in male users versus non-users but not female users versus non-users (HR 0.39, 95% CI 0.19-0.79; HR 0.82, 95% CI 0.29-2.27 respectively). Amongst patients with ulcerated tumours, lipophilic subtypes as well as low/moderate potency and high potency statins, significantly reduced recurrence risk (Table 3).

The association between long-term statin use and melanoma recurrence persisted in sensitivity analyses conducted in subgroups of patients: with ulcerated tumours (n=195); after excluding patients with a past history of melanoma (HR 0.10, 95% CI 0.02-0.42); after excluding patients who had a positive SLNB at diagnosis (HR 0.22, 95% CI 0.07-0.74).

Survival proportions

2-year RFS was 89% for long-term statins users, versus 85% for non-users (p=0.2) (Table 4). For patients with ulcerated tumours, 2-year survival differed significantly according to whether or not they were long-term users of statins (88% versus 74% RFS respectively, p<0.0001). Statin use had the greatest impact on 2-year RFS in males with ulcerated melanomas, where males who were not taking statins had a 65% RFS, versus a 91% RFS for regular users (p<0.0001).

Discussion

This study of 700 patients with tumour stage T1b-T4 primary cutaneous melanoma suggests a dramatic effect of long-term statin use commenced prior to diagnosis in significantly lowering risk of melanoma recurrence within 2 years of diagnosis compared to recurrence rate in non-users. Statins' apparent protective effect was greatest in male patients and those with ulcerated primary melanomas and did not differ by statin potency.

Based on findings here and elsewhere [11,14-17], we propose that tumours in people exposed to statins during melanoma development adopt a less aggressive phenotype. Statins prevent the conversion of HMG-CoA to mevalonate, thereby reducing downstream products necessary for critical cellular functions[10]. Disruptions of these processes in melanoma cells by statins have been shown to reduce growth and metastasis in-vitro[14,15], in animal[16,17] and cohort[11,19] studies. We propose that statins

render the tumour and its microenvironment less susceptible to tumour spread by inhibiting common pathways associated with tumour inflammation, ulceration and metastasis. In favour of this hypothesis is our previous finding that melanoma ulceration (a highly inflamed[12,24-26] and proliferative phenotype associated with higher recurrence and death[5]) is less common amongst long-term statin users[11]. In our current study we observed that statins' reduction of recurrence appeared strongest in ulcerated tumours, suggesting that in those developing ulcerated melanomas despite exposure to statins, the tumour and/or its environment were tempered by the drug. Limitations include the relatively short follow-up time, though our rates of first recurrence are consistent with reports from other populations[27]. It is possible that these findings could be explained by unrecognised confounding. The lack of dose-response relationship between statin use and disease recurrence also detracts somewhat from the biologic plausibility of our results. We were unable to validate patients' medication use though it is unlikely that factors such as recall bias was differential and could explain our results given the prospective nature of the study.

Statins' protective effect may be greater in males, as observed here and elsewhere[19]. In fact, statin use appears to overcome the prognostic disadvantage of males compared with females[19]. This may be partly explained by Rac1, the third most common somatic mutation in melanoma, being more common in men than women and studies demonstrate that statins counteract the biologic effect of this mutation[28]. It is also possible that males experience statins' anti-melanoma immune responses more strongly [29], due to reported differences in the immune system and response to immune therapies between males and females[30,31]. Alternatively, sex differences may be due to unknown confounding or chance.

The literature suggests that statins' anticancer effects may differ by subtype and potency. Lipophilic statin subtypes can penetrate the plasma membrane (e.g. rosuvastatin and pravastatin) and have been reported to decrease melanoma cell viability under in-vitro conditions, yet the required dose may exceed safe levels[32]. Our findings showed that lipophilic statins at commonly prescribed doses

significantly reduced melanoma recurrence. In contrast, the anticancer effects of lipophobic subtypes which are unable to penetrate the cell membrane (e.g. atorvastatin and simvastatin) have been more inconsistent[33]. Whilst not statistically significant, our results are consistent with mouse[34,35] and in-vitro[17] studies and suggest a protective effect in both lipophilic and lipophobic subtypes. Subgroup analyses were limited by small numbers and require validation in larger studies.

Strengths of this study were its prospective design and well-characterised cohort. Detailed tumour characteristics were obtained from histology reports and inter-observer agreement in reporting melanoma features is high in Australia[36]. We used disease recurrence as our primary outcome and all recurrences were confirmed histologically and/or radiologically. Statin use was self-reported, with high consistency in reporting between surveys and clinic letters[11]. Most statin users were long-term users, with few patients starting or stopping within the 3-year study period.

In conclusion, we have demonstrated that statin use commenced before and continued after melanoma diagnosis, may reduce the risk of disease recurrence. These findings strongly support further evaluation of the potential role of statins in improving the prognosis of high-risk melanoma.

Table 1. Baseline characteristics of 700 patients with primary melanoma in relation to 2-year disease recurrence

	Total (700) N (%)	**No recurrence (n=606, 83%) N (%)**	**Recurrence (n=94, 13%) N (%)**	**Chi-square P-value**
Patient factors				
Age (years)				
< 65	360 (51)	319 (53)	41 (56)	0.10
≥65	340 (49)	287 (47)	53 (44)	
Sex				
Male	410 (59)	345 (57)	65 (69)	0.03
Female	290 (41)	261 (43)	29 (31)	
Previous melanoma				
No	563 (80)	488 (81)	75 (80)	0.87
Yes	137 (20)	118 (20)	19 (20)	
Tumour factors				
Thickness (mm)				
≤1	121 (17)	115 (19)	6 (6)	<0.0001
>1 - 2	312 (45)	281 (46)	31 (33)	
>2 - 4	178 (25)	145 (24)	33 (35)	
>4	89 (13)	65 (11)	24 (26)	
Ulceration				
No	504 (72)	454 (75)	50 (53)	<0.0001
Yes	196 (28)	152 (25)	44 (47)	
Mitosis (no./mm^2)				
<1	88 (13)	83 (14)	5 (5)	<0.0001
1-3	296 (43)	277 (47)	19 (21)	
>3	300 (44)	233 (39)	67 (74)	
Subtype				
SSM[1]	278 (40)	253 (42)	25 (27)	0.01
Nodular	172 (25)	145 (24)	27 (29)	
Other[2]	118 (17)	103 (17)	15 (16)	
Not classified[3]	132 (19)	105 (17)	27 (29)	
Body site (location)				
Trunk	247 (35)	217 (36)	30 (32)	<0.0001
Head/neck	154 (22)	116 (19)	38 (40)	
Upper limb	143 (21)	136 (22)	7 (8)	
Lower limb	156 (22)	137 (23)	19 (20)	

1- Superficial spreading melanoma
2- Other: lentigo maligna (18%), desmoplastic (36%), naevoid (21%), spitzoid (4%), lentiginous (2%), acral lentiginous (7%), mixed (11%)
3- Not classified: unable to classify (15%), not stated (84%), other (1%)

Table 2. Baseline characteristics of patients with primary melanoma in relation to statin use

	Non-user (n=474) N (%)	Ever-user[5] (n=223) N (%)	Chi-square p-value (n=95) N (%)
Patient factors			
Age (years)			
< 65	298 (63)	62 (28)	<0.0001
≥65	176 (37)	161 (72)	
Sex			
Male	259 (55)	148 (66)	0.003
Female	215 (45)	75 (34)	
Previous melanoma			
No	383 (81)	177 (79)	0.66
Yes	91 (19)	46 (21)	
BMI			
<25	140 (32)	38 (18)	<0.0001
25-30	189 (43)	75 (36)	
>30	110 (25)	94 (46)	
Smoking			
Never/ ex-smoker	412 (92)	199 (93)	0.66
Current smoker	38 (8)	16 (7)	
Cardiovascular disease			
No	446 (94)	162 (73)	<0.0001
Yes	28 (6)	61 (27)	
Diabetes			
No	445 (94)	158 (71)	<0.0001
Yes	29 (6)	65 (29)	
Tumour factors			
Thickness (mm)			
≤1	80 (17)	41 (18)	0.65
>1 - 2	219 (46)	93 (42)	
>2 - 4	121 (25)	57 (26)	
>4	57 (12)	32 (14)	
Ulceration			
No	336 (71)	167 (75)	0.27
Yes	138 (29)	56 (25)	
Mitosis (no./mm^2)			
<1	54 (12)	34 (16)	0.31
1-3	203 (43)	93 (43)	
>3	210 (45)	90 (41)	
Subtype			
SSM[1]	192 (41)	86 (39)	0.15
Nodular	121 (25)	48 (22)	
Other[2]	70 (15)	48 (21)	
Not classified[3]	91 (19)	41 (18)	
Body site (location)			
Trunk	174 (37)	71 (32)	0.57
Head/neck	104 (22)	49 (22)	
Upper limb	92 (19)	51 (23)	
Lower limb	104 (22)	52 (23)	

1- Superficial spreading melanoma

2- Other: lentigo maligna (18%), desmoplastic (36%), naevoid (21%), spitzoid (4%), lentiginous (2%), acral lentiginous (7%), mixed (11%)

3- Not classified: unable to classify (15%), not stated (84%), other (1%)

4- missing=3

5- Ever user: any continuous 3 month period from 2 years before diagnosis until 1 year after diagnosis

Table 3: Risk of 2-year melanoma recurrence in long-term statin users

	Non-user	Non-user with recurrence	Long-term Statin user	Long-term statin user with recurrence	Non-user	Statin user HR (95% CI)	P-value
All patients[1]							
Statin use							
Unadjusted	493	71	204	22	1.00	0.73 (0.45-1.18)	0.20
Adjusted for age, sex	493	71	204	22	1.00	0.59 (0.36-0.97)	0.04
Adjusted[2]	493	71	204	22	1.00	0.53 (0.30-0.92)	0.02
Adjusted[3]	493	71	204	22	1.00	0.55 (0.32-0.97)	0.04
Stratified analyses[2]							
Sex							
Male	274	51	133	13	1.00	0.39 (0.19-0.79)	0.009
Female	219	20	71	9	1.00	0.82 (0.29-2.27)	0.70
Ulceration							
Non-ulcerated	351	34	153	17	1.00	0.91 (0.46-1.81)	0.79
Ulcerated	142	37	51	5	1.00	0.17 (0.05-0.52)	0.002
Combined							
Male, nonulcerated	184	20	99	11	1.00	0.98 (0.42-2.28)	0.96
Male, ulcerated	90	31	34	2	1.00	0.05 (0.006-0.43)	0.006
Female, nonulcerated	167	14	54	6	1.00	0.92 (0.26-3.19)	0.89
Female, ulcerated	52	6	17	3	1.00	0.23 (0.03-1.91)	0.17
Subgroup analyses (ulcerated tumours)							
Subtype							
Lipophilic[3]	142	37	30	3	1.00	0.15 (0.03-0.69)	0.01
Lipophobic[4]	142	37	14	2	1.00	0.30 (0.07-1.33)	0.11
Potency[5]							
Low/moderate	141	37	24	3	1.00	0.20 (0.04-0.93)	0.04
High	141	37	21	2	1.00	0.18 (0.04-0.78)	0.02

1. Missing=3
2. Adjusted for age, sex, BMI, diabetes, heart disease and smoking
3. Adjusted for age, sex, BMI, diabetes, heart disease, smoking, thickness, ulceration, mitotic rate, subtype, body site
4. Lipophilic: atorvastatin and simvastatin
5. Lipophobic: rosuvastatin, pravastatin
6. Missing dose=7

Table 4: 2-year survival proportions by long-term statin use, by sex and tumour ulceration

	2-year survival proportion	Log-rank p-value
Statin use		
Non-user	85%	0.20
User	89%	
Statin use, stratified by sex		
Male, non-user	81%	0.009
Male, user	89%	
Female, non-user	91%	
Female, user	88%	
Statin use, stratified by ulceration		
No ulceration, non-user	90%	<0.0001
No ulceration, user	89%	
Ulceration, non-user	74%	
Ulceration, user	88%	
Statin use, stratified by sex and ulceration		
Male, no ulceration, non-user	89%	<0.0001
Male, no ulceration, user	89%	
Male, ulceration, non-user	65%	
Male, ulceration, user	91%	
Female, no ulceration, non-user	92%	
Female, no ulceration, user	89%	
Female, ulceration, non-user	88%	
Female, ulceration, user	82%	

References

1. Guy GP, Jr., Machlin SR, Ekwueme DU, Yabroff KR. Prevalence and costs of skin cancer treatment in the U.S., 2002-2006 and 2007-2011. *Am J Prev Med.* 2015;48(2):183-187.
2. Whiteman DC, Green AC, Olsen CM. The Growing Burden of Invasive Melanoma: Projections of Incidence Rates and Numbers of New Cases in Six Susceptible Populations through 2031. *J Invest Dermatol.* 2016;136(6):1161-1171.
3. Karimkhani C, Green AC, Nijsten T, et al. The global burden of melanoma: results from the Global Burden of Disease Study 2015. *Br J Dermatol.* 2017;177(1):134-140.
4. Green AC, Baade P, Coory M, Aitken JF, Smithers M. Population-based 20-year survival among people diagnosed with thin melanomas in Queensland, Australia. *J Clin Oncol.* 2012;30(13):1462-1467.
5. Gershenwald JE, Scolyer RA, Hess KR, et al. Melanoma staging: Evidence-based changes in the American Joint Committee on Cancer eighth edition cancer staging manual. *CA Cancer J Clin.* 2017;67(6):472-492.
6. Mei Z, Liang M, Li L, Zhang Y, Wang Q, Yang W. Effects of statins on cancer mortality and progression: A systematic review and meta-analysis of 95 cohorts including 1,111,407 individuals. *Int J Cancer.* 2017;140(5):1068-1081.
7. Schachter M. Chemical, pharmacokinetic and pharmacodynamic properties of statins: an update. *Fundam Clin Pharmacol.* 2005;19(1):117-125.
8. Chou R, Dana T, Blazina I, Daeges M, Jeanne TL. Statins for Prevention of Cardiovascular Disease in Adults: Evidence Report and Systematic Review for the US Preventive Services Task Force. *JAMA.* 2016;316(19):2008-2024.
9. Fritz G. Targeting the mevalonate pathway for improved anticancer therapy. *Curr Cancer Drug Targets.* 2009;9(5):626-638.
10. Boudreau DM, Yu O, Johnson J. Statin use and cancer risk: a comprehensive review. *Expert opinion on drug safety.* 2010;9(4):603-621.
11. von Schuckmann LA, Smith D, Hughes MCB, et al. Associations of Statins and Diabetes with Diagnosis of Ulcerated Cutaneous Melanoma. *The Journal of investigative dermatology.* 2017;137(12):2599-2605.
12. Jewell R, Elliott F, Laye J, et al. The clinicopathological and gene expression patterns associated with ulceration of primary melanoma. *Pigment cell & melanoma research.* 2015;28(1):94-104.
13. Favero GM, M FO, Oliveira KA, et al. Simvastatin impairs murine melanoma growth. *Lipids Health Dis.* 2010;9:142.
14. Saito A, Saito N, Mol W, et al. Simvastatin inhibits growth via apoptosis and the induction of cell cycle arrest in human melanoma cells. *Melanoma Res.* 2008;18(2):85-94.
15. Glynn SA, O'Sullivan D, Eustace AJ, Clynes M, O'Donovan N. The 3-hydroxy-3-methylglutaryl-coenzyme A reductase inhibitors, simvastatin, lovastatin and mevastatin inhibit proliferation and invasion of melanoma cells. *BMC Cancer.* 2008;8:9.
16. Kidera Y, Tsubaki M, Yamazoe Y, et al. Reduction of lung metastasis, cell invasion, and adhesion in mouse melanoma by statin-induced blockade of the Rho/Rho-associated coiled-coil-containing protein kinase pathway. *J Exp Clin Cancer Res.* 2010;29(1).
17. Pich C, Teiti I, Rochaix P, et al. Statins reduce melanoma development and metastasis through MICA overexpression. *Front Immunol.* 2013;4(MAR).
18. Koomen ER, Joosse A, Herings RM, et al. Is statin use associated with a reduced incidence, a reduced Breslow thickness or delayed metastasis of melanoma of the skin? *Eur J Cancer.* 2007;43(17):2580-2589.
19. Livingstone E, Hollestein LM, van Herk-Sukel MPP, et al. Statin use and its effect on all-cause mortality of melanoma patients: a population-based Dutch cohort study. *Cancer Medicine.* 2014;3(5):1284-1293.

20. Nielsen SF, Nordestgaard BG, Bojesen SE. Statin Use and Reduced Cancer-Related Mortality. *N Engl J Med.* 2012;367(19):1792-1802.
21. Smithers BM, Hughes MCB, Beesley VL, et al. Prospective study of patterns of surgical management in adults with primary cutaneous melanoma at high risk of spread, in Queensland, Australia. *Journal of surgical oncology.* 2015;112(4):359-365.
22. Allan GM, Lindblad AJ, Comeau A, et al. Simplified lipid guidelines: Prevention and management of cardiovascular disease in primary care. *Canadian Family Physician.* 2015;61(10):857-867.
23. von Schuckmann LA, Celia B Hughes M, Lee R, et al. Survival of patients with early invasive melanoma down-staged under the new 8th AJCC edition. *J Am Acad Dermatol.* 2018.
24. Melnikova VO, Bar-Eli M. Inflammation and melanoma metastasis. *Pigment cell & melanoma research.* 2009;22(3):257-267.
25. Mantovani A, Allavena P, Sica A, Balkwill F. Cancer-related inflammation. *Nature.* 2008;454(7203):436-444.
26. Winnepenninckx V, Lazar V, Michiels S, et al. Gene expression profiling of primary cutaneous melanoma and clinical outcome. *Journal of the National Cancer Institute.* 2006;98(7):472-482.
27. Leiter U, Meier F, Schittek B, Garbe C. The natural course of cutaneous melanoma. *Journal of surgical oncology.* 2004;86(4):172-178.
28. Krauthammer M, Kong Y, Ha BH, et al. Exome sequencing identifies recurrent somatic RAC1 mutations in melanoma. *Nature genetics.* 2012;44(9):1006-1014.
29. Kwak B, Mulhaupt F, Myit S, Mach F. Statins as a newly recognized type of immunomodulator. *Nature medicine.* 2000;6(12):1399-1402.
30. Bouman A, Heineman MJ, Faas MM. Sex hormones and the immune response in humans. *Human reproduction update.* 2005;11(4):411-423.
31. Conforti F, Pala L, Bagnardi V, et al. Cancer immunotherapy efficacy and patients' sex: a systematic review and meta-analysis. *The Lancet Oncology.* 2018;19(6):737-746.
32. Maj M, Czajkowski R, Zegarska B, Kowaliszyn B, Pokrywczynska M, Drewa T. Anti-proliferative and cytotoxic activity of rosuvastatin against melanoma cells. *Advances in Dermatology and Allergology/Postępy Dermatologii i Alergologii.* 2016;33(4):257-262.
33. Urbich C, Dernbach E, Zeiher AM, Dimmeler S. Double-edged role of statins in angiogenesis signaling. *Circ Res.* 2002;90(6):737-744.
34. Tsubaki M, Takeda T, Kino T, et al. Statins improve survival by inhibiting spontaneous metastasis and tumour growth in a mouse melanoma model. *Am J Cancer Res.* 2015;5(10):3186-3197.
35. Zanfardino M, Spampanato C, De Cicco R, et al. Simvastatin reduces melanoma progression in a murine model. *Int J Oncol.* 2013;43(6):1763-1770.
36. Murali R, Cochran AJ, Cook MG, et al. Inter-observer reproducibility of histologic parameters of melanoma deposits in sentinel lymph nodes: implications for management of patients with melanoma. *Cancer.* 2009;115(21):5026-5037.